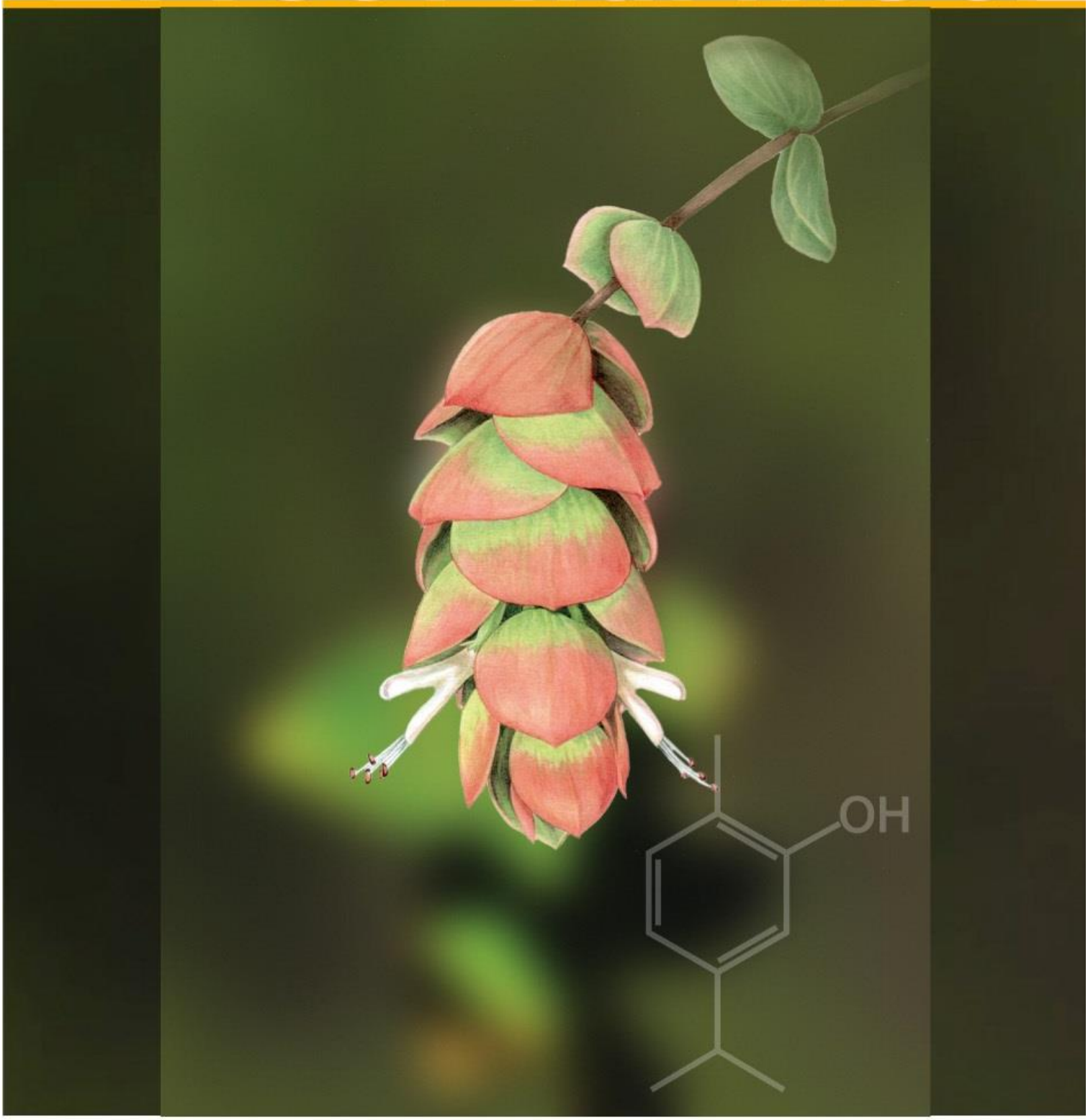
EMU
Journal of
Pharmaceutical
Sciences

EMUJPharmSci



Eastern Mediterranean University

EMU
Journal of
Pharmaceutical
Sciences

EMUJPharmSci

EDITORIAL BOARD

Editors-in-Chief
M. Fethi Şahin & F. Neriman Özhatay

Associate Editors
Mehmet İlktaç & Jale Yüzügülen & H. Ozan Gülcan

Section Editors

Gönül Şahin
Pharmaceutical Toxicology

Müberra Koşar
Pharmacognosy

F. Neriman Özhatay
Pharmaceutical Botany

Gülden Çelik & Mehmet İlktaç
Medical Microbiology

H. Cem Özyurt & Leyla Beba Pojarani & E. Dilek Özyılmaz
Pharmaceutical Technology

H. Ozan Gülcan
Pharmaceutical Chemistry

Emre Hamurtekin
Pharmacotherapy

Aybike Yektaoğlu & E. Vildan Burgaz
Organic and Analytical Chemistry

İmge Kunter
Biochemistry

Tuğba Erçetin
Pharmaceutical Biotechnology

Jale Yüzügülen
Pharmacology

Canan Gülcan
Pharmacoeconomy

Editorial Assistants
Sultan Öğmen Seven & Gizem Kinel & Açelya Mavideniz

Origanum cordifolium* **in cover picture was illustrated by Gülten Yeğenağa

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Advisory/Scientific Board

Prof. Dr. Melih Altan, Bezmialem University, Faculty of Pharmacy, Turkey

Prof. Dr. Ahmet Aydın, Yeditepe University, Faculty of Pharmacy, Turkey

Prof. Dr. Ayla Balkan, Hacettepe University, Faculty of Pharmacy, Turkey

Prof. Dr. Terken Baydar, Hacettepe University, Faculty of Pharmacy, Turkey

Prof. Dr. Berna Özbek Çelik, Istanbul University, Faculty of Pharmacy, Turkey

Prof. Dr. Tansel Ata Çomoğlu, Ankara University, Faculty of Pharmacy, Turkey

