PROCEEDINGS BOOK

Abstracts & Full Papers

5th ISPBS

The 5th International Symposium on
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April 26-28, 2019

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The Fifth International Symposium on Pharmaceutical and Biomedical Sciences

ISPBS – 5

PROCEEDINGS BOOK

ABSTRACTS & FULL PAPERS

April 26th – 28th, 2019

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Dear colleagues,

Having respected scientific board and organizing committee members from all over the world, ISPBS Symposium series started in 2016. The First Japan-Turkey International Symposium on Pharmaceutical and Biomedical Sciences (JATUSPAB) was organized by Kumamoto University, Kumamoto – Japan on October 2-3, 2016. This scientific organization was the first meeting of Turkish and Japanese scientists on our international collaboration and projects. In this meeting, more than twenty scientific works were presented orally or as posters.

Participants of the first meeting were from Turkey, Japan, Nepal, China, Egypt, Iraq and Sudan. After this successful meeting, organizing committee members from both sides, Turkish and Japanese scientists, decided to continue this valuable and fruitful scientific collaboration. Thus, "The Second Japan-Turkey International Symposium on Pharmaceutical and Biomedical Sciences, JATUSPAB-2" had been decided to be organized in Trabzon, Turkey during September 11-12, 2017. Although the title of the symposium covers Japan and Turkey, this valuable scientific meeting with unique social activities has brought together all scientists worldwide from all disciplines especially studying "Pharmaceutical and Biomedical" in Trabzon province of Turkey, where the green and blue meet. Indian Journal of Pharmaceutical Education and Research (IJPER), indexed with THOMSON REUTERS published a special issue covering some of the full papers selected after scientific evaluation. Then, the symposium, constituted the closing meeting of JSPS Bilateral Joint Research Project, was held on Kumamoto University, Kumamoto – Japan on March 17-19, 2017

That symposium was the fifth meeting series of ISPBS, and you can find abstracts of all the scientific works presented in ISPBS-5 in this PROCEEDINGS BOOK. We are proud to announce that selected full papers will be published in the contracted journals of the symposium after scientific evaluation. We are happy to invite ISPBS-5 participants to submit their full papers which were presented at the symposium 'Molecules', 'Annals of Phytomedicine', International Journal of Agriculture, Environment and Food Sciemces', 'Journal of Institute of Science and Technology-Iğdır University', and 'Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)'.

We would like to thank for their sincere supports of Turkish General Directorate of Forestry, TURKISH AIRLINES, Austrian Drug Screening Institite ÇAYKUR, Kilis 7 Aralık University, Khon Kaen University, Kumamoto University, Iğdır University, Rural Federal University of Rio de Janeiro (UFRRJ)-Brazil, AMAPMED, Association of Pharmaceutical Teachers of India, Cosmetic Producers and Researchers Associatons, Talya Herbal Compony, and MASMANA Olive Oils and all the other supporters. Moreover, organizing committee members would like to thank you all the participants their valuable scientific participation.

Organizing Committee hope that ISPBS-5 Symposium participants would have an amazing experience and unforgettable memories to take back their homes, and would like to thanks for all ISPBS-5 participants for their valuable contributions. We would like to remind you that ISPBS Symposium series will be organized every year. Hope to meet you in the sixth meeting series of ISPBS-5 in 2020 spring.

Sincerely,
Symposium Chairman

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PLENARY LECTURE

NUTRIREDOX: CONTROL OF OSTEOARTHRITIS PATHOGENESIS BY REDOX ACTIVE COMPOUNDS

Prof. Dr. Çimen Karasu

"Cellular Stress Response & Signal Transduction Research Laborotory"
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Osteoarthritis (OA) is a complex multifactorial degenerative disease of the joint that affects the cartilage of bones and other surrounding tissues [1]. It is characterized by severe pain in joint that increases by movement. Obesity, diabetes, past trauma, advancing age, female sex and genetics are the risk factor for this disorder. Articular cartilage comprises an extracellular matrix containing chondrocytes, responsible for synthesis of the extracellular matrix. In OA, the stress is characterised by an increase of pro-inflammatory mediator production by chondrocytes, including cytokines (IL-1β, TNF-α), reactive oxygen species, advanced glycoxidation/lipoxidation end products-protein adducts (AGEs/ALEs-adducts), and prostaglandins. This local inflammation induces redox dysregulation in the pathophysiology of OA and induces an increase in production of proteolytic enzymes (matrix metalloproteinases and aggrecanases) that will digest the cartilage matrix. The pharmacological treatment generally used for the treatment of the OA includes non-steroidal inflammatory drugs, opioids, acetaminophen, topicals and intra-articular injections of hyaluronic acid or corticosteroids, but all of them are the symptomatic interventions. In recent years, a wide range of evidence indicates that regulation of cellular redox signaling plays an important role in the management of OA pathogenesis. In this case, the activation of Nrf2 signaling pathway, which plays a regulatory role not only in oxidative stress, but also in inflammation, immunity and cartilage and bone metabolism, contributes to detoxification and protective processes in OA [2]. Recently, our lab carried out studies to determine the chondroprotective role of phytoactive molecules [e.g., polyphenols] able to preserve the articular cartilage and chondrocytes, in the context of development of OA because of their redox active and anti-inflammatory properties [3,4]. Our in vitro studies suggests that herbal polyphenols such as oleuropein, hydroxytyrosol, verbascoside, luteolin or colchicine, S-Allyl cysteine, exert a chondroprotective effect through actions such as anti-inflammatory, redox modulatory and anti-catabolic activity that are critical for mitigating OA disease pathogenesis and symptoms.

Keywords: Osteoarthritis, Primary Chondrocyte, Redox Signaling, Oxidative Stress, Plant Polyphenols

Acknowledgements: The Scientific and Technological Research Council of Turkey, TUBITAK, was supported the study by Grant No: 315S012.

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INVITED LECTURE

MULTI-TARGET OPPORTUNITIES IN THE ARACHIDONIC ACID CASCADE FOR TREATMENT OF INFLAMMATION

Erden Banoğlu[1], Burcu Çalışkan[1], Zehra Tuğçe Gür Maz[1], Abdurrahman Olgaç[1], Ulrike Garscha[2], Oliver Werz[2]

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Objective/Purpose: Arachidonic acid (AA) pathway is a central regulator of inflammatory response. Prostaglandin E_2 (PGE_2) produced by cyclooxygenases (COX)-1/2 and leukotrienes (LTB_4 and cys-LTs) produced by 5-lipoxygenase (5-LO) with the aid of 5-LO activating protein (FLAP) are considered to be mediators of the inflammatory response. For many years, the main strategy consisted in the inhibition of COX-1 and COX-2, in which their utility is somewhat limited due to potential gastrointestinal and cardiovascular toxicities, and expectations of having an effective and safer non-steroidal anti-inflammatory drugs (NSAIDs) have been only partially fulfilled. Emerging information has challenged some aspects of the original hypothesis indicating that single-target approach in the AA cascade could be replaced by multi-target approach, such as dual inhibition of microsomal PGE_2 synthase-1 (mPGES-1) and 5-LO and/or FLAP, producing more effective drug candidates lacking the adverse effects of single-target NSAIDs.
Materials and Methods: We have developed a combined ligand- and structure-based FLAP pharmacophore model for virtual screening of commercially available compounds (2.8 mio) followed by molecular docking and dynamics calculations for identification of novel chemotypes. The obtained hit compounds are synthetically modified to develop dual inhibitors of FLAP/5-LO and mPGES-1.
Results: Our medicinal chemistry efforts led to two final compounds, an isoxazole derivative 4-(4-chlorophenyl)-5-[4-(quinolin-2-ylmethoxy)phenyl]isoxazol-3-carboxylic acid (BRP-187) and a benzimidazole derivative, 5-{1-[(2-Chlorophenyl)methyl]-2-{1-[4-(2-methylpropyl) phenyl]ethyl}-1H-benzimidazole-5-yl}-2,3-dihydro-1,3,4-oxadiazole-2-thione (BRP-208) as multi-target inhibitors in the AA pathway affecting primarily FLAP and mPGES-1, and to a minor degree also 5-LO with in vivo efficacy in the animal models of inflammation.
Conclusion/Discussion: Taken together, BRP-187 and BRP-208 represent novel chemotypes of as multi-target inhibitors in the AA cascade and warrants further preclinical evaluation.
Keywords: Arachidonic acid, prostaglandin, leukotriene, inflammation, mPGES-1, FLAP(This study was supported by TUBITAK research grants 108S210 and 112S596).

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INVITED LECTURE

CHIRAL XANTHONE DERIVATIVES: SYNTHESIS, BIOLOGICAL ACTIVITIES AND ANALYTICAL APPLICATIONS

Madalena M. M. Pinto [1,2,*], Maria Elizabeth Tiritan [1,2,3,§], Carla Fernandes [1,2,§]

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Over the last decades, xanthone derivatives have been the core of diverse studies, essentially due to their wide range of biological and pharmacological activities [1]. Chiral derivatives of xanthones (CDXs), synthesized in our group, showed that several of their biological activities can depend on their stereochemistry. Thus, the enantioselective synthesis of CDXs is an important issue to obtain both enantiomers of CDXs for further biological activity screening.

Herein, a library of CDXs, synthesized using analogues of marine carboxyxanthone derivatives as chemical substrates linked to diverse chiral moieties, is presented. For the obtained pairs of enantiomers the following *in vitro* and *in silico* assays were conducted:

(i) growth inhibitory activity in three human tumor cell lines [2]; moreover, in an attempt to elucidate the mechanism of inhibition, DNA crosslinking capacity of the compounds was evaluated in some examples;

(ii) cyclooxygenase (COX-1 and COX-2) inhibition, where *in silico* studies were also evaluated [3];

(iii) inhibition of bacterial growth and biofilm formation on *Staphylococcus aureus*, *S. epidermidis* and *Pseudomonas aeruginosa* were evaluated.

(iv) additionally, human serum albumin (HSA) binding affinity was evaluated by spectrofluorimetry and in *in silico* studies [3], as well as by liquid chromatography (LC) using HSA as chiral stationary phase.

Enantioselectivity was observed in all studies. Other assays are ongoing and new CDXs are also being synthesized in our laboratory.

Besides the potential as new drugs, CDXs present structural features with interest as chiral selectors for LC. In this context, some of these small molecules were selected and bound to a chromatographic support for a new application as chiral stationary phases (CSPs) in LC [4]. The new xanthonic CSPs afforded promising enantioresolution results, high stability and reproducibility.

Accordingly, CDXs have important applications in the field of Medicinal Chemistry, not only as candidates for potential new drugs but also as analytical tools for enantioseparation in LC.

Keywords: carboxyxanthones; chiral derivatives of xanthones; bioactivity; chiral selectors

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INVITED LECTURE

STRATEGIC DESIGN OF DRUG CORE STRUCTURE: A BLEOMYCIN SPINOUT STORY

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Bleomycin is a 53-years old antitumor antibiotic isolated by Hamao Umezawa in 1966. It is still used to treat squamous cell carcinoma, malignant lymphoma, testicular cancer, ovarian cancer, head and neck cancer albeit the serious adverse effect of pulmonary fibrosis. Chemically it is a glycopeptide consisting of a non-natural peptide and a non-natural disaccharide and has molecular weight around 1,400. In order to reduce the adverse effect and improve the activity we undertook structural alteration of bleomycin by means of synthetic organic chemistry. Bleomycin molecule is an integrated entity of several functional segments. The pyrimidine part of bleomycin binds iron to activate molecular oxygen. Bithiazole-terminal amine part interacts with DNA. Overall bleomycin binds and cleaves DNA by activated oxygen. We elaborated new molecules by chemical restructuring of the bleomycin molecule.

We began with the iron core of the total structure of bleomycin and obtained the 1st generation iron-binding, oxygen-activating molecules consisting with aminoalanine, pyrimidine and histidine. Structural symmetrization of the 1st generation molecules afforded the 2nd generation oxygen-activating molecules characterized by histidine-pyridine-histidine structure. Further modification of the 2nd generation molecules resulted in diverse function, i. e., inhibition of zinc protein including zinc finger proteins and farnesyltransferase,[1), 2)] inhibition of NF-κB activation,[3)] increase of steady state expression of antiviral host factor APOBEC3G.[4), 5)] Recently, blockade of TGF-□/Smad signaling was achieved by a 2nd generation molecule ameliorated experimental skin fibrosis in mice.[6)] New functional molecules were thus obtained by molecular modification of bleomycin.

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INVITED LECTURE

SEARCHING FOR NATURAL PRODUCT ANTIMICROBIAL LEADS: LESSONS LEARNT FROM A SOUTHERN AFRICAN PERSPECTIVE

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South Africa is home to more than 3000 medicinal plant species and many of these have shown antimicrobial potential, thus adding to the scientific body of knowledge. As a researcher within the field for over 20 years, I would like to share the lessons learnt along the way.
Lesson 1.) Look at specific diseases and the related plant directives. Studies on medicinal plants for stomach ailments are frequently undertaken with little cognisance of neglected pathogens such as the *Helicobacter, Bacteroides* and *Clostridium* species which may yield excellent results not previously observed. A study on 43 plant species demonstrated that attention should not only be given to common pathogens such as *Escherichia coli.*
Lesson 2.) Look at topical relevant diseases in the country of study. Natural products demonstrating noteworthy activity against Listeriosis, a recent disease outbreak in South Africa, included *Withania somnifera* (MIC 20 µg/ml) and selected honey and propolis samples.
Lesson 3.) Ethnobotany as a source of traditional knowledge. Studies in northern Maputaland (KwaZulu-Natal) demonstrate how taking the ethnobotanical knowledge of using medicinal plants in combination to treat various infectious diseases validates the traditional use.
Lesson 4.) Looking for the single compound that provides excellent activity. The main compounds found in South African propolis are pinocembrin, galangin, and chrysin and with the use of MIC microdilution methods, minimum bactericidal activity, quorum sensing and cytotoxicity studies results will demonstrate the importance of looking at combinations to achieve efficacy.
Lesson 5.) Examining neglected allopathic antimicrobials with natural products. Antibiotics such as penicillin and even antimicrobial dyes are fast becoming redundant in the favour of newer antimicrobials. Penicillin combined with the common herbal tea Rooibos has been shown to act synergistically. Furthermore neglected dyes such as crystal violet and malachite green have shown to be synergistic when tested with *Gunnera perpensa* (fractional inhibitory concentrations of 0.02 against *Staphylococcus aureus* and 0.08 against *Candida albicans*).
Lesson 6.) Examining resistant strains. Examples from South African medicinal plants such as *Artemisia afra* followed by *Tetradenia riparia* and *Osmitopsis asteriscoides* respond better to resistant strains than conventional antibiotics.
Last but not least, lesson 7.) Examining the chemistry is an important link to bioactivity and studies have shown that different bioactivities are closely linked to the chemotype.
There is a move towards a more targeted approach of investigating antimicrobial activity leaving no doubt that following these lessons may yield novel findings that will help curb the ever increasing problem of antimicrobial resistance currently encountered.

INVITED LECTURE

EFFECT OF RAW SPIRIT IN "BOROVIČKA" BEVERAGE ON THE QUALITY OF THE PRODUCT

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Objective/Purpose: "Borovička" is a distilled spirit made from Juniper berries, produced by special fermentation of the *Juniperus communis* fruit. The Slovak distillation industry declares a need of approximately 500 tons of fresh fruits per year. Many companies do not have their own facility to collect juniper berries and to cultivate the shrubs and trees. Therefore they purchase plant raw material either from small local companies, which are organized a juniper fruit collection in all Slovak territory, or import the berries from Albania, Macedonia, Serbia, and Bulgaria where different species of the genus Juniper are very common. This is the main reason for the difficulties in controlling the raw spirit industry at every stage. Low-quality raw spirit results in low-quality alcohol product.
Material and Methods: Juniper fruits were collected from several wild plant populations in the town of Rožňava (Eastern Slovakia) and in the marshy mountain forest and national park of Dajti in Central Albania, to the East of Tirana. Four samples with a 10 g weight of dried juniper fruits from each investigated locality (Slovakia and Albania) were grounded in a blender. The essential oil from this raw-material was prepared by hydro-distillation (2 hours) in Clevenger-type apparatus according to the European Pharmacopoeia and a mixture of hexane was used as a collecting solvent. The analysis of the juniper essential oils was carried out using a Vega Series Carloerba Gas Chromatograph, connected to a Spectrophysics SP 4270 integrator. The following operating conditions were used: column: DB5, 30 m × 0.32 mm inner diameter (i.d.), film thickness: 0.25 mm, carrier gas: nitrogen, adjusted to a flux of 1 mL per min, injection and FID-detector temperatures: 220°C, respectively 250°C. Components were identified by their GC retention times, and the resulting values were comparable to those of literature. Oil component standards for comparison were supplied by Extrasynthese Ltd. (France).
Results: The GC/FID comparing of "Borovicka" distillates made in the *Juniperus communis* L. and *Juniperus oxycedrus* L. fruits had different results. Distillates from the Albanian raw material had higher contents of cumulative residues (0.40±0.10 % v/w), higher alcohols (1.80±0.10 g/0.1 l pure ethanol (p.e)), esters (0.70±0.10 g/0.1 l p.e), content of methanol (0.70±0.10 g/0.1 l p.e), aldehydes+ketones (0.10±0.02 g/0.1 l p.e) and Σ pinenes (0.50±0.10 g/0.1 l p.e). The flavor, smell, taste, and aroma differ from the original national "Borovicka" spirit. Aroma compounds in distilled spirits and liqueurs, their levels, and odor attributes are important for quality and authenticity. For example, a small amount of higher alcohols has a positive effect on the sensory properties of distillates. The chemical composition of the raw material of juniper berries plays an important role in the production of the "Borovička".
Conclusion/Discussion: Several authors show the negative effects of juniper berry oils on ethanol formation during the fermentation of juniper berry sugars. Juniper berry oils work as an inhibitor for the ethanol pathway while the formation of other by-products increases. In conclusion, for higher quality of this Slovakian national liquor, distillation companies need to select *Juniperus communis* L. fruits with the favorable pinene contents, as contributors of aroma, odor, lower essential oil quantity, and other inappropriate residues.

Acknowledgements: This research was supported by the Slovak Research and Development Agency (SRDA), the project: APVV-14-0843: "Research of possibilities of growing juniper (*Juniperus communis* L.) for the production of fruits".

ORAL PRESENTATIONS

ORAL PRESENTATION

A NEW SERIES OF MORPHOLINE-BASED THIAZOLYL-PYRAZOLINES AS DUAL EGFR AND HER2 INHIBITORS

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Objective: Epidermal growth factor receptor (EGFR) is a crucial member of receptor tyrosine kinase superfamily which also contains HER2, HER3 and HER4. EGFR is associated with tumor growth and progression including cell proliferation, impair of apoptosis, metastasis and angiogenesis. EGFR is widely amplified, mutated, or overexpressed in non-small-cell lung cancer. Besides, HER2 is also an important cancer biomarker and it is mainly overexpressed in breast cancer [1]. **Material and Methods:** 1-(4-Arylthiazol-2-yl)-3-(4-morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazolines (**3a-k**) were synthesized *via* the ring closure reaction of 2-bromo-1-arylethanones with 3-(4-morpholinophenyl)-5-(4-chlorophenyl)-1-thiocarbamoyl-2-pyrazoline (**2**), which was obtained *via* the cyclization reaction of 3-(4-chlorophenyl)-1-(4-morpholinophenyl)-2-propen-1-one (**1**) with thiosemicarbazide in the presence of sodium hydroxide. MTT assay was performed to evaluate the cytotoxic effects of all synthesized compounds on A549 human lung adenocarcinoma and MCF-7 human breast adenocarcinoma cell lines. Besides, tumor selectivity was also determined between Jurkat human leukemic T-cell line and human peripheral blood mononuclear cell (PBMC) line. Moreover, annexin V/ethidium homodimer III staining method was carried out to evaluate apoptotic effects of the most potent anticancer agents on A549 and MCF-7 cell lines. The most effective compounds were further investigated for their inhibitory potencies against eight kinases including EGFR, HER2, HER4, insulin-like growth factor 1 (IGF1R), insulin receptor (InsR), kinase insert domain receptor (KDR), platelet-derived growth factor receptors (PDGFR α, β). Erlotinib, an important EGFR-tyrosine kinase inhibitor, was used as a positive control for all *in vitro* assays. Molecular docking studies were performed to enlighten the binding modes of the most EGFR and HER2 inhibitors in the ATP binding sites of EGFR and HER2 (PDB ID codes: 4HJO and 3RCD, respectively). **Results:** 1-(4-(4-Fluorophenyl)thiazol-2-yl)-3-(4-morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (**3c**) and 1-(4-(4-cyanophenyl)thiazol-2-yl)-3-(4-morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (**3f**) were found as the most effective anticancer agents against both A549 (IC_{50}= 8.95±1.43 μM and 10.76±1.81 μM, respectively) and MCF-7 (IC_{50}= 9.59±1.95 μM and 8.05±1.47 μM, respectively) cell lines compared with erlotinib (IC_{50}= 22.35±2.84 μM μM and 8.24±1.37 μM, respectively). Compounds **3c** and **3f** showed significant tumor selectivity (IC_{50} > 300 μM and IC_{50}= 299.44±16.43 μM for PBMC line, respectively). Compounds **3c** and **3f** significantly induced apoptosis in A549 cells with 65% and 68%, respectively when compared with erlotinib (72%). Furthermore, compounds **3c** and **3f** showed significant apoptotic activity in MCF-7 cells with 78% and 51%, respectively when compared with erlotinib (66%). According to the results, compound **3f** was found as the most potent EGFR inhibitor in this series with an IC_{50} value of 4.34±0.66 μM when compared with erlotinib (0.05±0.01 μM), whereas compound **3c** did not show any significant inhibitory activity against EGFR (IC_{50}= 23.34±3.26 μM). Besides, compound **3f** showed significant inhibitory effects on HER2 with an IC_{50} value of 2.28±0.53 μM. However, compound **3c** exhibited moderate HER2 inhibitory activity with an IC_{50} value of 6.96±1.37 μM. Molecular docking studies indicated that compound **3f** showed high affinity to the EGFR site and formed substantial H-pi interactions with proper amino acid residues due to the presence of 4-cyanophenyl moiety and thiazole scaffold. On the other hand, compound **3c** did not show these crucial interactions but its thiazole ring binds to Thr766 through HOH4 bridge. Moreover, compound **3f** formed a key interaction with Asp863 and substantial H-Pi interactions with Cys805 and Leu852 in HER2 binding site, whereas compound **3c** only formed two interactions with Thr862 and Asp863. **Conclusion:** According to *in vitro* and *in silico* studies, compound **3f** stands out as a promising anticancer agent due to its dual EGFR and HER2 inhibitory activity.

Keywords: Thiazolyl-pyrazoline, anticancer activity, apoptosis, EGFR, HER2

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ORAL PRESENTATION

SECONDARY METABOLITES FROM THE CULTURE OF THE MARINE SPONGE-ASSOCIATED FUNGUS *PENICILLIUM ERUBESCENS* KUFA0220 AND THEIR ANTIBACTERIAL ACTIVITY

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Objective / Purpose: To investigate the secondary metabolites from the marine-derived fungus *Penicillium erubescens* KUFA 0220 as well as to evaluate the antibacterial activity of the isolated metabolites. **Material and Methods: The fungus** *Penicillium erubescens* KUFA 0220 was isolated from the marine sponge *Neopetrosia* sp., which was collected from the coral reef at Samaesan Island in the Gulf of Thailand. The crude of EtOAc extract of its culture, on the solid medium, was fractionated by column chromatography of silica gel and purified by crystallization, preparative TLC and Sephadex LH-20 column. The structures of the isolated compounds were established by extensive analysis of 1D and 2D NMR and HRMS. The absolute structures of the compounds were determined by X-ray analysis or by comparison of the calculated and experimental Electronic Circular Dichroism (ECD) spectra. The isolated compounds were tested against Gram-positive and Gram-negative reference strains and multidrug-resistant (MDR) strains from the environment. The capacity of the isolated compounds to interfere with the bacterial biofilm formation and their potential synergism with clinically relevant antibiotics for MDR strains were investigated **Results:** Five new compounds, including 1-hydroxy-12-methoxycitromycin (1), penialidin G (2), erubescenschromone A (3), erubescenschromone B (4) and 7-hydroxy-6-methoxy-4-oxo-3-[(1*E*)-3-oxobut-1-en-1-yl]-4H-chromene-5-carboxylic acid (5), were isolated **together** with thirteen known compounds, named citromycin (6), 12-methoxycitromycin (7), myxotrichin D (8), 12-methoxycitromycetin (9), anhydrofulvic acid (10), myxotrichin C (11), penialidin D (12), penialidin F (13), SPF-3059-30 (14), β-sitostenone (15), secalonic acid A (16), ergosterol 5,8-endoperoxide (17) and GKK1032B (18). Compounds **1, 3, 6-10, 13-14), 16 and 18 were tested** for their antibacterial activity against five reference bacterial strains consisting of three Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecium* ATCC 19434 and *Enterococcus faecalis* ATCC 29212) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), three multidrug-resistant isolates from the environment (MRSA *S. aureus* 66/1, VRE *E. faecium* 1/6/63 and *E. faecalis* B3/101) and a clinical isolate ESBL *E. coli* SA/2. **GKK1032B (18)** exhibited an *in vitro* growth inhibition of all Gram-positive bacteria, whereas **secalonic acid A (16)** showed growth inhibition of methicillin-resistant *S. aureus* (MRSA) and none of the compounds were active against Gram-negative bacteria tested [1]. **Conclusion / Discussion:** Marine-derived fungi, especially from the genus *Penicillium* have proved to be important sources of bioactive secondary metabolites, many of which exhibit cytotoxic and antibiotic activities [2]. New metabolites **1-5,** together **with the previously reported 6-18** were isolated from the solid culture of the marine sponge-associated fungus *P. erubescens* KUFA0220. Among the isolated metabolites, only **GKK1032B (18)** displayed significant antibacterial activity and showed a capacity to inhibit biofilm formation and synergistic effect. However, **secalonic acid A (16)** also showed growth inhibition of methicillin-resistant *S. aureus* (MRSA).

Keywords: *Penicillium erubescens*; marine sponge-associated fungus; *Neopetrosia* sp.; chromene, chromones; antibacterial activity.

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ORAL PRESENTATION

MECHANISMS OF ANTICANCER ACTION OF SESAMOL IN COLORECTAL CARCINOMA HCT116 CELLS

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Objective / Purpose: Antioxidants prevent carcinogenesis and tumor growth, while pro-oxidant agents increase the cellular ROS levels to cytotoxic levels, which can induce selective killing of cancer cells and also be therapeutically useful. Sesamol has been known to have important biological activities such as antioxidant and anticancer activity in various cancer cell types but not in colon cancer cells. Therefore, the objective of this research is to define the mechanism of anticancer action of sesamol in the colorectal carcinoma HCT116 cells.

Material and Methods: Antioxidation assays were determined from an elimination of the DPPH radical, ferric **reducing-antioxidant power** (**FRAP**), and $O_2^{\bullet -}$, and peroxyl radical scavenging activity. Intracellular level of $O_2^{\bullet -}$, H_2O_2 and GSH were determined by DHE, DCFH-DA, and CMF-DA assays, respectively. Antiproliferation was detected by neutral red assay. Cell cycle arrest and mode of apoptotic cells death were analyzed by flow cytometry.

Results: Sesamol (0.05, 0.25, 0.5, 2, 5, and 10 mM) showed reducing power and scavenged DPPH$^{\bullet}$, and $O_2^{\bullet -}$ radicals. ROO• radical scavenging of sesamol was increased at low concentrations (0.05 and 0.25 mM), but this activity was decreased at higher concentrations (0.5, 2, 5, and 10 mM). Sesamol at high concentrations (0.5, 1, 2, and 5 mM) suppressed cell viability via disruption of cell cycle progression, thereby causing S-phase arrest and inducing apoptosis—through the production of intracellular $O_2^{\bullet -}$, and mitochondrial dysfunction.

Conclusion / Discussion: The mechanisms underlying the anticancer action of sesamol is its pro-oxidant effect. In consequence, the endogenous ROS was generated and being a stimulus of the mitochondrial apoptosis pathway in human colon cancer HCT116 cells.

Keywords: Sesamol, anticancer, colon cancer, HCT116 cells, pro-oxidant, apoptosis.

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ORAL PRESENTATION

COMPARISON OF ANTIOXIDANT, ANTI-MMP -2,-9 AND ANTI-HYALURONIDASE ACTIVITY BETWEEN EXTRACTS FROM BLACK AND WHITE SESAME SEED CAKE (*SESAMUM INDICUM* LINN.)

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Objective / Purpose: Reactive oxygen species (ROS) is the significant factor that contribute to skin aging and also activate the matrix metalloproteinases (MMPs). Furthermore, ROS leads to the degradation of hyaluronic acid which is the crucial component of maintaining water in the skin. Sesame is a tropical annually herb in the Pedaliaceae family which is valued in edible and medicinal use. Due to the cold-pressed sesame oil industrial, sesame seed cake is a by-product that commonly used as a cattle feed. The aim of this study is to prepare the extracts from black and white sesame seed cake and investigate their antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity. **Material and Methods:** Black and white sesame seed cake were obtained from cold-press oil processing and extracted by solvents with different polarity using maceration method. The concentrated extracts were stored at -4 ^{o}C until use. The extracts were determined for total phenolic content (TPC) by Folin-Ciocalteu assay using a calibration curve of standard gallic acid. Antioxidant activity was determined using DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assay. Moreover, the matrix metalloproteinase -2 and -9 inhibition and hyaluronidase inhibition were evaluated by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). All of the experiments were performed in triplicate and data presented as a mean ± standard deviation (SD). **Results:** The ethanolic extract from both black and white sesame seed cake showed the highest %yield of 30.12% and 32.44%. The water extract of black sesame seed cake presented the highest TPC with the value of 237.13 ±3.39 mg of GAE /g of extract and followed by the methanolic extract of black (192.92 ±3.01 mg of GAE /g of extract). Whereas the water extract of white seed cake showed the highest TPC with value of 116.14 ±1.84 mg of GAE /g of extract. According to the antioxidant activities, almost the extracts of black seed cake exhibited the better DPPH radical-scavenging property and ferric reducing ability than the extracts of white seed cake. For the DPPH scavenging capability, the water extract from black seed cake showed the strongest antioxidant activity with IC_{50} value of 0.147 ±0.01 mg/ml, whereas, the same solvent extract from white seed cake presented IC_{50} value of 0.746 ±0.02 mg/ml. Furthermore, water extract from black seed exhibited the strongest reducing power value with FRAP value of 0.65 ±0.01 mM Fe (II) /g of extract weight and EC_1 value of 1.67 ±0.01mg/ml. In the part of Inhibition of MMP -2 and -9, the hexane extract of black seed cake exhibited the highest inhibitory activity at 48 h with the values of 85.20 ±0.50% (MMP-2) and 99.50 ±0.90% (MMP-9) while all extract of white seed cake showed no inhibitory activity. For the inhibitory of hyaluronidase, the extract from black seed cake showed the highest inhibitory activity at 48 h with the values of 99.94 ±0.81% inhibition whereas the extract from white seed cake presented no inhibition. **Conclusion / Discussion:** The results demonstrated that the extracts from black sesame seed cake possessed the higher ability of antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity than the extracts from white sesame seed cake that could be a valuable natural extract for further development into anti-aging cosmeceutical products.

Keywords: sesame seed cake, antioxidant, anti- matrix metalloproteinases -2,-9, anti-hyaluronidase

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ORAL PRESENTATION

DYNAMICS OF ANTIBODY TITERS IN ANTI-NEWCASTLE VACCINATED PIGEONS TREATED WITH A *CALENDULA OFFICINALIS* EXTRACT

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Objective/Purpose: Pigeons, especially competing ones, are exposed to numerous pathogens due to their broad circulation area. Enhancement of post vaccination immune responses represent an important goal of vaccination strategies, therefore various adjuvants were considered, including plant extracts such as *Calendula officinalis*. **Material and Methods:** The research was performed on two groups of adult racing pigeons, each vaccinated (0.2 ml, s.c.) against Newcastle disease with a different strain of Paramyxovius, LaSota (non-specific) or PMV1-RO96 (specific), while half of each group was orally treated with a *Calendula officinalis* alcoholic extract (0.2 ml/bird) for 7 days. The antioxidant activity of the marigold extract was determined by assessing free radical scavenging effect over DPPH (%) radical. Blood was sampled on days 0, 7 and 14 of the experiment and immunological tests included the monitoring the level and dynamics of antibody titres by hemagglutination inhibition test (HIT). Excel program was used to evaluate the statistical significance of the treatments' effects by group. **Results:** The radical scavenging activity of the alcoholic marigold extract was of 31.03±2.01%. In the *Calendula* treated, PMV1-RO96 vaccinated pigeons, the titres increased by day 14 with 47.95% against the water treated group while in the LaSota strain vaccinated pigeons showed a titre 39.98%. The final titres were similar in both groups but the intermediate decrease of 67.22% for the titres (day 7) was more pronounced in the group receiving the non-specific strain. **Conclusion / Discussion:** The relationship between antioxidant capacity and adjuvant effect of the alcoholic marigold extract could not be clearly established. The marigold extract influenced positively, but in a dissimilar way the dynamics of the specific humoral response in pigeons vaccinated against Newcastle disease.

Keywords: racing pigeons, marigold extract, antioxidant, antibodies, dynamics

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ORAL PRESENTATION

IS THE ANTIOXIDANT CAPACITY OF ALCOHOLIC PLANT EXTRACTS INDUCIVE OF DISSIMILAR *IN VITRO* IMMUNE RESPONSES IN WILD VERSUS DOMESTIC SPECIES?

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Objective / Purpose: Immunological effects of plants are exploited by traditional medicine worldwide. This paper aimed to investigate if there were differences in immunological effects of some alcoholic medicinal plant extracts which differ by antioxidant activity in different vertebrate classes, *Aves* and *Mammalia*, allowing the best choice of immune enhancing natural products. **Material and Methods:** The experiment was carried out on domestic (hens n=32 and dogs n=22) and wild representatives (farmed silver foxes n=30 and pheasants n=30) of *Aves* and *Mammalia* classes. The antioxidant capacity (free radical scavenging effect over DPPH,%) and the *in vitro* blastogenic capacity (BI%) of *Calendula officinalis, Arnica montana, Symphitum officinale, Echinaceea purpurea* and *Hippophae rhamnoides* alcoholic extracts were monitored. Excel program was used to estimate the significance of the differences. **Results:** The highest antioxidant activities were identified in *Hippophae rhamnoides* and *Echinacea angustifolia* extracts, which did not relate to the blastogenic capacity, except in chickens (43.87±14.8 and 43.87±14.8% respectively). *S. officinale* proved to have an intermediate antioxidant capacity, but the strongest stimulating activity only in hens (56.72±12.52%). In pheasants, dogs and foxes the responses were significantly ($p<0.001$) lower. Mammals showed negative responses to the extracts, foxes more than dogs ($p<0.001$), with only the *Hippophae rhamnoides* extract weekly stimulating immune cell growth. **Conclusion / Discussion:** Vegetal extract enhanced blast transformation was rather dependant on the class and degree of domestication than increased antioxidant activity in the tested species.

Keywords: plant extracts, antioxidant activity, *Aves*, *Mammalia*, domestication, cell-mediated immunity.

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ORAL PRESENTATION

TUBULIN-BINDING ANTI-CANCER POLYSULFIDES INDUCE CELL DEATH VIA MITOTIC ARREST AND AUTOPHAGIC INTERFERENCE IN COLON CANCER

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Colon cancer is a major cause of morbidity and mortality worldwide. Epidemiological studies revealed an inverse correlation between colon cancer risk and a garlic-rich diet. Natural organosulfur compounds confer protective effects against a wide range of cancer, including colon. Here, we studied the anti-colon cancer activity of garlic-derived diallyl/dibenzyl tetrasulfur (DATTS/DBTTS) derivatives. We validated the ability of DATTS/DBTTS to bind tubulin by MALDI-TOF mass spectrometry and its disruptive effect on the microtubule network by immunofluorescence. We selected cell lines with a defined genetic background including HT-29 (BRAF-mutation), SW480 and metastatic SW620 (both KRAS-mutation). Cell cycle analysis, Hoechst staining and western blots allowed to assess cell death. To further validate the anti-cancer activity of DBTTS, colony and spheroid formation assays as well as zebrafish xenografts were realised. To monitor autophagy, western blots, GFP-LC3 plasmid transfection and transmission electron microscopy (TEM) were conducted. All selected cell models were more sensitive to DBTTS than to DATTS. SW480 and SW620 were more susceptible to DBTTS than HT-29 cells. DBTTS induced mitotic arrest followed by cell death. Its anti-cancer activity was validated in 3D cell culture systems and in vivo. DATTS/DBTTS acted as a direct and reversible tubulin binder inducing microtubule disarrangements in cellulo. As tubulin alterations may affect autophagy progression, we evaluated the effect of DBTTS on autophagy. DBTTS induced LC3-II and p62 protein accumulation concomitantly with mitotic arrest in HT-29 but not in SW480 and SW620 cells. TEM analysis showed accumulation of pre-fusion complexes (phagophores and autophagosomes) indicating inhibition of the autophagic flux. Autophagy inhibitor bafilomycin A1 confirmed the inhibition of the autophagy flux by DBTTS in HT-29 cells. Immunofluorescence revealed p62 protein accumulation showing a dotted pattern, similar to the LC3-II puncta formation in HT-29 cells. Furthermore, silencing of p62 protein exacerbated cell death indicating a pro-survival role of p62 overexpression in DBTTS-treated HT-29 cells. Altogether, we showed here that DBTTS acts as an anti-cancer agent by targeting tubulin[1]. These results suggest that DBTTS targets colon cancer survival/death through autophagy interference depending on cell types with differential autophagy capacities and genetic signatures.

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ORAL PRESENTATION

IMMUNOLOGICAL AND ANTI-STRESS INFLUENCES OF A MARIGOLD TEA TREATMENT IN BOVINE

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Objective / Purpose: Stress of various origins represents an important factor impideing on vaccination results in bovine. This study aimed at investigating the anti-stress and immunological influence of the administration of a marigold tea in adult bovine, vaccinated against multiple antigens. **Material and Methods:** The research was carried out on treated bovine aged 3 to 5 years (n=20/group), which received marigold tea daily, for one week, compared to untreated controls. The animals were vaccinated with a multivalent vaccine on day 0 (Cattle Master). Blood was sampled on days 0, 7 and 14 of the experiment. The antioxidant capacity (scavenging effect over DPPH,%) for the marigold tea was quantified. The blast transformation test (SI%) was carried out to monitor the adaptive cell-mediated immunity. The significance of the differences was estimated by Excel program. **Results:** The antioxidant activity of the investigated aqueous extract was quite high (85.10±5.21%). The stress levels constantly decreased in marigold tea treated animals (1.09±0.047 to 0.064±0.02) and did not significantly vary in controls. The blast transformation test indicated lower SI% and also a prolonged response to vaccination in the treated group (40.65±14.91 to 79.82±3.34 versus 50.62±5.93 and 81.31±2.25%) by the third sampling. **Conclusion / Discussion:** The marigold tea exerted a stress lowering effect but prolonged the response to vaccination, when administered as tea to adult bovine.

Keywords: *Calendula officinalis*, water extract, bovine, N/L ratio, adaptive immunity

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ORAL PRESENTATION

DISCOVERY BIOLOGICAL ACTIVITY IN THE DISCARD OF THE FISHERY SHRIMP TRAWL FISHERIES

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Objective / Purpose: Bycatch of the shrimp trawling fishing has been an environmental issue during the past decades. For each kilogram of shrimp catch, 10 to 20 kg of other organisms are captured and discarded died in the ocean. Nevertheless, this reject can be an abundant source for the extraction of bioactive molecules, which may add value to these wastes. In this study, aimed to investigate the antioxidant activity of protein hydrolysates obtained from the crab most abundant from bycatch of shrimp fisheries in Brazil. **Material and Methods:** The species of crab *Hepatus pudibundus*, was collected in September 2017 in the region of Ubatuba, São Paulo, Brazil, by double-rig a commercial shrimp trawling. The muscle of the animals was hydrolyzed in a thermostated bath at 50 °C using two commercial enzymes: Alcalase and Protamex. Then, the hydrolysates were characterized and the *in vitro* antioxidant (against peroxyl radicals, DPPH radicals and the concentration of sulfhydryl groups associated with proteins (P-SH)) and antimicrobial activities were evaluated. **Results:** *Hepatus pudibundus*, when hydrolyzed with the enzyme Alcalase, have the lowest capacity to reduce the peroxyl radicals compared to the enzyme Protamex. No significant differences ($p > 0.05$) in the antioxidant activity against DPPH radicals between the samples hydrolyzed with the two enzymes. Additionally, the presence of sulfhydryl groups in the hydrolysates was observed, however, there were no statistical differences ($p > 0.05$) in the samples hydrolyzed with two enzymes. **Conclusion / Discussion:** In general, there were few differences between the muscle hydrolyzed with the enzyme Alcalase and Protamex, being that *H. pudibundus* presented some antioxidant activity. Thus, the present study demonstrated that the enzymatic hydrolysis of the crab most abundant from bycatch is an efficient technique that allows the release of peptides with antioxidant activity with potential in the use in the food industry.

Keywords: Bioactive peptides, enzymatic hydrolysis, antioxidant activity, antimicrobial activity, food preservation.

ORAL PRESENTATION

PRODUCTION OF PHYTOESTROGENS IN *GLOBULARIA TRICHOSANTHA* SSP. *TRICHOSANTHA*

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Objective/Purpose: Biotechnological approaches need to be utilized for the sustainable production of plant and its phytochemical content. The production of secondary metabolites is facilitated by different in vitro cultures. The current study aims the production of calli from *Globularia trichosantha* ssp. *trichosantha* and the quantitative analysis of gallic acid, caffeic acid, p-coumaric acid, taxifolin, rosmarinic acid, kaempferol, genistein, quercetin, biochanin A, formononetin, daidzein, shikimic acid in the calli and the plant by HPLC. **Material and Methods:** The seeds of the plant were sterilized and inoculated on MS medium without in vitro plant growth regulator. Hypocotyl, cotyledon, first leaf, epicotyl, apical meristem and root explants were taken from the 30-day-old aseptic seedlings germinated in vitro. Explants were then transferred to MS media for callus production together with various concentrations of plant growth regulators. Both herba and callus were analysed by HPLC. **Results:** The best callus production was recorded in the media containing 0.2 mg L^{-1} 2,4 - D + 0.1 mg L^{-1} BAP. The highest phytoestrogen contents were determined in the callus (529.589 µg g^{-1}), the fruit-seed (48.799 µg g^{-1}), the leaf (247.279 µg g^{-1}), the stem (44.751 µg g^{-1}), the roots (56.658 µg g^{-1}). The current study is important in the sense (1) that analysied phytoestrogens were quantitatively determined in calli and plant for the first time; (2) all the phytoestrogens in here were produced in the callus culture on average 5 times much more than the natural amount that the plant had. **Conclusion/Discussion:** The findings of this study suggest that *G. trichosantha* ssp. *trichosantha* has a strong potential for biomass production and phytoestrogen compounds in callus culture. Our data indicated that the production of phytoestrogens from *G. trichosantha* ssp. *trichosantha* will arise as the sources of knowledge in future studies.

Keywords: Callus production, *Globularia trichosantha* ssp. *trichosantha*, HPLC, phytoestrogen.

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ORAL PRESENTATION

TRANSPORTATION PATHWAYS OF SESAMOL IN MELANOMA (SK-MEL-2) CELLS

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Objective / Purpose: Sesamol is one of the potent antioxidant and anti-cancer agent found in sesame seed. Our previous studies showed that sesamol possessed antiproliferative effect in melanoma (SK-MEL-2) cells with less toxic to noncancerous Vero cells and inducing apoptosis in colon (HCT116) and lung (SK-LU-1) cancer cells. Sesamol has discerned potential cellular bioactivities, although it has high water solubility with low lipophilicity, which is unlikely to undergo passive transport. It, therefore, prompts us to determine the transportation pathway of sesamol. **Material and Methods:** A human malignant melanoma cell line (SK-MEL-2) and African monkey kidney epithelial normal cell line (Vero) were utilized as the *in vitro* models. The cellular transportation pathways of sesamol via the physiological mimicking condition, passive diffusion, and carrier mediated transportation pathway were evaluated. Since melanoma cells were known to overexpressed LAT1 influx protein, the effect of sesamol on LAT1 function was investigated in the presence and the absence of a competitive LAT1 inhibitor (L-leucine). The maximum velocity (Vm) was calculated to define the rate of sesamol transport. Quantitative analysis of sesamol was performed by using validated HPLC analysis. **Results:** The net uptake of sesamol into the SK-MEL-2 cells under physiological condition showed maximum velocity (V_m) of 881.84 pmol/min/mg protein, which was higher than the Vero cells (195.34 pmol/min/mg protein). As expected, sesamol was passively diffused through the SK-MEL-2 cells with relatively low Vm (195.89 pmol/min/mg protein). In the presence of LAT1 inhibitor, sesamol had significant low V_m (205.31 pmol/min/mg protein) compared to the V_m in the absence of LAT1 inhibitor. It is indicated the sesamol was transported via LAT1 influx proteins. **Conclusion/discussion:** LAT1 influx protein played pivotal role on mediated the intracellular transportation of sesamol in the SK-MEL-2 cells. The bioactivity of sesamol in the SK-MEL-2 may be facilitated by the LAT1 influx protein.

Keywords: Sesamol, LAT1, influx, melanoma, transportation, uptake mechanism.

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ORAL PRESENTATION

THE ANTIMICROBIAL AND GENOTOXIC EFFECTS OF A NEW SERIES OF QUINOXALINE-BASED HYDRAZONES

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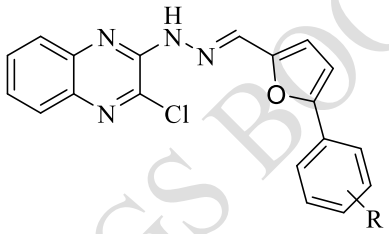
Objective: Quinoxaline nucleus has attracted a great deal of interest as a privileged scaffold in contemporary medicinal chemistry due to its unique physicochemical properties and diverse therapeutic applications [1]. In particular, quinoxaline derivatives have been reported as promising antimicrobial agents against a wide range of fungi and bacteria, including resistant ones. In antimicrobial drug discovery process, hydrazone is a privileged structural linker between various classes of aromatic and heteroaromatic rings such as benzene, furan, pyrrole, thiophene, pyridine, indole and quinoline with a unique feature of hydrogen bonding donor and hydrogen bonding acceptor regions [2]. In an effort to identify new antimicrobial agents, herein we designed and synthesized a series of quinoxaline-based hydrazones (Fig. 1) as potential antibacterial and antifungal agents.