Assoc. Prof. Dr. Silvia Dei, University of Florence, Department of Neuroscience, Italy

Prof. Dr. Deniz Songül Doğruer, Gazi University, Faculty of Pharmacy, Turkey

Prof. Dr. Benay Can Eke, Ankara University, Faculty of Pharmacy, Turkey

Prof. Dr. Mustafa Gazi, Eastern Mediterranean University, Faculty of Art and Sciences, TR. North Cyprus

Prof. Dr. Ali Hakan Göker, Ankara University, Faculty of Pharmacy, Turkey

Prof. Dr. Perihan Gürbüz, Erciyes University, Faculty of Pharmacy, Turkey

Prof. Dr. Huriye İcil, Eastern Mediterranean University, Faculty of Art and Sciences, TR. North Cyprus

Prof. Dr. Neşe Kırımer, Anadolu University, Faculty of Pharmacy, Turkey

Prof. Dr. İlkay Küçükgüzel, Marmara University, Faculty of Pharmacy, Turkey

Prof. Dr. Gülden Omurtag, Medipol University, Faculty of Pharmacy, Turkey

Prof. Dr. Feyyaz Onur, Lokman Hekim University, Faculty of Pharmacy, Turkey

Prof. Dr. Ayşe Mine Gençler Özkan,Ankara University, Faculty of Pharmacy, Turkey

Assoc.Prof. Dr. Cristina Salmeri, Palermo University, Scienze Chimiche e Farmaceutiche, Italy

Prof. Dr. Tolga Şahin, Inonu University, Faculty of Medicine, Turkey

Prof. Dr. Mehmet Tanol, Altınbas University, Faculty of Pharmacy, Turkey

Assoc. Prof. Dr. Halil Tekiner, Erciyes University, Faculty of Pharmacy, Turkey

Prof. Dr. Süreyya Ülgen, Biruni University, Faculty of Pharmacy, Turkey

Prof. Dr. Mert Ülgen, Acibadem University, Faculty of Pharmacy, Turkey

Prof. Dr. Elvan Yılmaz, Eastern Mediterranean University, Faculty of Art and Sciences, TR. North Cyprus