Compound	R	Compound	R
1	2-NO_2	**7**	3-CF_3
2	4-NO_2	**8**	4-Br
3	2-Cl	**9**	3,4-diCl
4	3-Cl	**10**	2,4-diCl
5	4-Cl	**11**	2,5-diCl
6	2-CF_3	**12**	4-Cl-2-NO_2

Figure 1. Compounds **1-12**

Material and Methods: 2-Chloro-3-(2-((5-arylfuran-2-yl)methylene)hydrazinyl)quinoxaline derivatives (**1-12**) were synthesized *via* the reaction of 2-chloro-3-hydrazinylquinoxaline with 5-arylfurfurals. After dissolution of the compounds with DMSO, Tween 80 was added and sonic agitator was used to encapsulate the substances. The liposomes were formed through a 0.45 µm sterile membrane filter to equalize the distribution of vesicles in sterile distilled water. Microdilution was performed on previously incubated microorganisms with standards prepared at an initial concentration of 256 µg/mL. Resazurin sodium was used as indicator. Sabaroud Dextrose Broth was used as a medium for yeasts and Mueller Hinton Broth was used as a medium for bacteria. Chloramphenicol and ketoconazole were used as antibacterial and antifungal agents, respectively. Minimum inhibitory concentrations (MIC) were found with the antibiogram test. Ames test was performed to determine the genotoxicity of the compounds using *Salmonella typhimurium t4* and *Vibrio fischeri* ATCC 7744. *In silico* Absorption, Distribution, Metabolism and Excretion (ADME) studies were also performed using the QikProp module of Schrödinger's Molecular modelling package. **Results:** Compounds **1-12** were found to be highly effective on *Pseudomonas aeruginosa* with a MIC value lower than 0.25 µg/mL when compared with chloramphenicol (MIC= 2 µg/mL). The results demonstrated that the antifungal effects of the compounds on *P. aeruginosa* did not depend on the aryl group at the 2nd position of the furan ring. On the other hand, the compounds were not effective on *Staphylococcus aureus*. It was also determined that these compounds did not show any antifungal activity against *Candida albicans* and *Aspergillus niger*. According to Ames test, the compounds were found to be non-mutagenic. Compounds **7**, **9**, **10** and **11** only violated one parameter of Lipinski's rule of five, whilst other compounds did not violate Lipinski's rule. Based on Lipinski's rule, these compounds can be considered as drug-like molecules. Compounds **1** and **2** did not violate Jorgensen's rule of

three, whereas other compounds only violated one parameter of Jorgensen's rule of three. The percentages of human oral absorption for the compounds were found to be high (93.60-100.00%). On the basis of these findings, all compounds are expected to have good oral bioavailability. **Conclusion:** According to the results, compounds **1-12** stand out as promising antibacterial agents against *P. aeruginosa* for further studies.

Keywords: Quinoxaline, hydrazone, antimicrobial activity, genotoxicity, pathogens

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ORAL PRESENTATION

SFX-BASED DRUG DEVELOPMENT FOR HIV ERADICATION

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Objective / Purpose: Main challenge of fight against HIV infection is to eliminate the latent viral reservoir from body. This reservoir is resistant to antiretroviral therapy and leads to viral rebound once the treatment is stopped. We have recently developed man-made derivative of IP_6 (inositol hexaphosphate) named L-HIPPO to suppress membrane localization of Gag and induced strong apoptosis of the host cell containing the un-budded viruses. The L-HIPPO was designed based on the fact that the HIV-1 MA domain of Gag mediates membrane binding through its interaction with inositol phospholipid PIP2 in the membrane. It has been shown that L-HIPPO has a MA-binding affinity 70-fold stronger than that of the less phosphorylated PIP2 analog. Here, we report first serial femtosecond X-ray crystallography (SFX) study of HIV-1 Gag MA as the complex with IP_6 using X-ray free-electron laser (XFEL) for the development of second generation of IP_6 derivative (Super-HIPPO) to eradicate HIV. **Metarials and Methods:** His_{10}-tagged HIV-1 MA gene was cloned into pRSF-1b vector and expressed in *E. coli* BL21(DE3) cells. MA was purified with a Ni-NTA column, and then the His_{10}-tag was cleaved off with TEV protease. Untagged MA was applied to a gel filtration column, Superdex 200 10/300. The fractions were concentrated, and then used in co-crystallization with IP_6 at room temperature by the hanging-drop method. Microcrystals were harvested, pooled and filtered through 40-micron Millipore mesh filter. A crystalline slurry of HIV-1 MA-IP_6 microcrystals was injected into the interaction region inside the front vacuum chamber at the LCLS CXI instrument using the coMESH injector. The SFX diffraction images collected at LCLS were processed using *Psocake* software. *CrystFEL*'s *indexamajig* program was used to index the crystal hits. **Results and Discussion:** MA-IP_6 microcrystals were obtained in the several conditions, then the conditions were optimized. The microcrystals in two different forms were used in the diffraction experiments and diffracted beyond 3.5 Å resolution. The crystal structures of MA-IP_6 were solved in the both cases by the molecular replacement method using the parameters and space group for these crystals obtained by synchrotron X-ray crystallography at a cryogenic temperature. Consistent with the prediction, the IP_6 molecules were interacted with residues in the highly basic region. The crystal packing of the two crystal forms will be discussed from the points of MA assemblies and their relation to IP_6 molecules. **Conclusion:** These results demonstrate the feasibility of conducting HIV-1 MA-IP_6 complex structural studies using XFELs, which hold great promise for a more comprehensive understanding of MA and IP_6 interaction. Moreover, crystallization trial on the MA-L-HIPPO is in progress.

Keywords: SFX, X-FEL, HIV-1 MA protein, IP_6, ambient temperature.

ORAL PRESENTATION

PENTACYCLIC TRITERPENE DERIVATIVES WITH SELECTIVE ANTI-PROLIFERATIVE EFFECT AGAINST CHRONIC MYELOGENOUS LEUKEMIA

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[2] Department of Bioorganic Medicinal Chemistry, School of Pharmacy, Kumamoto University, Kumamoto, Japan,
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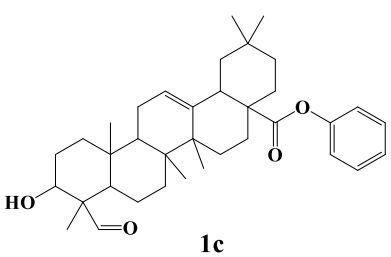
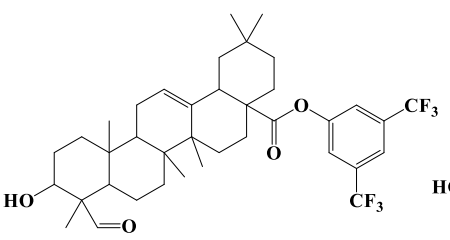
Objective / Purpose: Synthesis of pentacyclic triterpene derivatives with enhanced cytotoxic activity against chronic myelogenous leukemia K562 leukemic cells in particular. This is based on our previous finding that gypsogenin benzyl ester is a promising BCR ABL kinase inhibitor (IC_{50} 8.7μM). **Metarials and Methods:** We synthesized the benzyl esters of five different triterpenes namely, Asiatic acid, Betulinic acid, Glycyrrhetic acid, Oleanolic acid and hederagenin. Furthermore, different substituted benzyl esters of gypsogenin were prepared. All esters were produced through a direct reaction of the pentacylic triterpene with the adequate aryl bromide in presence of potassium carbonate. The afforded products were purified by flash column chromatography and confirmed by different spectroscopic methods. The synthesized compounds were evaluated against K562 cell line cancer cell line by MTT assay. Selectivity was assessed by measuring the effect on PBMC normal cells. The most selective compounds were selected for further apoptosis induction test and evaluated against BCR ABL kinase. **Results and Discussion:** We have found that derivatives PT5 and PT8 possess a remarkable inhibition effect on K562 cells proliferation (IC_{50} 4.78 and 3.1 μM, respectively). The presence of electron withdrawing substitutions at compound 1c benzyl ring enhanced its cytotoxic effect. Of interest, compound PT5 that have a high selectivity index (11) reflecting potentially less side effects. PT5 and PT8 demonstrated pronounced anti-proliferative activity against different leukemic genotypes and cervical cancer. They induced a pronounced apoptosis to K562 cells. Evaluation of those compounds against BCR ABL kinase is ongoing. **Conclusion:** New gypsogenin derivatives that surpass the parent compound cytotoxic effect have been synthesized confirming that gypsogenin represents an attractive nucleus that deserves more attention for anti- cancer drug development.

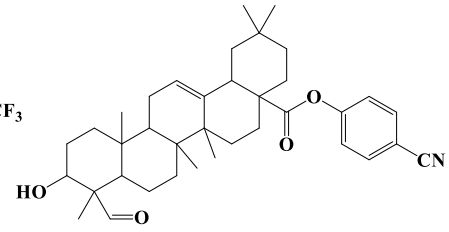
Keywords: Pentacyclic triterpenes, Gypsogenin, BCR-ABL tyrosine kinase, leukemia.



1c



PT5



PT8

ORAL PRESENTATION

PHYLOGENETIC INVESTIGATION ON *EUPHORBIA* L. SPECIES SUBGENUS *ESULA* PERS. SECT. *PITHYUSA* (RAF.) LÁZARO MEMBERS

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Objective / Purpose: In this study, molecular studies were conducted on *Euphorbia* L. subg. *Esula* Pers. sect. *Pithyusa* (Raf.) *Lázaro* taxonomic group members in Turkey. As a result of field studies conducted in the past years, the individuals collected in this section in our country were evaluated by phylogenetic analysis based on molecular data. The similarity to DNA sequences differences and suspicious species are tried to be determined, in this study. **Material and Methods:** DNA isolations were performed using the DNeasy Plant Mini Kit, following the manufacturer's instructions with some modifications. Second elution DNA extractions were used in the polymerase chain reactions. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) sequences and the trnL-F region of the chloroplast DNA (cpDNA) were used for molecular analysis. The PCR protocol of Shaw et al. (2007) was applied. Sequenced DNA was edited using Sequencer version 4.9. Molecular analysis was done using PAUP* software. **Results:** 637 characters were obtained from nrITS DNA data. 424 were constant, and 104 of 637 characters were parsimony-uninformative and 109 of them were informative for parsimony. 44 specimens have totally 1063 characters for trnL-F data were obtained. 940 of these characters are constant, 52 of them are parsimony-uninformative variable characters and 71 of them are parsimony-informative variable characters. **Conclusion / Discussion:** According to the ITS data, *E. terracine* added as an external group was separated from the other taxa. *E. falcata, E. cassia* and *E. gaillardotii* taxa, which were subsequently included into the sect. *Pithyusa* by Riina and colleagues were located as a separate group. The most important character in the morphological distinction is the seed characteristics. The taxa having smooth seed surfaces and rough ones were grouped separately in our ITS phylogenetic tree, except *E. petrophila*. E. petrophila is located with smooth ones. *E. yildirimlii* is very similar to *E. macroclada* according to DNA data. This supports the morphological similarity of two species. Thracian populations of *E. pannonica* differ from West Anatolian populations as morphologically, and molecular data confirm this separation.

Keywords: *Euphorbia*, molecular, nrITS, trnL-F

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ORAL PRESENTATION

INADEQUATE CALCIUM INTAKE BY THAI ADOLESCENTS: USE OF IBM TO INVESTIGATE

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Objective / Purpose: Calcium plays a very significant role in bone formation. The dietary reference intake (DRI) of calcium recommended for adolescents is 1,000 mg per day. Literature reviews showed that Thai adolescents in rural areas intook calcium at approximately one third of DRI.While several studies indicated insufficient intake of calcium among adolescents, it is not clear why calcium consumption was low. The aim of this study is to elicit the behavior and factors determining calcium consumption behavior among lower secondary students. **Material and Methods:**A qualitative study was conducted in one secondary schoolwhich represented the general characteristics of rural lower secondary schools in the Northeast of Thailand. Participants were 30 students from Grade 7-9, chosen by their instructors. Integrated Behavioral Model (IBM) was used to construct interview guidelines. Two rounds of face-to-face individual interviews were conducted for each participant. Prior to the first interview, a presentation on sources of calcium were provided to all participants. In the first round of interviews, data on the participants' attitude toward calcium consumption, influential persons,expressed attitudes of calcium consumption behavior, and perceived behavioral control, were collected. Two weeks later the second interview was conducted to collect data on knowledge about calcium, salient behavior, environmental constraints, and calcium consumption behavior. Content analysis was used to analyze data. **Results:**Fifteenparticipants were males. Mean ±SD ofBody Mass Index of participants Grade 7 were highest 20.7 ±6.26 kg/m^2.Eighteen participants had calcium containing food at least once in the past 24 hours. In the first interview, 12 participants intended to consume calcium containing food, but in the second interview only 7 actually consumed calcium containing food. Most of the participants had a positive attitude towards calcium consumption. Most participants expressed that calcium intake was supported by their parents. All of the participants had insufficient knowledge on sources of calcium and the amount of calcium required per day. Expressed barriers were unavailability of calcium containing foods at home or school, and limited budget to buy food. **Conclusion/Discussion:** The finding suggest that the IBM could be used to explain calcium consumption behavior among lower secondary students. Quantitative research should be conducted to quantify the finding of this study.

Keywords: health behavior, behavioral model, consumption, milk, students

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ORAL PRESENTATION

PROTECTIVE EFFECT OF SAFFRON PETALS (*CROCUS SATIVUS* L.) AGAINST ANTI-TUBERCULOSIS DRUGS INDUCED LIVER INJURY

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Objective / Purpose: The aim of the present study is to determine the phytochemical composition of Saffron Petals extract (SPE). It also investigates antioxidant and hepatoprotective activities of SPE anti-tuberculosis drugs (ATDs) induced Liver injury in rodent model. **Material and Methods:** Hepatic cellular injury was initiated by the administration of ATDs (100 mg/kg) followed by SPE at three different doses (100, 200, 400 mg/kg) for 2 weeks. At the end of the 2 week, rats were sacrificed and blood and liver samples were taken for serological, in vivo antioxidant studies, histological and immunohistochemistry examination parameters. The free radical scavenging activity of SPE was evaluated by DPPH, TPTZ and ABTS assay. The components present in SPE were identified by LC-ESI-MS/MS technique. **Results:** SPE exhibited significant DPPH, TPTZ and ABTS radical scavenging activities. The compounds identified by LC-ESI-MS/MS were found to be flavonoids and phenolic acids in nature. Treatment with SPE prevented oxidative stress by restoring the activity of antioxidant enzymes and decreasing the levels of liver toxicity markers SGOT, SGPT and LDH. Also, all the doses SPE showed significant decrease in inflammatory response via decreased over-expression of NF-kB, COX-2 and nitric oxide. **Conclusion / Discussion:** This study reveals that Saffron Petals a rich source of flavonoids, possess hepatoprotective potential against ATDs induced hepatic injury that may prove itself as a clinically useful natural product in management of drug induced liver injury.

Keywords: *Crocus Sativus*, LC-ESI-MS/MS technique, DPPH, hepatoprotective activity

ORAL PRESENTATION

BIOAPIGYN® OINTMENT FOR PELVIC MUSCLE TONUS VERSUS PELVIC FLOOR MUSCLE TRAINING FOR THE TREATMENT OF URINARY INCONTINENCE

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Objective / Purpose: The purpose of this work was the assessment of the clinical efficacy and safety of Bioapigyn® vaginal ointment for pelvic muscle tonus compared to pelvic floor muscle training in alleviation of the symptoms of stress, urge and mixed urinary incontinence and of vulvo-vaginal disorders in child-bearing and menopausal & postmenopausal women. **Materials and methods**: The experimental group consisted of 66 women was treated 28 days with Bioapigyn® ointment for pelvic muscle tonus (2.5 mL/day) which consisted of the following ingredients: honey; glycerol, *Cera flava*, oil extracts of *Capsella bursa-pastoris* L., *Urtica diodica* L., *Quercus robur* L., *Salvia officinalis* L., *Achillea millefolium* L., *Alchemilla vulgaris* L., *Calendula officinalis* L., *Matricaria chamomilla* L., *Plantago major* L., *Olea europaea* L. and essential oils *Melaleuca alternifolia*, *Timus vulgaris* ct. thymol and *Origanum vulgare*. The control group also consisted of 66 participants was subjected to pelvic floor muscle training during 28 days (five times a day). The degree of the incontinence and vulvo-vaginal disorders were assessed by ICIQ-UI SF score (ranging from 0 to 21), the volume of post voiding residual urine, perineometry, the total score of self-assessed vulvo-vaginal symptoms and vaginal pH determined before and after the treatment or training. **Results:** In the end of the treatment with Bioapigyn® ointment the mean value of ICIQ-UI-SF score decreased 54.9%, perineometry parameters increased between 31.5 and 34.3%., residual urine decreased for 76.9% and vaginal pH for 14.2%. All the symptoms of vulvo-vaginal disorders disappeared completely in all participants. The control group showed no changes in vaginal pH or the improvement concerning the vulvo-vaginal complaints. ICIQ-UI-SF score decreased 4.3%, residual urine volume for 9.1% while perineometry parameters increased between 4.3 and 8.3%. **Conclusion / Discussion**: Bioapigyn® vaginal ointment for pelvic muscle tonus alleviate the symptoms of incontinence by physical activity of the contraction and relaxation of smooth muscles of the pelvic floor due to the presence of the extract with smooth muscles contraction, smooth muscle relaxation and astringent properties resulting in the tightening and firming of the smooth muscles of the pelvic floor. Low pH, high osmolarity, high viscosity and greasiness as well as low water activity of Bioapigyn® ointment resulted in the alleviation, followed by complete disappearance of the symptoms of vulvo-vaginal complaints due to: the creation of unfavorable conditions for the growth, adhesion and multiplications of the pathogens; the creation of the protective coating on the vaginal mucosa enabling its recovery and preventing further irritation; alleviation of the vaginal dryness due to the presence of the humectants (glycerin, honey); preventing the pain during intercourse due to lubricating effect.

Keywords: urinari incontinence, vulvo-vaginal disorders, honey, herbal macerates

ORAL PRESENTATION

ANTICANCER ACTIVITY OF EXTRACT FROM TWIGS OF CAUCASIAN BEECH IN TURKEY

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Objective / Purpose: Structures of plant products are sometimes unprecedented, and these often show various biological activities. Plant extracts and chemicals isolated from plants have been widely used as drugs until now. Turkey is geographically unique, having huge fertile land located in the middle of Europe and Asia, thus intriguing plant products are expected to exist. However natural chemistry work using Turkish plants has not done everything. Aiming at development of new anticancer drugs based on Turkish plants, extracts with anticancer activity were explored.

Metarials and Methods: Various Turkish plants were collected, and more than 100 ethanol extracts were prepared to make a library. In the first screening, cytotoxic activities against human chronic myelogenous leukemia cell line K562 and human acute monocytic leukemia cell line THP-1 were examined by MTT assay after 3 days incubation. In the second screening, cytotoxicity against peripheral blood mononuclear cells (PBMC) from human healthy donors was examined by MTT assay. About the selected compound in the screenings, mechanism of cell death was examined. Furthermore, the activity of Turkish and Japanese samples was compared.

Results and Discussion: In the first screening, 4-5 samples hit (cell viability of less than 30%). Among them, 2 samples did not show cytotoxicity against PMMC (cell viability of more than 80%). One of them was well studied sample, in contrast there are almost no literatures about another sample, twigs of Caucasian beech (*Fagus orientals*). After treatment of cells with the sample, the cells were treated with annexin-V-FITC and ethidium homodimer III. Many cells were stained with only annexin-V. Western blotting of cells treated with the sample showed cleavage of Caspase-3. Furthermore, twigs of 2 kinds of beech in Japan were collected, and the same experiment was performed. Unlike Turkish beech, extract of Japanese beech did not show cytotoxicity.

Conclusion: We found ethanol extract of twigs of Caucasian beech in Turkey has selective cytotoxicity against cancer cell line by apoptosis. On the other hand, samples from Japanese beech did not show the same effect. Stem of beech is used for wood materials. The twigs have been wasted, thus this study will lead to effective utilization of wasted materials.

Keywords: Anticancer, Caucasian beech, Turkey

ORAL PRESENTATION

FLOW CYTOMETRY BASED ANTIPROLIFERATIVE, APOPTOGENIC AND CELLULAR DNA FRAGMENTATION ACTIVITIES OF GINGER (*ZINGIBER OFFICINALE*) RHIZOMES

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Background and Objective: Considering the side effects related with usage of synthetic chemotherapeutics, use of anticancer agents from medicinal plants with less or no side effects have been gained a great importance in recent decades. Ginger, the rhizome of *Zingiber officinale*, has been widely consumed as a spice and a common condiment for foods throughout the world. In addition, it has been used in traditional oriental medicine due to its multiple health benefits including antidiabetic and antiobesity effects, protective effect against ulcerative colitis and cardiovascular diseases, and also prevention potentials to cancer, that have been experimentally verified in various researches previously. **Material and Methods:** Based on wide range of beneficial pharmacological activities of ginger, this research aimed to evaluate possible anticarcinogenic and antiproliferative effects, associated with mechanism of carcinogenesis, of ginger methanolic-extract (GME) on human lung carcinoma (A549), non-small lung carcinoma (H1299), glioma (C6) cancer cells, and non-tumorous human umbilical vein endothelial cells (HUVECs). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed for assessment cancer prevention and antiproliferative activities of the GME. Apoptotic activity in GME treated cancer cell lines were determined by flow cytometry using apoptosis Detection Kit (Sigma-Aldrich). Cells were stained with Annexin V-Fluorescein isothiocyanate conjugate (AF) and propidium iodide (PI) solution to distinguish cell viability, early or late apoptosis, and necrosis. Acridine orange (AO)/ethidium bromide (EB) dual staining used for identification cells with damaged membrane and nucleus. Moreover, DNA fragmentation in apoptosis induced cancer cells were analyzed using Apoptotic DNA-Ladder Kit (Sigma-Aldrich), and DNA fragmentation were imaged after stained with Etidium bromide. **Results:** MTT results showed that the GME displayed remarkable decreases of cell growth in tested cancer cells through enhancing both apoptosis and necrosis in concentration and time dependent manner. The data obtained from the MTT assay were evaluated by one-way analysis of variance (ANOVA), and the IC_{50} values were found to vary from 2.16±0.08µg/mL to 8.53±0.62µg/mL at 24h ($p<0.05$) on the cancer cells. Flow cytometric analyses that were seem to be highly in correlation with MTT assay results, GME induced apoptosis (upon %90), whilst inhibited cell proliferation in the tested cancer cells. The GME-induced apoptosis in the cancer cells was also detected by cellular DNA fragmentation. Furthermore, antiproliferative and apoptotic activity results were confirmed under fluorescent microscope with GME treated and stained cancer cells when compared to untreated control cells. **Conclusions:** In conclusion, the findings of the research demonstrates that the edible rhizome extract of *Z. officinale* has significant anticarcinogenic and antiproliferative activities towards the tested cancer cells. It can be clearly suggested that further studies in animal models for the formulation of natural compounds could be conducted to elucidate chemopreventive and chemotherapeutic potentials of ginger.

Keywords: Annexin V-FITC; apoptosis; DNA fragmentation; flow cytometry; migration; *Zingiber officinale.*
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ORAL PRESENTATION

FORMATION OF LYSOZYME-CASEINATE HETEROPROTEIN COMPLEXES FOR IMPROVING THE THERMAL STABILITY OF LYSOZYME

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Objective / Purpose: Heteroprotein complexes has drawn more and more attention in recent years. The heteroprotein coacervates of sodium caseinate (CAS) and lysozyme (LYS) were prepared at pH 7. Through physicochemical and thermal dynamical study, the complexation behavior of CAS/LYS at different ratios and temperatures could be better understood. Thermal stability of lysozyme was hoped to be improved by formation of CAS/LYS complexes. **Material and Methods:** The formation of heteroprotein complexes of sodium caseinate (CAS) and lysozyme (LYS) at pH 7 was investigated by using turbidimetric analysis, particle size distribution and zeta potential at different CAS/LYS ratios. Moreover, isothermal titration calorimetry (ITC) was used to determine the type and magnitude of the energies involved in the CAS/LYS complexation process and evaluated the thermodynamic behavior of their complexation. Finally, the thermal stability of lysozyme in CAS/LYS complexes was evaluated by measuring the antimicrobial activity of lysozyme after spray-drying. **Results:** Our results indicated that the formation of a heteroprotein complexes of CAS/LYS was highly affected by CAS/LYS ratio. With increase of CAS/LYS ratio, the turbidity, particle size and lysozyme activity of CAS/LYS complexes were almost constant until CAS/LYS ratio reached 1.0. When CAS/LYS ratio exceeds 1.0, complexes size and lysozyme activity of CAS/LYS complexes drastically decreased and turbidity increased drastically. With the continued increase of CAS/LYS ratio, the turbidity and size slightly decreased, and lysozyme activity increased slightly. ITC results showed that the structuring stages were characterized by exothermic signals and were controlled by favorable enthalpy-change due to electrostatic interactions between both proteins. In addition, the interaction between two proteins is temperature-dependent and mainly entropy driven. Furthermore, through spray-drying process, lysozyme activity in CAS/LYS coacervates with ratio 1.0 was recovered more than 80% of its initial activity after adding calcium chloride. **Conclusion / Discussion:** Our data indicated that, when CAS/LYS ratio was below 1.0, limited caseinate chains could combine with abundant lysozyme molecules to form coacervates with big size (~ 100 μm). When CAS/LYS ratio reached 1.0, the caseinate chains were saturated with lysozyme, the structure of CAS/LYS complexes would change, shown as a significant increase of turbidity, decrease of particle size and lysozyme activity. These results were further confirmed with data from ITC tests. The CAS/LYS coacervates at ratio 1.0 were thermally stable against spray-drying operation, and one can assume that CAS covered the active sites in LYS to inhibit the inactivation of LYS. The present study provided useful information about CAS-LYS complexation and binding processes, which could facilitate their application in antimicrobial edible food packaging manufacturing.

Keywords: Lysozyme, Sodium caseinate, Heteroprotein, Isothermal titration calorimetry.

ORAL PRESENTATION

GLYCATION INDUCED STRUCTURAL ALTERATION OF BIOMOLECULES AND THEIR REVERSAL BY SOME ARTIFICIAL AND NATURAL COMPOUNDS

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Objectives/Purpose: Glycation induces changes in the structure of biomolecules like DNA and proteins. These alterations have been implicated in various diseases. The purpose of this study is to understand these structural alterations and their reversal by some artificial and natural compounds. **Material and Methods:** The *in vitro* glycation of DNA and proteins was carried out by incubating these samples with glycating agents like glucose/methyl glyoxal and/or natural test compounds for four weeks at 37 ºC. The desalted samples were used for further analysis. The structural alterations were analyzed with spectrophotometric and electrophoretic techniques. The amount of glycation products in the presence/absence of test compounds were measured by established methods like NBT assay, carbonyl content, Thioflavin T assays and total AGEs. **Results:** The spectrophotometric and electrophoretic results indicate that the structure of biomolecules changed during the process of glycation. The amount of glycation products also increased with the duration of the incubation. Test compounds like Thiamine (a vitamin), Thymoquinone (phytonutrient from black cumin), Eugenol and Curcumin caused reversal of structural alteration and decrease in the amount of glycation products. **Conclusion/Discussion:** This study clearly indicates that the structural alteration of biomolecules is induced by glycation and natural compounds can be used to reverse these structural alterations and prevent the accumulation of advanced glycation end products.

Keywords: Advanced Glycation end products (AGEs), Amadori products, Carbonyl content, Curcumin, Eugenol, Protein aggregation, Thiamine, Thymoquinone.

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ORAL PRESENTATION

NEUROPROTECTIVE AND COGNITIVE ENHANCEMENT POTENTIALS OF XANTHONE-ENRICHED FRACTION OF *GARCINIA MANGOSTANA* AND A-MANGOSTIN

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Objective: Mangosteen (*Garcinia mangostana*), also known as "The Queen of Fruit" has been used traditionally in Southeast Asia to treat against inflammation, skin infection, wound and diarrhea. The present study aimed to investigate the neuroprotective and cognitive enhancement potentials of xanthone enriched fraction (XEF) from *G. mangostana* and its major constituent, α-mangostin. **Material and Methods:** The neuroprotective effects of XEF and α-mangostin were studied in various stressors-induced neurotoxicity models in neuroblastoma cell lines and rat primary cells. Their effect on cognition was investigated in chronic cerebral hypoperfusion (CCH) rats, prepared by permanent bilateral common carotid arteries occlusion (PBCCAO). Two weeks after surgery, CCH rats were orally administered (single and 14 days repeated dose) with XEF and α-mangostin prior to locomotor activity and Morris water maze, long term potentiation (LTP) evaluation. **Results:** The stressors-induced neurotoxicity caused reduction in cell viability of 25 to 45%. At lower concentration range of 0.25-1μg/mL, XEF and α-mangostin showed significant and concentration dependent neuroprotection in all test models. Among them, α-mangostin showed the most promising neuroprotective effect, especially in the glutamate-induced neurotoxicity. The *in vivo* studies showed no effect on the rat's locomotor activity. However, α-mangostin (50mg/kg) and XEFGM (100mg/kg) significantly reversed the cognitive impairment induced by PBCCAO in the spatial learning test. In addition, α-mangostin also showed significant improvement in the reference memory. LTP results revealed that α-mangostin improved the basal synaptic transmission but has no improvement on the inhibition of LTP observed in CCH rats. Likewise, no changes were observed in the protein expressions of BDNF and CAMKIIα in hippocampus of the treated rats. **Conclusion:** The present study suggests that XEF and α-mangostin are potential protective agents against oxidative stress and excitotoxicity-induced neurodegeneration and ameliorated learning and memory deficits in CCH rats, worthy of further investigation.

Keywords: *Garcinia mangostana*; α-mangostin; Xanthone enriched fraction; Neuroprotective; Cognitive enhancement; Chronic cerebral hypoperfusion.

ORAL PRESENTATION

MEDICINAL AND AROMATIC PLANT PRODUCTION, TRADE AND FUTURE PERSPECTIVE ON TURKEY

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In order to solve the problems encountered in the culture of medicinal and aromatic plants, to take the research as a whole, to organize these studies and to provide coordination among the working people and institutions, in 1990, National Research Project of Medicinal and Fragrant Plants was initiated by Turkey Ministry of Agriculture and Forestry. The main purpose of these studies is to develop the registered varieties by cultivating new plant species for the production of standard and high quality raw materials required by different industries in medical, aromatic and dye plants in our country. Turkey due to plant genetic resources that have medicinal and aromatic plants in the culture, in terms of production and product development has an important place in the world.

Research institutes located in different regions of Turkey, start by selecting the appropriate type of culture to their ecology, engages in breeding and variety development work. Improvement studies have been made more efficient by integrating with quality studies to determine the active substances in plants. As a result of these studies, 41 cultivars of medicinal and aromatic plants belong to 15 species have been registered till now. The production areas of these species are increasing every year. In 2018, 300,000 tons of medicinal and aromatic plants were produced in approximately 100,000 hectares of land. 50,000 tons of this production was exported and 150 million US dollars of revenue was generated. In the following period, culture, breeding, cultivation and quality studies are continued and new varieties are developed and the production and usage areas of these varieties are brought into production. On the other hand, it is planned to give priority to export of processed products instead of raw material exports in order to get more share from the world herbal medicine market which is expected to reach US $ 5 trillion in 2050.

Key Words: Turkey, Medicinal and aromatic plants, production, trade

ORAL PRESENTATION

PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND NEUROPROTECTIVE PROPERTIES OF TURKISH OREGANO (*ORIGANUM ONITES* L.)

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Background and Objective: *Origanum onites* L. (fam. Lamiaceae), commonly known as 'kekik' in Anatolia, is an important medicinal plant that mainly used to manage for several ailments such as toothache, headache, high cholesterol, hypertension, diabetes, leukemia, stomach disorders, and bronchitis in Turkish traditional medicine. Although multiple biological activities of *O. onites* L. have been documented previously, this is the first research that has been performed with the plant growing under special conditions. **Material and Methods:** Therefore, the present study aimed to investigate the biological potential of aqueous and methanol extracts of the aerial parts from *O. onites* L. in terms of their enzyme inhibitory effects, antioxidant capacities, mineral contents and total polyphenolic compositions. Enzyme inhibitory assays were performed against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase enzymes. The total phenolic (TPC) and total flavonoid (TFC) contents were analyzed by the Folin-Ciocalteu method and aluminum chloride colorimetric assays, respectively, and K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, Pb mineral contents of the plant were also determined by Atomic Absorption Spectrometry. As for antioxidant activity, the extracts were tested by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) assays. **Results:** Overall, a strong correlation was observed between the phytochemical and mineral constituents of the plant and its biological properties. The aqueous extract possessed of higher TPC and lower TFC (102.18±0.32 mg g-1 extract as GAE and 36.17±0.14 mg g-1 extract as QE, respectively) compared to the methanol extract (85.90±0.16 mg g-1 extract as GAE and 45.53±0.22 mg g-1 extract as QE, respectively). The aqueous extract, which showed a higher TPC than that of the methanol extract, was found the most effective antioxidant in all assays. As for the neuroprotective potentials, both of the extracts showed the remarkable inhibition on cholinesterase enzymes with the values that ranged from 49.38±0.83% to 76.84±0.25%, whereas they displayed lower inhibition on tyrosinase enzyme. **Conclusion:** Taken together, *O. onites* L. with remarkable neuroprotective property, excellent antioxidant activities besides rich polyphenolic and mineral contents can be helpful to manage of oxidative stress-related diseases.

Keywords: *Origanum onites* L.; neuroprotection; enzyme inhibitory; antioxidant; polyphenolic; mineral content

ORAL PRESENTATION

GREEN SYNTHESIS OF CHITOSAN BASED NANOPARTICLES USING WATER EXTRACTS OF *CEPHALARIA BALANSAE* AND STUDY OF THEIR CYCTOTOXIC ACTIVITY

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Background: *Cephalaria* is an important genus of the Caprifoliaceae family which comprises 41 species spread out in Turkey and 24 of them are endemic [1]. Many studies concerning the chemical constituents and their biological activities of *Cephalaria* genus have been conducted in the literature. As it is clear from the chemical studies of this genus, this species has lots of natural compounds, including alkaloids, iridoids, flavonoids, phenolics and especially saponins that have different biological activities particularly antimicrobial, antifungal, cytotoxic, antioxidant, antidiabetic, anti-inflammatory and immunomodulatory activities [2-3]. Chitosan is the second most abundant found biopolymer in nature after cellulose that is a non-toxic, biocompatible, biodegradable, natural polysaccharide. It has great potential to use in many fields such as medical, food, agriculture and cosmetics [4]. Since nanotechnologies have become more popular in the pharmaceutical industry recently, combining these natural materials could be a good way to synthesize eco-friendly and nontoxic nanomaterials using for a safer alternative in the drug delivery system **Objectives:** The aim of this study was to synthesize and characterize of chitosan-based nanoparticles using water extracts of *Cephalaria balansae* and to evaluate their potential cytotoxic activity against cancerous esophageal, OE-33 and gastric adenocarcinoma, ACC-201 cell lines. **Materials and Methods:** The plant was collected from Antalya, Finike (22 km,1250 m) in July 2012, Turkey and was dried and grinded under suitable conditions. After that, water extracts of the plant were prepared. Chitosan [viscosity average molecular weight 20kDa, the degree of N-de-acetylation (75-85)] and sodium tripolyphosphate (STPP) were received from Sigma-Aldrich Company. Nanoparticles were prepared by ionic gelation method with STPP, that was prepared by doing optimize ratio between extract: chitosan and pH of chitosan solution. Also, $AgNO_3$ was added to nanoparticles to obtain the chitosan-Ag nanocomposites and the results were compared. OE-33 and ACC-201 cell lines were used for testing cytotoxicity by MTT method. **Results:** In this work, we reported on the synthesis of chitosan-based nanoparticles and chitosan-Ag nanoparticles using water extracts of *Cephalaria balansae.* The plant extract loaded chitosan nanoparticles showed particle size between 155 to 241 nm. The nanoparticles were characterized by UV-vis, Zeta-sizer, FTIR and SEM. According to activity results, the nanoparticle with the size of 219 nm showed an important inhibitory effect on cancerous OE-33 and ACC-201 cells with IC50 values of 13 and 14 µg/mL, respectively. **Conclusion / Discussion:** A simple green synthesis of chitosan-based nanoparticles using plant water extract has been successfully established in this study. The best particle size of chitosan nanoparticles and water extract was obtained as 162 nm average, with the polydispersity index of 0,361, while the best particle size of chitosan nanoparticles water extract with $AgNO_3$ was observed as 156 nm with the polydispersity index of 0,210. Previous studies have shown that nanoparticles with sizes less than 300 nm have a good ability to transport the body [5]. Thus, this eco-friendly method could be used for biomedical applications in future with their toxicity in OE-33 and ACC-201 cancerous cells.

Keywords: *Cephalaria balansae*, chitosan, nanoparticles, cytotoxic activity

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ORAL PRESENTATION

ISOLATION AND CHARACTERIZATION OF MAIN ACTIVE COMPONENTS RESPONSIBLE FOR ANTICANCER ACTIVITY OF *BAPTISIA TINCTORIA*

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Objective / Purpose: Cancer is the leading cause of deaths worldwide, and lung cancer accounts for major deaths annually. The global scenario accounts 17.8% of deaths due to lung cancer. It is reported that *Baptisia tinctoria* (L.) R. Vent. (Family – Fabaceae), a traditionally used plant, contains few components which have potential to be developed as anticancer drug. But no work has been carried out to identify these components. Thus, the present investigation was undertaken with an objective to isolate and characterize anticancer constituent(s) from *B. tinctoria* roots. **Material and Methods:** The crude extracts of *B. tinctoria* roots were prepared successively in order of increasing polarity by Soxhlation process. The crude extracts (chloroform and methanol extracts) and fractions of methanol extract (ethyl acetate fraction and remaining methanol extract) were evaluated for *in vitro* anticancer activity by MTT assay using human lung cancer cell line (A549). Gradient column chromatographic systems were developed for isolation of main active components from chloroform extract and ethyl acetate fraction, which were responsible for anticancer activity of *B. tinctoria* roots. **Results:** The chloroform extract and ethyl acetate fraction of methanol extract exhibited significant anticancer activity. Bioactivity-guided-fractionation of bioactive chloroform extract and ethyl acetate fraction led to the isolation of anticancer components, i.e., maackiain and trifolirhizin. **Discussion / Conclusion:** The present study establishes the anticancer potential of *B. tinctoria* roots. The activity is attributed to maackiain and trifolirhizin.

Keywords: Anticancer, Fabaceae, *Baptisia tinctoria*, Maackiain, Trifolirhizin

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ORAL PRESENTATION

CONTROLLED DELIVERY OF CIPROFLOXACIN FROM HALLOYSITE NANOTUBES

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Objective/Purpose: Ciprofloxacin (CIP) belongs to a family of antibiotics called fluoroquinolones and is used to treat a variety of bacterial infections. Halloysite ($Al_2Si_2O_5(OH)_4.2H_2O$ nanotube (HNT) is a two-layered aluminosilicate, with a predominantly hollow tubular structure [1-2]. CIP was loaded to halloysite nanotubes (HNTs) and encapculation efficiency and loading capacity of CIP were evaluated. Controlled CIP release from HNTs was investigated. **Material and Methods:** 100 mg of CIP was dissolved in 100 mL of 2% (v/v) acetic acid solution. 4 g of HNTs were added to the solution and this solution was mixed in sonicator (100 W) 30 mins. This solution was filtered through a vacuum filter. CIP encapsulated into the HNTs was allowed to dry for two days at room temperature. CIP encapsulated HNTs of 0.5 g were added to 100 mL of buffer solutions in the orbital shaker operated at 37ºC. CIP release from HNTs was investigated in HCl solution at pH 2.1, phosphate buffer solution (PBS) at pH 5.0 and at pH 7.4 simulating gastrointestinal system and blood through 24 hours. CIP concentration in the samples taken at certain time intervals from buffer media was measured in UV spectrofotometer. **Results:** The encapsulation efficiency and loading capacity of CIP into HNTs were found to be 88.3% and 17.3%, respectively. CIP absorbance vs concentration graphs were obtained in HCI and PBS buffer solutions at different pH values. The UV wavelength at which the CIP showed maximum absorbance for the CIP release profiles in HCl buffer was 268 nm. Maximum CIP absorbance values in PBS buffer solutions at pH 5.0 and at pH 7.4 were obtained as 283 and 289 nm, respectively. The CIP release efficiency in HCl solution at pH 2.1 simulating gastric media was determined as 27%. The CIP release percentages in PBS at pH 5.0 simulating intestinal tract and at pH 7.4 simulating blood were found to be 26% and 24%, respectively. **Conclusion/Discussion:** In this study it was shown that a high encapsulation efficiency and loading capacity of CIP was obtained into HNTs. Encapsulation of CIP into HNTs resulted in prolonged, sustained and controlled CIP release.

Keywords: Controlled drug delivery, ciprofloxacin, halloysite nanotubes.

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ORAL PRESENTATION

SETUP OF QAC-MICROENCAPSULES AND THEIR USE TO FIGHT BIOFILMS FORMED BY PATHOGENIC BACTERIA

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Objective: In this work we developed microcapsules that act as carriers of benzalkonium chloride (BAC) and dodecyltrimethylammonium chloride (DTAC). The goal is to allow microcapsules to penetrate to deep layers of biofilm and deliver the biocide. **Material and Methods:** Microcapsules were prepared with two different QACs: BAC and DTAC, then spray-dried by atomization. The microcapsules were prepared with two different emulsifier compositions: monolayer microcapsules (ML) stabilized with the sodium caseinate alone and bilayer microcapsules (LBL) stabilized with a mixture of sodium caseinate and pectin. The minimum inhibitory concentration (MIC) of free and micro-capsulated BAC/DTAC was investigated on food pathogenic bacteria such as *Salmonella enterica* CIP 8297, *Staphylococcus aureus* CIP 4.83, *Listeria monocytogenes* ATCC 35152 and *Escherichia coli*. CIP 54127. Antibacterial assays of our developed capsules were performed against biofilm formed by bacterial strains cited above and compared to BAC used under its free form. **Results:** Our results showed that MICs were significantly lower for encapsulated than for free BAC or DTAC. In addition, the disinfection efficacy of our formulated microcapsules was studied on 24h aged-biofilms formed at 30°C. Our results showed that the microencapsulation of both studied QACs used at their MICs reduced biofilm biomasses by up to 3 log. Free BAC and DTAC used at MICs had weaker antibiofilm effects when compared to microcapsules. One can assume the presence of two layers surrounding the QAC, in case of LBL, may result in a progressive and controlled release of the encapsulated molecule. Furthermore, less QACs quantities are required to observe greater antibiofilm when micro-capsulated. **Conclusion:** Several foodborne pathogens have developed intrinsic and acquired QACs resistance reducing the efficacy of these disinfectants. QACs are considered to have poor biodegradability, meaning that their excessive use is of major concern for the environment. To tackle these problems, the targeted disinfecting strategy was proposed in our work that unites efficient antibiofilm reduction with low QAC consumption. Therefore, our formulated QACs microcapsules seem to be a good strategy to apply in concerned sectors even when used at MICs. The application of such a QACs microcapsule-based delivery system can improve the surface disinfection procedures and reduce the generated chemical wastes.

Keywords: Pathogenic biofilm**,** benzalkonium chloride, dodecyltrimethylammonium chloride, anti-bacterial activity, micro-encapsulation

ORAL PRESENTATION

THE EFFECT OF *PIPER BETLE* LEAVE EXTRACTS ON WEIGHT, FOOD INTAKE AND BEHAVIOUR OF SD RATS BY USING ELEVATED PLUS-MAZE

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Objective: The clinical applications of weight loss management medicine are limited by their unwanted side effects on Central Nervous System (CNS). Interest in alternative medicine and plant-derived medication that affect the 'mind/behaviour' is growing. This study investigated the effect of an ethanolic extract of *Piper betle* (PB) leave on the weight, food intake and behaviour. **Materials and Methods:** Five groups of rat were given with high fat diet and treated with saline, phentermine and PB extracts, while another group of rats was given standard diet as a control. The behaviour were assessed by using Elevated Plus Maze (EPM) model. The studies were conducted with standard diet (SD) and high fat diet (HFD). Dried PB leaves were extracted with ethanol and phytochemical analysis was performed to reveal the presence of phytoconstituents. **Results:** HFD rats treated with PB (100, 300 and 500 mg/kg) showed no significant increase in body weight when compared to SD. Interestingly, HFD/PB(500 mg/kg) showed a reduction in food intake from the 14th week until the end of the studies, an increased in the percentage (%) of duration time spent in the open arms and arm entries in the centre square but decreased in the percentage (%) of duration of time spent in the close arms of EPM significantly ($P<0.05$) as compared to untreated HFD group. **Conclusion:** HFD/PB (500 mg/kg) showed the ability to suppress appetite and some anxiolytic properties with relatively lower sedative activities than that of phentermine and may have potential to be developed as weight loss agents.

Keywords: *Piper betle L.*, weight loss, food intake, elevated plus maze, anxiolytic properties.

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ORAL PRESENTATION

CHEMICAL COMPOSITION OF THE METHANOL EXTRACT OF VERBASCUM SPECIOSUM

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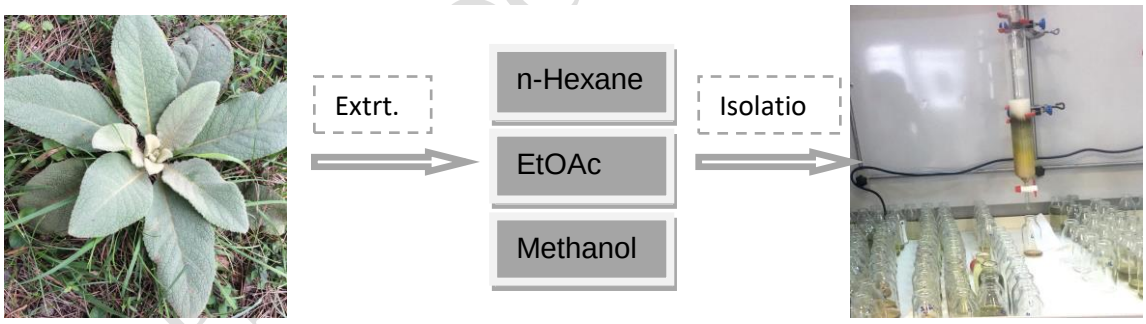
Abstract: The extracts, decoctions and infusions of *Verbascum*, commonly known as "mullein", have been used in traditional medicines for centuries in almost all parts of the world. To date, various alkaloids have been isolated from the aerial parts of some verbascum species, protoverbine, protomethine, verbaskine, anabasine, plantagonine, acetamide, verbacine, verballocine, verbasitie, verbascenine, verballoscenine, verbaskin, verbametrine, isovebametrine, verbamedine, verdoline and verbasine. Apart from its antifungalactivity, *V. speciosum* is a well-known herbused externally for treatment of wounds, cutsand skin disorders in the Black Sea region of Turkey. [1-3]

V. speciosum (Istanbul University Science Faculty Herbarium, Istanbul, deposition number 21530) leafs were collected in 2014 from Trabzon-Akçaabat region at 100 m altitude. The leaves were air dried at room temperature in a dark place and were thoroughly grounded. Powdered plant leafs (15g) were subjected to methanol extraction in 500 ml methanol using at room temperature. The extract was filtered through Whatman filter paper (no:1) and methanol was evaporated by using a rotary evaporator (BÜCHI Labortechnic AG, Flawil, Switzerland) at 40 °C.



Keywords: Plant extract, wound healing, *Verbascum*,

Acknowledgments: This document was given by the Turkish Patent Institute (TPE) withthetitle of "A Wound Remedial Herbal Product" TR2013 / 08599 and25418.32.

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ORAL PRESENTATION

ANTIPROLIFERATIVE EFFECTS OF SECONDARY METABOLITES ISOLATED FROM *CENTAURINUM ERYTHREAEA*

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Purpose: This study was carried out with the aim of isolation of secondary metabolites present in *Centaurinum erythreaea* by means of chromatographic methods and determination of structures by spectroscopic analysis as well as investigation of their anticancer potentials against breast adenocarcinoma (MCF-7) cells. **Material and Methods:** The plant materials were collected from Sipahiler Village, Derince, Kocaeli, Turkey (40°50′16.0″ N, 29°50′02.1″ E). The aerial parts of plant was extracted with methanol and chromatographed over Sefadex LH-20 and C18 packed columns. The structures of the isolated compounds were elucidated by spectroscopic methods (^{1}H-NMR, 2D-NMR, and MS data). Antiproliferative activities of the isolated secondary metabolites were investigated against MCF-7 cells by XTT assay and compared with 5-Fluorouracil (5-FU) and Vincristine, which are used as anticancer drugs. **Results:** Spectroscopic analysis results indicated that chromatographic process were achieve the isolation of three secondary metabolites. Thus, we here report the occurrence of 1,8-dihydroxy-3,5,6,7-dimethoxy xhantone- 3-*O*-rhamnopyranosyl-(1→6)-glucoside (1), ursolic acid (2) and centauroid A (3) compounds in *Centaurinum erythreaea* genus for the first time. XTT assay results showed that the isolated compounds showed good anti-proliferative effect in a dose-dependent manner between the range of 0.1 to 1 mg/mL. Among the compounds, the highest activity was exhibited by ursolic acid (2) with an IC_{50} value of 0.52 mg/mL. In addition, the compound 2 was found to display more effective than the positive controls (5-FU and vincristine) at all tested concentrations. **Conclusion:** The isolated compounds, especially ursolic acid (2), could be evaluated as a promising candidate for chemotherapeutic drugs in the experimental phase.

Keywords: *Centaurinum erythreaea,* seconder metabolites, chromatographic techniques spectroscopic methods, anticancer.

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ORAL PRESENTATION

TRADITIONAL USES, PHYTOCHEMICAL SCREENING AND BIOACTIVITIES OF A SPONTANEOUS SAHARIAN SPECIES

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Abstract:
The current study was to present *Matricaria pubescens* (Desf.) and its traditional uses. Also it was to investigate the antioxidant and the antibacterial activities of *M. pubescens* extracts. The antioxidant capacity and total phenolic content of *M. pubescens* extracts were measured utilizing Bleaching β-carotene assay (BCB) and Folin–Ciocalteu procedure, respectively. The highest phenolicand flavonïds contents were in that order 261.603± 0.033 µg gallic acid equivalent/mg extract and 259.666±0.024 µg quercetin equivalent/mg extract. While, values of relative antioxidant activity (AAR %) were between 21.74% and 41.74%. *M. pubescens* extracts were tested against Gram- and Gram+ pathogenic bacterial strains. It seems that *M. pubescens* has a particular efficacy against *E. cloacae* and *E. feacalis*.

Keywords: *Matricaria pubescens*, spontaneous plant, bioactivity, solvent extraction, Saharian zones.

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ORAL PRESENTATION

ISOLATION, CHARACTERIZATION AND ESTIMATION OF BIOACTIVE CONSTITUENTS OF *ACTAEA ACUMINATA* H. HARA

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Objective / Purpose: It has been estimated that neuropsychiatric disorders accounts for 14% of the global burden of disease. The roots of *Actaea acuminata* (Wall. ex Royle) H. Hara (Himalayan Baneberry; family - Ranunculaceae) has been recorded in old texts in the treatment of nervous disorders, scrofula, rheumatic fever, inflammation, rheumatism, cough and constipation. The methanol extract and ethyl acetate fraction of *A. acuminata* roots were previously reported to exhibit significant antianxiety, anticonvulsant and antidepressant activities. Thus, it was considered worthwhile to isolate, characterize and estimate bioactive constituents of *A. acuminata* roots.

Material and Methods: Bioactivity-directed fractionation scheme was followed to isolate bioactive constituents of *A. acuminata* roots. The isolated compounds were screened for various CNS activities such as antianxiety activity (Elevated Plus maze, Light / Dark model and Hole Board test), antidepressant activity (Forced swim test), sedative activity (Activity scores in Actophotometer) and anticonvulsant activity (MES-induced convulsion test). A HPTLC method was developed for quantitative determination of bioactive constituents in *A. acuminata* roots.

Results: The column chromatography of ethyl acetate fraction of *A. acuminata* roots using *n*-hexane, ethyl acetate and methanol in gradient system yielded five pooled fractions (F_1-F_5), which were evaluated for antianxiety, antidepressant, anticonvulsant and sedative activities. Amongst various fractions, only F_2 exhibited significant antianxiety, antidepressant and anticonvulsant activities with respect to control and statistically equivalent to the respective standard drugs. The column chromatography of bioactive F_2 led to isolation of four bioactive compounds, which were characterized as bergenin, gallic acid, acetyl bergenin and diacetyl bergenin. The content of bergenin and gallic acid in *A. acuminata* roots was estimated to be 0.8010 and 0.1242% w/w, respectively.

Conclusion / Discussion: The present study validates traditional claims of *A. acuminata* for CNS activities. Bioactive phenolic compounds (gallic acid, bergenin and acetylated derivatives), responsible for CNS activities, are isolated for the first time from *A. acuminata* roots.

Keywords: *Actaea acuminata*, Antidepressant, Anxiolytic, Bergenin, Gallic acid, Ranunculaceae

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ORAL PRESENTATION

DETERMINATION OF TOTAL PHENOLIC, FLAVANOID, CAROTENOID AND CHLOROPHYLL CONTENTS OF *NEPETA LAMIIFOLIA* WILD AND *NEPETA FISSA* C.A. MEYER COLLECTED FROM VAN DISTRICT OF TURKEY

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Objective / Purpose: *Nepeta* is a genus of annual or perennial herbs belongs to the *Lamiaceae* family which includes approximately 250 species. These plants are native to central and southern Europe, Asia, the Middle East, northern Africa, and to tropical mountains in Africa. *Nepeta* species are used in the traditional medicine of many countries. It has diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febrifuge, emmenagogue and carminative effects. **Material and Methods:** *Nepeta lamiifolia* and *Nepeta Fissa* C.A. were collected from Van district of Turkey. Total phenolic, flavanoid, carotenoid, chlorophyll contents were determined by using spectrophotometer device. **Results:** Total phenolic content was determined as 12,12 mg GAE/g plant for *Nepeta lamiifolia* Wild and 8,27 mg GAE/g plant for *Nepeta fisca* C.A.Meyer. Total flavanoid content was determined as 10,0 mg CE/g plant for *Nepeta lamiifolia* Wild and 4,89 mg CE/g plant for *Nepeta fisca* C.A.Meyer. The carotenoid content was determined as 22,64 mg/g plant for *Nepeta lamiifolia* Wild and 16,36 mg/g plant for *Nepeta fisca* C.A.Meyer. The chlorophyll content was determined as 46,58 mg/g plant for *Nepeta lamiifolia* Wild and 22,15 mg/g plant for *Nepeta fisca* C.A.Meyer. **Conclusion / Discussion:** The total phenolic and flavanoid contents of *Nepeta lamiifolia* Wild was detected as higher according to the some *Nepeta* species. So this plant can be studied for further detailed investigations.

Keywords: *Nepeta*, total phenolic content, flavanoid content.

ORAL PRESENTATION

CARBOXYMETHYL CHITOSAN: A POTENTIAL ACTIVE MOISTURIZING AGENT FOR COSMETIC APPLICATION

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Objective / Purpose: Chitosan has been regarded as an alternative natural polymer used for a variety of pharmaceutical and cosmetic applications so far]1, 2[. This study aimed to synthesize a water-soluble chitosan from shrimp chitosan in the form of carboxymethyl)CM(-chitosan in order to be an active moisturizing agent as well as to develop skin moisturizing creams containing CM-chitosan. **Material and Methods:** CM-chitosan was synthesized from shrimp chitosan through the incorporation of a carboxymethyl group using monochloroacetic acid under an alkaline condition. Moisture sorption isotherm as well as physical characteristics of the CM-chitosan including swelling index and melting point were then determined. Also, ultrastructural image of this compound, was visualized through a scanning electron microscope. *In vitro* pig skin shrinkage test as well as a measurement of pig skin hydration using Corneometer® were then carried out in order to evaluate the moisturizing effect of the obtained CM-chitosan compared with hyaluronic acid and propylene glycol. After that, moisturizing creams containing CM-chitosan, were developed and evaluated for their stabilities after 60 days of storage in various conditions. Eventually, performance test together with skin irritation test in 22 healthy volunteers, were performed. **Results:** Percent yield of CM-chitosan was 130.23 ± 7.25 %w/w indicating the increase in molecular weight of the material due to the addition of carboxymethyl group. By virtue of carboxymethyl group, an increase in hydrophilicity of chitosan, was observed. In addition, the synthesis of CM-chitosan in an alkaline solution, could reduce rigidity and crystallinity of the molecule. Swelling index and melting point of CM-chitosan were 500 mg/g and 174.8 ºC, respectively. Moreover, a superior moisturizing effect of 0.4% CM-chitosan above those of 0.2% hyaluronic acid and 5% propylene glycol was shown according to a lower shrinkage value and a higher percent effectiveness. The developed moisturizing cream containing CM-chitosan with pH 5.0 exhibited an excellent physical stability during storage. Interestingly, moisturizing efficacy in the volunteers of the formulation containing CM-chitosan, was better than those of hyaluronic acid and propylene glycol. This might be due to the fact that CM-chitosan, as a hygroscopic polymer, is capable of forming film onto the skin to prevent water loss along with absorbing water from the environment. **Conclusion / Discussion:** Our study successfully developed moisturizing creams containing CM-chitosan, which could be a promising active moisturizing agent derived from nature.

Keywords: Chitosan, Carboxymethyl chitosan, Skin moisturizer, Skin hydration measurement, Pig skin shrinkage test

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ORAL PRESENTATION

NOVEL CARBOXYMETHYLATED MERCAPTOTRIAZINOINDOLE DERIVATIVES AS POTENTIAL INHIBITORS OF ALDO-KETO REDUCTASE (AKR1B1) AMELIORATE HYPERGLYCEMIA MEDIATED AND 6-OHDA-INDUCED NEUROTOXICITY IN PC12 CELLS: *IN VITRO* HYPERGLYCEMIC PARKINSON'S DISEASE MODEL

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Background: A growing body of evidence were proposed linking altered glucose metabolism to the risk of progressive neurodegenerative disorders [1]. Insulin resistance and chronic hyperglycemia complications of Type 2 Diabetes mellitus have a higher risk of Parkinson's disease (PD) onset and share common features like (T2DM) dysregulated redox and inflammatory signaling pathways. Unfortunately, the molecular mechanisms underlying the interplay between T2DM and PD are still unknown [2]. In this study, we aimed to investigate the potential therapeutic effects of novel inhibitors of aldose reductases, the first enzyme of the polyol pathway and responsible for oxidative stress-induced inflammation in T2DM, on the pathogenesis of hyperglycemic-PD model. **Methods:** NGF-treated PC12 cells were used for mimicking the neuronal-like cell metabolism. We investigated the effects of new carboxymethylated mercaptotriazinoindole derivatives (CMTI, COTI) and Epalrestat (EPA) against neuronal injury mediated by 6-OHDA in PC12 cells under hyperglycemic (HG) conditions. For this purpose, cell viability/cytotoxicity analysis, ROS generation (DCFDA), inflammatory and nitrosative marker levels (iNOS, IL-1β, Tnf-α, 3-NT) were measured. In addition, total cellular antioxidant capacity and related effectors (SOD, CAT, GPx) were examined to support the potential redox modulatory effects of aldose reductase inhibitors. **Results:** NGF-treated PC12 cells exposed to 6-OHDA under HG conditions resulted in a loss of cell viability, increase in oxidative (ROS)-nitrosative stress (3-NT) levels and inflammatory markers (iNOS, IL-1β, Tnf-α). However, presence of EPA, CMTI and COTI significantly reversed these effects and potentiated the antioxidant capacity and its effectors (especially SOD). The newly synthesized derivatives showed more effective to the oxidative-stress related inflammation compared to commercially used EPA. **Conclusion:** Our results indicated that drugs used the treatment of T2DM and their derivatives could be a potential therapeutic approach for repositioning as multi-targeted therapy of hyperglycemic-PD.

Keywords: Hyperglycemia, PC12 cells, Parkinson Disesase's, Aldose Reductase inhibitors.

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 215S197).

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ORAL PRESENTATION

ANTIMICROBIAL ACTIVITIES OF SHELL AND CUP PARTS OF *QUERCUS COCCIFERA* L. FRUITS AGAINST OPPORTUNISTIC INFECTIOUS AGENTS

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Background: Acorns are the fruits of *Quercus coccifera* L., known as kermes oak, have been used against hemorrhoids, diarrhea, diabetes, kidney stones, and wound-healing remedy in the Anatolian folk medicine. In our previous research [1], neuroprotective effects of the ethanol and water extracts from the shell, cup and shelled acorn parts of acorn were explored through enzyme inhibition tests against acetylcholinesterase, and butyrylcholinesterase, antioxidant assays on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP), as well as total polyphenolic contents. **Material and Methods**: Taking our previous results on acorn fruits, the present study aimed to evaluate *in vitro* antimicrobial activities of the ethanol and water extracts from the shell, cup and shelled acorn parts of acorn (*Q. coccifera* L.), consumed as herbal coffee in some regions. *In vitro* antimicrobial activity of the extracts was assessed using agar diffusion test [2]. The bacterium inocula 100 µL in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37±2ºC for 24 hours); yeasts – on SDA agar (incubated at 35±2ºC for 48 hours). The extracts 20µL were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. Each antimicrobial assay was performed at least three times. As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923*; Escherichia coli* ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615. As a positive control were used ethanol; gentamicin (10 mg/disk) for Gram-negative bacteria, ampicilin (10 mg/disk) for Gram-positive bacteria, nystatin (100 UI) for *Candida*. We also used clinical strains of bacteria and yeasts isolated from the oral cavities of patients suffering from inflammatory periodontium. **Results and Discussion:** The study showed that aqueous extracts of acorn shell of acorn were established to be active against the reference and clinical strains of *S.aureus*, *Bacillus subtilis* and *Candida albicans.* All samples were ascertained to have an anti-staphylococcus activity; however the highest effect upon *S.aureus* was established when using aqueous extracts of acorn shells. The aqueous extracts of acorn cups and ethanol-aqueous extracts of acorn cup had a weak antimicrobial effect upon *Streptococcus pyogenes.* No antimicrobial effect of the studied extracts upon *E. coli* and *E. faecalis* was revealed in the current study. Neither sample showed an effect upon a probiotic strain of *Lactobacillus plantarum,* which fact indicates to a possibility of the use of *Quercus coccifera* -based products and probiotics as functional nutrition. **Conclusion:** Antimicrobial activities of different parts from acorn (*Q. coccifera* L.) have not been previously reported elsewhere. Accordingly, the results presented in this research could be the first report for the literature. Authors suggest that the results obtained from our laboratory studies could be useful scientific data for pharmaceutical industry as a natural antimicrobial agent.

Keywords: Acorn; *Quercus coccifera* L.; Antibacterial; Antifungal; Antimicrobial Agent

Acknowledgments: The authors would like to thank to Kilis 7 Aralık University, Central Laboratory for their technical support. Microbiology Laboratory at the Department of Genetics, Plant Physiology and Microbiology, Uzhhorod National University.

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ORAL PRESENTATION

SYNTHESIS OF COPPER NANOPARTICLES FROM WASTE ONION PEELS

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Objective/Pupose: Nanoparticles have extremely small dimensions and high surface area / volume ratio, so they have chemical and physical differences, such as catalytic reactivity, thermal and electrical conductivity, chemical stability, optical performance, antimicrobial activity, compared to larger particles of the same chemical composition. The physical and chemical processes used in the synthesis of nanoparticles have the disadvantages of producing many toxic substances that pollute the environment because they require high amounts of temperature and energy [1]. In recent years, in the synthesis of Au, Ag, Pt, Pd, Zn, Cu metal nanoparticles; biocompatible, non-toxic solvents, high cost-free, synthesized nanoparticles with good distribution, environmentally friendly biological synthesis methods are preferred [2]. In this study, copper nanoparticles (CuNs) were synthesized by the green, fast and environmentally friendly method using the extract of onion peel which is a waste material. CuNs find use in different areas such as nanoelectronics, magnetic devices, nanosensors, nanoprobes in medicines, pharmaceutical, cosmetic, catalytic and materials applications. **Materials and Methods:** Onionskeen and $CuSO_4.5H_2O$ were used as starting material for the synthesis of CuNs. To remove impurities, the collected onion peel wastes were washed several times with distilled water and then dried. To obtain onion extract, onion peel wastes were boiled in distilled water 30 mins. The extract obtained was stored by cooling and used in experiments. In the synthesis of CuNs, the solution volume of $CuSO_4.5H_2O$ was held constant at 50 mL, and the volume of the extract solution was changed between 10 mL and 90 mL. To determine the optimum conditions for CuNs synthesis, the temperature was varied in range of 20-80^oC, and the Cu solution concentration was changed in range of 1.0-8.0 M. Color of the solution changed from the blue color of Cu solution to black reveailing the formation of CuNs. Formation of CuNs was easily observed by UV-Vis spectroscopy. Maximum absorbance for CuNs was obtained at 450 nm. In addition, the characterization of CuNs was performed by SEM images and FTIR analyzes. **Results:** For CuNs synthesis, the optimum ratio of onion extract to $CuSO_4.5H_2O$ solution was determined as 70:50. The optimum copper concentration in solution and synthesis temperature were determined as 0.5 M and 20^oC, respectively. The characterization of CuNs was evaluated by the SEM images and FTIR spectra. **Conclusion:** CuNs production was performed by green synthesis method using onion waste peels. Both the environmentally hazardous agricultural and domestic wastes were eveluated, re-used, and the onion peel waste was shown to be suitable for the production of CuNs.

Keywords: Copper Nanoparticle, green synthesis, onion peel

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ORAL PRESENTATION

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING POMEGRANATE WASTE

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In recent years, metal nanoparticles have been used extensively in industrial fields due to their superior catalytic, magnetic and optical properties. Among metal nanoparticles, silver nanoparticles (AgNPs) are used in various fields due to their relatively inexpensive, highly conductive, and antibacterial properties[1]. The physical and chemical methods used in the production of AgNPs have high cost and toxic and hazardous chemicals that may have environmental and biological hazards in their structures. Recently, green synthesis has attracted great attention [2]. AgNPs have many potential applications in industrial fields involving antimicrobial agents, biological sensors, electronic devices, and waste water treatment. In this method, biological resources such as plant extracts, which are abundant and safe, are used. In this study, the production of AgNPs was performed by green synthesis using pomegranate (PM) waste.

PM waste and silver nitrate were used as a starting material for the synthesis of AgNPs. AgNPs formation was first observed by changing the color of the solution from yellow to dark brown. The Ag-extract solution was tested by a UV-Vis spectrophotometer, and maximum absorbance peak formation at 377 nm, which belongs to AgNPs,was observed. The characterization of AgNPs was performed by the SEM images and FT-IR analyses.

For the AgNPs synthesis, the optimum Ag concentration was determined as 10 mM, and the optimum temperature was 80oC.The formation of AgNPs was identified with the evaluation of SEM images and FT-IR spectra.

It was shown that both agricultural and domestic wastes are re-used and PM waste is suitable for the production of AgNPs.

Keywords: Silver nanoparticle, green synthesis, pomegranate waste.

ORAL PRESENTATION

SUSTAINED AND CONTROLLED ANTIBIOTIC RELEASE FROM CHITOSAN AND MAGNETIC CHITOSAN NANOPARTICLES

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Objective/Purpose: Chitosan (CTN) and magnetic chitosan (CTN-Fe_3O_4) nanoparticles (NPs) containing iron oxide were prepared as drug carrier by ionic gellation and co-precipitation techniques[1,2]. Metronidazole (MTZ) and tetracycline (TC) were chosen as model drugs. The magnetic property of CTN-Fe_3O_4 was evaluated by VSM measurements. MTZ and TC were loaded on CTN NPs and CTN-Fe_3O_4 NPs, respectively, and then controlled drug release was investigated. **Material and Methods:** The ionic gelation method was used for the synthesis of CTN NPs. The anionic cross linker, tripolyphosphate (TPP), is used in physically cross-linking and is added dropwise to CTN solution along with stirring and sonication. CTN coated Fe_3O_4 NPs were prepared using co-precipitation method. A certain amount of CTN was dissolved in acetic acid solution and pH of this solution was adjusted to 4.8 using NaOH. Ferrous ($FeCl_2.4H_2O$) and ferric ($FeCl_3.6H_2O$) reactive salts were added to the medium in N_2 atmosphere. Then ammonium hydroxide and TPP were added to the medium dropwise. After mixing, CTN coated Fe_3O_4 NPs were separated from the liquid phase using a neodymium magnet. After the MTZ-loaded CTN NPs were freeze-dried, the MTZ release of the CTN NPs was performed in 0.1 M HCl at pH 2.0 and in 0.1 M PBS at pH 7.4, similar to *in vivo* medium. TC release studies were also performed at 37 °C by adding 0.5 g/L CTN-Fe_3O_4 NPs in 100 mL PBS. **Results:** The encapsulation efficiency and loading capacity of MTZ on CTN NPs in HCl buffer at pH 2.0 were found to be 56.0% and 17.7%, respectively. The encapsulation efficiency and loading capacity of MTZ on CTN NPs in PBS buffer at pH 7.4 were determined as 41.3% and 19.7%, respectively. The MTZ release from CTN NPs in 0.1 M HCl at pH 2.0 and at 37°C simulating to gastric fluid was observed up to 20 hours and 44.0 % of the loaded MTZ was released in the medium. The MTZ release percentage in PBS at pH 7.4 was found to be 58.7%. The encapsulation efficiency and loading capacity of TC on CTN-Fe_3O_4 NPs in PBS buffer at pH 7.4 were found to be 80.0% and 7.3%, respectively. At the end of 24 hours,56.8 % of the loaded TC from CTN-Fe_3O_4 NPs released into the PBS. **Conclusion/Discussion:** High encapsulation efficiencies and loading capacities for MTZ and TC by CTN NPs and CTN-Fe_3O_4 NPs were obtained. A prolonged, sustained and controlled antibiotic release during 24 hours was observed using CTN NPs and CTN-Fe_3O_4 NPs.

Keywords: Controlled release, antibiotic, chitosan nanoparticles, chitosan-Fe_3O_4 NPs

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ORAL PRESENTATION

ENVIRONMENTAL POLLUTION DEGREE CHANGES THE BIOLOGICAL ACTIVITY OF NETTLE PLANT EXTRACTS IN CHICKENS

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Objective / Purpose: This study aimed to compare the changes in some biological parameters (body wight, total leukocyte numbers-TL and delayed type hypersensitivity – DTH) in 28 days old chickens treated with nettle extracts fro polluted and unpolluted areas. **Material and Methods:** The research was carried out (n=12/group) injected subcutaneously twice (days 0 and 7), with 0.5 ml of alcoholic nettle extracts harvested from both unpolluted (group III) and polluted areas (group IV), against 50^0 alcohol treated (group II) and untreated (group I) controls. Blood was sampled on days 0, 7 and 14, while wattle test to a homologous lymphocyte suspension was performed on day 14 of the experiment. The antioxidant capacity (scavenging effect over DPPH,%) of the nettle extracts was established. The significance of the differences was estimated by Excel program. **Results:** The antioxidant activity was reduced in plants from polluted areas (53.23 versus 79.08 %). There were no significant differences in weight gain/period between treated groups. As opposed to the slight changes in control groups, there was a constant increase of the TL in group III (from 15,400±3,421/mm^3 to 17,125±4,231/mm^3) and a decrease in group IV (17,611.1±2,401/mm^3 to 17,166.7±2,522/mm^3). The wattle test results after 48 h were similar in groups I, II and IV, the differences ranging between 0.23 and 0.28 mm, while the unpolluted nettle extract diminished the DTH to a difference of 0.07 mm. **Conclusion / Discussion:** The pollution exerted negative effects on the biological activity of the nettle extract reducing the weight gain and TL numbers, and increasing the wattle reactivity when compared to the unpolluted one.

Keywords: *Urtica dioica L.*, chickens, weight, adaptive immunity, wattle test

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ORAL PRESENTATION

INHIBITORY EFFECTS OF *HYPERICUM CERASTOIDES* (SPACH) N.ROBSON ON DIABETES-RELATED ENZYMES, AGE FORMATION AND MMP -2 AND -9

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Objective/Purpose: Various enzymes are known to take place in the regulation of blood glucose levels. Several natural products have been shown to possess antihyperglycemic activity by inhibiting those enzymes. Furthermore, it is quite well fact that increased levels of blood glucose are involved in protein glycation, resulting in the formation of advanced glycation end products (AGEs). AGEs are known to participate significantly in diabetic complications. Their formation generates malfunctions in various organs such as eyes, nerves, kidneys and heart. The boosted levels of AGEs in tissues are directly linked with the up-regulation of matrix metalloproteinases, an enzyme family responsible for the degradation of extracellular matrix. Due to such function, MMPs are related with inflammatory based diseases [1]. Different species of *Hypericum* (Hypericaceae) have been shown to inhibit different MMPs. There are also studies showing that certain *Hypericum* species inhibit diabetes-related enzymes such as α-amylase, α-glucosidase or protein tyrosine phosphatase 1B (PTP1B) [2]. Under the light of these information, this study was designed to investigate the inhibitory activities of *H. cerastoides* on α-amylase, α-glucosidase, PTP1B, MMP -2 and -9 as well as the formation of AGEs. **Material and Methods:** The plant samples were collected from Kayışdağı, İstanbul in 2017. After properly dried and powdered, they were extracted with 80% MeOH. The inhibition of α-amylase, α-glucosidase and PTP1B were spectrophotometrically measured. The inhibition of AGE formation was determined with specific fluorescence assay. The inhibition of MMPs was calculated with gelatin zymography. **Results:** 80% MeOH extract of *H. cerastoides* was found to possess considerable inhibitory activity on the enzymes mentioned. It also significantly inhibited the formation AGEs as well. **Discussion:** The findings of this study stated that *H. cerastoides* had a promising effect on hyperglycemia related inflammatory disorders. Naturally, future studies are required to fully explain the activity mechanism.

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POSTER PRESENTATIONS

POSTER PRESENTATION

ESSENTIAL OIL COMPOSITION OF *THYMUS VANDASII, THYMUS PANNONICUS* AND *THYMUS ATTICUS* FROM BULARIA

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Objective: The species of genus *Thymus* provoke substantial interest worldwide from phytochemical point of view, due to their diverse biological activities with potential for application in pharmaceutical, cosmetic and food industries. Very typical phenomenon for this genus is the chemical polymorphism, which is due both to the ecological factors and to genetic variations. Despite numerous publications on chemical content of *Thymus* species, the essential oil composition of the *Thymus* species in Bulgaria is poorly studied, or not studied at all. As a part of ongoing project, here in we report the chemical content of the essential oils of *Thymus vandasii, T. pannonicus* and *T. atticus.* **Material and Methods:** Air-dried aerial parts of *Thymus vandasii, T. pannonicus* and *T. atticus* were subjected to a micro distillation-extraction in a Likens-Nickerson apparatus for 2 hours using diethyl ether as a solvent. The obtained essential oils were analyzed by GC/MS. The individual components were identified by their RI and comparison of their mass spectra with those of NIST 14, WILEY and homemade MS databases. **Results:** The essential oils obtained from the aerial parts of *T. vandasii, T. pannonicus* and *T. atticus* were analyzed byGC/MS and 80 components in concentration more than 0.1%, representing 89.0-95.1% of the total oil were identified. It has been found that essential oils contained 5 main types of compounds – mono- and sesquiterpene hydrocarbons (MH and SH), their oxygenated derivatives (MO and SO) and aromatic compounds (AR). The results showed a significant difference in their chemical composition. Thus, oxygenated terpenoids were the main components in *T. vandasii* (69.2%) and *T. pannonicus* (69.9%). Among them, MO dominated in both samples (67.7 and 46.4%, respectively), while SO varied between 1.5 and 23.5% in *T. pannonicus* and *T. vandasii*, respectively. Geraniol was the main compound in both oils, followed by geranyl acetate (*T. pannonicus*) and linalool (*T. vandasii*). These oils differed also by the content of thymol, which was 5.4% in *T. pannonicus* and 0.5% only in *T. vandasii*. It has been found also that *T. pannonicus* oil contained relatively high amounts of elemol (8.0%). *T. atticus* essential oil displayed quite different profile and was very poor in the content of geraniol (< 1%). This oil was characterized by the presence of high amounts of aromatic compounds (24%) with thymol (8.4%) as a main one. It is worth to mention, that *T. atticus* oil contained the highest number of compounds, but none of them exceed 10%. In fact, the amounts of MH, MO and SH in this oil were almost equal. However, β-myrcene, thymol, germacrene D, caryophyllene oxide were the main components in this oil. **Conclusion:** This is the first report on volatile components in *T. vandasii*, as well as in *T. pannonicus* and *T. atticus*, collected from native populations in Bulgaria. The significant variety in chemical composition of the studied species required further investigations in order to complete the information on the relationship between the species in the genus *Thymus*, growing in Bulgaria.

Keywords: *Thymus vandasii, T. pannonicus, T. atticus* essential oil, GC/MS.

Acknowledgements: This work was supported by the NSF, Ministry of Education and Science, Bulgaria, Project DN 16/3.

POSTER PRESENTATION

VOLATILE CONSTITUENTS OF *INULA GERMANICA* L. AND *INULA BIFRONS* L. AERIAL PARTS

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Objective: Continuing our study on *Inula* species distributed in Bulgaria we have focused our attention on the chemical composition of essential oils obtained from aerial parts of *Inula germanica* L. and *Inula bifrons* L. To the best of our knowledge, the volatile constituents of these species have not been studied yet. **Material and Methods:** *Inula germanica* L. and *Inula bifrons* L. were collected from Belasitza and Rhodopes Mountains (Bulgaria), respectively. Air-dried plant material was subjected to a micro distillation-extraction in a Likens-Nickerson apparatus for 3 hours using diethyl ether as a solvent. The obtained essential oils were analyzed by GC-FID/MS. The individual components were identified by their RI and comparison of their mass spectra with those of NIST 14, WILEY and homemade MS databases. **Results:** The essential oils obtained from the aerial parts of *I. germanica* and *I. bifrons* were analyzed by GC-FID/MS and 140 components in concentration more than 0.1%, representing 98.3 and 97.6 % of the total oil were registered. It has been found that essential oils differed significantly in their chemical composition. Thus, *I. germanica* essential oil was rich in monoterpenoids (54.7%), while sesquiterpenoids dominated in *I. bifrons* essential oil (62.6%). Oxygenated monoterpenoids were dominating in *I. germanica* (53.5 %) with *cis*-carvyl acetate (20.7%) and 1,8-cineole (14.5%) as main components. The absence of monoterpene hydrocarbons and relatively low concentration of O-containing monoterpenes (1.8%) was characteristic feature for *I. bifrons* oil. In contrary, *I. bifrons* oil was found to be rich in sesquiterpene hydrocarbons (34.8 %) and O-containing sesquiterpenoids (27.8%). Among them, muurola-4,10(14)dien-1-ol (8.6%), δ-cadinene (7.7%), α- copaene (6.0%), β-selinene (5.9%) and Z-β-farnesene (5.8%) were the principal components. Significant amounts of fatty acids (12.3%) were also detected in *I. bifrons* oil, of which octanoic and tetradecanoic acids dominated. **Conclusion:** This is the first report on volatile components in *I. germanica* and *I. bifrons* aerial parts. The significant variety in chemical composition of the studied species required further investigations in order to complete the information on the relationship between the species in the genus *Inula*, growing in Bulgaria.

Keywords: *I. germanica* L., *I. bifrons* L., essential oil, GC-FID/MS.

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POSTER PRESENTATION

CHEMICAL AND BIOLOGICAL STUDIES OF *RHUS ALBIDA* SCHOUSB

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The present work was carried out in order to evaluate phytochemical and biological of the species Rhus albida Schousb which is a medicinal plant endemic to Morocco and the Canary Islands.
Rhus Albida Schousb commonly called zawaya belongs to the Anacardiaceae family. The phytochemical screening performed on leaves, fruit and underground stem or stolons, showed the presence of alkaloids in the case of stolons, catechic tannins in leaves and fruits and polyphenols in all organs.
The analysis by high performance liquid chromatography (HPLC) of methanol, ethyl acetate and aqueous extracts of all organs and polyphenols assay showed that the ethyl acetate extract has richness in phenolic compounds than other extracts.
On the other hand, the study of the antibacterial activity of the extracts by the method of bioautography showed significant inhibitory activity against gram-negative bacteria (*E. Coli* and *P. aeruginosa*), while the bacteria gram positive (S. aureus and E. hiriae) were resistant to the extracts. From plus l'incorporation of ethyl acetate and methanol extracts of the 3 organs in culture media of phytopathogens strains belonging to genus *Fusarium, Verticilium, Penicillium and Botrytis* has led to a significant inhibitory activity.

Keywords: Rhus Albida, Anacardiaceae , Phytochemistry ; Polyphenols, Activity, Morocco.

POSTER PRESENTATION

THE EFFECT OF THAI TRADITIONAL HERBAL "KLEEB BUA DAENG FORMULA" ON UNPREDICTABLE CHRONIC MILD STRESS-INDUCED COGNITIVE IMPAIRMENT IN MICE MODEL

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Objective / Purpose: Memory impairment is an important public-health problem. It lead to the causes of missing person and high cost for caregiver. Stress is an impact cause of memory impairment, that increasing oxidative stress leading to brain damage. The Thai traditional herbal formula Kleeb Bua Daeng) KBD(has been used for brain tonic in Chaophraya Abhaibhubeijhr hospital, Prachinburi, Thailand. This formula consists of 3 medicinal herbs, *Centella asiatica*, *Piper nigrum* and *Nelumbo nucifera*. In this study aimed to investigate learning and memory impairment behavior in mice on unpredictable chronic mild stress)UCMS(to clarify the learning and memory activity of KBD formula. The mechanism of action was determined by lipid peroxidation. **Material and Methods:** Mice were divided into 5 groups; 1(control+ vehicle, 2(UCMS+ vehicle, 3(UCMS+vitamin E)100 mg/kg/day(, 4(and 5(UCMS+KBD formula)100 and 500 mg/kg/day(. The UCMS has been used in animal model. UCMS paradigm involves the exposure of mice to a variety of relatively mild unpredictable stressors in a random order for 5 weeks. Three weeks after stating the UCMS procedure, mice were administered KBD or vitamin E. Learning and memory impairment behaviors were investigated using Y-maze test and novel objective recognition test, respectively. Oxidative stress level was investigated by measurement the concentration of malondialdehyde of lipid peroxidation. **Results:** The oral administration of KBD formula at dose 500 mg/kg was significantly increased % spontaneous alternation in Y-maze test and both of doses 100 and 500 mg/ kg were significantly increased the discrimination index in novel objective recognition test compared with UCMS group, dose dependent manner. The KBD was also significantly decreased concentration of malondialdehyde in both of hippocampus and frontal cortex.**Conclusion / Discussion:** These results suggested that UCMS caused oxidative stress and impaired learning and memory behaviors. KBD formula protect learning and memory impairment and reduced oxidative stress in brain. The mechanism of action such as enzyme activities and genes expression are in the further studies.

Keywords: Kleeb Bua Daeng formula, unpredictable chronic mild stress, learning and memory behaviors, oxidative stress

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POSTER PRESENTATION

STUDY AND SCREENING OF BACTERIA PRODUCING BIOPLASTIC

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Objective: The impacts of plastic waste for environment emerge as a world wild problem and a global challenge. These synthetic polymers are causing serious environmental problems due to their non-biodegradability. An escape from this ecological "dead-end" is gradual replacement of synthetic polymers with new biodegradabl materials. Polyhydroxyalkanoates (PHAs) present potential candidates to replace some conventional plastics. (PHAs) are natural, biodegradable, renewable and biocompatible biopolymers, accumulated intra-cellularly in bacteria as a carbon and/or energy storage. The present study was focused on the isolation of the polyhydroxyalcanoates accumulating bacteria belonging to the genus *Bacillus* isolated from different grass soil samples and waste water in West Algeria and the optimization of several parameters of PHA production. **Material and methods**: The visual screening for PHA production was investigated by staining with Sudan black. Different PHA positive *Bacillus sp* was identified. The effect of variation parameters such as cultural conditions and the nutritional requirements by use agro-food by-products as scrap dates, and sugarcane molasses was studied in bath culture. The polymer was purified and analyzed by Raman spectroscopy. **Result:** 30 *Bacillus. Sp* isolated from different samples. The visual screening for PHA production showed 10 cultures as PHA positive, classified as *Bacillus cereus*, *B. megaterium* and *B. mycoides*. The highest PHA accumulation (30% per mg cell dry matter) was obtained with cane molasses as sole carbon source in the culture medium at 2% of concentration. Whereas the highest biomass (0.98 g/l) was obtained at concentration of 5% must of dates scrap. **Conclusion:** New production process of biopolymers has been now becomes a major necessity. The production of PHA by bacterial fermentation represents a very interesting capital axis, view their resemblance to petrochemical-based plastic materials. The use of readily available cheap agro-industrial residues as the carbon sources may reduce the higher cost and improve economic bioplastic production process.

Keywords: Polyhydroxyalcanoates, by-product, bioplastic, Raman Spectrum.

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POSTER PRESENTATION

THE NOVEL SUPPLEMENT CONTAINING GINGER, RED DATE AND JEW'S EAR MUSHROMM PROTECTS AGAINST BRAIN DAMAGE AND MEMORY IMPAIRMENT FOLLOWING FOCAL ISCHEMIC STROKE IN OBESITY RATS

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Objective / Purpose: Despite the contunally rising in of stroke in obesity, the current therapeutic strategy against aforementioned condition is still limited. Therefore, the novel strategy is still essential. The polyherbal recipe containing ginger, red date and Jew's ear mushroom is reputed for the improvement of the key factors concerning the pathophysiology of stroke and obesity so the potential benefits of the recipe mentioned earlier on brain damage and memory impairment following ischemic stroke in obesity is investigated. **Materials and Methods:** Male Wistar rats, weighing 180-200 g, were induced obesity by high fat diet. Obese rats were orally given the recipe at doses of 150, 300 and 450 mg/kg BW once daily for 28 days prior to the induction of right middle cerebral artery occlusion (Rt.MCAO) and 21 days after the occlusion of Rt.MCAO. Then, the brain infarction volume, learning and memory, density of neurons in hippocampus were determined. The changes of AChE, Bax, oxidative stress status and eNOS in hippocampus were also determined. **Results:** All doses of the recipe improved brain infarction, hippocampal neuron density, and memory impairment. The reduction of AChE and oxidative stress status were observed in obese rats with Rt.MCAO. Low dose treatment decreased Bax while high dose treatment increased Erk and no change of eNOS was observed. **Conclusion/Discussion:** The polyherbal recipe containing ginger, red date and Jew's ear mushroom possesses the potential to improve brain damage and memory impairment in ischemic stroke with obesity condition. The principle mechanisms might occur via the suppression of AChE and oxidative stress.

Keywords: ginger, red date, Jew's ear mushroom, ischemic stroke, memory impairment

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POSTER PRESENTATION

NEUROPROTECTIVE EFFECT OF AQUEOUS EXTRACT OF ROSEMARY AGAINST ALUMINIUM-INDUCED NEUROLOGICAL DISORDERS

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Objective / Purpose: *Rosmarinus officinal L.* is one of the most important plants used in traditional Mediterranean diet and medicine, because of its high antioxidant activity and phenolic content. The Aim of this study is designed to investigate the neuroprotective effect of the Aqueous Extract of Rosemary (AER)-against Aluminum (Al)-induced neurotoxicity in young rats. **Material and Methods:** Al was administered intraperitoneally (at 60 mg/kg body weight, one times a week) and AER was given orally by gavage at a daily dose (150 mg/kg body weight/day) to rats for 45 days. **Results:** Al caused a deficit of memory performances, a significant increase of Acetylcholinesterase (AchE) activity and lipid peroxidation levels Thiobarbituric-acidreactivesubstances (TBARS).in addition, the histological study revealed that Al induce Necrosis with a decrease in the number of cellular units compared to control in the cerebral cortex and necrosis of pyramidal cells in the CA1 region of hippocampus. However, treatment with AER allowed recovering their working memory, inhibition of AchE activity and a decreased significantly TBARS and neuronal loss in the cerebral cortex and in the CA1 region of hippocampus. **Conclusion / Discussion:** Our findings suggested that aqueous extract of rosemary could improvement the memory which can be partially explained by its inhibition of AChE activity in rat brain; and also could to restore the neuronal degeneration induced by toxicity of Aluminium due to its antioxidant activities.

Keywords: Aluminum, *Rosmarinus officinal*, neurotoxicity, Memory.

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POSTER PRESENTATION

IN VITRO AND *IN VIVO* EVALUATION OF A NEW SERIES OF THIOSEMICARBAZONES AS POTENTIAL ANTI-INFLAMMATORY AGENTS

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Objective: Prostaglandins (PGs) generated from the arachidonic acid cascade by the action of cyclooxygenase (COX) isoenzymes are important mediators of inflammation associated with the pathogenesis and progression of cancer, arthritis, autoimmune, cardiovascular and neurological diseases [1,2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs across the globe. NSAIDs exert analgesic, antipyretic and anti-inflammatory effects through the inhibition of COX isoenzymes. However, the adverse effects of NSAIDs limit the long-term use of these agents and therefore extensive efforts have been devoted to the identification of new anti-inflammatory agents with improved potency and safety profile [2]. Due to the potential of thiosemicarbazones as anti-inflammatory agents, herein we designed a series of thiosemicarbazones as COX inhibitors for the management of inflammation. **Material and Methods:** New thiosemicarbazones (**1-12**) were synthesized *via* the reactions of 4-(aryloxy)benzaldehydes with 4-[4-(piperidin-1-ylsulfonyl)phenyl]thiosemicarbazide/4-[4-(4-morpholinyl)phenyl]thiosemicarbazide. Their *in vitro* inhibitory effects on COX-1 and COX-2 were determined using colorimetric COX inhibitor screening assay. MTT assay was performed to determine their cytotoxic effects on NIH/3T3 mouse embryonic fibroblast (healthy) cell line. The most effective COX inhibitors were also evaluated for their *in vivo* anti-inflammatory and antioxidant activities in lipopolysaccharide (LPS)-induced sepsis model. **Results:** The most potent and selective COX-1 inhibitor in this series was found as 4-[4-(piperidin-1-ylsulfonyl)phenyl]-1-[4-(4-cyanophenoxy)benzylidene]thiosemicarbazide (**3**) with an IC_{50} value of 0.98±0.04 µg/mL. On the other hand, 4-[4-(piperidin-1-ylsulfonyl)phenyl]-1-[4-(4-nitrophenoxy)benzylidene]thiosemicarbazide (**2**) was identified as a non-selective COX inhibitor (COX-1 IC_{50}= 7.25±0.35 µg/mL, COX-2 IC_{50}= 6.8±0.42 µg/mL) when compared with indometacin (COX-1 IC_{50}= 0.7±0.28 µg/mL, COX-2 IC_{50}= 11±5.66 µg/mL). Compounds **2** and **3** also showed low cytotoxic activity against NIH/3T3 cell line with IC_{50} values of 170±30 µg/mL and 125±5 µg/mL, respectively. According to *in vivo* studies, both compounds decreased myeloperoxidase (MPO) activities as well as nitric oxide (NO) and malonaldehyde (MDA) levels as compared to indometacin. The MDA levels of treatment groups (24.01 and 19.46 nmol/mL for compounds **2** and **3**, respectively) were lower than those in indometacin treated rats (32.81 nmol/mL). The decrease in MPO activities caused by compound **3** was more significant (3.75 fold) than that caused by indometacin (2.61 fold). It can be concluded that these compounds may act as anti-inflammatory and antioxidant agents. **Conclusion:** According to *in vitro* and *in vivo* studies, compounds **2** and **3** stand out as promising anti-inflammatory agents.

Keywords: Cyclooxygenase, thiosemicarbazone, anti-inflammatory activity

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POSTER PRESENTATION

EFFECT OF THE MEDICINAL PLANT' EXTRACTS (*SALSOLA SP*) ON THE CRYSTALLIZATION OF THE URINARY STONES STRUVITE

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Struvite is a magnesium ammonium phosphate mineral known to be a major problem of public health in urinary stones. Struvite precipitation occurs under certain conditions of pH, alkalinity, phosphorus, ammonium and magnesium concentrations. The search of inhibitors of crystallization is to prevent, slow or reduce one or another phase of crystallization in the formation of urinary calculi. Several natural and synthetic substances have been tested as inhibitors of phosphate salts crystallization. In the present investigation, the effect of aqueous and organic extracts of the medicinal plant, *Salsola sp* (CHENOPODIACEAE family) was studied in vitro on the growth and inhibition of struvite crystals.

Firstly, we have studied the crystallisation of struvite "in vitro" without inhibitors, flowed in second by use of inhibitors in order to explore the effect of the plant extracts on the crystallization of struvite. We have used an optical polarizing microscope to follow the evolution of the size of crystals and aggregates as a function of time. At the end of the experiment, the crystallization precipitate subjected to the IRTF spectroscopic analysis.

The results of the most of the organic and aqueous extracts have a significant inhibitory effect on the size of crystals and aggregates of struvite. Thus the aqueous and ethanol extracts have an important reduction (12 to 5-7 µm). We've observed a decrease in the size of the aggregates in the presence of all the extracts. This reduction is important for the ethanol extract (45 to 8µm), aqueous and methanol extracts (45 to 10-15µm).

Our result indicated that the extracts of the *Salsola sp* plant have an inhibitory effect on the crystallization of struvite, and deserves in the future, a deep study conduct on the effective extract phytochemical constituents as inhibitors.

Keywords: medicinal plants, *Salsola sp*, phosphate calculi, struvite, inhibitor.