Prof. Dr. Osman Yılmaz, Eastern Mediterranean University, Faculty of Art and Sciences, TR. North Cyprus


FACULTY OF
PHARMACY
Eastern
Mediterranean
University
"Virtue, Knowledge, Advancement"
THE WORLD UNIVERSITY RANKINGS 2019 TOP 800
• Top 600-800 in the world
• 7th in Turkey
• Only university from TRNC
www.emu.edu.tr

EMU Journal of Pharmaceutical Sciences
EMUJPharmSci

INSTRUCTIONS TO AUTHORS

EMU Journal of Pharmaceutical Sciences *(EMU JPharmSci)* covers the research on all aspects of Pharmacy presented as original articles, short reports and reviews.

EMU Journal of Pharmaceutical Sciences is published three times (March, July, November) in a year. It is an open access and peer-reviewed journal.

Original articles: These are limited to 15 typewritten pages in addition to supplementary materials (schemes, tables, figures, etc.).

Short papers: Short papers are limited to 5 typewritten pages and maximum of 2 supplementary materials (schemes, tables, figures).

Reviews: They are limited to 20 pages in addition to supplementary materials (schemes, tables, figures, etc.).

Article Submission

1) Contributions to **EMU Journal of Pharmaceutical Sciences** must be in English.
2) You will be guided stepwise through the creation and uploading of various files.

For further information please contact to the editor:
Prof. Dr. F. Neriman Özhatay (Editor in Chief)
Eastern Mediterranean University, Faculty of Pharmacy
Famagusta, North Cyprus
nerimanozhatay@emu.edu.tr nozhatay@istanbul.edu.tr

3) All manuscripts are subject to editorial review.
4) The title, author/authors name, surname, affiliation and address, correspondence address and the type of the article should be written on a separate sheet and attached to the first page of the manuscript.
5) The manuscripts should not be previously published or accepted for publication and should not be submitted or under simultaneous consideration for publication elsewhere.
6) The manuscripts are published in the order of final acceptance after review and revision.
7) If the manuscript is returned to authors for revision and the revised manuscript is not received by the editor within 2 months it will be treated as a new article.
8) If the manuscript is accepted and the proof is returned to the authors, corrected proofs should be sent to the editor within 5 days.

EMU
Journal of
Pharmaceutical
Sciences
EMUJPharmSci

PREPARATION OF THE MANUSCRIPT

In order to achieve uniform presentation and to avoid unnecessary delays, authors are requested to observe the following principles:

The manuscript should be prepared in MS Word format by using Times New Roman font (12 pt.) and double-spaced on one side of the paper with adequate margins (2.5 cm). Original drawings, figures, images etc. must be submitted with the original manuscript.

The original manuscript must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, key words, introduction, materials and methods, results and discussion, acknowledgements and references.

The reviews must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, key words, introduction, discussion, acknowledgements and references.

Pages should be numbered starting from the abstract page. Abbreviations must follow International rules and defined at their first mention in the text. The symbols should be selected in accordance with the international usage and defined where it is first used.

Title Page

Title: Must be short and informative and written in bold uppercase letters.

Authors: Names and surnames of the authors will be written in capitalized letter for the first letter of each word and the address of the author(s) should be linked by superscript numbers, and listed beneath the title. Corresponding author must be indicated (*) in the author names.

Example: Title **(13 pt.)**

HONEY PLANTS OF GUZELYURT (MORPHOU) IN NORTH CYPRUS

Authors (11 pt.)

Neriman Özhatay & Çağın Korkmazer *

Eastern Mediterranean University, Faculty of Pharmacy Famagusta, North Cyprus

Correspondence: E-mail of the corresponding author is written **(10 pt.)**.

cagintheking@gmail.com

Abstract

Briefly give the objectives, methods, results and conclusions in maximum 200 words **(11 pt.)**.

Key words

Authors must give 3- 6 key words which identify the subject covered by the paper **(11 pt.)**.

Introduction

Should indicate the subject of the article which is generally based on a brief interpretation of the related literature. The novelty and the aim of the study should be clearly stated.

EMU
Journal of
Pharmaceutical
Sciences
EMUJPharmSci

Materials and Methods

This part contains a brief and clear description of the materials and methods used. Subtitles can be given as appropriate.

For clinical trials carried on humans by applying drugs, the authors should have the approvals of the related local Ethical Committee. The mentioned approval, the protocol made with the human volunteers and their consent for the studies should be attached and mentioned in this part of the manuscript.

For experimental studies carried on animals, the authors should mention whether the institutional and national guide for care and use of laboratory animals was respected and also indicate the approval of the local Ethical Committee in this part of the manuscript.

For plant materials, herbarium name (or acronym), number, name and surname of the person who identified the plant materials should be indicated in this part of the manuscript.

Statistical analysis of the data and descriptive details of the chemicals used should be explained briefly as a sub-title in this section.

Results and Discussion (separate or together)

The data and results of the research (tables and figures) must be clearly and concisely defined and a comparison with related literature citations should be made as appropriate. Significant findings should be briefly summarized as a conclusion in the last paragraph.

Tables and Figures

Table and Figure titles should be short and informative **(10 pt.)**

Descriptive titles should be given at the top of the tables and at the bottom of the figures. Tables and Figures should be numbered consequently in the order of appearance within the text, referred as "Table 1" and Figure 1

Example:

Table 1. Disturbution of the new records for Turkish flora marked on the province map

Figures should be prepared with the highest resolution and embedded in the manuscript file. During the submission of the manuscript, figures should also be attached as separate files in "TIFF" or "JPEG" format.

Acknowledgements

Supporting institutions or individuals should be briefly acknowledged **(10 pt.)** just before the reference list.

References

Citation in the text should be by the author(s) surname and the publication date.

Examples: (Şahin 2000) – one author

(Şahin and Koşar 2000) – Two authors

(Şahin et al. 2000) – more than two authors

(Çelik and Özhatay 2000a, b) – More than one paper in the same year by the same author (s)

(Özhatay and Avcı 2000; Özhatay et al. 2001; Özhatay 2005) – listed by the earliest year first for multiple citations.

EMU Journal of Pharmaceutical Sciences
EMUJPharmSci

The references must be listed alphabetically in the references section. The names of the journals should be written in italics and volume numbers should be indicated in bold letters **(10 pt.)** Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations. The correctness of the references is belong to the authors.

Examples:

Journal article: Özhatay N, Kültür Ş, Gürdal B (2017). Check-list of additional taxa to the supplement flora of Turkey VIII *.İstanbul,J Pharm 47(1):31-46.*

Article by DOI: Özhatay N, Kültür Ş, Gürdal B (2017). Check-list of additional taxa to the supplement flora of Turkey VIII *.İstanbul,J Pharm* doi: 10.5152 /*İstanbulJPharm.2017.006*..

Book: Cotton CM, (1994).Ethnobotany Principles and Applications .John Wiley and Sons Ltd. England

Book chapter: Bonati A (1988). Industr and conservation of medicinal plants. In Akerele O., Heywood, V. and Synge H. (eds). *The Conservation Medicinal Plants*, p.141-148 Cambridge University Press UK

Dissertation (Thesis): Demirci S (2010). Andırın (Kahramanmaraş) İlçesinde Etnobotanik Bir Araştırma.Unpublished MScThesis (superviser Prof Neriman Özhatay), Istanbul University, Istanbul.

Research Report: Özhatay N, Akalın E, Yeşil Y, Demirci S, Güler N, Ersoy H (2010). Flora of Yıldız Mountains (Yıldız Mountains Biosphere Project Report Series No. 3), Prepared for the Ministry of Environment and Forestry, Ankara.
http://yildizdaglari.cevreorman.gov.tr/medialibrary/2010/07/Flora_full_report_en.pdf.
UNEP-WCMC (2009) Species suggested for review on the basis of the Analysis of 2007 (EC annual reports, SGR 49/8/3), Prepared for the European Commission. UNEP-WCMC, Cambridge.