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POSTER PRESENTATION

DESIGN, SYNTHESIS, *IN VITRO* AND *IN SILICO* EVALUATION OF NEW TRIAZOLOTHIADIAZINE DERIVATIVES AS POTENTIAL DIPEPTIDYL PEPTIDASE-4 INHIBITORS

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Objective: Dipeptidyl peptidase-4 (DPP-4) inhibitors, also known as gliptins, have come into prominence as incretin-based therapies in the armamentarium of oral antidiabetic drugs used for the management of type 2 diabetes [1]. Due to the significance of DPP-4 as a promising target for antidiabetic agents [2], herein new triazolothiadiazine derivatives were synthesized and investigated for their DPP-4 inhibitory effects. **Material and Methods:** New triazolothiadiazine derivatives (**2a-j**) were synthesized *via* the ring closure reactions of 2-bromo-1-arylethanones with 4-amino-5-(2-(pyridin-3-yl)ethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**), which was obtained *via* the solvent-free reaction of 3-pyridinepropionic acid with thiocarbohydrazide. Compounds **2a-j** were screened for their inhibitory effects on DPP-4 using a fluorescence-based method. Molecular docking studies were also performed to explore the possible binding mode of the most effective DPP-4 inhibitor in the active site of human DPP-4 (PDB code: 1X70) using Schrödinger's Maestro molecular modeling package. Furthermore, *in silico* Absorption, Distribution, Metabolism and Excretion (ADME) studies were performed using the QikProp module of Schrödinger's Molecular modelling package. **Results:** The most potent DPP-4 inhibitor in this series was found as 6-(4-bromophenyl)-3-(2-(pyridin-3-yl)ethyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**2e**) (IC_{50}= 78.00±1.41 μM). Molecular docking studies indicated that the pyridine ring formed π-π stacking and π-cation interactions with Tyr666 and Tyr662 residues in the active site of DPP-4, respectively. The triazole moiety of the triazolothiadiazine scaffold formed π-cation interaction with Arg125. According to *in silico* studies, compound **2e** only violated one parameter of Jorgensen's rule of three, whereas this compound did not violate Lipinski's rule of five. The percentage of human oral absorption of compound **2e** was predicted to be 100.00. On the basis of these findings, compound **2e** is expected to have good oral bioavailability. **Conclusion:** In the view of this study, the structural modification of the identified compound is on-going for the generation of new DPP-4 inhibitors with enhanced efficacy.

Keywords: Dipeptidyl peptidase-4, molecular docking studies, triazolothiadiazine.

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POSTER PRESENTATION

DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF THE PROTECTIVE EFFECT AGAINST METABOLIC SYNDROME OF PHYTOSOME CONTAINING THE COMBINED EXTRACT OF GINGER AND MULBERRY FRUIT

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Objective/Purpose: Currently, the novel therapeutic strategy against metabolic syndrome)MetS(is required. Due to the antimetabolic syndrome effect of mulberry and ginger together with the advantages of synergistic effect and encapsulation technique, we hypothesized that phytosome containing the extracts of mulberry fruit and ginger rhizome)GMP(should increase effective in protecting MetS. Therefore, we aimed to develop, characterize and evaluate the protective effect against MetS of GMP. **Materials and Methods:** The combined extract of ginger and mulberry was mixed with phospatidylcholine at ratio 1:1 and formulated as GMP. Then, it was characterized and determined *in vitro* biological activities. The potential doses of GMP ranging from 50, 100 and 200 mg/kg were orally given to MetS rats induced by HCHF diet for 21 days. **Results:** Our *in vitro* results showed the improvement of stability and biological activities related to MetS of the combined extract of ginger and mulberry fruit extract following the encapsulation by phytosome. In addition, our *in vivo* data revealed that GMP significantly improved lipid profiles, density and size of adipocyte, body weight, inflammatory markers such as TNF-α, IL-6 and PPAR-γ. **Conclusion/Discussion:** GMP is the potential supplement for managing MetS. The possible underlying mechanism might occur partly via the activation of PPAR-γ.

Keywords*:* phytosome, metabolic syndrome, ginger, mulberry fruit.

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POSTER PRESENTATION

INFERTILE ACTIVITY OF *DIPTEROCARPUS ALATUS* ROXB. EX G. DON OIL.

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Objective / Purpose: *Dipterocarpus alatus*, namely "Yang-na" in Thai, is an economic crop in Thailand. For Thai traditional use the oil part has been used for infertile in dogs and cats. But it lacks of the pharmacological activity. The chemical constituents contain in *D. alatus* are sesquiterpenes and triterpenes such as □-gurjunene, □-gurjunene, *s*-guaizulene and dipterocarpol [1-2[. The aimed of this study is to determine the infertile effect of *D. alatus* oil extract in male ICR mice model and its mechanisms. **Material and Methods:** Adult male mice were divided into five groups and receipted control or treatment for 5 weeks: group I)naeive, mice were received with 0.5 mL/kg/day of distilled water.(, group II)vehicle, mice were received with 0.5 mL/kg/day of corn oil.(, group III)mice were received with 50 mg/kg/day of *D. atatus* oil.(, group IV)Mice were received with 250 mg/kg/day of *D. atatus* oil.(, group V)Mice were received with 500 mg/kg/day of *D. atatus* oil.(. After treatment serums were collected for evaluation of testosterone level. Testis and vas deferens plus epididymis were for weighing. After treatment serums were collected for evaluation of testosterone level. Testis and vas deferens plus epididymis were for weighing. Mice sperm density were collected and calculated by Neubauer counting chamber under an optical microscope. Sperm viability and mortality were also assessed under an optical microscope. **Results:** There was no significant between the groups)P>0.005(in both of initial and final the body weight. Similarly, with the coefficient weight of reproductive organs such as testis and vas deferens plus epididymis showed no significant between the groups)P>0.005(. Sperm densities showed no significant differences between the control groups)naeive and vehicle(and treatment groups. The above result indicates that *D. atatus* with 50, 250 and 500 mg/kg/day had no effect on mice sperm density. The data was expressed as percentages of ratio sperm mortality and viability. The result showed significant differences between the control groups and treatment groups)$^{**}p$<0.001(with dose response manner between all treatment groups)$^{\#}p$<0.05, DA50 vs DA250, $^{\#\#}p$<0.001, DA50 vs DA500, DA250 vs DA500(. Testosterone level and histology are in process. **Conclusion:** *D. atatus* oil revealed the effect to decrease ratio sperm mortality and viability to male mice without effect to the sperm density and reproductive also body organ. The result showed the interesting data for pharmacological activity for infertility action of *D. alatus* oil and may be the candidate for developing infertility activity in the future. The further studies are androgen hormone and histology will be investigated.

Keywords: *Dipterocarpus alatus* Roxb. Ex G. Don, infertility, male reproductive

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POSTER PRESENTATION

CYTOTOXIC EFFECTS OF *HYMENOCALLIS LITTORALIS* ETHANOL EXTRACTS ON U937 CELL LINES

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Objective / Purpose: Leukemia is a hematological malignancy affecting myeloid and lymphoid lineages. Sixty thousand people are estimated to be diagnosed with leukemia each year. Other than chemotherapy and hematopoietic stem cell transplant, herbal therapy has become the alternative approach for leukemia treament due to its adequacy and minimal side effects. **Material and Methods:** In this study, we investigated the effects of *Hymenocallis littoralis* leaf and bulb extracts on U937 cell line. *Hymenocallis littoralis*, widely cultivated in Thailand, consists of several active components, such as lycorine, trispheridine, tazettine, flavonoids, alkaloids and volatile constituents. We treated U937 cell lines with 12 concentrations (1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 25 µg/mL) of *Hymenocallis littoralis* leaf and bulb extracts separately. The % cell viability was measure using XTT assay. The half maximal inhibitory concentration (IC50) was calculated using SigmaPlot program. Furthermore, flow cytometry was used to determine cell death pathway. **Results:** The results demonstrated that both bulb and leaf extracts of *Hymenocallis littoralis* can induce leukemic cell death in the concentration-dependent manner. The IC50 of U937 cell line treated with bulb and leaf extracts were 74.91µg/mL and 28.67 µg/mL respectively. **Conclusion / Discussion:** In conclusion, our finding indicates that *Hymenocallis littoralis* extracts have anti-leukemic activities. Further studies on mechanism, dosage and side effect should be done.

Keywords: *Hymenocallis littoralis*, Beach spider lily, cytotoxicity, leukemia, U937 cell line.

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EVALUATION OF ANXIOLYTIC AND SEDATIVE EFFECTS OF HIGH FIBER CASHEW DRINK IN METABOLIC SYNDROME RATS

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Objective / Purpose: Metabolic syndrome (MetS) is reported to be a risk factor of many deleterious disorders such as cardiovascular diseases. Moreover, it is also associated with neuropsychiatric disorders, including neuropsychiatric disorders such as anxiety and insomia. It has been demonstrated that the antioxidant and dietary fiber can protect against MetS, anxiety and insomia. Based on these pieces of information, the beneficial effect of high fiber of cashew drink was focused. Therefore, the purpose of this study is to determine the effect of this cashew drink against anxiety and insomnia in animal model of metabolic syndrome rats induced by high fat diet (HFD). **Material and Methods:** Male Wistar rats weighing 200-250 g were induced obesity by HFD for 12 weeks and were orally given the high antioxidant high fiber of cashew based functional drink at doses of 1, 10 and 100 mg/kg BW for 14 days. Then, the determination of the anxiety and sedative effects were assessed. The determinations of oxidative stress status including brain levels of malondialdehyde, and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and GABA-Transaminase (GABA-T) were also investigated at the end of study. **Results:** The high fiber cashew drink at dose of 1 mg/kg BW significantly increased number in open arms and all doses of high fiber cashew drink significantly increased time spent to open at day7 and day 14. All doses of the high fiber cashew drink decreased sleep onset but only the high dose showed the increased sleep time. The improved oxidative stress markers were observed in all treatment groups. Interestingly, the decreased GABA-T activity was observed only in the low dose treatment group. **Conclusion / Discussion:** The high fiber cashew drink is the potential functional drink to provide prophylactic effect against anxiety and insomnia. However, further studies concerning toxicity and possible active ingredients are essential.

Keywords: anxiety, insomnia, cashew, dietary fiber

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POSTER PRESENTATION

SYNTHESIS OF NOVEL GYPSOGENIN DERIVATIVES

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Objective: Gypsogenin compound, a natural saponin derived from *Gypsophila* plant species, is obtained from the plant roots. Gypsogenin compound in pentacyclic triterpene structure has important biological activities such as anticancer, antimicrobial, antioxidant, antithyrosine kinase activity. Therefore, we aimed to obtain new bioactive gypsogenin derivatives. **Material and Method:** The starting material Gypsogenin aglycone was combined with different amines compounds by using sodium triacetoxy borohydride in DCE at room temperature. Purification was carried out using chromatographic methods. **Conclusions:** Up to now, in our continuous research, the elucidation of the synthesized compounds (**1-3**) was determined by IR, UV, ^{1}H NMR, APT and LCMS analysis. **Conclusion / Discussion:** In the last part of the study, biological activities of new compounds will be investigated.

Keywords: Gypsogenin, semi synthesis.

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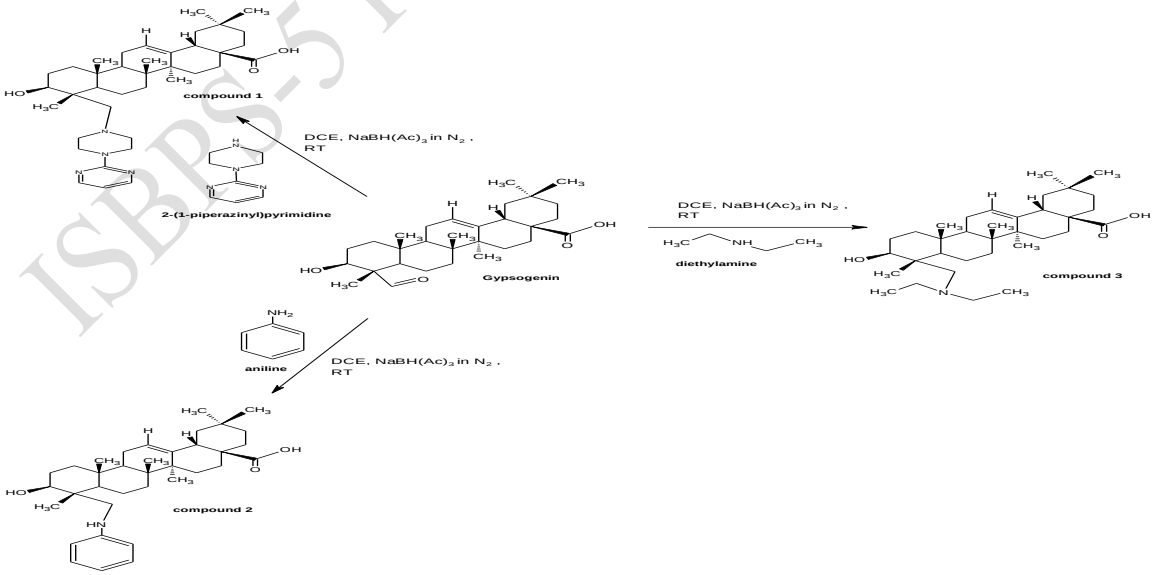

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POSTER PRESENTATION

INVESTIGATION OF ADSORPTION AND DELIVERY CONDITIONS OF THE OIL OF THYME ON POLYMERIC NANOPARTICLES

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Material and Methods: Essential oils, also called volatile and ether oils, are fragrant oily mixtures that are obtained from plant material. Since the Middle Ages these natural products have been widely used for bactericidal, virusidal, fungicidal, antiparasitic, medical and cosmetic purposes. Especially in recent years, due to the potential dangers of synthetic additives, the use of these oils in food, beverages, pharmaceuticals, perfumery, cosmetics and agriculture has increased as consumers increasingly demand natural ingredients. According to literature researches, antibacterial properties of the carvacrol molecule contained in thyme oil appeares to be more effective than other essential oils. p(HEMA-MATRP) nanoparticles were characterized by Zetasizer, FT-IR and SEM. In addition to these, the nanopolymer put into the cellulose membranes carried out release behaviors at high speeds in the first minutes and then at low speeds. It was observed that they carried out high rates of substance release. **Objective/Purpose:** In this study, 2-Hydroxyethyl Methacrylate-N-methacryloyl-L-tryptophan p(HEMA-MATRP) nanoparticles were synthesized by the surfactant- free emulsion polymerization technique. **Results:** The zeta-size results of the p(HEMA-MATRP) nanoparticle was found to be 191,1 nm and PDI: 0.031. FT-IR spectra showed that MATRP monomer was successfully added to the p(HEMA) nanoparticle structure. In optimum conditions (time, carvacrol concentration and temperature), carvacrol adsorption experiments with nanoparticles were carried out. A maximum of 13417,07 mg carvacrol binding was calculated under at a concentration of 7,5 mg/mL carvacrol in ethanole for 15 minutes at 25°C. pH and temperature experiments were performed to investigate the release conditions of nanopolymers bound to carvacrol under optimum conditions. **Conclusion / Discussion:** Our data indicated that the synthesized nanoparticles has a great surface area and carvacrol binding characteristics. Carvacrol release studies show that this nanopolymeric material is the useful material for medical purposes.

Keywords: Carvacrol, nanoparticles, controlled release systems.

POSTER PRESENTATION

ANTIOXIDANT, ANTI-PROLIFERATIVE AND IMMUNOMODULATORY ACTIVITIES OF *PLEUROTUS ABALONUS*, AN EDIBLE MUSHROOM IN U937 CELL LINE

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Objective / Purpose: *Pleurotus abalonus* (PA) has been reported to have potential treatment and prevention benefits in various diseases such as diabetes (1), acquired immune deficiency syndrome (2) and cancers (3). Mushroom derived immunomodulators become interested area of research in nowadays. We determined the effects of PA aqueous extracts on U937, with particular regard to their anti-proliferative activities and the immunomodulatory properties of the PA in phorbol 12-myristate 13-acetate (PMA)-treated U937 cells. Moreover, antioxidation activity and phytochemical contents of PA were also revealed. **Material and Methods:** PA powders were extracted with sterile water at 4, 22, 50 and 100 °C. After PA treatment, viability and cytotoxicity were determined by MTS and LDH assays, respectively. Total phenolic contents and total antioxidant activity were determined by Folin-Ciocalteu and ABTS assays, respectively. The expressions of cytokine mRNAs including TNF-α, IL-1β and IL-10 in PMA-treated U937 cells were measured by the quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR). **Results:** After 72 h. treatment, PA4, PA22 and PA50 displayed anti-proliferative effect against U937 cells with IC50 of 1.86 ± 0.03, 1.24 ± 0.23 and 0.65 ± 0.18 mg/ml, respectively. The released LDH confirmed their cytotoxicity. Interestingly, PA100 displayed no inhibitory effect with approximately 100% viability at all tested conditions. In addition, all PA contained various phenolic contents ranging from 32.98±0.18 to 78.38±0.14 gallic acid equivalent mM/kg dry mass. All crudes exhibited antioxidant activity ranging from 187.29±13.16 to 339.05±25.81 trolox equivalent mM/kg dry weight. In this report, PA100 induced the production of pro-inflammatory cytokine mRNAs including TNF-α and IL-1β, but not the anti-inflammatory cytokine, IL-10 in PMA-treated U937 cells. **Conclusion / Discussion:** Our data indicated that the hot water extract from *Pleurotus abalonus* showed promising anti-oxidant and immunomodulatory activity in human cell line. The mushroom appeared as the source of promising candidate and deserves future direction for immunomodulatory applications.

Keywords: *Pleurotus abalonus*, antioxidation, anti-proliferation, immunomodulatory activity, cytokines

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POSTER PRESENTATION

BIOAPYGIN® HERBAL OINTMENT AND PESSARIES COMPARED TO ACIDOSALUS® PESSARIES AND VAGINAL PROBIOTIC IN THE TREATMENT OF VULVO-VAGINAL DISORDERS

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Objective / Purpose: The objective of the study was assessment of the clinical efficacy and safety of a 10-day treatment of vulvo-vaginal disorders with Bioapigyn® vaginal ointment and pessaries compared to Acidosalus® pessaries and vaginal probiotic. **Materials and methods**: 124 females were randomly selected into four groups (each of 31 participant) and treated once a day for ten days with: A) Bioapygin® vaginal pessaries (one pessary per day); B) 2.5 mL of Bioapygin® vaginal ointment; C) Acidosalus® vaginal pessaries (one pessary per day); D) 2.5 mL of Acidosalus® vaginal probiotic. All the patients were subjected to gynecological examination, measurement of vaginal pH, Pap test, native (wet) mount preparation and KOH test, self-assessment and clinical assessment of the symptoms at baseline and following the therapy. **Results**: At baseline all 124 participants had abnormal pH values up to 7.5. The total score for the severity of signs and symptoms stated by a patient (vaginal discharge, vaginal odour, itching, burning, vaginal dryness) ranged from 2 to 13 and signs observed at gynaecological examination (oedema, erythema, excoriation, erosion, vaginal discharge) from 0 to 4. The severity of signs and symptoms stated by the patient decreased up to 87%, signs observed at gynaecological examination up to 95% and vaginal pH up to 11%. There was no significant difference in the treatment efficiency among four tested products. There were no adverse effects reported during the study and monitoring period for neither of the product. **Conclusion/Discussion**: Both Bioapygin® and Acidosalus® products were highly efficient in alleviation of the symptoms of nonspecific vulvo-vaginal disorders caused by elevated vaginal pH. The products created unfavorable conditions for pathogenic growth, adhesion and replication through the lowering of vaginal pH value, promoting the growth of lactobacilli, creating the environment with low water activity and creating a protective layer on the damaged mucosa that creates a physical barrier to the entrance of the pathogens into the cells and enables the recovery of the vaginal mucosa.

Keywords: Bioapygin, Acidosalus, vulvo-vaginal disorders, vaginal pH, herbal extracts, honey

POSTER PRESENTATION

THE PERFORMANCE OF BIOAPIFIT® ANTI HEMORRHOIDAL OINTMENT COMPARED TO ACIDOSALUS® SUPPOSITORIES IN THE TREATMENT OF HEMORRHOIDAL DISEASE

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Objective / Purpose: Haemorrhoids are a very common anorectal condition defined as the symptomatic enlargement and distal displacement of the normal anal cushions. Haemorrhoidal disease correlate positively with age and more than half of the population of both genders aged 50 years and older will develop haemorrhoidal disease during their lifetime. Besides, the venous circulation disorders, chronic constipation, sedentary lifestyle, a diet low in fibbers are important risk factors for the occurrence of haemorrhoids. The primary objective of this study was assessment of the efficacy of Bioapifit® anti hemorrhoidal ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Achilea millefolium* L., *Plantago major* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of hemorrhoids of grade 1 to 3 while the second goal was the comparison of its treatment potential with Acodosalus® suppositories applied under the same condition. **Materials and methods**: The experimental group consisted of 40 participants was treated with Bioapifit ointment applied externally three times a day onto clean perianal area and rectally once a day for 10 consecutive days. The control group also consisted of 40 participants was treated with Acodosalus® suppositories are applied rectally once a day (before bedtime) for 10 consecutive days. The evaluation of the patients before and following the therapy was done in terms of pain (0-10), defecation discomfort (0-10), bleeding severity (0-4), anal itching severity (0-4) and overall subjective discomfort (0-10). For statistical evaluation Statistica 11.0 software package was employed. **Results:** Ten days external and rectal application of Bioapifit® ointment resulted in significant decrease of all the symptoms of haemorrhoidal disease at third day of the treatment while in the end of the treatment overall subjective discomfort decreased for more than 96%. Clinical cure was observed in 85% of the patients. Acidosalus® suppositories applied rectally for ten days also resulted in significant decrease of all the symptoms while in the end of the treatment overall subjective discomfort decreased for app 77%. Clinical cure was confirmed in 67.5% of the patients. None of the patients in either Bioapifit or Acidosalus group experienced any adverse effect during the treatment and follow up period for both medical devices. **Conclusion / Discussion:** Physical parameters like low pH, high osmolarity/low water activity, high viscosity, greasiness and coating effect as well as lubricating effect of Bioapifit® anti hemorrhoidal ointment resulted in the alleviation of the symptoms of haemorrhoidal disease such as bleeding, itching, irritation and pain as well as wound infection due to: the creation of the protective coating on the damaged perianal and rectal mucosa enabling its recovery and preventing further irritation; the creation of unfavorable conditions for the growth, adhesion and multiplications of the pathogens (low pH, high osmolarity, low water activity, coating effect); alleviation of pain and discomfort during defecation due to lubricating effect.

Keywords: hemorrhoidal disease, honeybee's products, herbal macerate.

POSTER PRESENTATION

THE PERFORMANCE OF BIOAPIFIT® WOUND CARE OINTMENT IN THE TREATMENT OF PRESSURE ULCERS

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Objective / Purpose: A pressure ulcer is defined as localized injury to the skin and/or underlying tissue usually over a bony prominence, as a result of pressure, or pressure in combination with shear. According to the type of injury the ulcers could be categorized from stage 1 (Non-blanchable erythema of intact skin) to stage 4 (Full-thickness skin and tissue loss). The objective of this study was assessment of the efficacy of Bioapifit® wound care ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Plantago major* L., *Achilea millefolium* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Symphytum officinale* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of stage II and III pressure ulcers. **Materials and methods**: 50 participants (24 males and 26 females) with total 72 ulcers of stage II and III were treated 28 days with Bioapifit wound care ointment. The ointment was applied on the wound twice a day and covered with bandage during the whole course of the study. The healing process was assessed by Pressure Ulcer Scale for Healing (PUSH) tool ver. 3.0. once a week. This tool evaluates the ulcer surface area (length x width) scored from 0 (no ulcer present) to 10 when the surface area of the ulcer exceeded 24 cm²; the quantity of exudates scored from 0 to 3 (0-no exudates present, 1-light, 2-moderate, 3-heavy); the type of tissue scored from 0 to 4 (0-closed wound, 1-epithelial tissue, 2-granulation tissue, 3-slough, 4-necrotic tissue). The PUSH score ranges from 0 (completely closed wound with no exudates) to maximum 17 with ulcer surface higher than 24 cm² with heavy exudates and the presence of necrotic tissue. For statistical evaluation Statistica 11.0 software package was employed. **Results:** At baseline mean value and standard deviation of the PUSH score for ulcer surface area, quantity of exudate, type of tissue and the total score were 8.5±0.8, 1.4±0.8, 2.8±0.4 and 12.6±1.9, respectively. All the mentioned values decreased significantly after only seven days of the treatment ($p<0.00001$). Moreover, significant decrease of wound pH was also observed. All infected wounds were found sterile after seven days of the treatment. Further treatment resulted in linear decrease of PUSH parameters reaching zero values after 28 days of the therapy. Slough disappeared after 14 days of the therapy and epithelial tissue was obtained on the edge of 51 of 72 ulcers (70.8%). Complete closure of all ulcers following three weeks of the treatment was found in 30% of the patients while after four weeks all the ulcers healed completely in all 50 participants. No side-effects were observed during the course of the study. **Conclusion / Discussion:** Four weeks of the topical treatment with Bioapifit® wound care ointment resulted in complete closure of all ulcers with mean healing time of 25.9 days. Those results could be attributed to the ingredients with pH adjusting, high osmolarity, high astringency and coating potential that accelerated wound closure, exhibited bactericidal effect and prevented further wound infection.

Keywords: pressure ulcers, PUSH tool, honeybee's products, herbal macerate.

POSTER PRESENTATION

OCIMUM TENUIFORUM PROTECTS AGAINST NEURODEGENERATION AND MEMORY IMPAIRMENT IN ANIMAL MODEL OF MENOPAUSE WITH OBESITY

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Objective / Purpose: Dysfunctions of blood vessel and brain, the important disorders in post-menopausal women, are increasing their importance and require the effective therapeutic strategy. In this study, the effect of *Ocimum tenuiflorum* or Kaphrao on the dysfunctions of brain and blood vessels in animal model of obese postmenopausal women were explored due to its benefits on memory impairments and atherosclerosis. **Materials and Methods:** Female Wistar rats, weighing 180-200 g, were induced experimental menopause by bilateral ovariectomy and then they were induced obesity with high-fat diet. Ovariectomized (OVX) rats with obesity were fed with high fat diet (HFD) containing 5% Kaphrao for 8 weeks. The assessments of spatial memory and biochemical profiles, oxidative stress status and apoptosis in hippocampus together with the density of foam cells, expressions of ICAM and VCAM and oxidative stress status in internal carotid artery were performed. **Results:** The results showed that Kaphrao significantly improved spatial memory, serum triglyceride, densities of survival neuron, apoptosis and oxidative stress status in hippocampus. It also decreased foam cell, densities of ICAM and VCAM positive cells and oxidative stress status in internal carotid artery. **Conclusion/Discussion:** Kaphrao can improve neurodegeneration and memory impairment in animal model of menopause with obesity. The mechanism might occurr partly via the improvement of oxidative stress status, and apoptosis whereas the improvement of endothelial dysfunction occurred partly via the improvement of oxidative stress, adhesion molecules and foam cell formation.

Keywords: *Ocimum tenuiflorum*, memory, cerebral blood vessel, menopause, obesity.

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POSTER PRESENTATION

CAROTENOID AND BETA-CAROTENE CONTENT IN *SCHLEICHERA OLEOSA* FRUIT AND ITS ANTI-OXIDANT ACTIVITY

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Objective / Purpose: This research aimed to determine the content of some active compounds which was total carotenoid and beta-carotene content and some antioxidant activities from each part in the fruit of this plant. *Schleichera oleosa* (Lour) Oken is a plant in family Sapindaceae, generally found in some area of Thailand. The information and scientific data about this plant are not enough to confirm the medicinal use especially the fruit of this plant which commonly consumed. **Material and Methods:** The crude extract from Peel, juice and seed part of fruit were determined for total carotenoid and beta-carotene content. Total carotenoids content was determined by the spectrophotometric method at 450 nm and beta-carotene content was determined by HPLC with UV-visible detector. The antioxidant activity was evaluated by FRAP and ORAC assay. **Results:** Total carotenoid content was 343.57, 74.27 and 11.90 mg/g extract equivalents to beta carotene in peel, juice and seed part in respectively. The amount of beta-carotene by HPLC method was 0.15 and 0.44 mg/g extract in peel and juice but could not be detected in seed by this method. The antioxidant activity from each part of this fruit by FRAP assay were shown as trolox equivalent which were 173.10 and 62.79 mg trolox equivalent per g extract in peel and juice but could not detected this activity in seed part. The antioxidant activity from ORAC assay was shown as trolox equivalent which were 784.76, 518.65 and 283.09 mg trolox equivalent per g extract for peel, juice and seed in respectively. **Conclusion / Discussion:** It could be concluded that peel extract from the fruit of this plant showed the maximum content of carotenoid content but the highest beta-carotene content was shown in juice part. The maximum antioxidative activities by FRAP and ORAC assay were shown in peel which complied with the carotenoid content. This information could be used for further study about the product development of this kind of fruit and value added for consuming in the future.

Keywords: *Schleichera oleosa, antioxidant activity, FRAP, ORAC.*

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POSTER PRESENTATION

VACCINIUM VITIS-IDAEA L., PICKED IN BULGARIA *INDICATE IN VITRO* ANTITUMOR ACTIVITY

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Objective: Cancer is a serious problem for modern medicine, considering late diagnosis and inadequately effective therapy. Along with efforts to understand the complex genetic and epigenetic factors that trigger a carcinogenic process, it is also necessary to analyze the potential natural active substances that may delay or even stop the progression of carcinogenesis [1, 2]. A promising candidate are Bulgarian cranberries from high mountain plant populations, which are rich in phenolics and anthocyanins and have proven beneficial effects on human body [3]. The present study aims to evaluated *in vitro*, antitumor activities of total methanol extracts and purified fractions (B-nonanthocyanin / C- anthocyanins) of *Vaccinium Vitis-Idaea L.*, picked in Bulgaria on human cervical (HeLa) and breast (MCF7) cancer cell lines, as well as to examine some of the mechanisms underlying them. **Materials and methods:** Four methanol extracts and respective number purified fractions (B-nonanthocyanin / C- anthocyanins) of Bulgarian cranberry were used. Antitumor effect was established by Trypan Blue method and MTT cell viability assay. Assessment of apoptotic activity was performed using DNA fragmentation method. **Results:** The results from MTT analyses showed that B- nonanthocyanin fractions of Bulgarian cranberry have well expressed inhibitory effect on survival of tested tumor cells. The observed effect dependent of the dose administered and were stronger in relation with the high-mountain populations and HeLa cell line. The integrity of the extracted DNA from treated survival cells indicates possible apoptosis mechanisms under the action of biologically active ingredients from cranberries. **Conclusion:** Evaluation of antitumor activities of Bulgarian cranberries using modern molecular methods, could contribute to establish the natural substances useful for human health in general and modern oncology.

Keywords: Cranberries, antitumor activity, HeLa, MCF7.

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POSTER PRESENTATION

INTERACTION OF *TILIACORA TRIANDRA*)COLEBR.(DIELS ON CYTOCHROME P450 AND P-GLYCOPROTEIN ACTIVITY IN RAT

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Objective / Purpose: The combination uses of herbs or dietary supplements with conventional drug may results to the potential for herb-drug interaction. *Tiliacora triandra*)Colebr.(Diels has long been used as an herbal remedy in folklore medicine, but there remain insufficient of the herb-drug interaction. This study was to investigate the influences of **aqueous** extracts of *T. triandra*)TTA(on rat hepatic cytochrome P450)CYP(and P-glycoprotein)P-gp(activity in everted intestine. **Material and Methods:** A single daily doses of TTA)0.5, 1.0 and 2.0 g/kg bw(were orally administered to rat treated groups)n=6/group(, respectively for 7 days. The alkoxyresorufin *O*-dealkylation)AROD(activities of CYP1A and CYP2B, CYP3A4-catalyzed testosterone 6β-hydroxylation and P-gp activity using rhodamine-123 as substrate were examined. **Results:** Significant inhibition of the CYPs activities; CYP1A, CYP2B and CYP 3A4 were reported. The alteration of P-gp activity was determined as dose dependent manner. The P-gp activity was decreased when compared to TTA and P-gp inhibitor; Verapamil)100 µg/kg bw(treated groups, respectively. **Conclusion / Discussion:** These results indicated that the **aqueous** extracts of *T. triandra* might interfere on herb-drug interaction. However, the mechanism of action requires further study.

Keywords: cytochrome P450, P-glycoprotein, everted intestine, *Tiliacora triandra*

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POSTER PRESENTATION

"OA" ,THE NOVEL SUPPLEMENT, IMPROVES BRAIN INFARCTION, BRAIN EDEMA AND BRAIN DYSFUNCTION FOLLOWING CEREBRAL ISCHEMIA IN ANIMAL MODEL OF METABOLIC SYNDROME

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Objective / Purpose: Metabolic syndrome (MetS) can increase the prevalence of stroke and exacerbates the severity of stroke related pathophysiology. Brain infarction and brain edema following ischemic stroke are the most serious factors contributing to death in stroke. Based on the neuroprotective effect of black rice and dill together with the synergistic effect, we aimed to determine whether the supplement containing the extracts of black sticky rice and dill could improve brain infarction and brain edema in MetS condition.

Material and Methods: OA at doses of 0.5, 5 and 50 mg/kg BW were orally given to male Wistar rats which were induced MetS by high-fat high-carbohydrate diet (HFCD) for 16 weeks and induced ischemic-reperfusion injury for 90 minutes. The animals were determined neurological score every 7 days. At the end of experiment, brain infarction volume and brain water content, Peroxisome proliferator-activated receptor gamma (PPARγ), phosphorylation of Erk, and endothelial nitric oxide synthase (eNOS) in cortex together with vascular cell adhesion molecule 1 (VCAM-1) in common cerebral artery were determined.

Results: All doses of OA extract significantly improved brain infarction volume, brain water content, neurological score and Erk phosphorylation in cortex. The reduction of VCAM1 was also observed at all doses treatment whereas the increase in PPARγ and eNOS level were observed only in MetS rats with cerebral ischemia which received OA at doses of 0.5 and 5 mg/kg BW.

Conclusion / Discussion: OA could mitigate brain damage, and brain dysfunction following stroke in MetS condition. The possible underlying mechanism occurs partly via the enhanced PPARγ, Erk phosphorylation and eNOS expression in cerebral cortex together with the suppression of VCAM1 expression in cerebral vessel.

Keywords: cerebral ischemia, metabolic syndrome, black sticky rice, dill.

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POSTER PRESENTATION

EFFECT OF *DIPTEROCARPUS ALATUS* ROXB. EX G. DON LEAVES EXTRACT ON UNPREDICTABLE CHRONIC MILD STRESS INDUCED DEPRESSION IN ICR MICE AND ITS MECHANISMS

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Objective / Purpose: Chronic stress involves the over activation of hypothalamic-pituitary-adrenal axis)HPA axis(which leads to release corticosterone and over production of free radicals which resulting in neuronal cell damage. These maybe involved in unpredictable chronic mild stress)UCMS(induced depression and cognitive impairment. *Dipterocarpus alatus*, commonly known as "Yang-na", is an economic crop in Thailand. Previous studies on Yang-na leaves extract have revealed the major flavonoids are apigenin, kaempferol, and quercetin, which have antioxidant properties]1[. This study aimed to investigate the effect of *Dipterocarpus alatus* leaves extract on UCMS induced depression in male ICR mice model and its mechanisms. **Material and Methods:** Mice were divided into 5 groups; 1(non-stress + vehicle 2(UCMS + vehicle 3(UCMS + vitamin E)100 mg/kg(4(UCMS + yang-na)100 mg/kg(5(UCMS group + yang-na)500 mg/kg(. After, 3rd week of exposing the various stressors, mice were received the treatment at 4th-6th week. Anhedonia behavior was determined using sucrose consumption test and hopeless behaviors were determined using forced swimming test)FST(and tail suspension test)TST(. After finished the behavioral test, blood samples were collected in order to investigate the corticosterone)CORT(level. **Results:** UCMS mice expressed the depressive behaviors when compared with non-stress mice. UCMS treated with Yang-na showed the significantly reduced depressive behaviors when compare with UCMS treated vehicles as similar as treated with imipramine. UCMS mice also induced the increasing level of CORT in serum when compared with non-stress mice. Yang-na treated UCMS mice showed significantly decrease CORT level. *Dipterocarpus alatus* also showed the inhibitory effects on monoamine oxidase activity *in vitro*. **Conclusion:** Taken together Yang-na leaves extract can improve cognitive impairment symptoms, and decreased oxidative stress induced by UCMS. The possible mechanism may be involved in the antioxidant effect of apigenin, kaempferol and quercetin which are the major constituents in Yang-na leaves extract. These results showed that Yang-na leaves extract provides health benefit and may be the candidate for developing antidepressant in the future.

Keywords: *Dipterocarpus alatus* Roxb. Ex G. Don, UCMS, depression, corticosterone

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POSTER PRESENTATION

EFFECTS OF QUERCETIN ON OXIDATIVE AND INFLAMMATORY STRESS PATHWAYS IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Background: Osteoarthritis (OA) is one of the most common and progressive chronic degenerative disease. While the exact mechanisms driving the development of OA remain poorly understood, the main hallmarks were extracellular matrix degeneration, subchondral bone thickening, osteophyte formation and joint space narrowing [1]. The major contributory factors in the progression of OA are aging, oxidative stress, and the expression of inflammatory and catabolic gene in articular chondrocytes [2]. Previous studies demonstrated that quercetin prevents proteoglycan destruction and attenuates oxidative stress-induced apoptosis in rat chondrocytes [3]. The main objective of this study was to investigate the anti-inflammatory/chondroprotective and redox modulatory effects of Quercetin (QUE) in human osteoarthritic chondrocytes (OACs). **Methods:** Primary chondrocytes were isolated from the joint cartilages of OA patients undergoing total knee arthroplasty (grade 4, mean age= 66 years, BMI: 29.7 ± 4.4 kg/m2). The alterations in cell proliferation (MTT), adhesion profile (RTCA-iCELLigence System), reactive oxygen species generation (ROS), lipid hydroperoxide levels (LPO), HNE-protein adduct levels (HNE), AGE-protein adduct levels (AGE), 3-nitrotyrosine levels (3-NT), glutathione peroxidase activity (GPx), and inflammatory progenitors ICE/caspase-1 (ELISA) were determined. **Results:** QUE decreased cell viability of OACs in a concentration dependent manner. However, at lower concentrations (1-100 nM) QUE increased adhesion profile of OACs for 24 h. Intracellular ROS levels were significantly decreased after QUE treatment, on the contrary no comparable effects had been found on the end product levels of oxidative stress (LPO, HNE, AGE, 3-NT) and inflammatory progenitors. **Conclusion:** Our results suggest that the QUE treatment of OACs is not sufficiently effective in overcoming severe OA, even at low concentrations, at least *in vitro*.

Keywords: Osteoarthritis, Quercetin, Oxidative stress, Inflammation, Chondrocytes

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S012).

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POSTER PRESENTATION

ICARIIN INHIBITS INFLAMMATORY DEGENERATION IN HUMAN OSTEOARTHRITIC CHONDROCYTES VIA MODULATION OF REDOX STRESS-INDUCED PATHWAYS

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Objective: Osteoarthritis (OA) is the most common form of arthritis, caused by the inflammation, breakdown, and eventual loss of cartilage in the joints. Currently, the process underlying osteoarthritis cannot be reversed. There is need to find safe and effective drugs that can reduce the inflammation and regulate the redox stress-ınduced pathways in the pathogenesis of OA disease.[1] Icariin is a flavonoid, derived from several species of plants. Previous studies indicated that, icariin is able to regulate cellular metabolism in human synoviocytes and reduces cartilage degeneration in a mouse model.[2,3] According to this, we aim to determine intracellular mechanisms in which icariin is beneficial against OA pathology. **Methods:** Chondrocytes were isolated from OA patients undergoing total knee arthroplasty.[4] 30 days after isolation, chondrocyte cells were treated with different concentration of icariin. Cell viability was assessed using MTT colorimetric assay. The proliferation rate (RTA-ICelligence system) and reactive oxygen species (ROS) formation **(DCFH-DA assay) were analyzed.** Inflammatory markers such as IL-1B, IL-6, TNF-α (Luminex Assay) and caspase-1/ICE (ELISA) levels were determined. Redox disturbances were also examined for lipid hydroperoxides (LPO), glutathione peroxidase (GPx) and 4-HNE-, AGE-adduct levels by ELISA. **Results:** Icariin, at 10-100 nm concentrations, improved the viability and proliferation in OA chondrocytes. These findings are accompanied by decreased ROS formation, decreased lipid hydroperoxides and inhibited advanced lipoxidation end products protein adducts level (HNE-adducts). AGE-adducts did not change by icariin treatment. Icariin can also improve inflammation by partly modifying the levels of IL-1B, IL-6, TNF-α and caspase-1/ICE. **Conclusion:** The results indicate that ROS-induced lipid peroxidation and HNE-adduct formation mediates inflammatory degeneration in OA cartilage that can be modulated by intervention with icariin.

Keywords: Icariin, Osteoarthritis, Primary Chondrocyte, Redox Stress

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S012).

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POSTER PRESENTATION

NOVEL ALDO-KETO REDUCTASE INHIBITORS GIVE RISE TO HAVE A GREAT POTENTIAL ON REGULATING HYPERGLYCEMIA EXCERBATED $A\beta_{1-42}$ NEUROTOXICITY IN PRIMARY HIPPOCAMPAL NEURONS

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Background: Alzheimer's disease (AD) is characterized by progressive loss of neuronal loss, impaired neuronal metabolism, redox signalling pathways related to neuroinflammation leading to cognitive deficits, and changes in behavior and daily life [1]. Recent studies showed that AD is a risk factor for Diabetes mellitus (DM) and several neurodegenerative mechanisms, such as mitochondrial dysfunction, inflammation, oxidative stress, are connected in both pathologies [2,3]. Thus, developing novel therapeutic strategies for attenuating both pathologies are urgently needed to treat neurodegenerative disorders in DM. In this point of view, we tended to design a new therapeutic approach for targeting multiple common pathways in DM and AD, via novel aldo-keto reductase inhibitors. **Methods:** Primary hippocampal cultures were prepared from embryos (E17-19) from Wistar albino rats. For the hyperglycemia conditions, cells were exposed with different concentration of high glucose (50, 75 100, 125 and 150 mM) for 48 and 72 hours. To induce amyloidogenic toxicity cells were incubated with 500 nM oligomeric $A\beta_{1-42}$ for 24 h under 150 mM glucose conditions. After all cells were incubated with glucose (150 mM, 48 h), co-treated with various concentrations (1, 5, 10, 50 μM) new carboxymethylated mercaptotriazinoindole derivatives (CMTI, COTI) and Epalrestat (EPA) followed by co-treatment with 500 nM oligomeric $A\beta_{1-42}$ aggregates for 24 h. Cell viability and membrane integrity was determined by MTT and NRU assays, respectively. **Results:** Primary hippocampal cells showed apoptotic features, such as swelling, inhibition of axonal outgrowth, hyperosmolarity damage under 150 mM glucose and $A\beta_{1-42}$ exposure increases cell death significantly. However, our in vitro treatment protocol with EPA, CMTI and COTI disrupted the neurotoxicity mediated by long term high glucose mediated $A\beta_{1-42}$ induced injury via regulating membran permeability (NRU), reducing ROS levels and increased cell viability (MTT). **Conclusion:** This preliminary data revealed that aldose-keto reductase inhibitors might be a potential approach to handle with hyperglycemia induced $A\beta_{1-42}$ neurotoxicity, at least in vitro.

Keywords: Hyperglycemia, Hippocampal neurons, Alzheimer Disesase's, Aldose Reductase inhibitors.

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 215S197)

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POSTER PRESENTATION

ENDOGENOUS CANNABINOIDS EXERTS NEUROPROTECTIVE FUNCTIONS AGAINST AB$_{1-42}$-MEDIATED INJURY IN HIPPOCAMPAL NEURONS UNDER HYPERGLYCEMIC CONDITIONS

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Background: Alzheimer's disease (AD) is a multifactorial neurodegenerative diseases, linked to various mechanisms with different regions of the brain [1]. Senile plaques and neurofibrillary tangles are the most hallmark lesions in the brain of AD in addition to neuronal cell loss [2]. Recent studies suggest that oxidative stress (OS)-induced damage may play an important role in the initiation and progression of AD pathogenesis. However, current treatment regimes are unable to modulate multiple mechanisms involved in AD [3]. The endocannabinoid system (ECs) has been associated with several pathological and physiological conditions like neuronal plasticity, neurodegeneration, inflammatory response, circadian rhythm, redox homeostasis. The multi-targeted effectiveness of ECs emerged a as potential approach to treat AD [4]. So, we intend to investigate the neuroprotective action mechanisms of arachidonylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) which might be closely linked to redox-mediated signalling pathways. **Methods:** To investigate the role of AEA and 2-AG on hyperglycemia, we designed a high glucose (150 mM, 48h) induced neurotoxicity model in primary hippocampal neurons. Afterwards, palmitic acid (PAM), ER-stress inducer, was added to the cultures to induce lipotoxicity for the short term. On the parallel experiments, Aβ_{1-42} (oligomeric form) was exposed to cultures under high glucose condition to induce hyperglycemic-AD model. Fort he protection experiments, cells were treated with AEA or 2- AG for 6 h, then co-treated with 150 mM glucose (24 h) and followed by incubation with 500 nM oligomeric Aβ_{1-42} (24 h) or PAM (last 4 h) in high glucose conditions. MTT, NRU assays were held to evaluate neurotoxicity and intracellular ROS generation levels were measured by DCFH-DA protocol. **Results:** 2-AG increased cell viability/proliferation at lower concentrations but both AEA and 2-AG have cytotoxic effects at relatively high concentrations (>10 μM). In addition, both agents significantly reversed the glucotoxicity mediated cell loss, however AEA was unable to alter the glucolipotoxicity compared to 2-AG. On one hand, both agents reduced intracellular ROS levels in hyperglycemic-AD model but on the other hand only 2-AG significantly increase cellular proliferation/viability at relatively lower concentrations. **Conclusion:** Our results indicated that AEA and 2-AG have antioxidant effect at relatively lower concentrations however 2-AG has promising effects on cell proliferation/viability that should be take in consideration for further studies.

Keywords: Endocannabinoid system, Alzheimer Disesase's, Neuroprotection, AEA, 2-AG,

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S088)

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POSTER PRESENTATION

THE SYNTHETIC CANNABINOID, CP 55,940, HAS A POTENTIAL REDOX MODULATORY EFFECT ON Aβ1-42-MEDIATED INJURY IN HIGH GLUCOSE-INDUCED NEUROTOXICITY IN HIPPOCAMPAL NEURONS

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Background: Diabetes mellitus (DM) has given rise to a risk of developing Alzheimer's disease (AD) and the neurodegeneration related to taupathies and amyloidogenesis have a strong correlation with the DM-related cognitive dysfunction [1]. Recent studies are trying to elucidate the molecular mechanisms underlying the neurodegeneration pathways in AD associated with DM, or *vice versa* [2]. The endocannabinoid system (ECs) is one of the systems that involves in both central and peripheral physiological processes. CP 55,940 is an synthetic cannabinoid agonist that reduces the short-term mitochondrial dysfunction and oxidative stress linked to excitotoxicity [3]. However, long term neurodegenerative or neuroprotective effects have not been elucidated yet. In this point of view, we aimed to clarify the potential long term effects of CP 55,940 on long term high glucose and $A\beta_{1-42}$ exposed neurotoxicity model. **Methods:** To investigate the role of CP 55,940 on hyperglycemia induced $A\beta_{1-42}$ mediated toxicity, primary hippocampal neurons were incubated with 150 mM glucose for 24 h, followed by incubation with 500 nM oligomeric $A\beta_{1-42}$ under the same conditions. For the protection experiments, cells were treated with CP 55,940 for 6 h, then co-treated with 150 mM glucose (24 h) and followed by incubation with 500 nM oligomeric $A\beta_{1-42}$ (24 h) in high glucose conditions. MTT, NRU and DCFH-DA analysis were held for the nerutoxicity assessment. **Results:** CP 55,940 increased cell viability/proliferation at lower concentrations (1-500 nM) under long term hyperglycemic conditions. Moreover, CP 55,940 showed dramatic significant reduction in cellular ROS levels ($p<0.01$) but this reduction has not been reflected to increment of cell viability. **Conclusion:** Understanding the mechanistic action of CP 55,940 on mitochondria might provide new insights into more effective therapeutic approaches for oxidative stress related disorders.

Keywords: Endocannabinoid system, Hyperglycemia, $A\beta_{1-42}$, Neuroprotection, CP 55,940

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S088)

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POSTER PRESENTATION

EFFICACY OF COLD-EXTRACT KAFFIR-LIME SHAMPOO AND TREATMENT FOR ANTI-DANDRUFF

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Objective / Purpose: The purpose of prospective study was to investigate the efficacy of cold-extract kaffir-lime shampoo and treatment for their anti-dandruff activities. **Materials and Methods:** 52 volunteers who lived in Chonburi participated in this study. The volunteers were randomly assigned into four different groups. Group 1, 2, 3, and 4 were treated by Cold extract Kaffir lime shampoo, Kaffir lime treatment, Kaffir lime shampoo and treatment, and Standard Anti-Dandruff shampoo (2% Ketoconazole), respectively. The volunteers were followed-up twice after the two-week period of use of the products. Aramo-ASW machine was used to detect the efficacy of the products. The endpoints were scalp status, hair loss, scalp sensitivity, keratin of scalp, scalp sebum, hair density, hair thickness, hair pore status, and cuticle status. The data were analyzed and evaluated using Paired t-test and ANOVA. **Results:** The data were analyzed and evaluated using Paired t-test and ANOVA. This study showed that the anti-dandruff efficacy of the cold-extract kaffir-lime shampoo, the cold-extract kaffir-lime treatment and the combination of the shampoo and the treatment were not significantly different from each other and from Standard Anti-Dandruff shampoo (2% Ketoconazole). **Conclusion / Discussion:** In this review, we summarize the current knowledge on the efficacy of cold-extract kaffir-lime shampoo and treatment for their anti-dandruff activities that kaffir-lime oil can exhibit potent anti-dandruff activities with non-inferiority study to 2% Ketoconazole shampoo and may be applied to potential alternative candidates for natural haircare product.

Keywords: kaffir-lime, Anti-dandruff

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POSTER PRESENTATION

BIOLOGICAL ACTIVITIES OF *SCABIOSA HOLOLEUCA* BORNM.

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Objective: The genus *Scabiosa (Dipsacaceae* family*)*, comprising about 100 species, occurs mostly in the Mediterranean region. There are 30 *Scabiosa* species in the Flora of Turkey [1]. *Scabiosa* means "scabies" [2]. Up to date, chemical investigations of *Scabiosa* species have mainly revealed the presence of saponins, flavonoids, coumarins, and iridoid glucosides [3, 4]. *Scabiosa hololeuca* Bornm. is a perennial woody plant. The extinction risk of the generation in nature is considered very high. *Scabiosa hololeuca* is known as 'uyuzotu' in Turkey [5,6]. **Material and Methods**: In the present study, the aerial parts of *S. hololeuca* were collected from Bozan (Eskişehir) planting area. The leaves of *S. hololeuca were* subjected to accelerated solvent extraction (ASE) to get sequentially non-polar and polar extracts: *n*-hexane (HE), methanol (ME) and water (WE) extracts. The yields of HE, ME and WE extracts were found to be as 0.85%, 7.31% and 20.31% respectively. The free radical scavenging activity of the extracts was evaluated using DPPH and TEAC assays. The fatty acid content of the seeds of *S. hololeuca* was investigated after methylation with boron trifluoride (12.5% inmethanol) using GC-FID/MS technique. **Results**: The highest antioxidant activity was detected for WE (IC_{50} 0.38 ±0.03 mg/mL,) and ME (IC_{50} 0.79 ±0.04 mg/mL) extracts. However, HE showed low antioxidant activity (34.58 ±0.7 %). The highest TEAC values were obtained for the aqueous (2.72 ±0.12 mM) and methanol (1.28 ±0.02mM) extracts. Total phenolic content was determined by Folin-Ciocalteu method. The highest total phenol content was demonstrated for ME and WE (0.24±0.02 mg GAE/g, 0.34 ±0.01mg GAE/g). The present work is the first report about antioxidant activity and total phenolic content of *Scabiosa hololeuca.* Methyl nonadecanoate (27.6%), methyl palmitate (16.6%), methyl linoleate (11.6%), methyl linolenate (9.4%), methyl oleate (9.3%), methyl behenate (5.6%), and methyl stearate (5.4%) were found as major fatty acids. In the polar extracts C-dihexoylapigenin, quercetin glucoside an caffeoylquinic acid have been identified. **Conclusion / Discussion:** The water and methanolic leaf extracts of *S. hololeuca* were found to be valuable source of effective antioxidants.

Keywords: *Scabiosa hololeuca*; extract; accelerated solvent extraction (ASE); DPPH; TEAC; phenolic acid.

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POSTER PRESENTATION

INVESTIGATION OF PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF *GYPSOPHILA LARICINA* SCHREB. EXTRACTS

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Objective: *Gypsophila* is the third biggest genus in Caryophyllaceae family in the Flora of Turkey [1]. *Gypsophila* taxa have very large economic value due to use in medicine, pharmaceutical industry, food, cosmetics and chemical industry [2]. *Gypsophila laricina* Schreb.(Syn= *G. sphaerocephala* Fenzl ex Tchihat.) is a perennial species which usually grows on dry slopes and limestone rocks at elevations of 500-2000 m. *G. laricina* is entirely glabrous or bracts and calyx glandular-hairy [3, 4]. **Material and Methods**: In the present work, the aerial parts of *G. laricina* were collected from Bozan (Eskişehir) planting area. The dried leaves of *G. laricina* were extracted with accelerated solvent extraction (ASE) method with hexane (HE), methanol (ME) and water (AqE), at 70°C and 100°C, 103 bar and during 18 min. The free radical scavenging activity was evaluated using DPPH and TEAC assays. The total phenol contents was spectrophotometrically determined with Folin-Ciocalteau Reagent, the flavonids content was determined with $AlCl_3$ reagent. The fatty acids were isolated from the fruits with fatty aid extraction kit and esterified with BF_3 reagent for subsequent GC-FID/MS analysis. **Results**: The extracts yield values were calculated as 0.7% for HE, 1.3% for ME, and 7.7% for AqE. The highest antioxidant activity was detected for AqE (IC_{50} 0.627 ±0.015 mg/mL) and ME (IC_{50} 0.286±0.003 mg/mL) extracts. HE extract was detected as inactive. The highest values of Trolox equivalent antioxidant capacity (TEAC) were found for ME and AqE extracts (2.90±0.02 mM and 2.33±0.06 mM, respectively). The total amount of phenols was also found to be high in the ME (0.542±0.005 mgGAE/$g_{kuru\ ekstre}$) and AqE (0.336 ±0.005 mgGAE/$g_{kuru\ ekstre}$). The hexane extract contained the lowest total amount of phenol (0.038±0.002 mgGAE/$g_{kuru\ ekstre}$). The total amount of flavonoid was found to be 0.408±0.012 mgRE / $g_{kuru\ ekstre}$ in the ME. Methyl 8-hydrohyoctadecanoate (46.7%), methyl nonadecanoate (5.2%), methyl linoleate (4.5%), and hentriacontane (49.6%) were found as major lipophilic compounds of *G. laricina*. **Conclusion / Discussion:** The aqueous and methanol extracts of *G. laricina* are found to be good source of effective antioxidants.

Keywords: *Gypsophila laricina*; extract; ASE; DPPH; TEAC; TPC; TFC; fatty acid extraction

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POSTER PRESENTATION

SCREENING OF BIOLOGICAL ACTIVITY OF THE LEAF EXTRACTS OF *AZADIRACTA INDICA* A. JUSS. (GEED HINDI) FROM SOMALIA

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Objective: This study was undertaken for screening biological activity of *Azadirachta indica* A. Juss. growing in Somalia. **Material and Methods**: The leaves of *A. indica* were collected in Somalia in 2019 year. The leaves have been subjected to screening for biological activities and phytochemical profile. The leaf was extracted with accelerated solvent extraction (pressurized liquid extraction) method using sequentially *n*-hexane, methanol and water as solvents. Maceration with water was also applied to get the folk medicine preparation. The essential oil was obtained by hydrodistillation of the leaves in Clevenger type apparatus. The chemical profiles of the oil and extracts were investigated with GC-FID/MS techniques. The biological profiles of the oil and extracts were studied using microtiter plate assays: DPPH and TEAC. The total phenol contents (TPC) were determined as gallic acid equivalent using Folin-Ciocalteau reagent. The total flavonoids (TFC) were determined using $AlCl_3$ as complex giving agent. **Results**: The sesquiterpenes predominated in the leaf essential oil. The highest free radical scavenging potential was determined for aqueous extract obtained with ASE method (IC_{50}: 0.284±0.06). However, hexane extract (IC_{50}: 1.459±0.035mg/mL) demonstrated low activity, while the essential oil was found to be inactive in this test. In the experiment all the samples have shown activity towards to ABTS radicals with highest TEAC values for aqueous extracts (2.53±0.23 mM) obtained with ASE. TPC values were found to be between 0.108±0.005 GAEmg/mL and 0.139± 0.005 GAEmg/mL. TFC values were determined as highest in aqueous extracts (0.052 and 0.047 REmg/mL) and lowest in hexane extract (0.003 REmg/mL). **Conclusion / Discussion:** The study provides information on the leaf biological and phytochemical profile of *Azadirachta indica* native to Somalia.

Keywords: *Azadirachta indica*, essential oil, extract, activity.

POSTER PRESENTATION

BIOLOGICAL ACTIVITIES AND CHEMICAL PROFILES OF *SATUREJA HORTENSIS* ESSENTIAL OILS FROM DIFFERENT LOCALITIES

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Objective: The genus *Satureja* (Lamiaceae) comprises of 15 species in Flora of Turkey. *Satureja hortensis* L., which is the only species cultivated in Turkey, is an annual plant and its fresh and dry leaves are used as spices, fresh vegetables, and preservative folk medicine. The species has commercial importance for Turkey and is also cultivated. The essential oils have earlier been investigated for biological activities and composition [1-4]. **Material and Methods**: *Satureja hortensis* Landraces collected from different location were grown at Isparta ecological conditions. In the present study, the essential oils of the plants have been hydrodistillated in Clevenger apparatus for subsequent investigation for chemical profile, free radical scavenging and anti-diabetic activities. The chemical composition of the oils was investigated with GC-FID/MS method. The oils were also tested for free radical scavenging activities and inhibition of porcine pancreatic α-amylase enzyme. **Results**: Gas chromatographic analysis resulted with carvacrol as the main constituent of the essential oils. The variability of chemical profile of the oils was revealed with planar chromatography technique (HP TLC). The essential oils demonstrated good free radical scavenging activities against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. **Conclusion / Discussion:** The essential oils of the different landraces in *S. hortensis* contained at least 40% carvacrol. The contents of thymol, *p*-cymene and □-terpinene in the oils varied according to landraces origin of the plant. The oils were found to be the good source of effective antioxidants. However, antidiabetic activity was not noteworthy.

Keywords: *Satureja hortensis*; essential oil; DPPH; TEAC; carvacrol, HP TLC.

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POSTER PRESENTATION

MEMORY-VITALIZING EFFECT OF *BONGARDIA CHRYSOGONUM* L. AS INHIBITORS OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE

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Bongardia chrysogonum L. (Berberidaceae), a Turkish medicinal plant, is widely utilized for the treatment of epilepsy, haemorrhoids, urinary tract infections and gastrointestinal disorders in traditional medicine. The current study was carried out to assess its potential memory enhancing effect through enzyme inhibition tests as well as antioxidant test systems. The aerial parts and tuber of *B. chrysogonum* L. were extracted with ethanol, water and chloroform solvents, and subjected to enzyme inhibitory assays against acetylcholinesterase (AChE) and butyrylcholinsterase (BChE), which are related to pathogenesis of Alzheimer's disease. Antioxidant activity of the extracts was analyzed by four *in vitro* methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC). Phytochemical compositions of these extracts were also determined spectrophotometrically. The richest extracts in total phenols and flavonoids were found to belong to the chloroform extract of the tuber and the ethanol extract of the leaf (215.09±0.53 mg g-1 extract as GAE and 240.74±1.01 mg g-1 extract as QE, respectively). According to the results of antioxidant assays, almost all the extracts obtained from different parts of the plant exhibited remarkable antioxidant activities, among them the best antioxidant capacity was observed in the leaf extracts. As regards to enzyme inhibition potentials, the highest enzyme inhibitory activity was measured in the water extracts of tuber (83.81±0.33% and 62.14±0.60% inhibition on AChE and BChE, respectively), while the lowest enzyme inhibition was determined in the chloroform extract of the tuber (43.86±0.90% and 27.63±1.09%, inhibition on AChE and BChE respectively). To the best of our knowledge, we herein disclose the first evaluation of in vitro neuroprotective effect of the aerial parts and tuber extracts obtained from *B. chrysogonum* L. determined by inhibition of AChE and BChE enzymes along with antioxidant activity test systems such as DPPH, ABTS, FRAP and CUPRAC in the current study. Thus, the data obtained from this work could be the first report for the literature.

Keywords: *Bongardia chrysogonum* L., neuroprotection, polyphenolic content, enzyme inhibitory, antioxidant.

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POSTER PRESENTATION

DESIGN, SYNTHESIS AND *IN VITRO* EVALUATION OF NEW TRIAZOLOTHIADIAZINE DERIVATIVES AS POTENTIAL ANTITUMOR AGENTS

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Objective: Lung cancer (LC) is the most common cause of cancer-related mortality in both men and women across the globe [1]. On the other hand, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality [2]. Despite substantial advances in therapeutic approaches, there is an urgent need to identify new anticancer agents for the treatment of LC and CRC. For this purpose, we designed a series of indole-based triazolothiadiazines as new candidates for the management of LC and CRC. **Material and Methods:** New triazolo[3,4-*b*]-1,3,4-thiadiazines (**2a-g**) were synthesized *via* the ring closure reactions of 2-bromo-1-arylethanones with 4-amino-5-((5-methoxy-2-methyl-1*H*-indol-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**), which was obtained *via* the solvent-free reaction of 5-methoxy-2-methyl-3-indoleacetic acid with thiocarbohydrazide. MTT assay was performed to determine their cytotoxic effects on A549 human lung adenocarcinoma and Caco-2 human colorectal adenocarcinoma cell lines. Furthermore, the most potent compounds were evaluated for their effects on apoptosis, caspase-3 activation and mitochondrial membrane potential. **Results:** Among these compounds, 6-(4-chlorophenyl)-3-[(5-methoxy-2-methyl-1*H*-indol-3-yl)methyl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**2e**) was found as the most effective agent on A549 and Caco-2 cell lines with IC_{50} values of 10±2.83 µg/mL and 14±2.83 µg/mL, respectively when compared with cisplatin (IC_{50}= 5.50±0.71 µg/mL and 7.75±0.35 µg/mL for A549 and Caco-2 cell lines, respectively). MTT assay indicated that the chloro substituent enhanced anticancer activity against A549 and Caco-2 cell lines. Compound **2e** showed more significant apoptotic activity against Caco-2 cell line than A549 cell line. According to flow cytometric analysis, compound **2e** induced apoptosis through caspase-3 activation in Caco-2 cell line. This compound also caused more significant mitochondrial membrane depolarization in Caco-2 cell line than A549 cell line.**Conclusion:** According to the *in vitro* studies, compound **5** stands out as a promising anticancer agent for further *in vitro* and *in vivo* studies.

Keywords: Apoptosis, anticancer activity, caspase-3, triazolothiadiazine.

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POSTER PRESENTATION

SYNERGISTIC EFFICACY OF HERBAL PRODUCTS AND ANTIBIOTICS COMBINATIONS AGAINST CLINICAL STRAINS OF *ESCHERICHIA COLI* ISOLATED FROM POULTRY

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Objective / Purpose: This study was aimed to evaluate the *in vitro* antimicrobial efficacy of certain herbal products and antibiotics combinations against *Escherichia coli* clinical strains isolated from poultry. **Material and Methods:** a preliminary assessment of essential oils and/or ethanolic extracts derived from several herbal species belonging to the following families: *Lamiaceae, Asteraceae, Hypericaceae, Umbelliferae* and *Geraniaceae* was conducted using the classical diffusion method Kirby Bauer. Selected herbal products were further investigated to establish the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations using the broth microdilution technique; finally, these products were checked in combination with tetracycline, neomycin and ampicillin, respectively against the reference strains *Escherichia coli* ATCC 10536 and clinical strains recovered from chicks with septicemia (n=5) and adult chicken with polyserositis (n=5). **Results:** The overall analysis of the data indicated promising valences for the antimicrobial potential for both ethanolic extracts and essential oils. Bacterial strains and herbal products type dependent susceptibility differences were recorded, with the most active product being the essential oils > ethanolic extracts derived from *Thymus vulgaris* L. > *Coriandrum sativum* L., *Pelargonium graveolens* L.Her. > *Lavandula angustifolia* Mill. > *Rosmarinus officinalis* L> *Salvia officinalis* L. > *Melissa officinalis* L. Synergistic antibacterial activity with antibiotics was recorded only for essential oils derived from *Thymus vulgaris* L., *Salvia officinalis* L. and *Melissa officinalis* L. **Conclusion / Discussion**: Further *in vitro* and *in vivo* research (aiming the mechanism of action and the potential cytotoxicity) are intended to ensure the development of herbal based therapeutic products.

Keywords: herbal products, antibiotics, *in vitro* combined antimicrobial efficacy, *E. coli*, poultry

POSTER PRESENTATION

POTENTIAL HEALTH BENEFIT OF RICE SPROUT FROM RICE VARIETIES RD 6 AND BLACK GLUTINOUS RICE

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Objective / Purpose: Rice (*Oryza* sp.) is an important food crop in Thailand and many parts of the world. The most popular part for edible is the grain, where the aerial part of rice sprout is less consumed. The aerial part of rice is majorly composed of leave, which is an important source of photosynthesized compounds. Here we determined the potential health benefits of the rice sprout from two cultivated rice varieties—RD 6 and black glutinous rice (BGR)—as they represent the popular edible white and black rice grains in Thailand, respectively. **Material and Methods:** The rice sprouts (20 g) of RD 6 and BGR varieties at age of 20-25 days were extracted thrice by using methanol with 1% hydrochloric acid (100 ml). The extract solvent was removed by rotary evaporator and then freeze dried to obtain dry residue. Total phenolic content was determined by using Folin-Ciocalteu method. Then chlorophyll and total anthocyanin were determined spectrophotometrically. **Results:** The height of rice sprouts of RD 6 and BGR varieties at age of 20-25 days were of 15-18 cm, respectively. BGR gave higher %yield (8.37% w/w fresh weight) than RD 6 (8.00% w/w fresh weight). Total phenolic content of RD 6 and BGR were 1.97 and 1.83 mg of gallic acid equivalence per gram extract, respectively. Based on UV absorbance scanning at the specific wavelength, both RD 6 and BGR varieties contained majorly chlorophyll A (400-450 and 650-700 nm) and chlorophyll B (590-600 nm). RD 6 showed slightly higher chlorophyll content than BGR varieties. As expected, BGR showed slightly higher anthocyanin content than RD 6 varieties. **Conclusion / Discussion:** The rice sprouts of RD 6 and BGR varieties possess relatively active constituents. At the young age, the leaves of both varieties produced not significantly different of the total phenolic content, chlorophyll and anthocyanin. More experiments are on process to identify and quantify the phytoconstituents of these two rice varieties at different ages.

Keywords: Rice, health benefit, sprout, RD 6, black glutinous rice

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POSTER PRESENTATION

SEARCH ON NEUROBIOLOGICAL EFFECT OF BROWN COFFEE AND CHICORY COFFEE EXTRACTS THROUGH CHOLINESTERASE AND TYROSINASE ENZYME INHIBITION

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Objective / Purpose: Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) have a progressive nature and today, no cure is available, yet. The most prescribed drug class for AD is cholinesterase (ChE) inhibitors at the moment. On the other hand, tyrosinase (TYR) is the chief enzyme in melanin biosynthesis and inhibition of TYR is becoming an important target for PD treatment. Our extensive previous studies on various coffees samples from plants such as terebinth, date, etc pointed out to great potential of their enzyme inhibitory activity regarding neuroprotection. In the current work, we, thus, aimed to search neuroprotective potential of the ethanol extracts prepared from herbal coffees of brown rice (*Oryza sativa* L.) and chicory (*Cichorium intybus* L.). Chicory coffee is made of the roots of the *C. intybus* by roasting and grinding them. Brown rice coffee is prepared with brown rice that has been deeply roasted to draw out a unique coffee flavor. **Material and Methods:** The coffee samples used in this study were purchased from supermarkets in Japan (brown rice) and Iran (chicory). ChE and TYR inhibitory activity potentials of the ethanol extracts prepared from the mentioned coffee samples were screened using ELISA microtiter assay at 2 mg/mL stock concentration. **Results:** Based on our results, the ethanol extract prepared from chicory coffee had a modest TYR inhibitory activity (29.05%), while the brown rice coffee extract was found to be inactive. On the other hand, they were both inactive against sister ChE enzymes in the tested concentration. **Conclusion / Discussion:** Our current data on chicory and brown rice coffee extracts revealed that they do not have neuroprotection through TYR and ChE inhibition. Various other mechanisms by different experimental methods can be suggested for further investigation.

Keywords: Cholinesterase, tyrosinase, enzyme inhibition, coffee, neuroprotection.

POSTER PRESENTATION

DISCOVERY OF A NEW CLASS OF SIRT2 INHIBITORS WITH ANTIPROLIFERATIVE EFFECT BASED ON S-TRITYL L CYSTEINE SCAFFOLD

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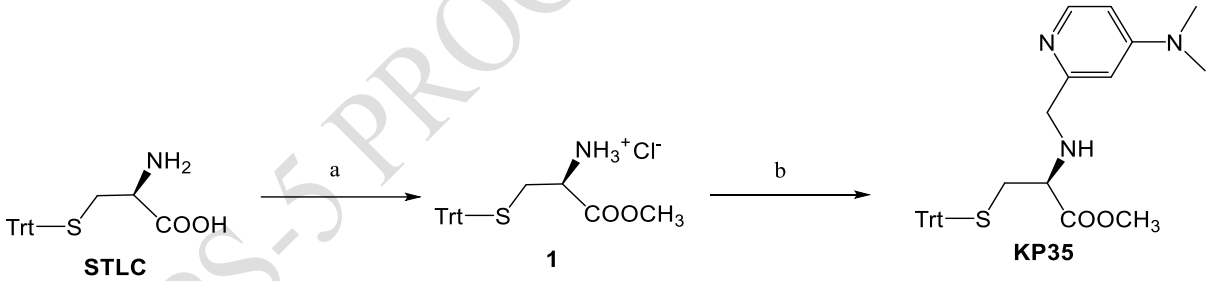
Objective / Purpose: Design of new class of SIRT2 inhibitors based on S trityl L cysteine that possesses antiproliferative effect against breast cancer cells.

Materials and Methods: Recent studies indicated that $SIRT_2$ is also involved in the pathogenesis and development of several cancers. Herein, we designed and synthesized new class of $SIRT_2$ inhibitors based on *S*-trityl-L-cysteine scaffold. Our design is dependent on our previous discovery of S trityl L Histidine as SIRT2 inhibitors. The new derivatives were confirmed by different spectral methods and evaluated against SIRT2 inhibition by electrophoretic mobility shift assay. Selected compounds were evaluated for their cytotoxicity against MCF-7 breast cancer cells.

Results and Discussion: Compound KP35 has IC_{50} 10.8 µM for $SIRT_2$ inhibition. Moreover, it inhibits proliferation of MCF7 and leukemic cell lines. KP35 is a drug-like and a promising small molecule that can be a candidate for fighting some types of cancers.



Scheme 1: a) $SOCl_2$, MeOH b) p dimethylamino picolinaldehyde, $NaBH(OAc)_3$, rt 3h.

Conclusion: We have developed a new class of SIRT2 inhibitors with a lead compound of IC_{50} 10.8 µM. It showed a considerable activity against MCF-7 and other leukemic cells. KP35 possesses drug-like properties and represents a promising hit anti-cancer agent.

Keywords: SIRT2, STLC, breast cancer, leukemia.

POSTER PRESENTATION

BIOLOGICAL ACTIVITIES OF THE EXTRACTS OBTAINED BY ACCELERATED SOLVENT EXTRACTION METHOD FROM *ONOBRYCHIS TOURNEFORTII* DESV. (FABACEAE)

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Objective: The genus *Onobrychis* Adans. belonging to Fabaceae family is represented by 162 species in the world, and is densely distributed in the Anatolia-Iran- Caucasian triangle [1]. A recently published article emphasized that *Onobrychis* species are important legumes and a good source of protein. The species have ecological effects such as nitrogen fixing and improving the soil structure [2,3].

Material and Methods: In the present study, the aerial parts of *Onobrychis tournefortii* were collected from Yapıldak (Eskişehir) in forestland. The leaves of *O. tournefortii* were subjected to accelerated solvent extraction (ASE) to get sequentially non-polar and polar extracts: *n*-hexane (HE), methanol (ME) and water (WE) extracts. The free radical scavenging activity was evaluated using DPPH and TEAC assays. Total phenolic content was determined by Folin-Ciocalteu method. In addition, the seeds of *Onobrychis tournefortii* were subjected to fatty acid extraction.

Results: The yields of HE, ME and WE extracts were found to be as 0.54%, 9.58% and 17.58% respectively. Methyl oleate (31.5%), methyl linoleate (28.7%), methyl palmitate (23.8%) and methyl stearate (5.3%) were found as major fatty acids. In the extracts apigenin pentoside, caffeic acid derivative, quercetin neohesperioside, rutin, quercetin glucoside, luteolin/kaempferol glucuronide, phenylethanoid glycoside similar to samioside have been identified.

The highest antioxidant activity was detected for WE (IC_{50} 0.52 ±0.027 mg/mL,) and ME (IC_{50} 0.51 ±0.005 mg/mL) extracts. However, HE showed low activity (1.48 ±0.024 mg/mL). The highest TEAC values were obtained for the aqueous (2.96±0.004 mM) and methanol (2.85 ±0.02 mM) extract. The highest total phenol content was demonstrated for ME and WE (0.45 ±0.014 mg GAE/g, 0.79 ±0.03 mg GAE/g).

Conclusion / Discussion: The present work revealed that aquous extract of *Onobrychis tournefortii* is the good source of effective antioxidants. The fruits contaned a valuable fatty acids.

Key Words: *Onobrychis tournefortii*; extract; accelerated solvent extraction; DPPH; TEAC; fatty acid.

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POSTER PRESENTATION

NEUROBEHAVIORAL DEVELOPMENTAL TOXICITY OF SPROUTED SEEDS EXTRACT OF TRIGONELLA FOENUM GRAECUM (L.) IN MICE.

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Abstarct: Previous results from our laboratory have demonstrated that sprouted fenugreek has shown a reproductive toxic effect on mice. This study was undertaken to complete those data and to determine the behavioral teratogenic effects on the mouse offspring of dams orally administered by lyophilized aqueous extract of sprouted organic fenugreek seeds during all the period of pregnancy.

Material and Methods: 40 females where divided in five groups and administered orally by aqueous germinated seeds extract of fenugreek at doses of 0 (control), 200, 500, 800 and 1000 mg/kg/day respectively. Offspring were submitted to behavioral tests since the birth to the 21postnatal day. Surface righting reflex, Cliff avoidance and rotarod test.

Results: offspring from treated dams showed a significantly lower success in righting reflex and cliff avoidance test. The rotarod test reveals that prenatally treated group remained on the rod for a significant short time than control.

Conclusion: these results indicate that prenatal exposure to sprouted fenugreek seeds produced a delay of early response development and impaired locomotors coordination.

Keywords: Developmental toxicity, sprouted seeds, fenugreek, neurobehavior

FULLTEXT PAPERS

FULL TEXT-PAPER

COMPARISON OF ANTIOXIDANT, ANTI-MMP -2,-9 AND ANTI-HYALURONIDASE ACTIVITY BETWEEN EXTRACTS FROM BLACK AND WHITE SESAME SEED CAKE (*Sesamum indicum* Linn.)

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Abstract

Objective: To prepare the extracts from black and white sesame seed cake and investigate their antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity. **Materials and methods**: Black and white sesame seed cake were obtained from cold-press oil processing and extracted by solvents with different polarity using maceration method. The extracts were determined for total phenolic contents and tested for antioxidant activities using DPPH and FRAP assay. Moreover, the matrix metalloproteinase -2 and -9 inhibition and hyaluronidase inhibition were evaluated by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). **Results**: The water extract of black sesame seed cake presented the highest TPC with the value of 237.13 ±3.39 mg of GAE /g of extract and followed by the methanolic extract of black seed cake (192.92 ±3.01 mg of GAE /g of extract). The water extract also gave high free radical scavenging and ferric reducing ability with the IC_{50} ,FRAP value and EC_1 value of 0.147 ±0.01 mg/ml, 0.65 ±0.01 mM Fe (II) /g of extract weight, 1.67 ±0.01mg/ml, respectively. Moreover, the extract from black sesame seed cake at 1.0 mg/ml also exhibited the highest MMP-2,-9 and hyaluronidase inhibition of 85.20 ±0.50%, 99.50 ±0.90%, and 99.94 ±0.81% inhibition, respectively whereas the extract from white seed cake presented no inhibition. **Discussion and conclusion**: The results demonstrated that the extracts from black sesame seed cake possessed the higher ability of antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity than the extracts from white sesame seed cake that could be a valuable natural extract for further development into anti-aging cosmeceutical products.

Keywords : sesame seed cake, antioxidant, anti- matrix metalloproteinases -2,-9, anti-hyaluronidase

INTRODUCTION

Skin aging is one of the biological aging phenomenon which is a major dermatologic concern in the modern society. Aged skin presents the appearance of sagging and wrinkling and skin discoloration.[1] The production of ROS and the generation of oxidative stress may cause damage to the skin structure and functions.[2-4] Moreover, ROS production inhibits collagen synthesis as a result from the activation

of matrix metalloproteinases (MMPs). MMPs are able to degrade various components of extracellular matrix (ECM) proteins including collagen, fibronectin, elastin, and proteoglycans. The degradation of ECM might contribute to a feature of premature skin aging.[5-9] Hyaluronic acid (HA) is one of the ECM molecules that involved in skin moisture. HA is degraded into smaller HA fragments (HAF) by hyaluronidases (HYAL) leading to lose function of maintaining water in the skin. Moreover, HA can also interact with free radicals such as ROS leading to its degradation.[10-12] Therefore, many studies that related to skin aging were aim to inhibit the production of free radicals, promote collagen synthesis and reduce HA degradation.[10,12-13]

The bioactive natural compounds represent an important role in prevention of age and oxidative stress-related diseases including skin aging. Polyphenol compounds, commonly found in various edible and nonedible plants are widely investigated for their antioxidant properties because of their ability to prevent tissue damage from ROS.[14-16] Sesame is an oilseed plant in the Pedaliaceae family which is one of the oldest and most traditional oilseed crops. In recent years, cold-pressed sesame oil has become commercially popular that cause the waste product called sesame seed cake. Several studies established antioxidant activity and anti-inflammatory that related to its phenolic compound especially lignans and lignan glycoside.[17-19] Nevertheless, the biological activity of the sesame seed cake associated with skin aging have been rarely investigated. Therefore, the aim of this study is to prepare the extracts from black and white sesame seed cake and investigate their antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity.

MATERIAL AND METHODS

Plant materials

Two different colored varieties were used in the study including black sesame seed (received from Mae Tang farm in Chiang Mai, Thailand) and white sesame seed (purchased from local market in Chiang Mai, Thailand). The black and white sesame seed cake were obtained from cold-press oil processing and were dried by hot-air oven for 24 hours at temperature of 45 ºC. The seed cake was ground into fine powder and kept at 4 ºC until further use.

Extraction

The black and white sesame seed cake were extracted by solvents with different polarity including hexane, ethyl acetate, 95% ethanol, methanol and water by maceration method. The concentrated extracts was stored at -4 ºC until use.

Biological activities assay

Determination of total phenolic content of the sesame seed cake extracts[20]

The black and white sesame seed cake extracts were determined for total phenolic by Folin-Ciocalteu assay using a calibration curve of standard gallic acid. Briefly, the different concentrations of the extracts were mixed with the Folin-Ciocalteu reagent. Then, Na_2CO_3 7.5% w/v was added and the mixtures were incubated for 30 min in the dark condition. The absorbance was measured at 765 nm using spectrophotometer. The concentration of total phenolic contents in all extracts were presented as gallic acid equivalent (GAE) in milligram per gram dry sample.

Determination of antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [20-21]

The different concentrations of extracts and standard were mixed with 167 μM DPPH• in methanol and incubated in the dark at room temperature for 30 min. At the endpoint of reaction, the mixtures will be measured at 520 nm using spectrophotometer microplate reader. The percentage of inhibition will be calculated by the equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Whereas; $A_{control}$ is the absorbance of the control reaction

A_{sample} is the absorbance of test sample

Determination of antioxidant activity with ferric reducing antioxidant power (FRAP) assay[21]

The extracts were determined for FRAP value using a calibration curve of standard ferrous sulphate. Brifly, the FRAP reagent was prepared by the mixture of acetate buffer, TPTZ solution, $FeCl_3$ solution and DI water. Each concentration of the extracts was mixed with FRAP reagent and were then incubated at room temperature for 5 min. At the endpoint of reaction was measured at 593 nm by spectrophotometer microplate reader. The regression equation from the standard curve was used to calculate the FRAP value of each sample.

Determination of Matrix metalloproteinase -2 and -9 inhibition by gel electropholysis[22-23]

3T3 Cell Line murine was seeded in T75 Flasks and then add DMEM, high glucose. Cells were incubated at 37°C and secreted MMP-2 and MMP-9 in the cell supernatant before using gel electropholysis to analyze. Various concentration of samples were added in 96-well plate with the secretion of MMP-2 and MMP-9. The mixtures were incubated at 37°C for 24 and 48 hours. After incubation, the mixtures were collected and mixed with 2x dye and will be kept in 4°C for next step.

For gel electrophoresis, resolving gel and stacking gel were prepared into casting chambers. Next, the mixture with 2x dye were loaded into stacking gel and the protein was separated by electrophoresis. After the running finish, the gel was stained with staining buffer (CooMassie blue) and determine the results using imageJ program.

Determination of hyaluronidase inhibition by gel electropholysis[24]

In briefly, various concentration of samples were mix with hyaluronidase. The mixtures were incubated at 37°C for 24 and 48 hours. After incubation, the mixtures were collected and mixed with 2x dye and were kept at 4°C for next step.

For gel electropholysis, resolving gel and stacking gel was prepared into casting chambers. Next, the mixture with 2x dye was loaded into stacking gel and the protein was separated by electrophoresis. After the running finish, the gel was stained with staining buffer (Alcian blue 8GX) and determined the results using imageJ program.

Statistical analysis

All of the experiments were performed in triplicate and data presented as a mean ± standard deviation (SD). The data were subjected to analysis of variance using T-test at 95% confidence level ($p < 0.05$) by SPSS version 17.0.

RESULTS

Percentage yields of the extracts

Figure 1 shows the percentage yields of the black and white sesame seed cake extract by different solvent using maceration method. The ethanolic extract from both black and white sesame seed cake showed the highest %yield of 30.12% and 32.44%. The extracts from white seed cake presented slightly higher percentage yields than those from the black seed cake

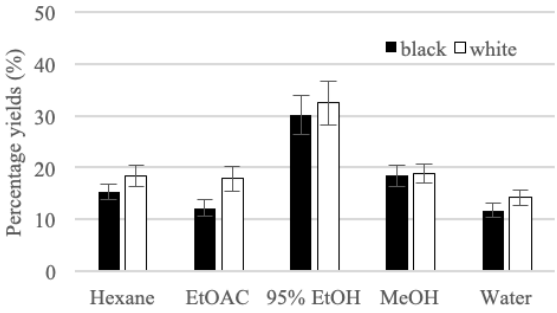

Figure 1. Percentage yields of the extracts from black and white sesame seed cake

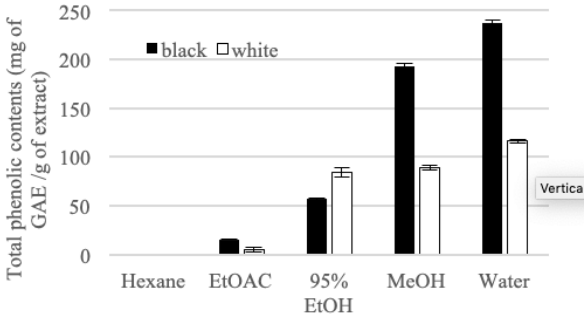

Figure 2. The total phenolic contents of the extracts from black and white sesame seed cake

Total phenolic contents of the extract

Total phenolic contents of the extracts from black and white sesame seed cake are demonstrated in Figure 2. The water extract of black sesame seed cake presented the highest TPC with the value of 237.13 ±3.39 mg of GAE /g of extract and followed by the methanolic extract of black (192.92 ±3.01 mg of GAE /g of extract). Whereas, for white seed cake, the water extract showed the highest TPC with value of 116.14 ±1.84 mg of GAE /g of extract. The extracts from black seed cake exhibited higher phenolic contents than those from the white seed cake.

Antioxidative activities

DPPH radical scavenging activity

According to the antioxidant activities, almost the extracts of black seed cake exhibited the better DPPH radical-scavenging property and ferric reducing ability than those from white seed cake. The water extract from black seed cake showed the strongest DPPH scavenging capability with IC_{50} value of 0.15 ±0.01 mg/ml, whereas, the water extract from white seed cake presented IC_{50} value of 0.75 ±0.02 mg/ml.

Ferric reducing antioxidant power (FRAP) activity

Water extract from black seed cake exhibited the strongest reducing power value with FRAP value of 0.650 ±0.01 mM Fe (II) /g of extract weight and EC_1 value of 1.670 ±0.01mg/ml followed by the methanolic from black seed cake. On the contrary, almost the extracts from white seed cake presented a weaker reducing power. (showed in Table 1.)

Anti-MMP -2 and -9 activities

The inhibition of MMP -2 and -9, MMP -2 and -9 activity appeared as a white clearing on a dark blue background as shown in fig 3. The hexane extract of black seed cake exhibited the highest inhibitory activity at 48 h with the values of 85.20 ±0.50% (MMP-2) and 99.50 ±0.90% (MMP-9) while all extract of white seed cake showed no inhibitory activity as shown in Table 1.

Anti-hyaluronidase activity

The inhibitory of hyaluronidase activity also showed a white clearing on a light blue background as shown in fig 4. The extract from black seed cake presented the highest inhibitory activity at 48 h with the values of 99.94 ±0.81% inhibition whereas the extract from white seed cake showed no inhibition as shown in Table 1.

Table 1. Comparison of the antioxidant activities, anti-MMP-2 and -9 activities and anti-hyaluronidase activity of the extracts from black and white sesame seed cake

Samples		IC_{50} of DPPH radical scavenging (mg/ml)	FRAP		% MMP-2 inhibition (at 1.0 mg/ml)	% MMP-9 inhibition (at 1.0 mg/ml)	% hyaluronidase inhibition (at 1.0 mg/ml)
Part used	Extraction solvent		FRAP value (mM Fe (II) /g of extract weight)	EC_1 (mg/ml)			
Black sesame seed cake	Hexane	NA	NA	NA	85.20 ±0.50	99.50 ±0.90	NA
	Ethyl acetate	1.74 ±0.06	0.09 ±0.01	11.53 ±0.02	60.79 ±1.02	59.72 ±0.34	97.40 ±0.76
	Ethanol	0.97 ±0.02	0.15 ±0.01	8.00 ±0.02	NA	NA	NA
	Methanol	0.19 ±0.01	0.48 ±0.01	2.38 ±0.01	56.67 ±0.82	79.97 ±1.42	99.94 ±0.81
	Water	0.15 ±0.00	0.65 ±0.01	1.67 ±0.01	NA	80.11 ±0.91	NA
White sesame seed cake	Hexane	NA	NA	NA	NA	NA	NA
	Ethyl acetate	1.75 ±0.06	0.06 ±0.01	27.06 ±0.02	NA	NA	NA
	Ethanol	1.41 ±0.09	0.10 ±0.01	12.52 ±0.02	NA	NA	NA
	Methanol	1.16 ±0.02	0.19 ±0.01	6.41 ±0.01	NA	NA	NA
	Water	0.75 ±0.02	0.18 ±0.01	6.11 ±0.01	NA	NA	NA

Standards

Ascorbic acid	0.04 ±0.01	1.55 ±0.01	0.06 ±0.01	NA	NA	NA
Trolox	0.01 ±0.00	1.38 ±0.01	0.07 ±0.01	ND	ND	ND
BHT	0.11 ±0.01	0.98 ±0.01	1.32 ±0.01	ND	ND	ND
Gallic acid	ND	1.34 ±0.01	0.08 ±0.01	91.94 ±0.91	94.88 ±1.04	NA
Sesamin	ND	ND	ND	NA	NA	NA
Sesamol	ND	ND	ND	NA	NA	NA

NA, No activity; ND, No determine; IC_{50} value (mg/ml) was the concentration of the sample that inhibited 50% of the DPPH radicals; FRAP value was expressed in terms of mM Fe (II) /g of extract weight using ferric chloride standard curve; EC_1 was the concentration of antioxidant with a reducing effect equivalent to 1 mmol/L Fe(II); ± standard deviation was use to present the mean values of three determinations.

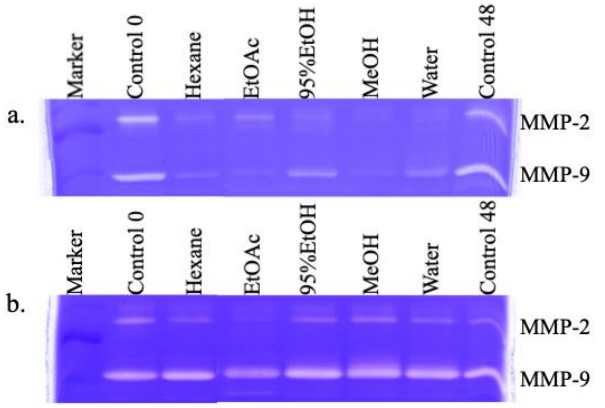

Figure 3. Detected MMP-2,-9 by SDS-PAGE; a. Black sesame seed cake extracts; b. White sesame seed cake extracts

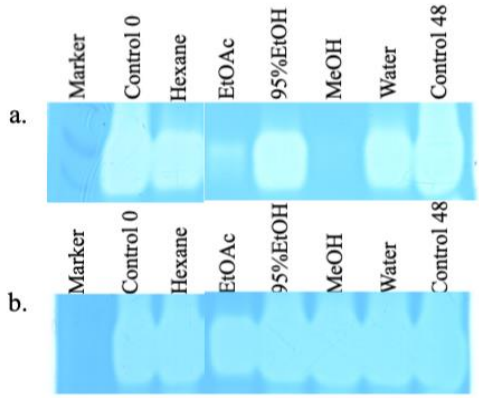

Figure 4. Detected hyaluronidase by SDS-PAGE; a. Black sesame seed cake extracts; b. White sesame seed cake extracts

DISSUSSION AND CONCLUSION

The results demonstrated that all the extracts from black sesame seed cake possessed the higher ability of antioxidant, anti-MMP -2,-9 and anti-hyaluronidase activity than the extracts from white sesame seed cake. The result of antioxidant activities including DPPH scavenging and ferric reducing ability were correlate with the amount of total phenolic contents of each extract. The extract with high phenolic compounds also presented great scavenging ability. Moreover, the extracts from black sesame seed cake revealed MMP -2 and -9 inhibition that corresponding to anti-aging activity due to the collagen and elastin fibers are degraded by MMP -2 and -9. Additionally, the extract from black seed cake exhibited the highest hyaluronidase inhibitory activity that may reduce the hyaluronic acid degradation which improve skin dehydration. Therefore, the extracts from black sesame seed cake could be a valuable natural extract for further development into anti-aging cosmeceutical products.

ACKNOWLEDGEMENTS

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FULL TEXT-PAPER

SYNTHESIS OF COPPER NANOPARTICLES FROM WASTE ONION PEELS

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Abstract

Nanoparticles have extremely small dimensions and a high surface area/volume ratio, so they have chemical and physical differences, such as catalytic reactivity, thermal and electrical conductivity, chemical stability, optical performance, antimicrobial activity, compared to larger particles of the same chemical composition. The physical and chemical processes used in the synthesis of nanoparticles have the disadvantages of producing many toxic substances that pollute the environment because they require high amounts of temperature and energy. In recent years, in the synthesis of Au, Ag, Pt, Pd, Zn, Cu metal nanoparticles; biocompatible, non-toxic solvents, high cost-free, synthesized nanoparticles with good distribution, environmentally friendly biological synthesis methods are preferred. In this study, copper nanoparticles (CuNPs) were synthesized by the green, fast and environmentally friendly method using the extract of onion peel which is a waste material.

Onion peel and $CuSO_4.5H_2O$ were used as a starting material for the synthesis of CuNPs. The color of the solution changed from the blue color of Cu solution to black revealing the formation of CuNPs. Formation of CuNPs was easily observed by UV-Visspectroscopy. In addition, the characterization of CuNPs was performed by SEM images and FTIR analyses.

For CuNPs synthesis, the optimum ratio of onion extract $CuSO_4.5H_2O$ solution was determined as 70:50. The optimum copper concentration in solution and synthesis temperature were determined as 0.5 M and 20^oC, respectively.

In this study, both the environmentally hazardous agricultural and domestic wastes were evaluated, re-used, and the onion peel waste was shown to be suitable for the production of CuNPs.