Electronic resources: (2014) World Nuclear Association. Radioisotopes in Medicine, http://www.world-nuclear.org/info/inf55.html,www.world-nuclear.org/info/inf55.html. Accessed 13.10.2014.
Treglia G, Ceriani L, Sadeghi R, Giovacchini G, Giovanella, L. (2014) Relationship between prostate-specific antigen kinetics and detection rate of radiolabelled choline PET/CT in restaging prostate cancer patients: A meta-analysis, *Cli Chem Lab Med*. http://www.reference-global.com/toc/cclm/current Accessed 16.09.2014

EMU Journal of Pharmaceutical Sciences
EMUJPharmSci

CONTENTS

Investigation of *Streptococcus pyogenes* carriage among pharmacy students in North Cyprus……1
Sahar Haddad, Mehmet Ilktac*, Sultan Ogmen, Gulden Celik

Screening the cholinesterase inhibitory potential of some (1E, 4E)-1.5-diphenylpenta-1.4-dien-3-one derivatives ……7
Acelya Mavideniz, Amirhossein Fallah, Foroogh Koshravi, Farimah Ahdno, Mehmet Arter, Tugba Ercetin, Mustafa Fethi Sahin, Hayrettin Ozan Gulcan*

Outer and inner morphological characteristics of Saturea and Thymbra taxa exported as Oregano from Turkey II 13
Narin Sadıkoglu*, Neriman Ozhatay

Adsorption of iron, lead, paracetamol, imipramine on natural polymers…….33
Gönül Şahin*, Sonia Sanajou, Reihaneh Behnoush, Ehsan Bahramzade

Analgesic nephropathy.....55
Gonul Sahin*, Sonia Sanajou, Hananeh Kordbacheh

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Investigation of *Streptococcus pyogenes* carriage among pharmacy students in North Cyprus

Sahar Haddad, Mehmet Ilktac*, Sultan Ogmen, Gulden Celik

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

Streptococcus pyogenes is one of the most frequently detected bacterial agent of pharyngitis and skin infections that may result in the late complications of rheumatic fever and glomerulonephritis. The aim of the study was to investigate *S. pyogenes* carriage among pharmacy students in a university in Cyprus.

Throat samples were inoculated onto blood agar which was incubated at 37 °C for 48 hours. Gram positive, catalase negative beta hemolytic cocci which were sensitive to bacitracin and resistant to trimethoprim-sulfamethoxazole were identified as *S. pyogenes*.

A total of 140 healthy students were included in the study. 77.1% of students were Iranian, 5% each were Syrian and Iraqi, 4.3% were Nigerian and 8.6% were from other nationalities. Five (3.6%) students, all Iranian, were found to be *S. pyogenes* carriers. 4.6% of Iranian students were determined to carry *S. pyogenes*.

The study is the first study in North Cyprus reporting the low rate of group A beta hemolytic *Streptococcus* carriage in young adults in Turkish Republic of North Cyprus.

Keywords

Carriage, *S. pyogenes*, students, throat.

Article History

Submitted: 11 June 2019 Accepted: 29 July 2019 Published Online: 11 September 2019

Article Info

***Corresponding author:** Mehmet Ilktac, email: mehmet.ilktac@emu.edu.tr

Research Article:

Volume: 2 Issue: 1 September 2019 Pages: 1-6

©Copyright 2019 by EMUJPharmSci – Available online at dergipark.org.tr/emujpharmsci.