Keywords: Copper Nanoparticle, green synthesis, onion peel

INTRODUCTION

Due to their specific properties, such as size, structure, and distribution, nanoparticles have differences such as catalytic reactivity, thermal conductivity, non-linear optical performance, and chemical stability [1].

Nanoparticles, which have these beneficial properties, are applied in potential areas such as cosmetics, biomedicine, electronics, chemical industry, optics, space industry, energy science, light emitters, food, food packaging, catalysis, information storage, chemical detection and imaging, drug distribution, non-linear optical devices, mechanical and biological labeling [2].

Production of nanoparticles can be realized using physical, chemical and biological methods [4]. However, physical methods are not preferred because they require the useof expensive and space-consuming equipment, high temperature, and pressure [3]. On the other hand, chemical methods are also disadvantageous due to the release of many toxic substances that will pollute the environment [4].

The biological synthesis method has been preferred in recent years since it is not toxic and expensive and is an environmentally friendly method in which synthesized nanoparticles demonstrate a good distribution [5].

Microorganisms, live plants, and plant extracts are used in the biosynthesis of metallic nanoparticles such as Au, Ag, Pt, Pd, Zn, and Cu. Enzyme, flavonoid, protein and antioxidant compounds naturally synthesized from microorganisms and plants act as reducing and stabilizing agents [6].

Since copper nanoparticles (CuNps) have excellent physical and chemical propertiesamong metal nanoparticles, are more cost-effective than gold and silver, and can interact easily with other particles, they have been reported to be used as sensors, catalysts, magnets, and antimicrobial agents [7].

When the literature is examined, CuNPs are reported to be synthesized from plants such as *Calotropisgigantean*, *Punicagranatum*, *Ocimum sanctum*, *Azadirachtaindica*, *Citrus medica*, and *Alliumcepa* [8].

In this study, CuNPs were synthesized by a fast, environmentally friendly and cost-effective green method using the extract of onion peel which is a waste material.

MATERIAL AND METHOD

Preparation of Onion Peel Extract

The copper metal solution used in the study was prepared with $CuSO_4.\ 5H_2O$ (Sigma-Aldrich). Onion peels were used as a reducing agent in the synthesis of CuNPs, and the peels were obtained from a local market in Sivas. After onion peels were collected and washed twice with distilled water, they were separated into small pieces and dried to removethe moisture. 10 g of dried onion peels were boiled by stirring in 1000 ml of distilled water for 30 min, and the peels were extracted. The extract brought to room temperature was filtered using Whatman No. 1 filter paper and stored at +4 ^oC for use in the synthesis of CuNPs.

Green Synthesis of Copper Nanoparticles

In order to determine the optimum conditions in the synthesis of copper nanoparticles, the metal concentration was changed between 0.1-0.8 M, the amount of extract between 10-90 mL, the temperature between 20^oC-80^oC, and the mixing time between 30-180min. The synthesized CuNPs were separated by centrifuging (Hitachi, CR22N) at 1000 rpm for 10 min.

Characterization of Copper Nanoparticles

The Uv-Vis spectrophotometer analysis of the synthesized CuNPs was performed in the research laboratory of Cumhuriyet University Chemical Engineering Department. The absorbance scans of CuNPs were examined by a UV-visible double-beam spectrophotometer (UV-2600, Shimadzu) in the wavelength range of 200-900nm. In order to identify the morphological structures of the synthesized CuNPs, a Scanning Electron Microscope (SEM) (Tescan MIRA3, XMU), and to determine their

molecular structure, a Fourier Transform Infrared Spectrometer (FT-IR) (Bruker Model, Tensor II) were used. SEM and FT-IR analyses were performed at the Advanced Technology Application and Research Center of Cumhuriyet University.

RESULTS

SEM Images

The size and structure of the CuNPs obtained by the green synthesis method using onion peel extract were analyzed by SEM. The SEM image presented in Figure 1 demonstrates that CuNPs are spherical and their diameters change between 114.55 and 287.93 nm.

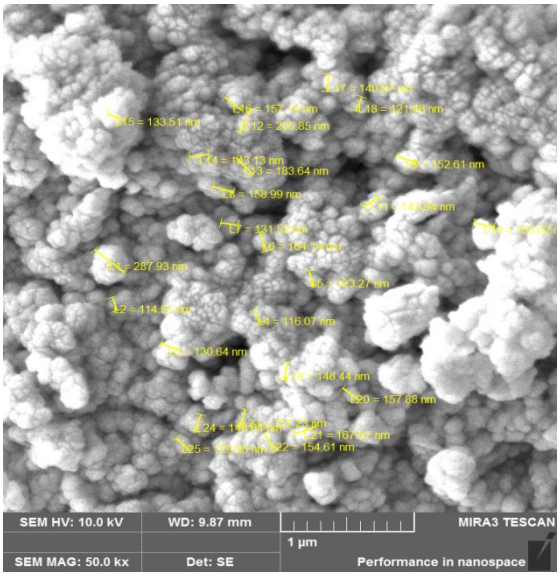

Figure 1. SEM images of CuNPs synthesized with onion peel extract

FTIR Spectra

FTIR analysis was performed to determine the molecules which were effective in reducing, in the CuNPs synthesis with onion peel. The peaks observed at 3207 cm^{-1} and 2926 cm^{-1} in the spectrum presented in Figure 2 correspond to C-H stretching. The peak observed at 1590 cm^{-1} demonstrates the presence of aromatic groups. The peaks observed at 1267 cm^{-1} and 1165 cm^{-1} correspond to C-O stretching.

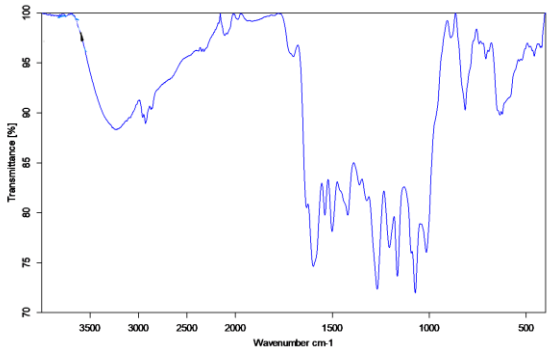

Figure 2. FTIR spectrum of CuNPs synthesized with onion peel extract

Effect of Cu concentration

UV-Vis absorption spectroscopy of the nanoparticles synthesized by mixing equal amounts of the extract and Cu metal solution was presented in Figure 3. When the Cu solution and the onion peel extract were mixed, a color change from blue to black was observed in the solution. With UV-Vis spectroscopy of the solutions obtained by changing the Cu solution concentration between 0.1M-0.8M, Cu concentration with the maximum absorbance was determined. The maximum absorbance was observed at 456 nm, and this result is consistent with the literature [9]. The optimum copper solution concentration was determined as 0.5M.

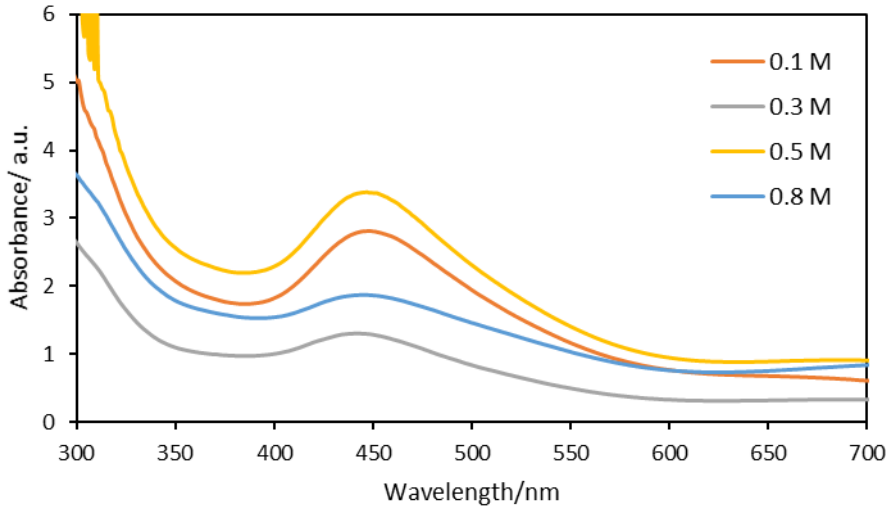

Figure 3. UV-Vis spectroscopy of CuNPs synthesized at different Cu concentrations Absorbance-Wavelength curves (Cu: Onion extract 1:1, T = 20 ºC).

Effect of extract amount

The synthesis of CuNPs was examined by adding different amounts of onion peel extract (10-90 mL) to 50 ml of Cu(II) solution (0.5 M). The optimum extract amount was determined by analyzing the obtained mixtures by UV-Vis spectroscopy. It was determined that absorbance increased with the increase in the amount of extract from 10 ml to 50 ml, but the same absorbance value was obtained in 70 ml and 90 ml (Figure 4). For the use of a smaller amount of extract, 70 ml was determined as the optimum extract amount.

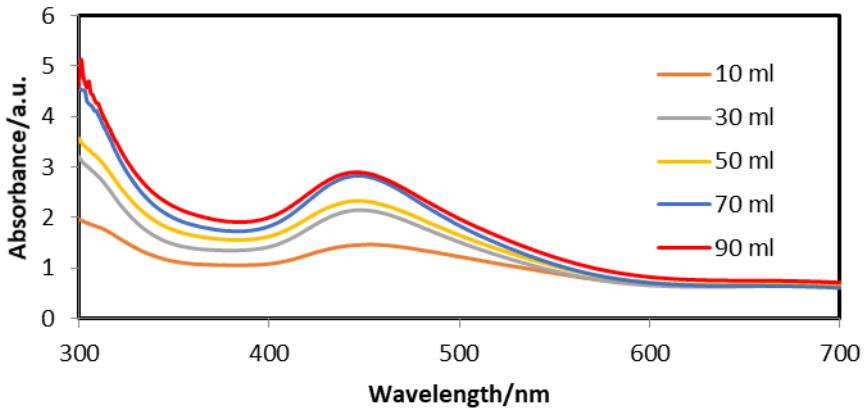

Figure 4. UV-Vis spectroscopy of CuNPs synthesized in different onion peel extract amounts Absorbance-Wavelength curves (T = 20 ºC).

Effect of reaction time

In nanoparticle synthesis, the reaction time is an important parameter. In order to investigate the effect of reaction time on CuNPs formation, analysis with UV-Vis spectroscopy was performed at different reaction times (30, 60, 90, 120, and 180 minutes). In the studies conducted to determine the reaction time, the copper concentration was set at 0.5 M, and the mixing ratio of onion extract and the copper solution was set at 1:1. Figure 5 presents the Absorbance-Wavelength curves obtained at different reaction times for the synthesis of CuNPs. The optimum reaction time was determined as 180 minutes.

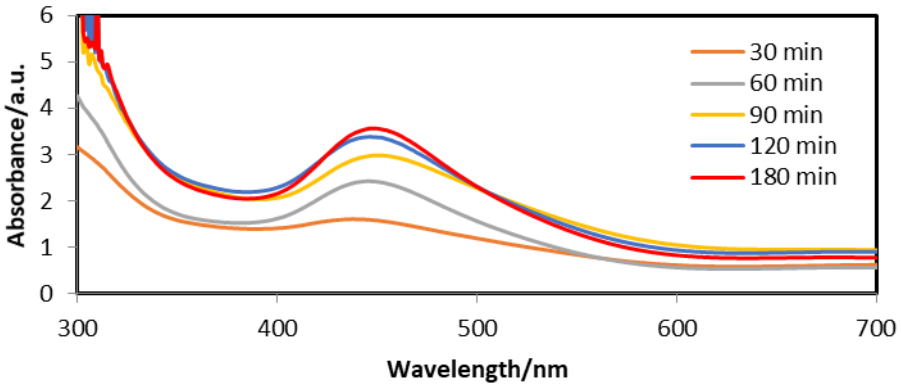

Figure 5. UV-Vis spectroscopy of CuNPs synthesized at different reaction times Absorbance-Wavelength curves (Cu: Onion extract 1:1, T = 20 ºC).

Effect of reaction temperature

In order to determine the optimum temperature in the synthesis of Cu NPs, 0.5 M 50 ml Cu solution was mixed with 70 ml of onion extract (Cu: Onion extract, 50:70), and studies were carried out at 20, 30, 50, and 80 ºC. The prepared solutions were analyzed by UV-Vis spectroscopy. The highest absorbance of CuNPs was obtained at 20 ºC, and the optimum temperature was determined as 20 ºC (Figure 6).

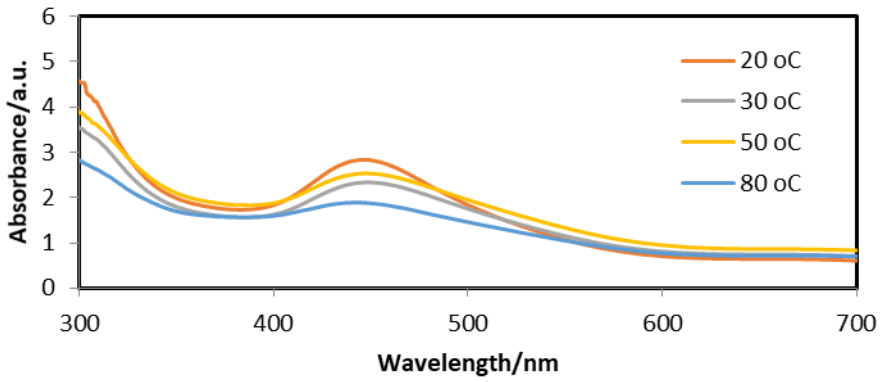

Figure 6. UV-Vis spectroscopy of Cu NPs synthesized at different temperatures Absorbance-Wavelength curves

CONCLUSION:

By using the onion peel extract as a reducing agent, the green synthesis of CuNPs was performed using an environmentally friendly and low-cost method. For the synthesis of CuNPs, 0.5 M Cu concentration, 70 ml:50 ml onion extract: copper ratio, 180 min reaction time, and 20 ^{o}C reaction temperature were determined as optimum values. In the characterization of CuNPs, it was identified that the particle size changed between 114-287 nm as a result of SEM analysis.

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FULL TEXT-PAPER

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING POMEGRANATE WASTE

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Abstract

In recent years, metal nanoparticles have been used extensively in industrial fields due to their superior catalytic, magnetic and optical properties. Among metal nanoparticles, silver nanoparticles (AgNPs) are used in various fields due to their relatively inexpensive, highly conductive, and antibacterial properties[1]. The physical and chemical methods used in the production of AgNPs have high cost and toxic and hazardous chemicals that may have environmental and biological hazards in their structures. Recently, green synthesis has attracted great attention [2]. AgNPs have many potential applications in industrial fields involving antimicrobial agents, biological sensors, electronic devices, and waste water treatment. In this method, biological resources such as plant extracts, which are abundant and safe, are used. In this study, the production of AgNPs was performed by green synthesis using pomegranate (PM) waste.

PM waste and silver nitrate were used as a starting material for the synthesis of AgNPs. AgNPs formation was first observed by changing the color of the solution from yellow to dark brown. The Ag-extract solution was tested by a UV-Vis spectrophotometer, and maximum absorbance peak formation at 377 nm, which belongs to AgNPs,was observed. The characterization of AgNPs was performed by the SEM images and FT-IR analyses.

For the AgNPs synthesis, the optimum Ag concentration was determined as 10 mM, and the optimum temperature was 80^{o}C.The formation of AgNPs was identified with the evaluation of SEM images and FT-IR spectra.

It was shown that both agricultural and domestic wastes are re-used and PM waste is suitable for the production of AgNPs.

Keywords: Silver nanoparticle, green synthesis, pomegranate waste.

INTRODUCTION

Due to their unique properties and biological applications, nano-sized particles are intensively studied to develop their synthesis and application areas [1,2]. Nanoparticles are synthesized by various methods physically, chemically and biologically [3].

Besides being expensive, the physical and chemical methods used in the synthesis of nanoparticles have environmental and biological hazard potential since chemicals used are toxic and hazardous [4].

Physical synthesis includes methods such as removal with laser, exposure to high energy radiation, and lithography. Chemical synthesis includes methods such as chemical reduction, electrochemistry, and photochemical reduction. Chemicals, used in chemical synthesis, cause toxic formation by attaching to the particle surface and prevent the use of particles in the medical field [5].Furthermore, these methods are expensive and time-consuming methods. Therefore, an environmentally and economically suitable synthesis method is required for nanoparticle production.

As a result, it was aimed to develop an environmentally friendly, energy efficient, convenient, easy and fast method for the preparation of silver nanoparticles (AgNPs) without using harmful and toxic chemicals. Therefore, methods for the synthesis of nanoparticles from microorganisms and plants have been developed [6,7]. Since the use of microorganisms in nanoparticle synthesis is more costly and time-consuming compared to plants, nanoparticle synthesis with plants is preferred more [8].

Inorganic metal nanoparticles such as silver, gold, copper, zinc, titanium, and nickel can be synthesized with different parts of the plant, such as root and leaf. The extract obtained from the root or leaf of the plant provides the formation of nanoparticles by reducing the metal ions.

In this study, the production of AgNPs from PM waste was carried out with green synthesis. This method is environmentally friendly, single-step, low cost and non-toxic for human health. The characterization of synthesized AgNPs was performed with UV-Vis, SEM, and FTIR.

MATERIALS AND METHODS

Collection of the plant materials and preparation of the extract

Used PM wastes were washed with distilled water to remove dirtiness and dried until the moisture was removed entirely. 10 g of dry PM waste was boiled in 200 ml of distilled water for 30 minutes, cooled and extracted and stored in the refrigerator at 4°C for use in the nanoparticle synthesis.

Synthesis of AgNPs

$AgNO_3$ (Sigma-Aldrich) was used for the synthesis of AgNPs. In the synthesis of AgNPs, the extract-metal solution ratio was kept constant at (2:1), and by changing the temperature in the range of 20°C-80°C, metal concentration in the range of 1-10 mM, and mixing time in the range of 30-180 min, optimal conditions for AgNPs synthesis were determined. The synthesized nanoparticles were separated by centrifuging (Hitachi, CR22N) at 10.000 rpm for 10 minutes.

Characterization of AgNPs

UV-Vis spectrophotometer analyses of the synthesized AgNPs were performed in the research laboratory of Cumhuriyet University Chemical Engineering Department. The absorbance scans of AgNPs were examined by a UV visible double-beam spectrophotometer (UV-2600, Shimadzu) in the wavelength range of 200-900 nm. In order to identify the morphological structures of the synthesized AgNPs, a scanning electron microscope (SEM) (Tescan MIRA3, XMU), and to determine their molecular structure, a Fourier Transform Infrared Spectrometer (FT-IR) (Bruker Model, Tensor II) were used. SEM and FT-IR analyses were performed at the Advanced Technology Application and Research Center of Cumhuriyet University.

RESULTS

SEM Images

The SEM micrograph for the AgNPs synthesized by the reduction of $AgNO_3$ using PM waste extract is presented in Figure 1. SEM analysis was performed to determine the morphological structure and dimensions of AgNPs. It was determined that the average particle size of the synthesized AgNPs of spherical structure ranged between 50-200 nm.

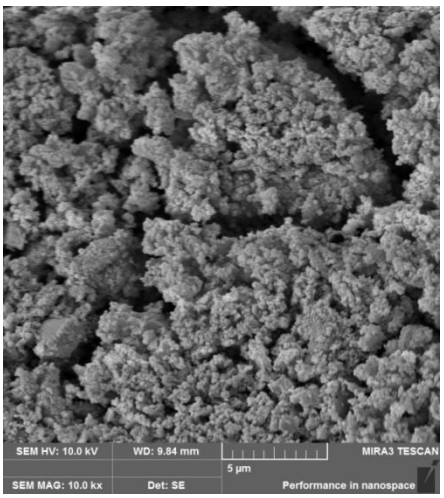

Figure 1. SEM images of AgNPs

FTIR Spectra

FTIR analysis was performed to determine the molecules that were effective in reducing Ag^+ ions to AgNPs with PM waste. The bands at 3371 cm^{-1} and 2924 cm^{-1} in the spectrum given in Figure 2 correspond to the stretching of primary and secondary amines, which are abundant in plants and which are organic derivatives of ammonia.

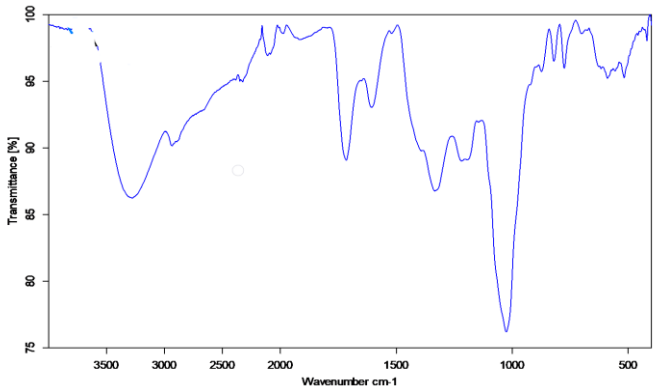

Figure 2. FTIR spectrum of AgNPs synthesized using PM waste extract

The effect of silver nitrate concentration

By keeping extract amount constant at 5 ml, the UV-Vis absorption spectroscopy of the nanoparticles synthesized with silver nitrate solution at different concentrations was presented in Figure 3. The UV-Vis spectroscopy results of the solutions, obtained by changing silver nitrate concentration between 1 mmol/L -10 mmol/L, were evaluated, and the wavelength at which they displayed the maximum absorbance and the optimum nitrate concentration were determined. The maximum absorbance was observed at 377 nm, and the optimum silver nitrate concentration was determined as 10 mM.

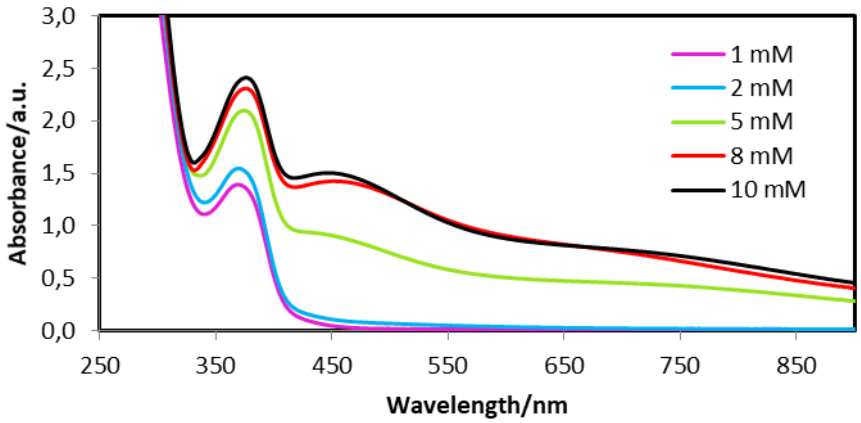

Figure 3. UV-Vis spectroscopy of AgNPs synthesized at different silver nitrate concentrations Absorbance-Wavelength curves

Effect of reaction time

In nanoparticle synthesis, the reaction time is an important parameter. In order to investigate the effect of the reaction time on the formation of nanoparticles, analyses were performed with UV-Vis spectroscopy at different reaction times at the constant 10 mM $AgNO_3$ concentration. When silver nitrate and PM waste extract were mixed, the solution color changed from yellow to brown over time. When the reaction time increased from 30 minutes to 180 minutes, an increase in absorbance peak intensity was observed (Figure 4). The optimum reaction time for the production of AgNPs was determined to be 180 minutes.

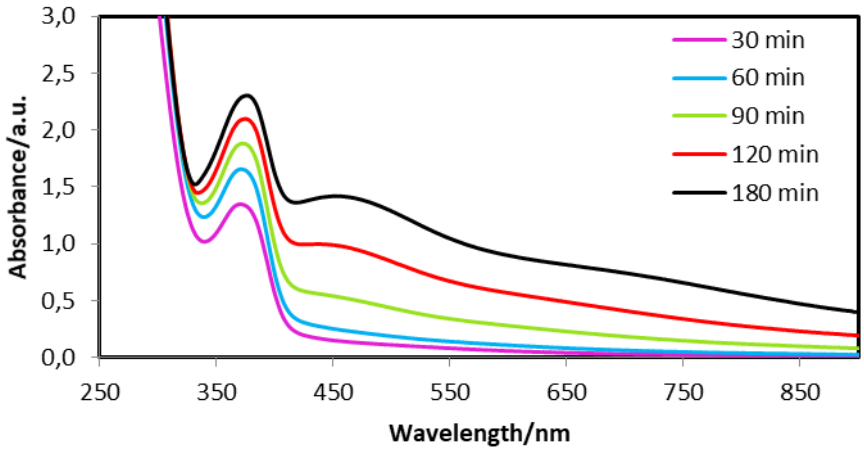

Figure 4. UV-Vis spectroscopy of AgNPs synthesized at different reaction times Absorbance-Wavelength curves (C_{AgNO3}: 10 mM, T: 20 ºC).

Effect of reaction temperature

Another important parameter in nanoparticle synthesis is temperature. In order to determine the optimum temperature of AgNPs, studies were carried out at 20, 30, 50 and 80 ºC in 5 ml extract amount and at 10 mM silver nitrate concentration. The amount of AgNPs in mixtures prepared for determination of the optimum temperature was determined by UV-Vis spectroscopy. Figure 5 displays the spectroscopy results of AgNPs synthesized at different temperatures. As the temperature increases, the increase in absorbance is observed. Obtaining a higher nanoparticle yield has been reported at high reaction temperatures, and therefore, the optimum reaction temperature was determined as 80 ºC [9].

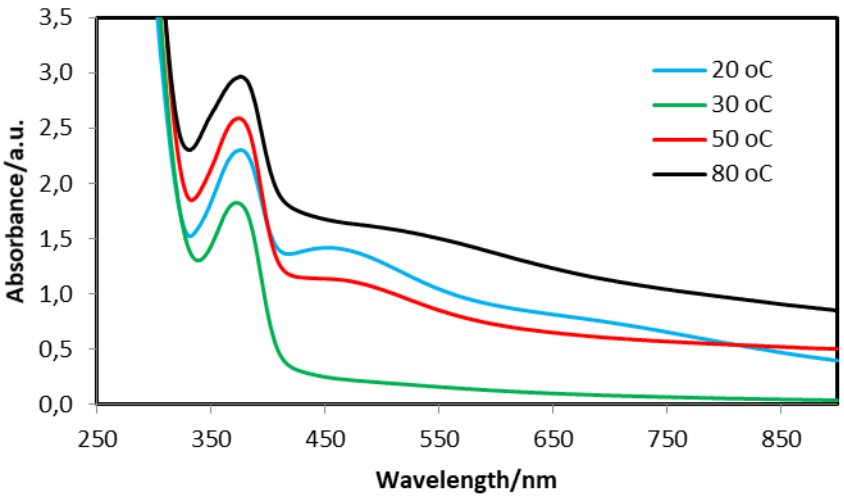

Figure 5. UV-Vis spectroscopy of AgNPs synthesized at different temperatures Absorbance-Wavelength curves. (C_{AgNO3}: 10 mM)

DISCUSSION AND CONCLUSION

PM waste extract was used as a reducing agent, and AgNPs were synthesized with a simple, fast, and environmentally friendly method. For the synthesis of nanoparticles, the reaction time of 180 minutes and 10 mM silver nitrate concentration were determined as optimum values. The increase in the reaction temperature results in an increase in the nanoparticle yield, and the optimum reaction temperature was determined as 80 °C. In the characterization of the synthesized AgNPs, it was determined that the particle size changed between 50-200 nm as a result of SEM analysis. In the FTIR spectrum, primary and secondary amine groups, which are effective in reducing Ag^{+} ions, were determined.

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FULL TEXT-PAPER

CONTROLLED DELIVERY OF CIPROFLOXACIN FROM HALLOYSITE NANOTUBES

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Abstract

Ciprofloxacin(CIP) belongs to a family of antibiotics called fluoroquinolones and is used to treat a variety of bacterial infections. Halloysite ($Al_2Si_2O_5(OH)_4.2H_2O$) nanotube (HNT) is a two-layered aluminosilicate, with a predominantly hollow tubular structure. CIP was loaded to halloysite nanotubes (HNTs) and encapculation efficiency and loading capacity of CIP were evaluated. Controlled CIP release from HNTs was investigated.

CIP encapsulated HNTs of 0.5 g were added to 100 mL of buffer solutions in the orbital shaker operated at 37°C. CIP release from HNTs was investigated in HCl solution at pH 2.1, phosphate buffer solution (PBS) at pH 5.0 and at pH 7.4 simulating gastrointestinal system and blood through 24 hours. CIP concentration in the samples taken at certain time intervals from buffer media was measured in UV spectrofotometer.

The encapsulation efficiency and loading capacity of CIP into HNTs were found to be 88.3% and 17.3%, respectively. Maximum CIP absorbance values in HCl buffer at pH 2.1, PBS buffer solutions at pH 5.0 and at pH 7.4 were obtained as 268 nm, 283nm and 289 nm, respectively. The CIP release efficiency in HCl solution at pH 2.1 simulating gastric media was determined as 27%. The CIP release percentages in PBS at pH 5.0 simulating intestinal tract and at pH 7.4 simulating blood were found to be 26% and 24%, respectively.

In this study it was shown that a high encapsulation efficiency and loading capacity of CIP was obtained into HNTs. Encapsulation of CIP into HNTs resulted in prolonged, sustained and controlled CIP release.

Keywords: Controlled drug delivery, ciprofloxacin, halloysite nanotubes

INTRODUCTION

Controlled drug release is of great importance with its ability to increase a therapeutic effect at the determined dose. Therefore, carrier nanomaterials are developed for the delivery of an appropriate dose of a drug to the target site within a certain period of time.

Halloysite nanotubes (HNTs) have been commonly used in recent years in biomedical implants, drug release systems, and tissue engineering applications due to their high strength, low weight, and capability to deliver functional pharmaceuticals. HNTs are among naturally occurring clay minerals

and are composed of aluminum and silicon oxide with a hollow tubular structure and a 10-15 nm double layer ($Al_2Si_2O5(OH)_{4.nH2O}$). HNTs used for commercial purposes have an external diameter of 50-100 nm, an internal diameter of 10-20 nm, and a length of 0.5-2.0 μm [1]. The internal octahedral alumina layer is connected to the tetrahedral silica layer to form a cylindrical structure. HNTs are also preferred because of having a large specific surface area, hollow and layered structure, and being easily modified by treatment with chemicals to increase the adsorption capacity. When HNT contacts with water, it may swell and forms highly stable colloidal suspensions. This makes its separation from the aqueous phase very difficult [2,3].

Drug release from HNTs starts from the desorption of an active agent (drug) on external surfaces, and it is released from the ends of the nanotubes to the media. This is followed by the release of the drug, the access to the surface of which is delayed due to diffusion limitations in the pores, from the ends of the nanotubes. This very specific morphological structure ensures that the delayed release of the drug occurs, and prevents the explosive release of some very useful drugs.

In this study, CIP antibiotic used in drug release systems from HNTs is one of the synthetic fluoroquinolones and is from the antibiotic family with a wide gram-negative spectrum [4]. It is used in systemic disorders such as respiratory system, typhoid, urinary tract infection, bone, joint, respiratory tract and gastrointestinal infections [5]. CIP can be dissolved in dilute acid solutions, and its pKa values are between 5.61 and 6.18. It has variable secondary amine groups. It is a high molecular weight drug that is insoluble in water [6].

In this study, encapsulation efficiency and loading capacity of CIP in HNTs nanotubes were investigated. Then, release profiles in different buffer solutions mimicking the *in vivo* system were obtained.

MATERIAL AND METHODS

100 mg of CIP was dissolved in 100 mL of 2% (v/v) acetic acid solution. 4 g of HNTs were added to the solution and this solution was mixed in sonicator (100 W) 30 mins. This solution was filtered through a vacuum filter. CIP encapsulated into the HNTs was allowed to dry for two days at room temperature. CIP encapsulated HNTs of 0.5 g were added to 100 mL of buffer solutions in the orbital shaker operated at 37ºC. CIP release from HNTs was investigated in HCl solution at pH 2.1, phosphate buffer solution (PBS) at pH 5.0 and at pH 7.4 simulating gastrointestinal system and blood through 24 hours. CIP concentration in the samples taken at certain time intervals from buffer media was measured in UV spectrofotometer.

RESULTS

The amount of the free drug in a 5 ml drug solvent, which was not encapsulated in HNTs, was determined by using the calibration lines of CIP in different buffers. The encapsulation efficiency of CIP in HNTs was calculated using Equation (1) (Table 1).

$$\text{Encapsulation efficiency } (EE)\% = \frac{Initial\ amount\ of\ drug - free\ drug}{Initial\ amount\ of drug} \times 100 \qquad [1]$$

Table 1. CIP encapsulation efficiencies of HNTs.

Encapsulation efficiency (%)	88.3

Drug loading capacity per total unit weight of particle was calculated using the following Equation (2).

$$\text{Drug Loading Capacity \%} = \frac{Initial\ amount\ of drug - Free drug}{Total\ amount\ of\ drug + total\ amount\ of HNTs} \times 100 \quad [2]$$

Drug loading capacity per total unit weight of particle is presented in Table 2.

Table 2. CIP loading capacity of HNTs.

CIP loading capacity (%)	17.3

CIP release profiles from HNTs were examined by changing the types of buffer. The reason for examining CIP release profiles in buffer solutions at different pHs is to create media similar to in-vivo media. HCl solution representing gastric fluid at pH 2.1, phosphate buffer solution (PBS) representing large intestine media at pH 5.0, and PBS solution representing the small intestine and blood at pH 7.4 were used to obtain CIP release profiles.

The concentration of CIP was changed between 5-30 mg/L, and CIP calibration graphs were obtained in different buffer solutions (Figure 1). The UV wavelength at which CIP showed the maximum absorbance for the release profiles in HCL buffer was found to be 268 nm. Other wavelengths at which CIP showed the maximum absorbance were found to be 283 nm in PBS buffer at pH 5 and 289 nm in PBS buffer at 7.4

0.5 g of CIP-loaded HNTs were added to the media containing 100 ml of buffer solution, and CIP release profiles were monitored for 24 hours in shaking incubators set to 37°C. The samples taken in tubes from buffer solutions containing CIP-loaded HNTs at specific time intervals (5, 15, 30, 60, 120, 180, 1440 min) were centrifuged. The amounts of CIP released in the liquid medium were analyzed at the optimum wavelength determined for the buffer used via Spectrophotometer Genesys 10S UV-Vıs spectrophotometer. The releases of CIP from HNTs in different buffers were calculated using Equation (3).

$$Cumulative\ Release\ \% = \frac{Cumulative\ amount\ of\ released\ drug\ at\ t\ moment}{Initial\ amount\ of\ drug} \times 100 \quad [3]$$

The release profiles obtained are presented in Figure 2.

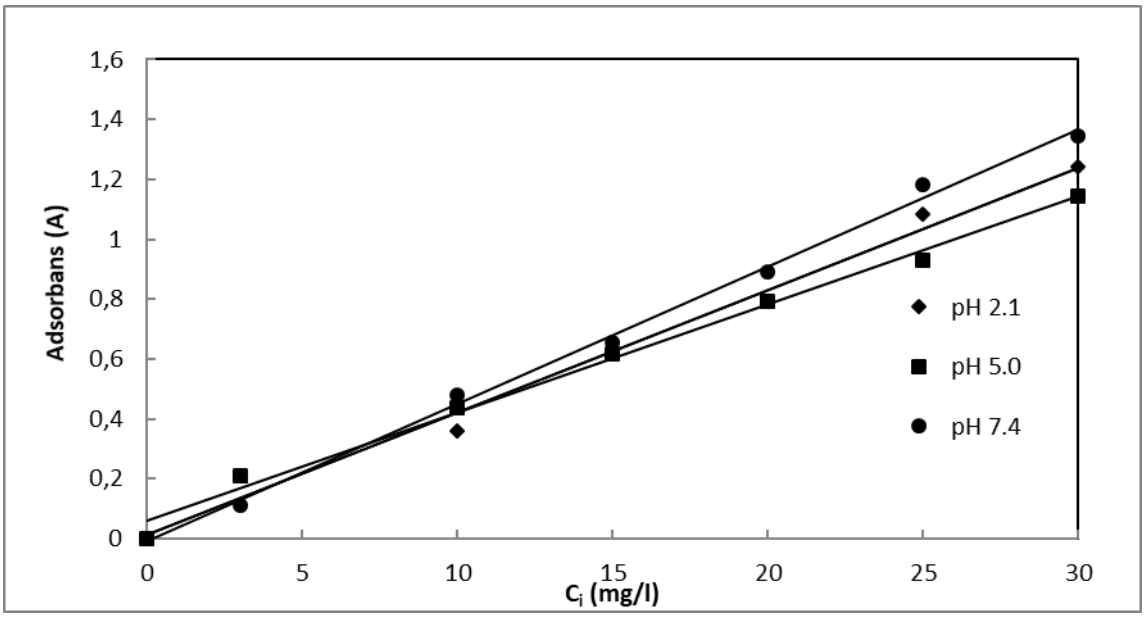

Figure 1. CIP calibration graphs in pH 2.1 HCl buffer, pH 5.0 and pH 7.4 PBS buffers.

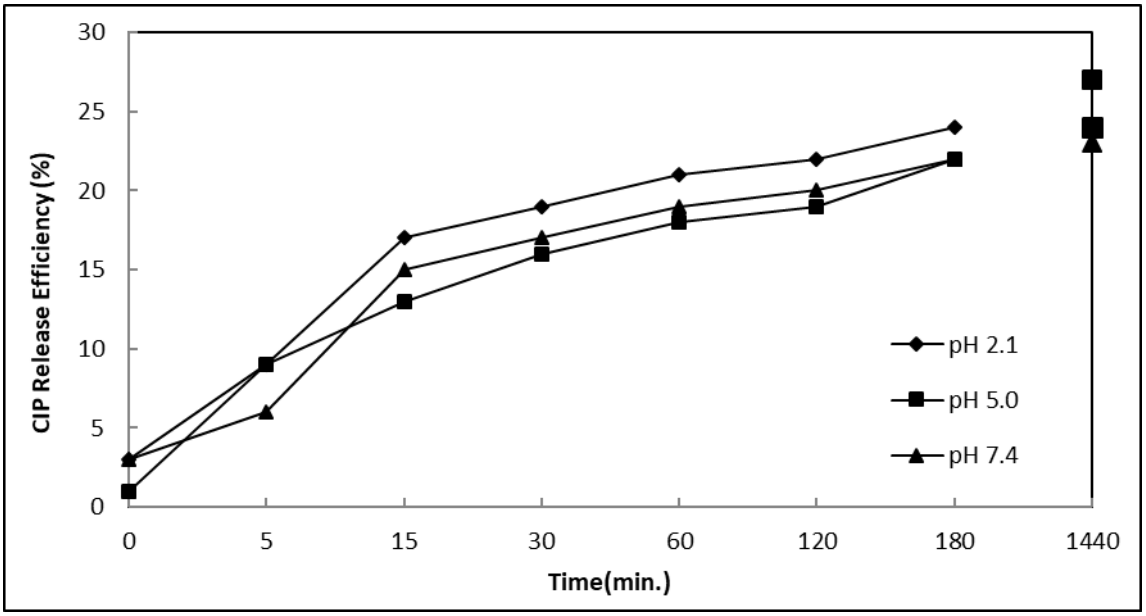

Figure 2. Time-dependent *in vitro* release profiles of CIP from HNTs in pH 2.1' HCl, pH 5.0 and 7.4 PBS buffers.

According to the drug release profiles from HNTs, CIP is released in a controlled manner within approximately 3 hours. CIP release after 24 hours from HNTs in HCl buffer solution at pH 2.1 simulating the gastric fluid was found to be 27%. CIP release from HNTs in PBS buffer solution with pH 5.0 representing the descending and ascending colons of the large intestine was found to be 23%. CIP release from HNTs in PBS buffer solution with pH 7.4 simulating the blood and small intestine (distal small intestine) was found to be 24%.

DISCUSSION AND CONCLUSION

In this study it was shown that a high encapsulation efficiency and loading capacity of CIP was obtained into HNTs. Encapsulation of CIP into HNTs resulted in prolonged, sustained and controlled CIP release. It appears that HNTs maintained long-term and controlled CIP release even in severe acidic pH of the stomach due to their powerful physical properties and clay structure.

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FULL TEXT-PAPER

SUSTAINED AND CONTROLLED ANTIBIOTIC RELEASE FROM CHITOSAN AND MAGNETIC CHITOSAN NANOPARTICLES

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Abstract

Chitosan (CTN) and magnetic chitosan (CTN-Fe_3O_4) nanoparticles (NPs) containing iron oxide were prepared as drug carrier by ionic gellation and co-precipitation techniques. Metronidazole (MTZ) and tetracycline (TC) were chosen as model drugs. The magnetic property of CTN-Fe_3O_4 was evaluated by VSM measurements. MTZ and TC were loaded on CTN NPs and CTN-Fe_3O_4 NPs, respectively, and then controlled drug release was investigated. The encapsulation efficiency and loading capacity of MTZ on CTN NPs in HCl buffer at pH 2.0 were found to be 56.0% and 17.7%, respectively. The encapsulation efficiency and loading capacity of MTZ on CTN NPs in PBS buffer at pH 7.4 were determined as 41.3% and 19.7%, respectively. The MTZ release from CTN NPs in 0.1 M HCl at pH 2.0 and at 37ºC simulating to gastric fluid was observed up to 20 hours and 44.0 % of the loaded MTZ was released in the medium. The MTZ release percentage in PBS at pH 7.4 was found to be 58.7%. The encapsulation efficiency and loading capacity of TC on CTN-Fe_3O_4 NPs in PBS buffer at pH 7.4 were found to be 80.0% and 7.3%, respectively. At the end of 24 hours,56.8 % of the loaded TC from CTN-Fe_3O_4 NPs released into the PBS. High encapsulation efficiencies and loading capacities for MTZ and TC by CTN NPs and CTN-Fe_3O_4 NPs were obtained. A prolonged, sustained and controlled antibiotic release during 24 hours was observed using CTN NPs and CTN-Fe_3O_4 NPs.

Keywords: Controlled release, antibiotic, chitosan nanoparticles, chitosan-Fe_3O_4 nanoparticles

INTRODUCTION

The aim of controlled drug delivery systems is to ensure that the drug is properly delivered to the site of action and administered at the desired dosage, to increase its effect on the target site and to minimize unwanted side effects. Therefore, carrier materials are developed for the delivery of the required amount of the drug to the target site in a given time. Recent developments in nanotechnology have shown that nanoparticles have great potential due to their unique physicochemical and biological properties as drug carriers.

Therefore, many drug delivery systems have been developed so far. Drug delivery systems are desired to be biocompatible and biodegradable, on the other hand, not to have a toxic effect on living organisms. Biopolymeric materials are preferred to provide these properties [1]. Important properties of CTN, such as renewability, non-toxicity, biodegradability, and biocompatibility have made it a quite remarkable biopolymer. CTN is obtained by alkaline deacetylation of chitin, which is a long-

chain biopolymer of N-acetyl-D-glucosamine. CTN is a linear polysaccharide that is composed of randomly distributed β– (1-4) linked D-glucosamine and N-acetyl-D-glucosamine. The use of magnetic nanoparticles in drug targeting systems has a significant potential for the treatment of infectious diseases and cancer [2]. The most important property of magnetic nanoparticles is that they can be targeted directly to the organ intended to be treated by applying an external magnetic field. Furthermore, a small size of magnetic nanoparticles and the fact that they can gain drug delivery capacity through surface modification are other important properties required for controlled and targeted drug release [3].

In this study, MTZ and TC antibiotics with broad-spectrum antimicrobial activity against bacteria causing various diseases were chosen as model drugs [4]. The loading and release of MTZ antibiotic to CTN NPs and the loading and release of TC antibiotic to CHT-Fe_3O_4 NPs were examined.

MATERIAL AND METHODS

The ionic gelation method was used for the synthesis of CTN NPs. The anionic cross linker, tripolyphosphate (TPP), is used in physically cross-linking and is added dropwise to CTN solution along with stirring and sonication. CTN coated Fe_3O_4 NPs were prepared using co-precipitation method. A certain amount of CTN was dissolved in acetic acid solution and pH of this solution was adjusted to 4.8 using NaOH. Ferrous ($FeCl_2.4H_2O$) and ferric ($FeCl_3.6H_2O$) reactive salts were added to the medium in N_2 atmosphere. Then ammonium hydroxide and TPP were added to the medium dropwise. After mixing, CTN coated Fe_3O_4 NPs were separated from the liquid phase using a neodymium magnet. After the MTZ-loaded CTN NPs were freeze-dried, the MTZ release of the CTN NPs was performed in 0.1 M HCl at pH 2.0 and in 0.1 M PBS at pH 7.4, similar to *in vivo* medium. TC release studies were also performed at 37 ˚C by adding 0.5 g/L CTN-Fe_3O_4 NPs in 100 mL PBS.

RESULTS

The encapsulation efficiency of MTZ in CTN NPs was calculated using Equation 1. MTZ encapsulation efficiency values of CTN NPs contained in different buffers are presented in Table 1.

$$\text{Encapsulation efficiency } (EE)\% = \frac{Initial\ amount\ of\ drug - free\ drug}{Initial\ amount\ of drug} \times 100 \quad [1]$$

Table 1. MTZ encapsulation efficiencies of CTN NPs in different buffer solutions.

Encapsulation efficiency (%)	PBS buffer	HCl buffer
	41.33	55.96

Furthermore, drug loading capacity per total amount of particle was calculated using Equation 2. The values obtained in different buffers for drug loading capacity are presented in Table 2.

$$\text{Drug Loading Capacity \%} = \frac{Initial\ amount\ of drug - Free\ drug}{Amount\ of\ drug + amount\ of\ NPs} \times 100 \quad [2]$$

Table 2. MTZ loading capacities of CTN NPs in different buffer solutions.

Drug Loading Capacity (%)	PBS buffer	HCl buffer
	19.71	17.67

MTZ was effectively loaded in CTN NPs, and loading efficiency was evaluated by changing the loading pH with different buffers. Accordingly, the maximum loading capacity was obtained at pH 7.4 in potassium phosphate buffer.

The process of loading TC in CTN coated iron oxide nanoparticles was performed by adding 0.5 g/L nanoparticle into a 100 mL drug solution containing 50 mg/L TC at pH 5.0. The solution was mixed by an orbital mixer for 30 hours. TC measurements were performed in the samples taken from the media at certain time intervals, and the time-dependent change of the TC amount adsorbed per unit CTN-Fe_3O_4 NPs weight was examined (Figure 1). The encapsulation efficiency was calculated based on Equation 1, and the drug loading capacity was calculated based on Equation 2 (Table 3).

Table 3. TC encapsulation efficiency and loading capacity of CTN-Fe_3O_4 NPs in PBS buffer.

Encapsulation efficiency (%)	40.32
Drug Loading Capacity (%)	80.04

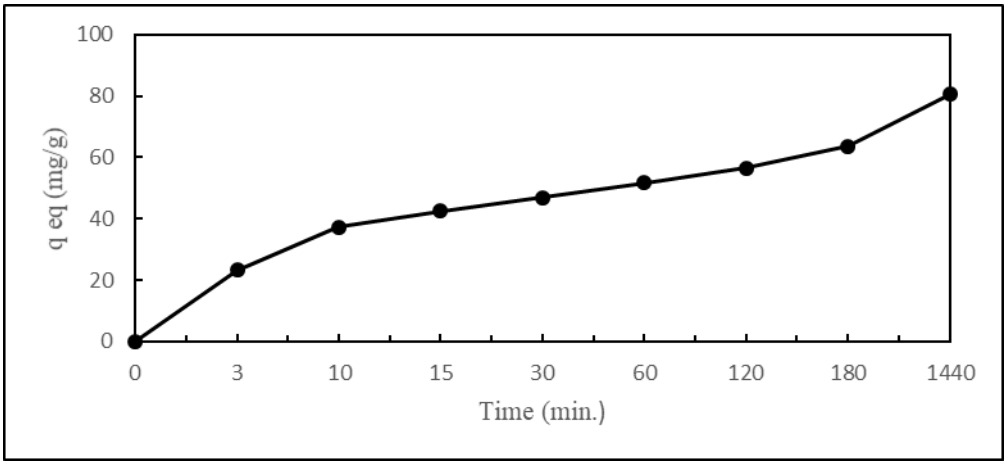

Figure 1. TC loading profile of CTN-Fe_3O_4 NPs.

MTZ release profiles from CTN NPs were obtained in different buffer solutions. 0.1 M Phosphate-Buffer Solution (PBS) at pH 7.4 representing the intestine and blood, and hydrochloric acid (HCl)

solutions at pH 2.0 representing the gastric fluid were used to simulate *in vivo* media. Release experiments were performed at 37ºC, the body temperature.

After adding 10 mg of MTZ-loaded CTN NPs to the flasks containing 100 ml of PBS buffer, the flasks were placed in a shaking incubator operated at a constant temperature of 37 °C. 2 ml of samples taken in Eppendorf tubes from the medium at certain time intervals (1,2…….,20 hours) were centrifuged at 5000 rpm for 5 minutes. Then, MTZ released in the liquid part was analyzed at a wavelength of 320 nm in the Genesys 10S UV-Vıs spectrophotometer. Similarly, CTN NPs loaded with 10 mg MTZ were added to 100 ml HCl acid solution, and the above experimental steps were repeated. The samples in the HCl buffer were analyzed at 278 nm at which the maximum absorbance of MTZ was observed.

The release of MTZ from CTN NPs in different buffers was found by using Equation 3. The release profiles obtained are presented in Figure 2.

$$Cumulative\ Release\ \% = \frac{Cumulative\ amount\ of\ released\ drug\ at\ t\ moment}{Initial\ amount\ of\ drug} \times 100 \qquad [3]$$

After freezing and drying of TC loaded magnetic CNT-Fe_3O_4 NPs, TC release was performed in 0.1 M PBS with a pH of 7.4 to be similar to the *in-vivo* medium. Release studies were performed in a shaking incubator operated at a constant temperature of 37 °C after adding 0.5 g/L TC loaded CNT-Fe_3O_4 NPs to flasks containing 100 ml PBS buffer. The samples taken from the medium at certain time intervals (1,2…….,20 hours) were centrifuged at 5000 rpm for 5 minutes to analyze the controlled release of TC from CNT-Fe_3O_4 NPs. TC concentration in the supernatant was analyzed at a wavelength of 360 nm in the Spectrophotometer Genesys 10S UV-Vıs spectrophotometer.

In the media containing phosphate buffer, up to 20 hours of drug release from CHT-Fe_3O_4 NPs was observed. The controlled release profile obtained was presented in Figure 3.

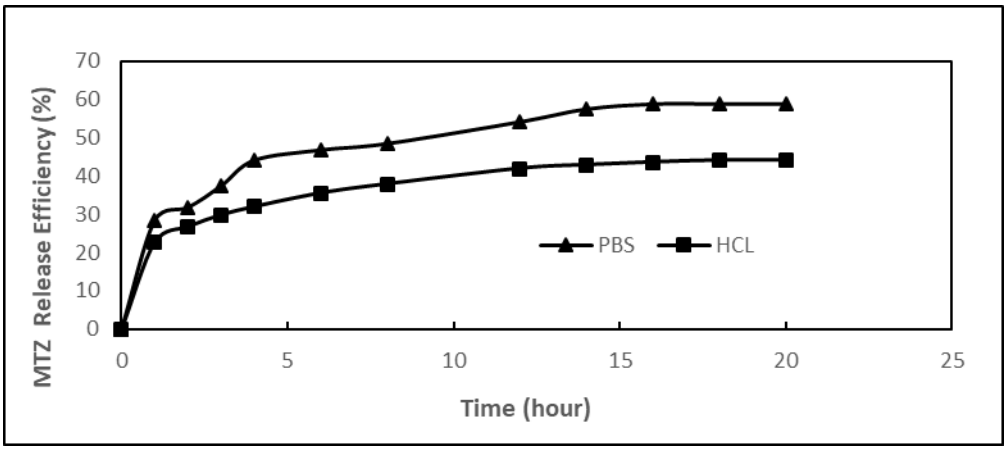

Figure 2. *In vitro* release profiles of MTZ from CTN NPs in different buffer solutions.

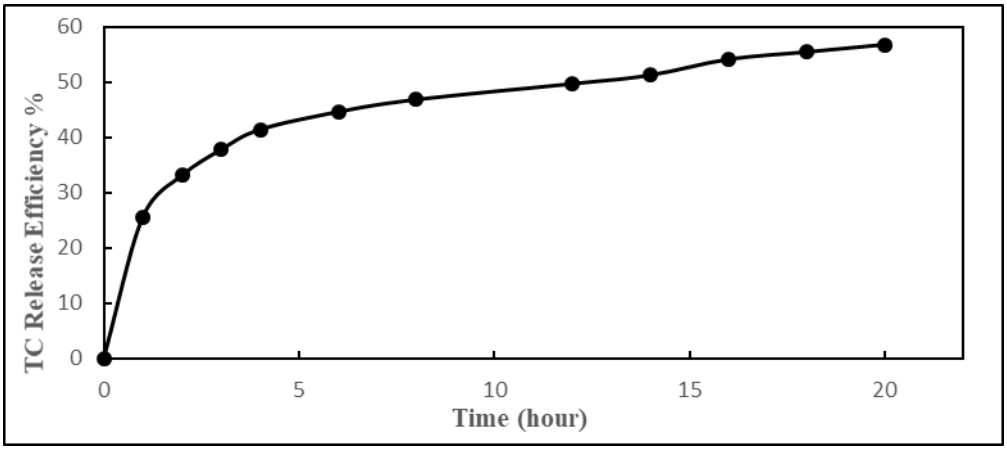

Figure 3. *In vitro* release profiles of TC from CNT-Fe_3O_4 NPs in PBS buffer solution.

DISCUSSIONS AND CONCLUSION

The *in vitro* drug release study of CTN NPs was followed by the first rapid release of MTZ at the beginning and then its continuous release. This first rapid release, which is defined as the "explosion effect," occurs with desorption of MTZ localized on the surface of nanoparticles. The first rapid release may have resulted from the rapid dissolution of drug crystals or from the rapid hydration of nanoparticles due to the hydrophilic nature of the CTN. The slower and controlled release occurred after the first rapid release. The release of the drug is mainly affected by ionic interactions between CTN chains, which depends on the intensity of cross-linking set during the formation of the CTN network.

The first rapid release of TC from CNT-Fe_3O_4 NPs may have occurred as a result of the rapid dissolution of drug crystals, similar to MTZ. Then, the slow and controlled TC release continued for 20 hours. The controlled release occurred with the slow desorption of TC localized on the inner surface of CNT-Fe_3O_4 NPs.

Drug release profiles from both CTN NPs and CNT-Fe_3O_4 NPs reveal that it can effectively sustain the release of MTZ and TC. The continuous and controlled drug release from nanoparticles is important because it allows the drug to remain on the surface of the release site for a long time, to increase the bioavailability of the drug and to extend the therapeutic effect.

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FULL TEXT-PAPER

PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND NEUROPROTECTIVE PROPERTIES OF TURKISH OREGANO (*ORIGANUM ONITES* L.)

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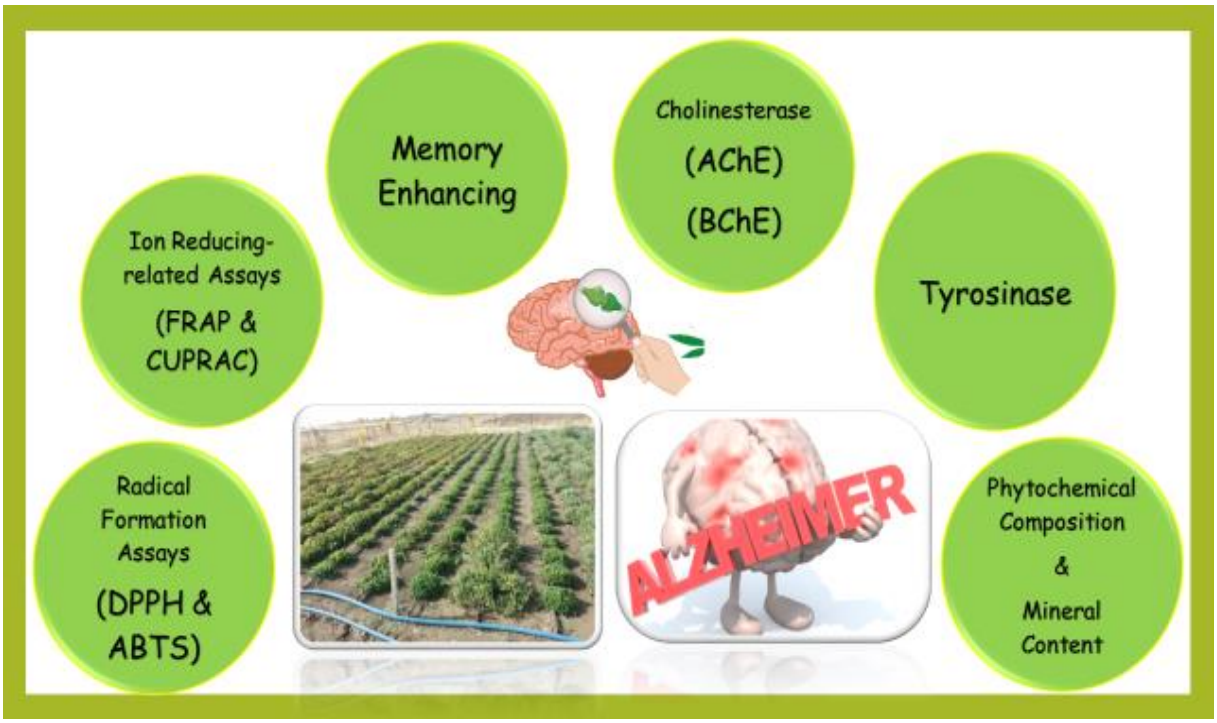
Background and Objective: *Origanum onites* L. (fam. Lamiaceae), commonly known as 'kekik' in Anatolia, is an important medicinal plant that mainly used to manage for several ailments such as toothache, headache, high cholesterol, hypertension, diabetes, leukemia, stomach disorders, and bronchitis in Turkish traditional medicine. Although multiple biological activities of *O. onites* L. have been documented previously, this is the first research that has been performed with the plant growing under special conditions. **Material and Methods:** Therefore, the present study aimed to investigate the biological potential of aqueous and methanol extracts of the aerial parts from *O. onites* L. in terms of their enzyme inhibitory effects, antioxidant capacities, mineral contents and total polyphenolic compositions. Enzyme inhibitory assays were performed against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase enzymes. The total phenolic (TPC) and total flavonoid (TFC) contents were analyzed by the Folin-Ciocalteu method and aluminum chloride colorimetric assays, respectively, and K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, Pb mineral contents of the plant were also determined by Atomic Absorption Spectrometry. As for antioxidant activity, the extracts were tested by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) assays. **Results:** Overall, a strong correlation was observed between the phytochemical and mineral constituents of the plant and its biological properties. The aqueous extract possessed of higher TPC and lower TFC (102.18±0.32 mg g-1 extract as GAE and 36.17±0.14 mg g-1 extract as QE, respectively) compared to the methanol extract (85.90±0.16 mg g-1 extract as GAE and 45.53±0.22 mg g-1 extract as QE, respectively). The aqueous extract, which showed a higher TPC than that of the methanol extract, was found the most effective antioxidant in all assays. As for the neuroprotective potentials, both of the extracts showed the remarkable inhibition on cholinesterase enzymes with the values that ranged from 49.38±0.83% to 76.84±0.25%, whereas they displayed lower inhibition on tyrosinase enzyme. **Conclusion:** Taken together, *O. onites* L. with remarkable neuroprotective property, excellent antioxidant activities besides rich polyphenolic and mineral contents can be helpful to manage of oxidative stress-related diseases.

Keywords: *Origanum onites* L.; neuroprotection; enzyme inhibitory; antioxidant; polyphenolic; mineral content

Graphical Abstract



1. INTRODUCTION

Origanum onites L. (fam. Lamiaceae), commonly known as 'kekik' in Anatolia, is mainly native to Asia-Tropical, Asia-Temperate, Africa and Europe. In Turkey, a leading country in oregano trade in the World, the genus *Origanum* is represented by 22 species and 32 taxa, 21 being endemic and the ratio of endemism in the genus is 65.2%. Among the genus, *Origanum onites* L. is known as the dominant species (Marrelli et al., 2018; Mahomoodally et al., 2018). Oregano plays a primary role in the global trade of culinary herbs. Apart from their worldwide culinary use as food flavorings for centuries. *Origanum* species are utilized in food industry, alcoholic beverages, culinary, perfumery, and folk medicine. It has also been used in the treatment of several ailments in Turkish folk medicine such as toothache, headache, high cholesterol, hypertension, diabetes, leukemia, stomach disorders, and bronchitis (Semiz et al., 2018).

The formation of Reactive Oxygen Species (ROS), such as superoxide ion (O_2), hydroxyl radical (OH) and Hydrogen peroxide (H_2O_2), have often been reported to induce DNA damage, protein carboxylation, and lipid peroxidation, causing a variety of chronic health disturbances and diseases, including cancer, ageing, Parkinson's disease, Alzheimer's disease, and cardiovascular diseases. Recent research indicates that several herbal plants can offer alternative sources of dietary ingredients to promote human health and might open promising opportunities for the treatment of a wide range of troublesome diseases and infections (Sun et al., 2016; Sahoo et al., 2018) . Although, there are so many methods for combatting neurodegenerative diseases and disorders, they cannot always provide effective treatments and mediations. Hence, an extensive research on developing new treatment strategies against these disorders are still needed to cure nowadays. Since ancient times, natural products (NPs), originated from natural sources such as plants, have been used for the cure and

treatment of many diseases in Anatolian folk medicine 'herbal therapies' (Gezici and Sekeroglu, 2019a; Awasthi et al., 2016; Godyń, et al, 2016).

So far, we have investigated various of medicinal plants using in vitro antioxidant, anticancer, antiproliferative, anticholinesterase, etc. experiments, which aimed to contribute to the finding of new herbal products for prevention and treatment of cancer and neurodegenerative diseases (Gezici, 2019; Gezici and Sekeroglu, 2019a; Gezici and Sekeroglu, 2019b; Sekeroglu et al., 2018a; Sekeroglu et al., 2018b; Gezici, 2018; Senol et al., 2018; Gundogdu et al., 2018; Karik et al., 2018; Belkhodja et al., 2017; Gezici et al., 2017; Sekeroglu et al., 2017; Akgunlu et al., 2016; Orhan et al., 2013; Sekeroglu et al., 2012; Orhan et al., 2012, etc.). Taking our previous researches on medicinal plants and plant-derived natural products, the current study was undertaken to evaluate total polyphenolic and mineral contents, in vitro antioxidant effects, neuroprotective and enzyme inhibitory activities of the extracts from aerial parts of *Origanum onites* L. Due to it has medicinal and economic importance, multiple biological activities of *O. onite*s L. have been documented previously. So far, no detailed studies on neuroprotective properties have been performed with extracts Turkish oregano growing under special conditions. Thus, this assessment may help us to find new potential sources as natural neuroprotective agents.

2. MATERIAL AND METHODS

2.1. Collection of Plant Material

The plant was growing by Prof. Dr. Murat Tuncturk at Medicinal and Aromatic Plants Garden, Van Yuzuncu Yil University, and the voucher specimen was deposited in the herbarium Van Yuzuncu Yil University, Van-Turkey. Taxonomic classification of the plant was given in the Table 1.

Table 1. Taxonomic classification of the plant and plant sample in the Medicinal and Aromatic Plants Garden

Kingdom:	*Plantae*
Subkingdom:	*Tracheobionta*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Subclass:	*Asteridae*
Order:	*Lamiales*
Family:	*Lamiaceae*
Genus:	*Origanum*
Species:	*onites L.*

2.2. Crude Extract Preparation

To prepare crude extracts, air dried samples (50 g) of the aerial parts of the plant were individually extracted with 250 ml methanol (70%) and distilled water for 2 days at the room temperature, as described in our previous research (Gezici and Sekeroglu, 2019b). The extracts yields (w/w%) are given in the Figure 1. Extraction yields of the methanol and water extracts of the aerial parts of the plant were determined as 15.824% and 9.822% (w/w), respectively.

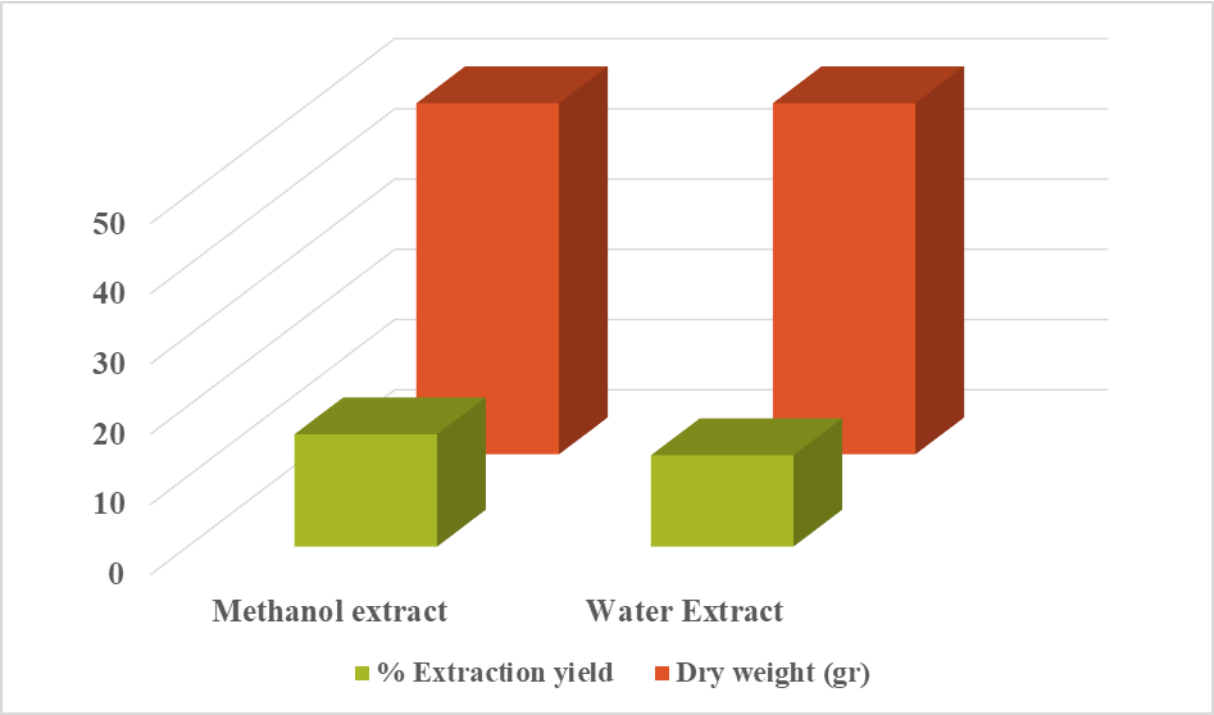


Fig. 1. Extraction yield (w/w) of the plant

2.3. Determination of Total Polyphenolic Contents

Phenolic compounds in total were determined in accordance with slightly modified Folin-Ciocalteau's method (Singleton and Rossi, 1965; Gezici and Sekeroglu, 2019). Absorption was measured at 760 nm at a using a 96-well microplate reader (VersaMax Molecular Devices, USA). Total flavonoid content of the extracts was calculated by aluminum chloride colorimetric method (Woisky and Salatino, 1998; Gezici and Sekeroglu, 2019b). A number of dilutions of quercetin were obtained to prepare a calibration curve. Absorbance of the reaction mixtures was measured at wavelength of 415 nm with a using a 96-well microplate reader (VersaMax Molecular Devices, USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g-1 extract), respectively.

2.4. Determination of Mineral Contents

The presence of mineral elements such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), cadmium (Cd), and lead (Pb) in the aerial parts of the plant were determined by Atomic Absorption Spectrometry (AAS, Perkin-Elmer 2280) (Sekeroglu et al., 2017).

2.5. Antioxidant Activity Assays

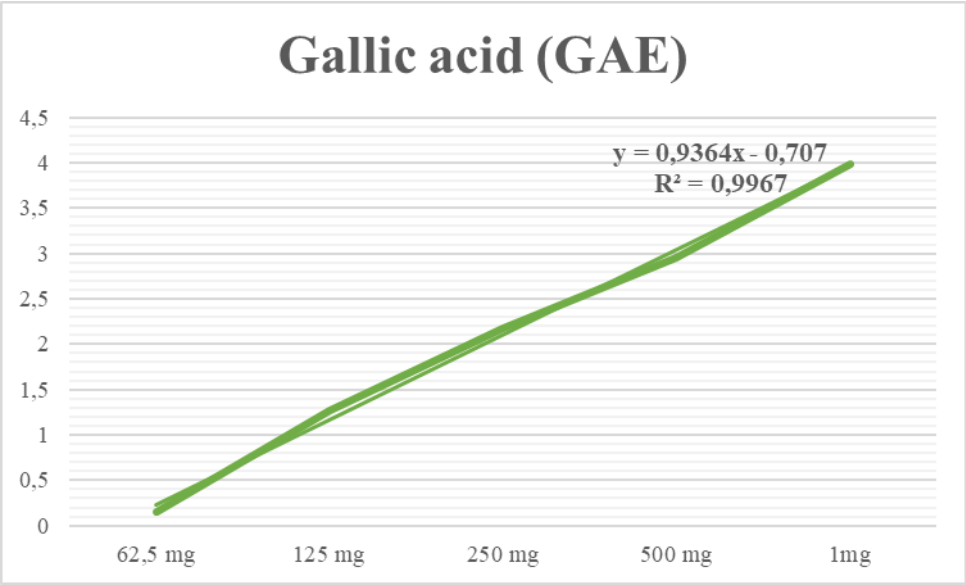
Since oxidative damage is one of the major factors contributing to both cancer and neurodegeneration, *In vitro* methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) were performed to reveal antioxidant activity of the extracts (Gezici et al., 2017; Sekeroglu et al., 2017; Gundogdu et al., 2018; Gezici and Sekeroglu, 2019b).

2.6. Enzyme Inhibition Assays

Neuroprotective activities of the extracts on AChE, BChE, and tyrosinase were evaluated in the current study. AChE and BChE inhibitory activity of the samples was measured by slightly modified spectrophotometric method of Ellman et al. (1961). Electric eel AChE (EC 3.1.1.Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1. Sigma, St. Louis, 7 MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5´-Dithio- bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents, conditions and calculations were same as described in our previous publication (Senol et al., 2018; Gezici and Sekeroglu, 2019b). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. The experiments were done in quarduplicate. Galanthamine hydrobromide (Sigma, St. Louis, MO, USA) was used as the reference drug. Inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) was determined using the modified dopachrome method with L-DOPA as substrate (Sekeroglu et al., 2012).

3. RESULTS AND DISCUSSION

Phenolic compounds such as phenolic acids and flavonoids are reported to be involved in various biochemical activities like antioxidant, antimicrobial, antithrombotic, antiartherogenic, antiinflammatory, anticarcinogenic and antimutagenic. Total polyphenolic compositions of the extracts were identified spectrophotometrically in the present study. Regarding of total phenol and flavonoid quantities, the aqueous extract possessed of higher TPC and lower TFC (102.18±0.32 mg g-1 extract as GAE and 36.17±0.14 mg g-1 extract as QE, respectively) compared to the methanol extract (85.90±0.16 mg g-1 extract as GAE and 45.53±0.22 mg g-1 extract as QE, respectively). Gallic acid and quercetin equivalent as commercial standards for total phenolic phenolic and flavonoid contents were shown in the Figure 2.



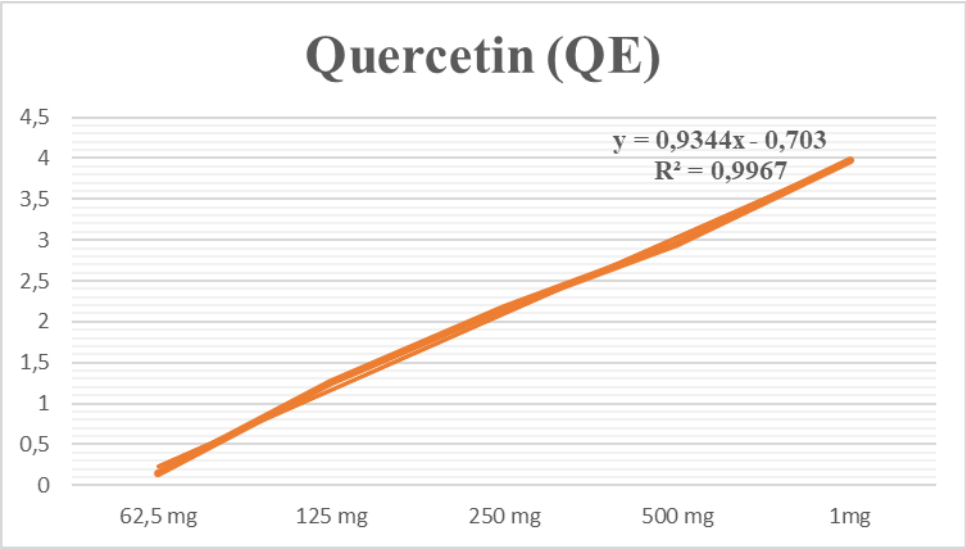


Fig. 2. Gallic acid and Quercetin Equivalent

K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, Pb mineral contents of the plant were also determined in the presented research. Among them, Ca and K were found as major minerals, whereas Cd and Pb were found the minor elements. The mineral values were represented as mean±Sd in the Table 2.

Table 2. Mineral contents (mg/kg) of *O. onites* L.

K (g/kg)	**Ca (g/kg)**	**Mg (g/kg)**	**Fe (mg/kg)**	**Zn (mg/kg)**
13,50±0,71	14,47±1,59	4,71±0,20	2830,31±127,54	44,74±2,16
Cu (mg/kg)	**Mn (mg/kg)**	**Cd (mg/kg)**	**Pb (mg/kg)**	
10,46±1,13	122,64±4,62	0,13±0,02	0,58±0,61	

Antioxidant activity of the extracts obtained from the aerial parts of the plant were evaluated by using radical scavenging against DPPH and ABTS radicals, and ion reducing antioxidant power on FRAP and CUPRAC. According to antioxidant assays, all the extracts obtained from aerial parts of *O. onites* L. exhibited remarkable scavenging activities on DPPH, FRAP, ABTS, and CUPRAC, comparing the standard antioxidants.

The aqueous extract, which showed a higher TPC than that of the methanol extract, was found the most effective antioxidant in all assays. Scavenging activity of the aqueous extract on DPPH were determined as 85.69±1.04 mg TEs/g extract, when it was found as 78.91±0.71 mg TEs/g for the methanol extract. Antioxidant capacity against ABTS was determined as higher in the water extract (93.06% inhibition) than that of the methanol (82.16 % inhibition). The results were summarized in the Figure 3.

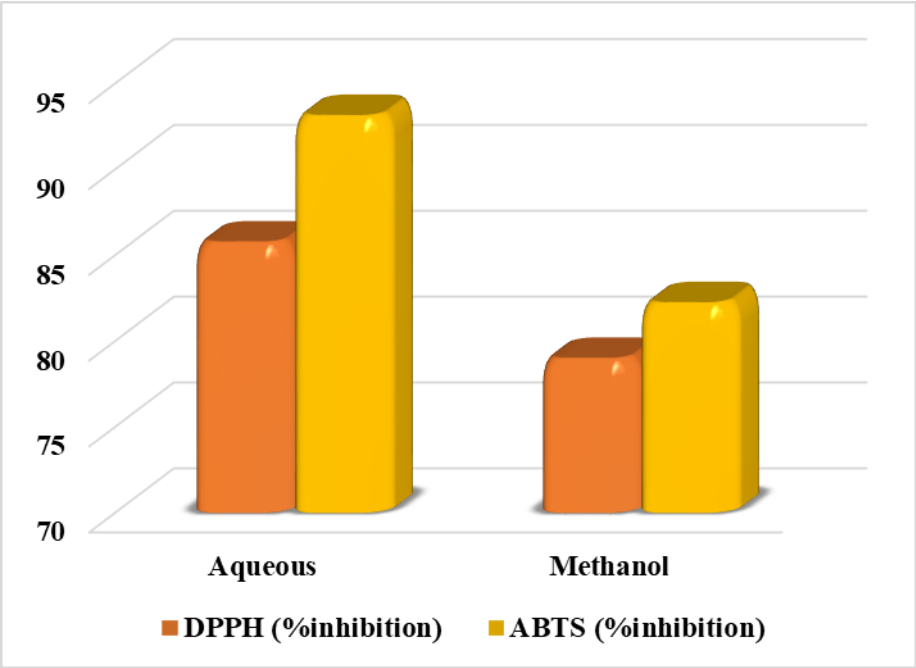


Fig. 2. Antioxidant Activity Resuls of the Extracts

Neuroprotective activity of the extracts was assessed through enzyme inhibition assays on cholinesterase enzymes. The aerial parts the plant were extracted with water and methanol solvents, and subjected to enzyme inhibitory assays on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase which are closely related to pathogenesis of neurodegenerative disease. As for the results, both of the extracts exhibited the higher cholinesterase inhibitory activity on AChE and BChE with the values that ranged from 49.38±0.83% to 76.84±0.25%, whereas they displayed lower inhibition on tyrosinase enzyme. In general, a significant correlation was found between the total antioxidant capacities and neuroprotective potentials of the tested extracts.

4. CONCLUSION

Overall, a strong correlation was observed between the phytochemical and mineral constituents of the plant and its biological properties. In the light of the findings of results, it is exceedingly important to indicate that, *O. onites* L. with remarkable neuroprotective property, excellent antioxidant activities besides rich polyphenolic and mineral contents can be helpful to manage of oxidative stress-related diseases. Our data indicated that the extracts from Turkish oregano appear to be a natural source having promising inhibitory molecules, are worth to conduct further *in vivo* investigations that is under further investigation by our group.

ACKNOWLEDGMENTS

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FULL TEXT-PAPER

CARBOXYMETHYL CHITOSAN: A POTENTIAL ACTIVE MOISTURIZING AGENT FOR COSMETIC APPLICATION

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Abstract

This study aimed to synthesize a water-soluble chitosan from shrimp chitosan in the form of carboxymethyl)CM(-chitosan for being an active moisturizing agent and to develop skin moisturizing creams containing CM-chitosan. Moisture sorption isotherm as well as physical characteristics of the CM-chitosan were then determined. Also, a morphology of this compound, was visualized through a scanning electron microscope. *In vitro* pig skin shrinkage test and a measurement of pig skin hydration using Corneometer® were then carried out for evaluating the moisturizing effect of this compound compared with hyaluronic acid. After that, moisturizing creams containing CM-chitosan were developed and evaluated for their stabilities after 60 days of storage. Performance test and skin irritation test in 22 healthy volunteers were also performed. By virtue of carboxymethyl group, an increase in hydrophilicity of chitosan was observed. In addition, an alkaline solution, used in the synthesis process, could reduce rigidity and crystallinity of the molecule. Swelling index and melting point of CM-chitosan were 500 mL/g and 174.8 ^{o}C, respectively. Interestingly, the superior moisturizing effect of 0.4% CM-chitosan above those of 0.2% hyaluronic acid was shown. The moisturizing cream containing CM-chitosan exhibited an excellent physical stability during storage. Moreover, moisturizing efficacy in the volunteers of the cream containing CM-chitosan, was comparable to hyaluronic acid. This might be due to the fact that CM-chitosan is capable of forming film onto the skin to prevent water loss along with attracting water from the environment. From our study, CM-chitosan could be an alternative moisturizing agent derived from nature.

Keywords: Chitosan, Carboxymethyl chitosan, Skin moisturizer, Skin hydration measurement, Pig skin shrinkage test

INTRODUCTION

Chitosan has been regarded as an alternative natural polymer used for a variety of pharmaceutical and cosmetic applications so far (Kumar, 2000). This oligopolysaccharide is commonly derived from deacetylation and hydrolysis of chitin, which is mainly found in the shells of arthropods and even insects. Several researches denoted that chitosan exerted a number of biological activities such as antimicrobial, antioxidation, anti-obesity, anti-inflammation and anti-hypertension (Muanprasat *et al*., 2017). Vitalities of chitosan in cosmetic skin cares were widely established due to its abilities of UV-protection, emulsifier, stabilizer, along with viscosifier (Aranaz *et al*., 2018). Unfortunately, the major drawbacks of using natural chitosan are its low solubility in water, which is able to be improved only in an acidic solution, as well as incompatibility with anionic molecules. As a result, a modified chitosan in the form of Carboxymethyl (CM)-chitosan, has been invented since then (Chen *et al.*, 2003; Rachtanapun *et al.*, 2012). The use of CM-chitosan in pharmaceutical formulation as a wound healing agent and a drug-targeting agent, has been gradually expanded. However, a skin moisturizing effect of CM-chitosan has been rarely investigated. Thus, our study aimed to synthesize CM-chitosan from shrimp chitosan in order to be an alternative moisturizing agent as well as to develop a skin moisturizing cream containing CM-chitosan.

MATERIALS AND METHODS

1. Synthesis of CM-chitosan

CM-chitosan was synthesized according to the modified methods of Chen *et al.*, (2003) and Rachtanapun *et al.*, (2012). Initially, shrimp chitosan (25 g) was dispersed into an alkaline solution consisting of sodium hydroxide: isopropanol: DI water (1:8:2) and continuously stirred at 25 ^{o}C for 1 h. Monochloroacetic acid was then sufficiently added into the mixture and stirred for 30 min. The mixture was then dried using hot-air oven at a temperature of 50 ^{o}C for 4 h. Solid material was subsequently dispersed in methanol. Glacial acetic acid served as a pH-adjuster to neutralize the pH value of the mixture. The material was then rinsed 5 times by 70% ethanol to remove some contaminants. Lastly, the material was rinsed by absolute ethanol and dried at 50 ^{o}C for 18 h. Percent yield was calculated using the following equation (1);

$$\text{Yield (\%w/w)} = \frac{\text{Weight of CM-chitosan (g)}}{\text{Weight of Chitosan (g)}} \times 100 \qquad (1)$$

2. Determination of moisture sorption isotherm of the CM-chitosan

The obtained CM-chitosan (2 g) was dried at 50 ^{o}C for 3 h and stored within desiccators for 7 d. Saturated salt solutions including LiCl, $MgCl_2$, $Mg(NO_3)$, NaCl, KCl, and KNO_3 presenting accurate relative humidities of 11, 33, 52, 75, 86, and 93%, respectively, were added into the desiccators. The CM-chitosan was weighed every 24 h until equilibrium. Percent equilibrium moisture (%ECM) was then calculated following this equation (2);

$$\text{\% ECM} = [\frac{We}{Wi}(Mi + 1) - 1] \times 100 \qquad (2)$$

Where, We is the weight (g) of CM-chitosan at the equilibrium, Wi is the weight (g) of CM-chitosan at the initial and Mi is the relative humidity at the initial. Moisture absorption isotherm was expressed as a relative graph between %ECM and water activity (a_w) of each salt solution.

3. Determination of physical characteristics of the CM-chitosan

Swelling index of CM-chitosan was evaluated following the method described in British Pharmacopeia 1998. Briefly, CM-chitosan (20 mg) was scattered onto water surface in a graduated cylinder (10 mL). The cylinder was then gently shaked every 10 min, 6 times. After 3 h, the volume (mL) of swollen CM-chitosan was measured. Swelling index was then calculated using the following equation (3);

$$\text{Swelling index} = \frac{\text{Volume of swollen CM-chitosan (mL)}}{\text{weight of CM-chitosan (1g)}} \qquad (3)$$

Melting point of CM-chitosan was additionally determined by Differential Scanning Calorimetry (DSC) using this condition; pan volume of 30 µL (B014-3016), heat flow rate of 10 mW/min and temperature range of 50 – 450 ^{o}C.

4. Structural morphology of the CM-chitosan

Scanning electron microscope (SEM) was performed in order to directly visualized morphologies of shrimp chitosan and CM-chitosan.

5. In vitro moisturizing testing of the CM-chitosan

Pig skin shrinkage test and determination of skin hydration using Corneometer®, following the method of Kassakul *et al.* (2014), were carried out in order to evaluate *in vitro* moisturizing effect of CM-chitosan (0.2% and 0.4% aqueous solutions) comparing with 5% propylene glycol (PG), 5% butylene glycol (BG), 5% glycerin, and 0.2% hyaluronic acid (HA).

1) Pig skin shrinkage test

Pig skins, using frank area skins from three different piglets, were freshly obtained from market, Chiang Mai, Thailand. The entire hair was completely shaved and fat-absorbed using tissue paper. The pig skins were cut into 3 × 3 cm square shape and the area (cm^2) was coded as A1. Also, the skins were weighed and expressed as W1. The skins were soaked in the tested moisturizing solutions as described above for 2 h. A pig skin without soaking served as a control. Then, the soaked skins were dried using hot-air oven at 60 ^{o}C for 90 min. The reduction in weight and area of the soaked skin compared with those of the initial were determined and expressed as differential values.

2) Determination of pig skin hydration using Corneometer®

The increases in skin hydration of pig skin after applying the tested moisturizing solutions were determined using Corneometer®. Skin hydration of plain skin was firstly measured and each tested solution (100 µL) was then topically applied by cotton bud. After 15 min and 30 min of incubation at 25 ^{o}C, the later skin hydration was determined. Percent effectiveness was then calculated using the following equation (4);

$$\%\ \text{Effectiveness} = \frac{\text{Skin hydraton after moisturizing} - \text{Skin hydration at initial}}{\text{Skin hydration at initial}} \times 100 \qquad (4)$$

6. Development of moisturizing creams containing CM-chitosan

Oil-in-water emulsion was prepared using a conventional hot process. Briefly, an oil phase consisting of squalane, cetyl alcohol, beeswax, glyceryl monostearate, jojoba oil, cyclomethicone, stearyl

alcohol, vitamin E acetate, ceteareth-25, triethanolamine, stearic acid, was heated by water bath to 70 ^{o}C. In the meantime, an aqueous phase containing propylene, glycerin, DMDM hydantoin, water as well as CM-chitosan was heated to 75 ^{o}C. Then, the oil phase was gradually added to aqueous phase with continuously stirring until the emulsion are completely formed.

7. Stability testing of the moisturizing cream containing CM-chitosan

The formulations were stored under various conditions including an accelerated condition (6 cycles of heating-cooling; 4 ^{o}C for 48 h followed by 45 ^{o}C for 48 h: 1 cycle), room temperature, 4 ^{o}C, and 45 ^{o}C for 60 d. Their physical appearances such as color, sedimentation, and phase separation together with the viscosity and pH value of the formulations were evaluated and compared with those of the initial.

8. Primary skin irritation and performance testing in human volunteers

The tests in 22 human volunteers were ethically approved by ethic committee on Human Rights Related to Human Experimentation of Chiang Mai University, Thailand. Firstly, primary skin irritation was investigated following the standard method describing in OECD guideline. The tested samples (200 µL) including creams containing PG, HA, or CM-chitosan along with negative control (DI water) and positive control (1% w/w sodium lauryl sulfate: SLS) were individually loaded into a fin chamber and applied onto volunteers' backs for 4 h. After removal of the fin chamber for 1, 24, 48, and 72 h, skin erythema and edema were observed and calculated based on Draize scoring system (OECD TG 404 2002). Skin irritation of each sample was subsequently graded according to Primary irritation index (PII) (Auletta 2004).

In addition, human volunteers, who showed no skin irritation, were included in the performance testing. The samples were topically applied onto different skin areas. After 15, 30, and 60 min of incubation at 25 ^{o}C, 75% relative humidity, the skin hydration of each area was measured using Corneometer®. Percent effectiveness was lastly calculated using the equation (4).

RESULTS AND DISCUSSION

In our study, CM-chitosan was synthesized through the incorporation of carboxymethyl group under an alkaline condition. As a presence of carboxymethyl group, an increase in percent yield (130.23 ± 7.25 %w/w) was observed. Water sorption isotherm as shown in Figure 1, demonstrated that a rapid increase in %ECM was in accordance with an increase in a_w. This result could be interpreted that CM-chitosan was a hygroscopic polymer, which is corresponding to the studies of Rachtanapun *et al*., (2009).

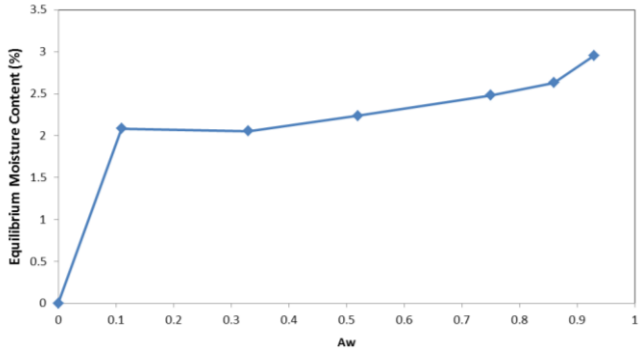
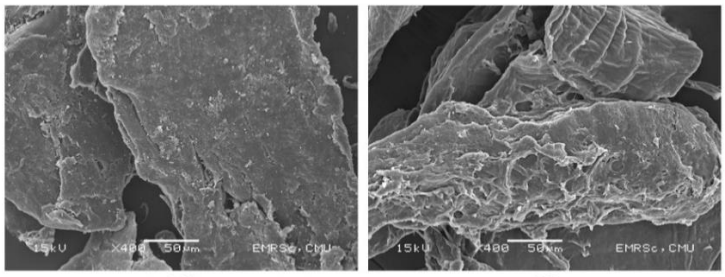


Figure 1 Water sorption isotherm of CM-chitosan

Swelling index of CM-chitosan was compared to different common cosmetic polymers as shown in Table 1. The results presented that CM-chitosan could be easily swollen, which was comparable to HPMC and SCMC. Interestingly, higher swelling index of CM-chitosan than that of HPMC within 1 h indicates a faster swelling rate of CM-chitosan. Besides, swelling index of CM-chitosan was also higher than that of MC, squid chitosan, and shrimp chitosan. This might be interpreted by its morphology (Figure 2). The structural morphologies of chitosan and CM-chitosan illustrates that the apparent decreases in rigidity, crystallinity and smoothness of the compound's texture, might be due to a strong alkaline condition, offered superior swelling and water absorption capacities of this compound above shrimp chitosan (Rachtanapun *et al*., 2012). As a result, an alkaline condition also played a crucial role in this process. Additionally, DSC thermogram of CM-chitosan illustrated that the melting point onset of this compound was 174.8 ^{o}C with the peak of 176.6 ^{o}C (Data not shown).

Table 1 Swelling index of the CM-chitosan compared with HPMC, SCMC, MC and shrimp chitosan

Types of polymers	**Swelling index (mL/g)**	
	1 h	3 h
CM-chitosan	500	500
HPMC	40	500
SCMC	500	500
MC	< 25	< 25
Shrimp chitosan	< 25	< 25



(A) (B)

Figure 2 Structural morphology of (A) Shrimp chitosan and (B) CM-chitosan visualized through Scanning electron microscope (SEM)

The moisturizing effect of CM-chitosan was compared with those of common cosmetic moisturizing agents. Figure 3 demonstrated the differential values (weight and area) of pig skins after soaking in the different moisturizing solutions and drying. Higher differential value directly indicates a lower efficacy in the prevention of moisture evaporated from the skin. Plain skin presented the highest value since there is not any moisturizer to protect the water loss. The efficacy of CM-chitosan as a moisturizer was dose-dependent. It is worth noting that the efficacy of 0.4% CM-chitosan was better than those of 5% PG, 5% BG, and 0.2% HA. However, the measurement of skin hydration should be carried out in order to precisely confirm this efficacy.

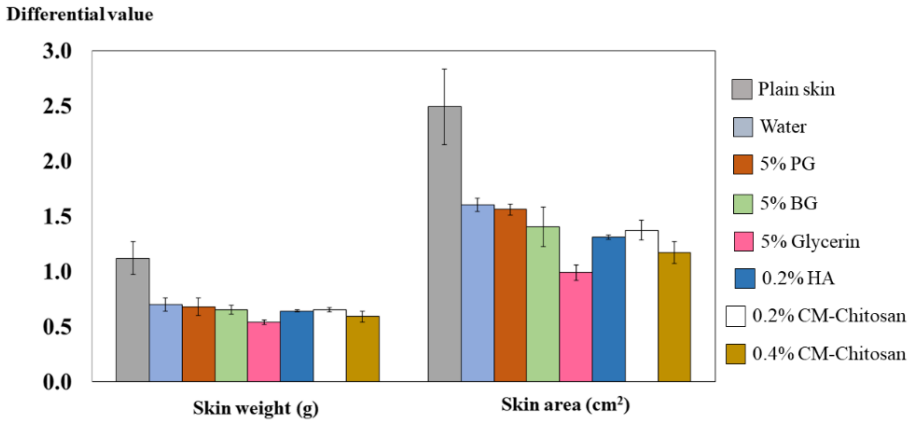


Figure 3 Differential values of pig skin weight and area after soaking in different moisturizing solutions

Percent effectiveness evaluated by Corneometer® (Figure 4) was in a good agreement with the results of shrinkage test. Skin hydration was significantly increased after application of 0.4% CM-chitosan compared with plain skin. After 15 min, %effectiveness of 0.4% CM-chitosan was additionally higher than those of 5% PG, 5% BG and 0.2% HA. By increasing in the incubation times, the decreases in %effectiveness were observed in all groups. This might be due to water evaporation from the skin with times.