INTRODUCTION

Streptococci which are Gram positive cocci arranged in chains or pairs are classified according to their hemolytic characteristics (alpha, beta, gamma) and to the C-polysaccharide present in their cell wall. Although many species of *Streptococcus* are normal flora in various parts of human body, some serogroups of beta hemolytic streptococci, especially group A *Streptococcus* (GAS, *S. pyogenes*) and group B *Streptococcus* (*S. agalactiae*), cause important infections in human. *S. pyogenes* cause pyogenic infections, with a characteristic tendency to spread, as opposed to staphylococcal skin infections which are generally localized. GAS is also responsible for the non-suppurative complications, acute rheumatic fever (ARF) and acute glomerulonephritis (AGN), which may develop following upper respiratory tract and skin infections related with *S. pyogenes*. GAS associated diseases and complications which mainly affect children continue to have devastating effects on public health and the national economy. The prevalence of GAS pharyngitis in school aged children with sore throat was reported to be 37%. In addition to the infection it causes, GAS can also be carried in the upper respiratory tract without any symptoms. 15-32% of school-aged children and 25% of household contacts of children with *S. pyogenes* pharyngitis were reported to be GAS carriers. GAS transmits from person to person with close contact via inhalation of organisms in large droplets or by direct contact with respiratory secretions. Carrying GAS in the throat asymptomatically has been shown to have little or no effect in the development of ARF. Likewise, GAS carriers have been shown to have a minor role in transmitting the disease. However, role of GAS carriage in the development of invasive diseases has not clearly been excluded yet. Twelve of 152 household contacts of patients with invasive GAS infection were reported carry the same strain that had infected the index patient. Eradication of GAS in the carriers is not suggested by health authorities except for those who have familial history of ARF or in the presence of *S. pyogenes* outbreaks (Demuri and Wlad 2014; Martin 2016; Moloi 2015; Davies *et al*. 1996).

Cyprus has recently been an attractive country in Eastern Mediterranean to students for undergraduate and graduate studies of foreign students. Taking into account the variation in the rate of people living in different parts of the world carrying the agents of various infectious diseases, it is inevitable that such an increase in the number of foreign population in a country may lead to the change of the epidemiology of some

infectious diseases locally. To our knowledge, studies related with the carriage of *S. pyogenes* in foreigners and in young adults are limited in Cyprus. The present study was undertaken to determine the rate of GAS carriage among students from different nationalities studying pharmacy in Eastern Mediterranean University in Cyprus.

MATERIALS AND METHODS

Pharmacy students without any clinical symptoms of upper respiratory tract were included in the study. After the informed consent form had been signed, students were requested to fill out a questionnaire in order to determine the demographic characteristics of the students and the presence of any risk factors.

Throat samples were taken from the posterior pharyngeal wall and tonsils using a sterile cotton swab without swabbing the cheeks, tongues, lips or other areas of the mouth. Swabs were immediately inoculated onto Colombia agar containing 5% sheep blood (Biomérieux, France) and media were incubated at 37 °C under the atmosphere containing 5% CO_2 for 48 hours (Spellerberg and Brandt 2015).

After incubation, dome-shaped beta hemolytic colonies, with a diameter of $\geq$ 0.5 mm were further identified by Gram staining, catalase test, 0.1 international unit bacitracin and trimethoprim-sulfamethoxazole sensitivity. Catalase negative beta hemolytic streptococci which were found to be sensitive to bacitracin and resistant to trimethoprim-sulfamethoxazole were identified as *S. pyogenes* (Spellerberg and Brandt 2015).

RESULTS

In total, 140 (Confidence Level (CL) 95%, Confidence Interval (CI) 7.7%) students were included in the study. Of 140 students, 52 (47.1%) and 88 (62.9%) were male and female, respectively. Ages of students were determined to range from 17-42 and the mean age was calculated to be 21. 77.1% of students were Iranian, 5% of each were Syrian and Iraqi, 4.3% were Nigerian and 8.6% were from other nationalities.

Five (3.6%) of 140 students were found to carry *S. pyogenes* in their upper respiratory tract. All of the carriers were found to be Iranian and the rate of the carriage among Iranian students was determined to be 4.6%. Demographical data related to the 5 *S. pyogenes* carriers are given in the Table 1.

Table 1: Demographical data associated with five *S. pyogenes* carriers.