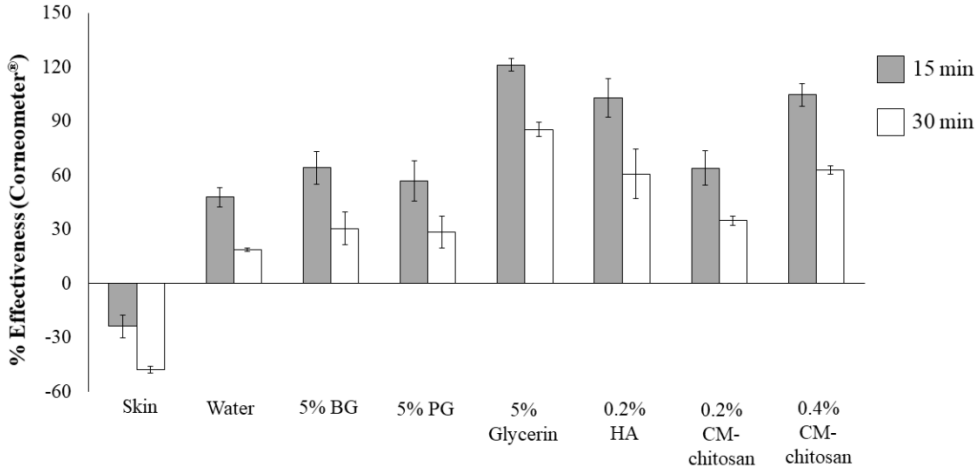


Figure 4 Percent effectiveness of different moisturizing solutions in pig skin measured by Corneometer® after 15 and 30 min of applications

Cream containing CM-chitosan in a concentration of 0.4% was consequently developed due to the most effective concentration according to the results of *in vitro* moisturizing evaluation. The obtained cream presented an excellent physical appearance with pH 5.5, which is suitable for skincare cosmetic. Besides, the cream leaved no greasy and absorbed easily through the skin. After 60 d of stability testing, no phase separation occurred. In addition, the viscosity of CM-chitosan cream was not significantly changed compared to the initial (Data not shown). This could be assumed that CM-chitosan cream showed a good stability profile.

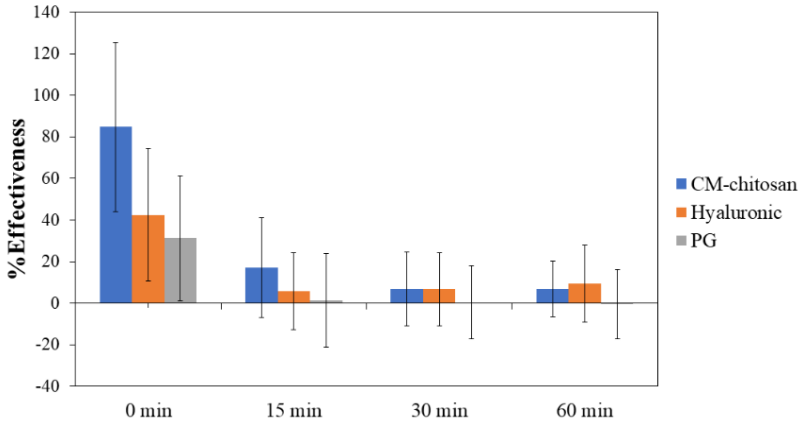


Figure 5 Percent effectiveness of three moisturizing creams in human volunteers at the initial, after 15, 30 and 60 min of applications

Prior to evaluating efficacy of the formulations, it is necessary to identify skin irritation profile (Auletta 2004). After 72 h of the observation, PII value of 1% SLS was 2, whereas PII values of all samples were 0, indicating that all creams were safe. Figure 5 illustrates the percent effectiveness of cream containing CM-chitosan compared with creams containing HA and PG in 22 human volunteers. At the initial, CM-chitosan exhibited the greatest effect, among all formulations. However, the decreases in %effectiveness were observed in all groups during 1 h. It is worth mentioned that moisturizing effect of CM-chitosan was comparable to that of HA, which is one of the most expensive moisturizers for cosmetology and better than PG. These might be due to the fact that CM-chitosan and HA are capable of forming the film onto skin surface together with highly water attracting from the atmosphere, while PG, as a humectant, conversely exerts a hygroscopic property.

CONCLUSION

Our study successfully synthesized CM-chitosan that exhibited its outstanding skin moisturizing capability in comparable to hyaluronic acid. Moreover, the moisturizing cream containing CM-chitosan demonstrated not only safe for skin but also an excellent efficacy in improving skin hydration.

ACKNOWLEDGEMENTS

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FULL TEXT-PAPER

TRADITIONAL USES, PHYTOCHEMICAL SCREENING AND BIOACTIVITIES OF A SPONTANEOUS SAHARIAN SPECIES

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Abstract

The current study was to present *Matricaria pubescens* (Desf.) and its traditional uses. Also it was to investigate the antioxidant and the antibacterial activities of *M. pubescens* extracts. The antioxidant capacity and total phenolic content of *M. pubescens* extracts were measured utilizing Bleaching β-carotene assay (BCB) and Folin–Ciocalteu procedure, respectively. The highest phenolic and flavonïds contents were in that order 261.603± 0.033µg gallic acid equivalent/mg extract and 259.666±0.024 µg quercetin equivalent/mg extract. While, values of relative antioxidant activity (AAR %) were between 21.74% and 41.74%. *M. pubescens* extractswere tested against Gram- and Gram+ pathogenic bacterial strains. It seems that *M. pubescens* has a particular efficacy against *E. cloacae* and *E. feacalis*.

Keywords: *Matricaria pubescens*, spontaneous plant, bioactivity, solvent extraction, Saharan zones.

INTRODUCTION

Herbal medicine is one of the oldest in the world. It represents an interesting alternative to treat and cure without creating unwanted side effects. Nowadays, modern and folk medicines are intimately linked because the molecular models of most of the drugs in the market originate from the plant. Substitute synthetic antioxidants, is also one of the important aims of several industries such as food processing and cosmetic. They are looking for natural antioxidants (Xu D.P. et al., 2017, Mlcek J. et al., 2016, Krishnaiah D. et al., 2011), that are strong and save. Among the highly sought natural products, appear the polyphenols of plants. They are widely used as vascular protective (Li A.N. et al., 2014), antioxidants (Huang D.J. et al., 2005, Scalbert A. et al., 2005). Previous research works (Xie Y. et al., 2015, Bilto YY. et al., 2012) have proved their microbiological activities also. On the other hand, the mysterious world of plants is still inexhaustible; scientists with traditional practitioners have to exchange knowledge to save species that could be benefic for human being. (Hostettmann K. and Marston A., 2002). This work represents a contribution to the phytochemical and biological investigations on the Asteraceae *Matricaria pubescens* (Desf.). It is a spontaneous species, from the southern of Algeria, famous and widely used in medicine and traditional practices in these regions (Hammiche V., Maiza K., 2006). Research works done on this species exhibited the presence of flavonoids and coumarins (Gherboudj O. et al., 2012). The aim of this study is to present a Saharan spontaneous plant from Algeria as well as the checking of the antioxidant and antibacterial activities of some extracts prepared from this species.

PRESENTATION AND TRADITIONAL USES OF *M. pubescens* (Desf.)

M. pubescens (photo 1) is an annual spontaneous plant. Because of recent climatic alterations, its period of vegetation and flowering has changed. Instead of March, currently it begins to appear from the end of January, just after the rainy season and does not last very long, its habitat is reduced to sandy-clay depressions and wadi beds. It is a North African endemic species, it is found in Western Sahara, Morocco, Tunisia, Algeria and Libya. (http://www.catalogueoflife.org/annualchecklist). It is named by Garetoufa and/or ouazouaza (Maiza K. et al. 2003). Tuaregs calls it Aynasnis. *M. pubescens* is a medicinal plant highly appreciated by the inhabitants of the Saharan zones; it is widely used in traditional preparation and folk medicine (Hammiche V. and Maiza K., 2006, Ould El Hadj M. D. et al., 2003). According to field questionnaires, local uses are to relieve gastro-intestinal disorders, biliary calculus pain and dysmenorrhea. In addition, it is applied to treat eye diseases, kidney disease and rheumatism. Besides, they apply their preparations based on this plant to fight against dryness and pain related to teething in children. Frequently, it is used in the case of scorpion bites. Women of these regions use the crushed aerial parts of *M. pubescens* as a filter for the heated goat's butter. It will be pleasantly flavored and better preserved after cooling. They also add the powdered plant to the traditional soup (Tchicha, Harira) to give the food a very pleasant smell. It can be added to tea. It is harvested and marketed on a large scale in the weekly markets of these regions. Nomads do not report the plant as toxic (Hammiche V. and Maiza K., 2006, Ould El Hadj M. D. et al., 2003).

Photo 1: *Matricaria pubescens* (Desf.).

MATERIAL AND METHODS

Plant materıal: The species *M. pubescens* (Desf.) is collected during the flowering period, from southeastern Algeria. The botanical identification was made by Dr K. Maiza a pharmacist at the University of Algiers and Dr O. Ouled Belkhir agronomist at the University of Ouargla. The dried aerial parts were reduced to powder and then used in all experiments.

Preparation of extracts: 25 g of plant powder were macerated three times (24 h x3) in 100 ml of methanol 70%. The combined solutions were evaporated to afford MeOH extract. This procedure was repeated on new plant amounts to achieve the ethyl acetate and butanol extracts, after successive extractions with ethyl acetate (50 ml x3) then butanol (50 ml x3) of the hydromethanolic aqueous phases. To be closer to the traditional preparation, the aqueous extracts (aqous), in turn, was the result cold solution of the decoction for 1 hour of 50 g of plant powder in 100 ml of boiling water. All the prepared extracts were dried then stored at 4°C until use. The hydrodistillation of the aerial parts of *M. pubescens* using cleavenger apparatus during 3h gives its essential oil (E.O).

Estimation of phenols contents: Standard methods were applied to calculate the contents of phenols and flavonoids in *M. pubescens* prepared extracts. For the first group, it was used the folin Ciocalteu reagent and ascorbic acid as a standard. For the second one Aluminum chloride was the reagent and the quercetin was the reference in the calibration curve. (Xu DP. Et al., 2017)

Antibacterial assay: The samples prepared from aerial parts of *M. pubescens* were tested against the following Gram- and Gram+ bacterial strains: *E. cloacae* ATCC 13047; *P. mirabilus* ATCC 49452; *K. pneumonia* ATCC 70060; *E. coli* ATCC 25922; *S. aureus* ATCC 25923; *E. feacalis* ATCC 29212. The evaluation of the antibacterial activity of the different extracts was performed by the solid medium diffusion method (Balouiri M. et al., 2016). Dimethylsulfoxide (DMSO) was used to prepare solutions of ethyl acetate and butanol extracts and E.O (100 µg/ml). For the antibiograms, 10 µl of the prepared solutions were deposited on sterilized paper disks. The disk containing DMSO was used as blank. Three dishes were prepared for each test. The diameters (mm) of inhibition's zones were measured and saved.

RESULTS AND DISCUSSION

Extraction yields and phenols contents: Figure 1 illustrates values yields, polyphenol and flavonoids contents of *M. pubescens* Extracts. Methanol 70% and water have extracted very important quantities of polar compounds from *M. pubescesns* where their extracts showed the highest yields, whereas the obtained essential oil was the lowest (0.1%). The notable phenolic (261.603± 0.033µg EAG/mg ext.) and flavonoïds (259.666±0.024 µg equivalent quercetin/mg ext.) contents were calculated in the case of ethyl acetate extract.

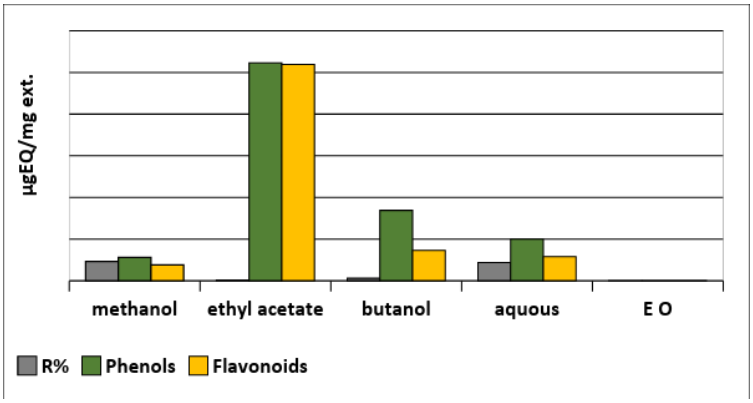

Fig 1: Percentage yields, polyphenol and flavonoids contents of *M. pubescens* extracts.

Bleaching β-carotene assay (BCB): *M. pubescens* extracts were submitted to verify their antioxidant capacity against the β-carotene oxidation. The oxidation of linoleic acid generates peroxyl free radicals that will then oxidize the highly unsaturated β-carotene (Kartal N. et al., 2007). However, the presence of antioxidants in the extracts to be tested will minimize the oxidation of β-carotene and hydroperoxides formed in the β-carotene / linoleic acid system will be neutralized. Thus, the rate of degradation of β-carotene depends on the antioxidant capacity of these extracts. Following results showed on the figure 2, nearly all extracts acted with moderate activities in this test, except *M. pubescens* ethyl acetate extract that differentiated itself compared to the other extracts by a relative antioxydant activity (AAR) estimated at 41.31%. Probably, among its constituents there is a significant concentration of polyphenols and/or flavonoids with low polarity as antioxidants which can

reduce the oxidation of lipid components in food and/or in cell membranes or inhibit conjugated dienes hydroperoxides derived from the oxidation of linoleic acid which are known to be carcinogenic. (Kartal N. et al., 2007, Rajeev B. et al., 2012).

However, most of other tested extracts were exhibited very close (AAR) (Fig. 3) that varied from 21.74% to 26.09%, they were less active to prevent β-carotene discoloration than the first extract and BHT but significant similarities were noted with Tocopherol behavior.

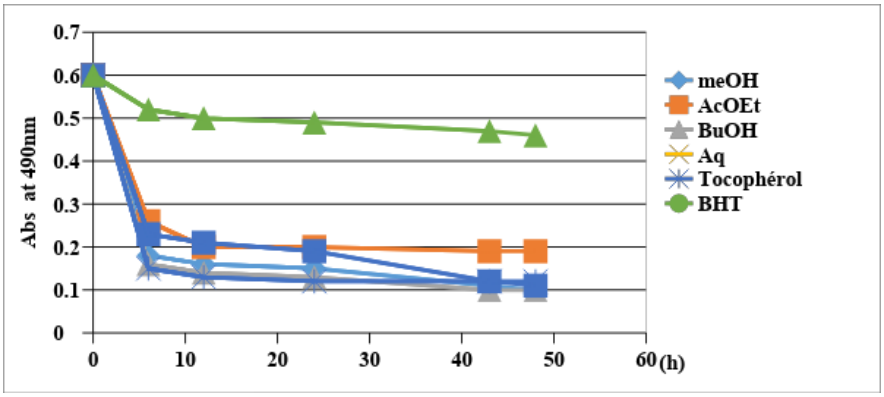

Figure 2: Kinetic of bleaching β-carotene at 490 nm.

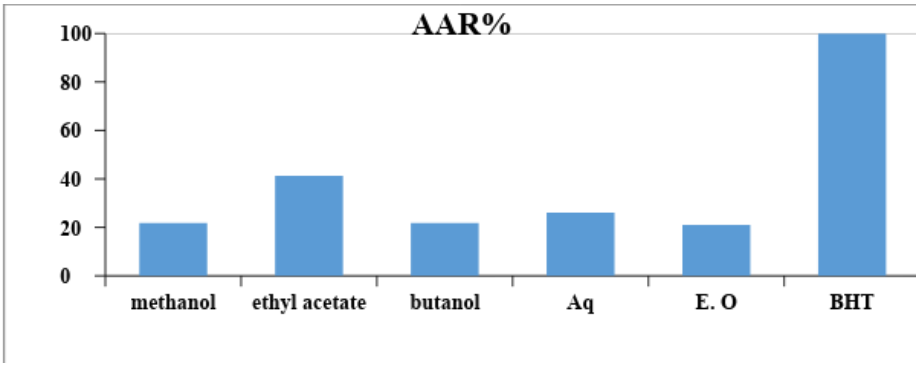

Fig. 3: Relative antioxidant activity (AAR %) of *M. pubescens* extracts.

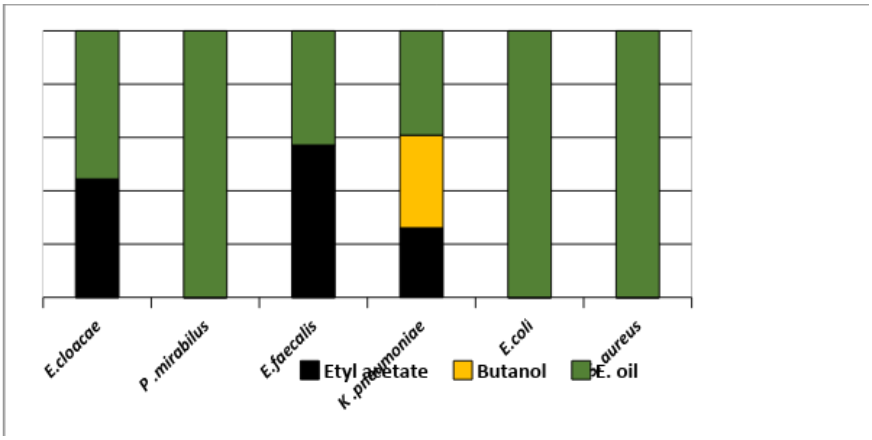

Fig.4: Bacterial sensitivity vis-à-vis *M. pubescens* extracts.

Antibacterial assay: Different actions were registered for the tested samples. As it can be seen on the figure 4, the essential oil extracted in this work has exhibited the strongest effect against *P. mirabilus*, *E. coli* and *S. aureus*. However, *E. cloacae*, *K. pneumoniae*,*E. feacalis* were more and/or less sensitive to E. O. Ethyl acetate extract has presented a moderate action on *E. cloacae*, *E. feacalis* and only a slightly effect face to *K. pneumonia*. In the same way, only a weak sensitivity to the third extract was evaluated for *K. pneumonia*, the other bacterial microorganisms were resistant to butanol extract. It is clearly illustrated that the difference in chemical compositions of the extracts has a great influence on the sensitivity and /or resistance of the bacterial microorganisms tested. Purification of the active compounds should explain these effects.

CONCLUSION

In general, obtained results allow to conclude that these extracts especially ethyl acetate extract, appears able to contribute in the resolution of one of some main problems in food processing and aliments conservation, by the reduction of linoleic acid oxidation. In addition, these conclusions explain perhaps, the traditional use of *M. pubescens* for preserving goats butter by the autochthonous in Saharan regions. Moreover, antibacterial activity against tested pathogenic bacteria was promising in our research for natural antibacterial alternatives.
It seems that *M. pubescens* has favorable resource with strong bioactivities. So, further investigations were suggested to be carried out in pharmacology, cosmetics and food industries.

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FULL TEXT-PAPER

GREEN SYNTHESIS OF CHITOSAN BASED NANOPARTICLES USING WATER EXTRACT OF *CEPHALARIA BALANSAE* AND STUDY OF THEIR CYTOTOXIC ACTIVITY

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Abstract

Nanoparticle synthesis has been ongoing to be developed as a green method to use it in the drug delivery system. Considering the great potential of chitosan as bioactive agents, we aimed to synthesize and characterize of chitosan-based nanoparticles using water extract of *Cephalaria balansae* and to evaluate their potential cytotoxic activity against cancerous esophageal, OE-33 and gastric adenocarcinoma, ACC-201 cell lines by MTT method. The nanoparticles were characterized by UV-vis, Zeta-sizer, FTIR and SEM. The nanoparticles showed particle size between 65 to 280 nm. All nanoparticles were also monitored to detect their size stability for 5 weeks by Zeta-sizer. According to the activity results, the nanoparticle with the size of 182 nm showed an important inhibitory effect on cancerous OE-33 and ACC-201 cells with IC_{50} values of 13 and 14 µg/mL, respectively. Thus, this eco-friendly method could be used for biomedical applications in future with their toxicity in OE-33 and ACC-201 cancerous cells.

Keywords: Green synthesis, Chitosan, nanoparticles, *Cephalaria balansea*, cytotoxic activity

1. INTRODUCTION

Nanotechnology is the application of science to control matter at the molecular level. Research and applications of nanotechnology, especially in the field of medicine, have attracted great attention recently. There are many treatments today that take a lot of time and are also very expensive. Using nanotechnology's advantages, quicker and much cheaper treatments can be developed. Nano-sized particles and molecules have structures and dimensions that differ from those of conventional small-molecule drugs, so they can be a potential alternative, with a unique biological effect for the treatment of many diseases [1]. Several anti-cancer drugs including paclitaxel, doxorubicin, 5-fluorouracil and dexamethasone have been successfully formulated using nanomaterials [2].

The size of nanoparticles ranges from 10 nm to 1000 nm. Due to their small size and large surface area they exhibit unique chemical and physical properties. Nanoparticles can be prepared both from natural and synthetic polymers. Chitosan is the second most abundant found biopolymer in nature after cellulose that is a non-toxic, biocompatible, biodegradable, natural polysaccharide and easily available [3]. Since chitosan has these kinds of important properties and shows more effectivity in nanoparticle form, it has been used for drug delivery as well as tissue engineering, food packaging and waste-water treatment [4]. The first chitosan nanoparticles were described in 1994 and an anticancer drug carried by chitosan nanoparticles was obtained. After that, many optional ways to synthesize of chitosan

nanoparticles were tried. Methods for the preparation of chitosan nanoparticles can be emulsification that is the first tried way, ionic gelation and reverse micellization [4-6]. The simplest and widely used preparation method is ionic gelation. One of the advantages of this method using tripolyphosphate (TPP) which is a non-toxic crosslinker, the chitosan nanoparticles have good stability with this crosslinker. This method gives a simple procedure, cost-effective, environment-friendly and more stable materials [7]. Therefore, it allows to green synthesis. The most acceptable way between nanotechnology and green chemistry is the use of bioactive compounds for the synthesis of nanoparticles. For this reason, we chose a medicinal plant called *Cephalaria balansae* as a bioactive extract besides chitosan. Natural products using plants have received considerable attention because of their potential to treat various diseases. *Cephalaria balansae* is a plant with containing biologically active compounds, which belongs to the Caprifoliaceae family that has been used in traditional medicine for many years due to its antioxidant, cytotoxic, antidiabetic, antimicrobial and antifungal activities [8-10]. The recent investigations and findings in biological activity studies of this genus have mostly focused on immunomodulatory properties [11]. *Cephalaria balansae* has been also reported that it has cytotoxic, hemolytic and immunomodulator activities. The constituents in *Cephalaria balansae* extract are flavonoids, alkaloids, iridoids, lignans and especially saponins [12]. To the best of our knowledge, the present study is the first report about syntheses of chitosan nanoparticles using *Cephalaria balansae* extract.

In another part of the study, silver chitosan nanoparticles have been produced using silver metals. Ag-nanoparticles have been found to be an effective agent in the treatment of cancer [1]. The potential of silver nanoparticles as an anti-cancer agent could open new doors in the field of medicine. Moreover, when silver nanoparticles are combined with chitosan, the new nanocomposite obtained is one of the rare composite materials which can be used as a biosensor and treatment in some specific cancer cells [13]. Overall, in the light of all these findings, production of new nanoparticles using a mix of chitosan, plant extract and silver metal has seen a worth doing research to analyze their cytotoxic activity.

2. MATERIALS AND METHODS

2.1. Materials: Chitosan (Medium moleculer weight, 75-85% deacetylated), tripolyphosphate (TPP), silver nitrate ($AgNO_3$) were purchased from Sigma-Aldrich. *Cephalaria balansae* Raus. was collected from Antalya-Elmali, Finike highway 22 km, at about 1250 m altitude in July 2012 (36° 32′ 45.2″ N, 29° 59′ 11.1″ E). A voucher specimen has been deposited (No: R.S. Gokturk 7525) at the Herbarium Research and Application Centre of Akdeniz University.

2.2. Methods:

2.2.1. Preparation of plant extract: The plant extract was prepared from *Cephalaria balansae***.** The aerial part of plant was first extracted with methanol at room temperature for overnight and then concentrated under reduced pressure (40ºC). This residue was extracted with *n*-BuOH: H_2O (1:1) solvent system. The water phase was seperated and was filtered. This water extract was used for synthesis of chitosan nanoparticles and Ag-chitosan nanocomposites (CNPs/Ag CNPs) in following steps.

2.2.2. Preparation of Chitosan nanoparticles by *Cephalaria balansae* extract: Chitosan nanoparticles were prepared based on ionic gelation method which is cross-linking of chitosan with TPP [14]. First, 2 mg/ml acetate buffer was prepared then acetic acid was added until pH 3. Chitosan (2 mg/ml) was dissolved in a solution of acetate buffer and stirring using a magnetic stirrer at 50 ºC for 30 minutes. TPP was dissolved in deionized water at a concentration of 0.5 mg/ml. *Cephalaria balansae* water extract was dissolved at 2 mg/ml in water and stirred until homogenous solution occur.

After preparing solution of chitosan, TPP and *Cephalaria balansae* water extract, were filtered through PVDF membrane syringe filter (pore size 0.22 μm). 0.5 mL water extract solution was put in microtube and 0.5 mL chitosan solution was added. Thereafter the mixture was homogenized using vortex for 20 seconds. 0.25 ml TPP solution was added into the homogenous mixture and then immediately homogenized again using vortex for 20 seconds.

2.2.3. Preparation of chitosan/Ag nanocomposites by *Cephalaria balansae* extract: Chitosan/Ag nanocomposites (CS/Ag NCPs) were synthesized by a green method using *Cephalaria balansae* extract. 2 mL of TPP (1% in H_2O) was added to 40 mL of chitosan solution (2 mg/mL in acetic acid solution 2%) and stirred at 50°C for 30 min. Then 1 mL of $AgNO_3$ (0.01 M) was added dropwise to solution mixture, in a short time 2 ml of *Cephalaria balansae* extract (1 mg/ml) was added quickly and stirred at 70°C for 90 min. The solution was centrifuged (12000 rpm; 30 min) and the pellet was washed twice with deionized water to remove excess. This method was also performed by without TPP to see the effect of TPP on the nanoparticles [15-16].

Additionally, blank chitosan nanoparticle (without extract) was prepared using the same method both for chitosan nanoparticles and chitosan/Ag nanocomposites. The nanoparticles were first ultrasonicated and then analyzed by zeta-sizer. The size of nanoparticles was monitored for 5 weeks by zeta-sizer.

2.2.4. Characterization: The absorbance spectra of particle solutions were examined by UV–vis spectrophotometry (Optizen-Pop). The Zetasizer (Malvern Zetasizer, Nano-ZS) was used to determine the average size and polydispersity index of nanoparticles. Optical rotations and FTIR spectra were recorded on a Rudolph Research Analytical Autopol I automatic polarimeter and on an ATI Mattson Genesis Series Fourier transform infrared spectrophotometer, respectively. The particle size and surface morphology of chitosan nanoparticles were examined by scanning electron microscope (SEM) with a Thermo Fisher Scientific FEI/ Apreo S.

2.2.5. Preparation for studying *in vitro* cytotoxicity assay of nanoparticles: ACC 201 (human gastric adenocarcinoma) and OE33 (human caucasian oesophageal carcinoma) which were obtained from ATCC cell lines were used for the cytotoxicity assay. All cell lines were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 with 10% fetal bovine serum and 0.1% penicillin/streptomycin (Serox GmbH, Germany). All cells were incubated in 95% humidified atmosphere of 5% CO_2 at 37˚C. Samples' cytotoxicity effect was detected with MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide) assay which determines the activity of mitochondrial reductase of viable cells calorimetrically [17-18]. Cell lines were cultivated at 1×105 cells/ml initial cell concentration in 96 well plates. After 24 hours, the cells were treated with samples at different concentrations (0.5, 5 and 50 μg/ml) and doxorubicin was used as positive control (20, 2 and 0.2 μg/ml). After 48 hours of treatment optical densities (OD) of the dissolved material were determined at 570 nm (refrence filter 620 nm) with UV visible spectrophotometer. Spectrophotometric measurement was used for determining the percentage of living cells. Half maximal inhibitory concentration (IC_{50}) is a measure of the 50% inhibition on cell viability. The determined IC_{50} concentrations of the samples reduce cell growth by 50% under laboratory conditions. For this purpose, GraphPad Prism 5 software (San Diego, CA, USA) was used for the calculation of the IC_{50}.

3. RESULTS AND DISCUSSION

3.1. UV-Visible spectroscopy: UV-Visible spectroscopy is a simple and quite a sensitive technique that can be used to detect the formation of nanoparticles. UV-Vis absorption spectra of the nanoparticles were recorded in the wavelength over the range of 200-500 nm with Optizen-Pop

spectrophotometer. The measurements were accomplished at room temperature using quartz cuvettes and the blank is pure water. Nanoparticles were diluted with water and analyzed. The UV–vis absorbance spectra of pure chitosan nanoparticle gives a peak at 270 nm which is a characteristic peak of chitosan and the other significant peak is around 320 nm refers to chitosan nanoparticle [19-20]. Ahmed et al. [21] have proved that chitosan and olive leave extract have lower energy difference, more conjugated system and more stable than chitosan after calculating their energy gap difference.

The nano size Ag-chitosan nanocomposites have unique band which is called a localized surface plasmon resonance (SPR). This peak absorption could be observed between 380 nm to 420 nm [22-23]. This range is changeable depending on some parameters such as concentration of nanoparticles or shape of nanoparticles. According to experimental studies of Duggal [2] in which synthesis and characterization of silver nanoparticles have been studied report when the concentration of silver nanoparticles increases, the peak absorption decreases. Agarwal et al. [3] have reported that chitosan/Ag nanoparticles with approximately spherical morphology give a peak at around 380 nm. In this study, plant extracted Ag-chitosan nanocomposites appearance in the electronic absorption spectrum of a band is located at 370 nm. In addition to that, Ag^{+} ion absorption bands which is located between 200 and 230 nm still appeared in our spectrum. So, this situation demonstrates that in the synthesized nanoparticle, the presence of silver ion solutions can be assumed to residue as a low concentrated. It is obvious that the reduction capacity of chitosan is related to the concentration of silver salt in nanoparticles. Overall, we could observe the basic correlation between chitosan, extract and silver by using UV-vis spectroscopy but the interaction between silver ions and polar groups of chitosan and extract can be more understood in FTIR spectrum.

3.2. Fourier transform-infrared spectra analysis (FTIR): The FTIR spectra of chitosan nanoparticles are shown in Fig.1. Almost all Ag-chitosan nanoparticles are given approximately the same vibrations, there are some stretching which are proven the relation between chitosan, extract, $AgNO_3$ and TPP. The main characteristic absorption peaks of chitosan are observed in all samples, such as vibration of the amino group (NH_2), O=C-NHR, and amine NH symmetric. The bands between 3.246 cm^{-1} and 3.259 cm^{-1} were related to the vibrations of amino groups from NH-amine and OH groups in chitosan nanoparticles. The peak at 2924 and 2853 cm^{-1} correspond to C-H and C-N stretching respectively.

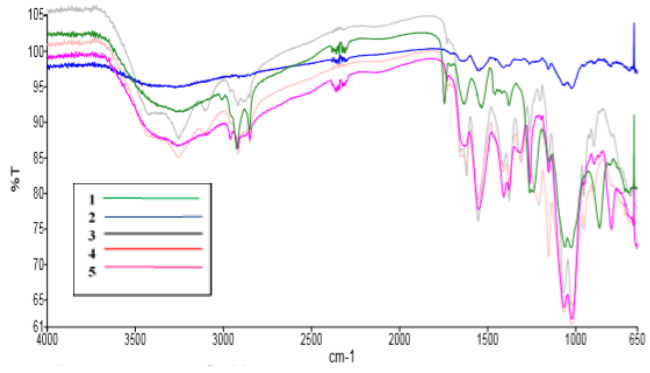

Fig.1. FTIR spectra of chitosan nanoparticles (NPs). Spectrum numbers are the same order with the **Table 1** sample numbers.

The main difference between chitosan-Ag nanoparticles with/without TPP is the reduction in stretching of O=C-NHR and O-H / N-H band. This could be qualified that when TPP is used as a crosslinker, it has a strong interaction with ammonium group of chitosan and more hydrogen bonding in chitosan-TPP nanoparticle. The other noticeable effect of TPP is that adding extract to the Ag-chitosan nanoparticles the stretching of O-H and NH_2 get shifted from lower wave number region to higher wave number region. This difference could be observed an amide linkage between chitosan and

extract [24]. As can be seen from Table 1, when the presence of Ag nanoparticles in the chitosan, the hydroxyl peak reduces.

Table 1. Fourier-transform infrared (FTIR) spectra bands for chitosan nanoparticles

Sample No	Sample Name	TPP	Wavenumbers (cm−1)		
			O-H	O=C-NHR	NH_2
1	Chitosan NPs (blank)	+	3256	1636	1534
2	Chitosan-Ag NPs	+	3246	1607	1528
3	Chitosan-Ag NPs	-	3255	1619	1553
4	Chitosan-Ag-extract NPs	+	3256	1620	1550
5	Chitosan-Ag-extract NPs	-	3259	1630	1553

3.3. Particle size and polydispersity index analysis (Zetasizer): Size distribution and polydispersity index of the nanoparticles (NPs) were analyzed by using the Zetasizer and results are shown in Fig.2. This represents the average size of chitosan, chitosan-extract, Ag-chitosan and Ag-chitosan-extract nanoparticles which are 182, 162, 194 and 156, respectively. The polydispersity index (PDI) which describes the distribution of particle size was found less than 0.5 for all nanoparticles. When the number of polydispersity index is smaller, the size of the particles is more uniform. Therefore, the polydispersity index affects the particles' characteristic and it is expected to be less than 0.5 [25]. According to Wazed et al. [26] results, silver loaded nanoparticles have higher PDI than chitosan nanoparticles. This evidence was interpreted as surface chelation of silver ions with chitosan nanoparticles. Our results are shown in similarity with this study. The chitosan NPs were dispersed in deionized water and observed over five weeks in order to evaluate their stability with comparing their average size and PDI by Zetasizer.

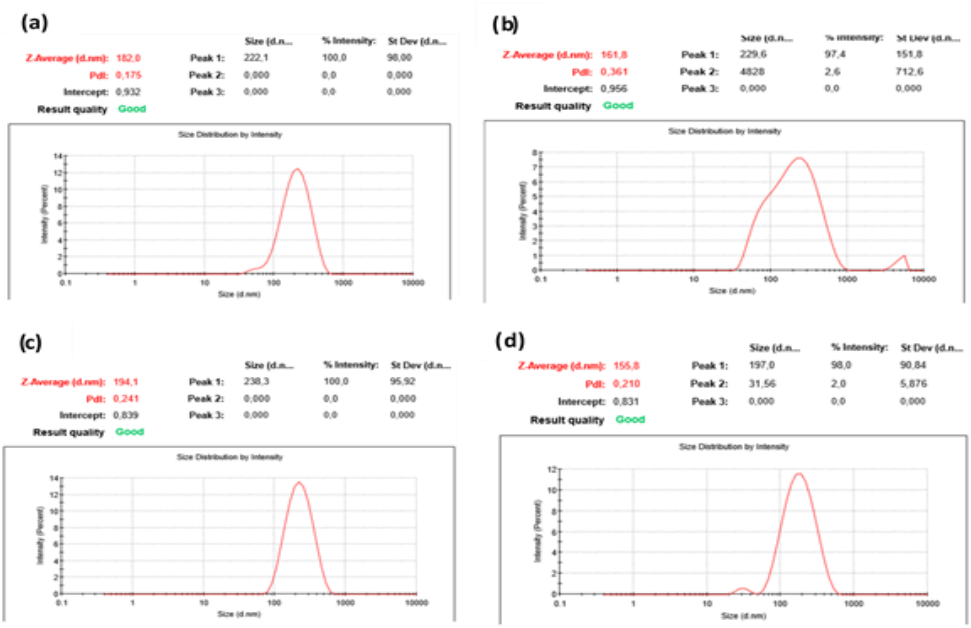

Fig. 2. The average size and polydispersity index (PDI) of chitosan nanoparticles.
(a) Chitosan NPs
(b) Chitosan-extract NPs (c) Ag-chitosan NPs
(d) Ag-chitosan-extract NPs

It is obvious from Fig.3. Ag-chitosan nanoparticles which are synthesized by using TPP crosslinker are effective in stabilizing the synthesized particles for five weeks, whereas some unexpected very small particles could appear in Ag-chitosan nanoparticles without TPP after some weeks. This may be owing to increased intra cross-linking between chitosan and TPP. Also, the PDI value of all Ag-chitosan based nanoparticles increased after 5 weeks. This difference may be the reason of non-freezing of nanoparticles since freeze-drying is a widely used process for drying NPs to improve their stability. Nemrawi et al. [27] showed the effect of freeze-drying for the stability of the CS NPs at the beginning and the end of the experiment. They proved that freeze-drying is likely to decrease the NPs size because it prevents the nanoparticles from agglomeration. Moreover, the PDIs were lower after freeze-drying and the surface charges were higher. If we have had compared the PDIs after freezing, we would have seen different results.

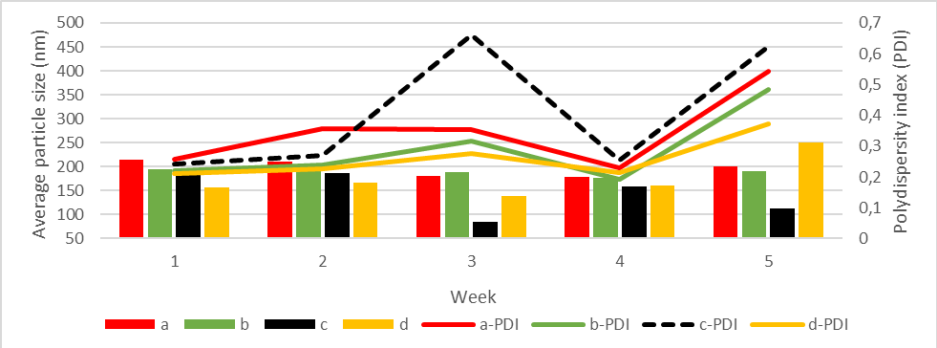

Fig. 3. The average size and polydispersity index (PDI) of chitosan nanoparticles in five weeks.
(a) Chitosan NPs, (b) Chitosan-extract NPs, (c) Ag-chitosan NPs, and (d) Ag-chitosan-extract NPs

3.4. Morphology Analysis-Scanning electron microscope (SEM): Scanning electron microscope (SEM) was used to observe the surface morphology of chitosan nanoparticles. Fig.4a and Fig.4b show representative SEM images of chitosan and Ag-chitosan nanoparticle samples. The images of the chitosan nanoparticle reveal that they are not well dispersed, and the shape of particles are mostly spherical or nearly cubic like shaped in Fig.4a. Fig.4b demonstrates the shape of Ag-chitosan nanoparticles which are very homogenous morphology and spherical in shape. Also, the morphological structure of chitosan nanoparticles is exposed to the large particle size and agglomerated state whereas, Ag-chitosan nanoparticles don't show any agglomeration. Chitosan nanoparticles have porous surface due to agglomeration attributes. Although this situation may be a negative effect, in biomedical applications this can however be turned into an advantage [20]. For example, the porous nature and agglomeration capabilities of the chitosan nanoparticles provide them useful as a critical chitosan-based bio-nano pesticide [28].

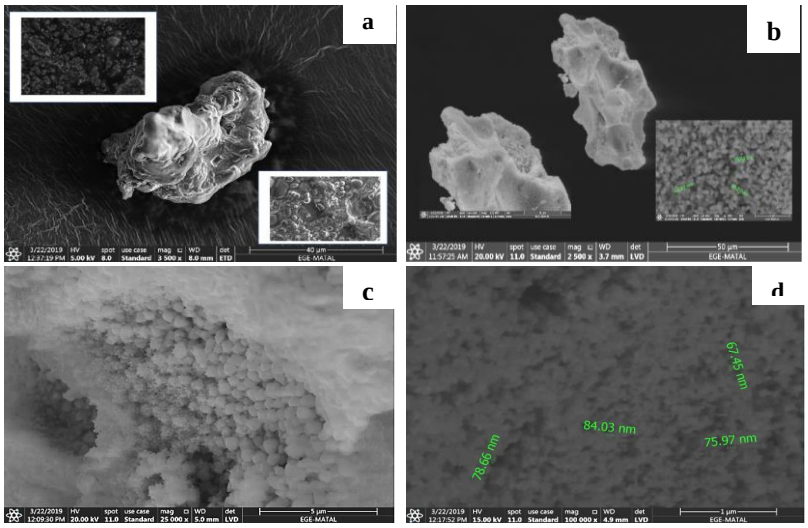

Fig. 4. SEM images (a)Chitosan nanoparticle (b)Chitosan-Ag nanoparticle (c)Chitosan-Ag-extract nanoparticle (d)Chitosan-Ag-extract nanoparticle with TPP

The size of Ag-chitosan nanoparticles was found in ranges varying from 60 to 180 nm. SEM images of Ag-chitosan-extract nanoparticles demonstrated that nanoparticles dispersed as a spherical shape and there is no agglomeration. Mostly the size, surface charge, and composition among particles have affected the value of the aggregation states of nanoparticles [29]. Fig.4d shows that using TPP as a crosslinker in the synthesis of the chitosan nanoparticles makes nanoparticles reasonably more homogeneous. This could be attributed to the presence of strong crosslinking between chitosan and TPP. The average particle size of Ag-chitosan-extract nanoparticles is less around 65-90 nm. Although there is some limitation of SEM to determine the internal structure of nanoparticles, it can still make available reliable information regarding the purity and the degree of particle aggregation [29]. Nanoparticles can find varied shapes including spherical, triangular, cubic, oval, helical, rod and prism. In this study, almost all the nanoparticles were a spherical shape. The size and shape of the nanoparticles is an important limitation that affects their performance [30]. For instance, Bartczak et al. [31] studied gold nanoparticles of four different shapes which are spherical, rod-shaped, hollow and silica-gold core-shell particles. According to the results of this study; the cellular uptake of spherical particles was the highest whereas the hollow shape particles was the lowest. The present study also shows that spherical particles are more stable and useful.

3.5. *In vitro* Cytotoxicity analysis: The synthesized chitosan-based nanoparticles were analyzed by MTT for their toxicity on cancer cells which are ACC 201 and OE33 cell lines. According to the cytotoxicity test results **(**Fig. 5-6**),** chitosan NPs have an inhibitory effect on both ACC 201 and OE33 cell lines and the IC50 value determined on the ACC201 cell line was 14.06±2.59µg/ml and 12.815±2.81µg/ml on the OE33. The chitosan-extract nanoparticles did not show inhibition of viability on the ACC 201 cell line, whereas the IC50 value on the OE33 cell line was determined to be 39.805±3.29µg/ml. In the case of Ag-chitosan nanoparticles with extract loaded, the result displayed that this sample has more inhibitory effect on ACC 201 than OE33. Also, the IC50 value on the ACC 201 cell line was found at a concentration of 55.1±2.94µg/ml, whereas the inhibition concentration was not achieved on the OE33 cell line. As a result of the MTT study, it can be said that the OE33 cell line has a higher resistance to most of the samples than that of the ACC 201 cell line.

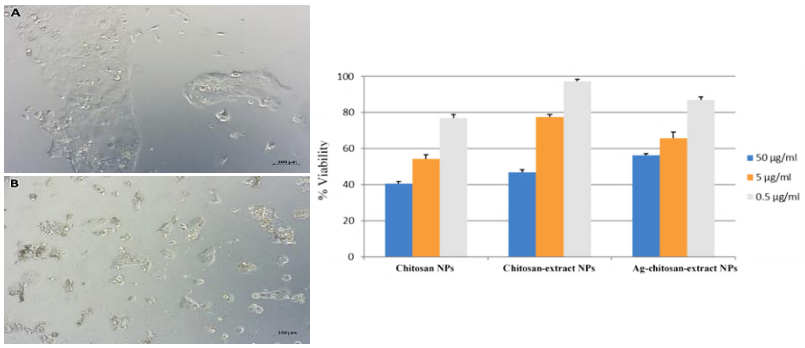

Fig. 5. Cytotoxic activities of nanoparticles on OE33 cell line. [Doxorubicin was used as positive control]
A. Doxorubicin cell image B. Chitosan NPs cell image

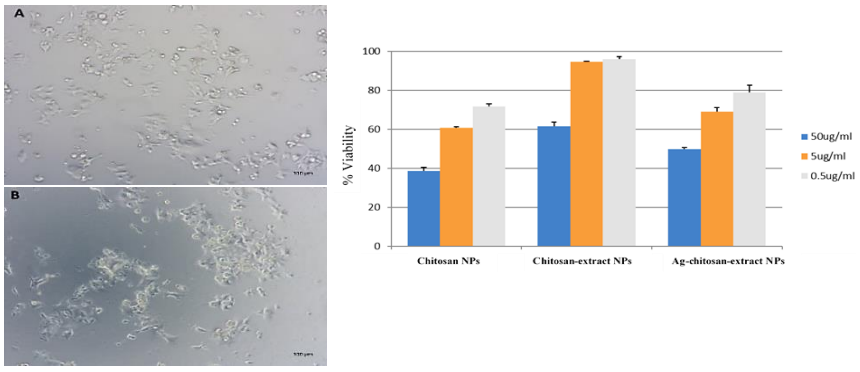

Fig. 6. Cytotoxic activities of nanoparticles on AC 201 cell line. [Doxorubicin was used as positive control], A. Doxorubicin cell image B. Chitosan NPs cell image

4. CONCLUSION

Chitosan, chitosan-extract, Ag-chitosan and Ag-chitosan-extract nanoparticles were prepared, and their structures were characterized by UV-vis, Zeta-sizer, FTIR and SEM. Water extract of *Cephalaria balansea* was used for the synthesis of chitosan nanoparticles by using the ionic gelation technique. The method is simple, cost effective and less time consuming as well as ecofriendly. The ionic gelation technique requires TPP as a crosslinker. To understand the role of TPP in synthesis, the method was also progressed without TPP. The results showed that using TPP increases the stability and homogeneous of nanoparticles. The prepared chitosan nanoparticles were also combined with silver ion to enhance their properties. The data clearly show that silver coated chitosan nanoparticles are smaller in size. The shape of nanoparticles was found spherical shape with the size from 65 nm to 280 nm. Previous studies have shown that nanoparticles with sizes less than 300 nm have a good ability to transport the body [32]. For this reason, those which are bioactive from the synthesized nanoparticles could have potential in biomedical applications. From FTIR spectra, it was revealed that the synthesized nanoparticles are enclosed by chitosan having functional groups of amines, carboxylic acids, alcohols, and esters. Based on cytotoxicity experimental results, only chitosan nanoparticles showed efficient cytotoxic activity against ACC 201 and OE33 cancer cells whereas the chitosan nanoparticles combined with extract or silver did not show significant activity. The results obtained here can be supported by further experimentation. For instance, the cytotoxic potential of chitosan nanoparticles can be investigated under *in vivo* condition. Moreover, pure compounds isolated from plants can be used in the synthesis of nanoparticles instead of using plant extract.

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