Age/Sex	Living in a crowded home	Hospitalization	Use of antibiotics within 3-6 months	Previous infection with *S. pyogenes*	Contact with someone with *S.pyogenes* infection	Chronic disease	Immunosupression	Heavy smoker	Frequency of hand-washing in a day	Group sports	Sharing clothes and home utensils	Living in dormitory
21/Female	No	No	No	No	No	No	No	No	More than 5 times	basketball	No	No
20/Female	No	No	Yes	No	No	No	No	No	2-4times	No	No	No
20/Female	No	No	No	No	No	No	No	No	2-4times	No	No	No
21/Male	No	No	No	No	No	No	No	yes	2-4 times	No	No	No
23/Female	No	No	No	No	No	No	No	No	2-4 times	No	No	No

DISCUSSION

S. pyogenes is a facultative anaerobic, Gram positive coccus that is responsible for important suppurative infections and non-suppurative complications. Pharyngitis related with *S. pyogenes* is still an important public health problem worldwide with an average of hundred millions of cases annually (Sanyahumbi *et al*. 2016). *S. pyogenes* carriage in children has been well investigated worldwide. In a systematic review done in 2010, the pooled prevalence of *S. pyogenes* carriage in throat samples of asymptomatic children younger than 18 in different countries were reported to be 12% (CL 95%; CI 9–14%) (Shaikh *et al*. 2010). In another systematic review including 5-15 year old children in African countries, the prevalence of carriage was reported to be 6% (CL 95%, CI 6-11%) (Moloi 2015). In a study, including a total of 1893 throat samples from 1-6 year old healthy children in 13 day-care centers, the carriage rate was reported to be 4.8% in Turkey (Sevinc and Enoz 2008). In some other studies, as high as 15–20% of the school aged children who were asymetomatic were reported to be carriers of *S. pyogenes* (Schwartz *et al*. 1981; Shulman 1994). In spite of the presence of many studies investigating the asymptomatic carrier state of GAS in children, data related with the carriage in young adults are limited. In a study performed in Poland on 205 healthy adults between 18 and 44 years old, only three (1.5%) adults were reported to carry GAS (Bura *et al*. 2016). Levy *et al*. (2015) reported the asymptomatic carriage rate of GAS as 9.6%, lower than that of our study, among students aged 18-27 years. In parallel to the result of our study, asymptomatic pharyngeal GAS colonization of adult population was reported to be less than 5% (Spellerberg and Brandt 2015; Bura *et al*. 2016).

In Cyprus, studies related with *S. pyogenes* are very limited with only one study in which the strains isolated from pharyngitis or scarlet fever cases were serotyped and their antibiotic resistance rates were reported (Koliou *et al*. 2007). To our knowledge, the present study is the first study reporting the carriage rate of *S. pyogenes* in young adults in our country.

Although the enrollment of limited number of adults is an important limitation, the present study, together with the finding of low level of carriage rate among young adults, is the first study related with GAS carriage in our region. Large scale studies are needed in the field to clarify the epidemiology of GAS carriage in Cyprus and in Eastern Mediterranean.

REFERENCES

Bura M, Michalak M, Padzik M, Gowin E, Celczyńska-Bajew L, Mozer-Lisewska I (2016). The carriage of potentially pathogenic β-haemolytic streptococci (β-HS) in healthy adult inhabitants of Wielkopolska, Poland. *Fam Med Prim Care Rev* **3**:221-224.

Davies HD, McGeer A, Schwartz B, Green K, Cann D, Simor AE, Low DE (1996). Invasive group A Streptococcal infections in Ontario, Canada. *New Engl J Med* **335**(8):547-54.

Demuri GP, Wlad ER (2014). The group A streptococcal carriage state reviewed: Still an enigma. *J Ped Infect Dis Society* **3**(4):336-42.

Koliou M, Ioannou Y, Efstratiou A, Hannidou N, Pieri V, Alexandrou M, Soteriades ES (2007). Circulating serotypes and antimicrobial sensitivity of *Streptococcus pyogenes* isolates from children in Cyprus. *Clin Microbiol Infect* **13**:645-647.

Levy RM, Leyden JJ, Margolis DJ (2015). Colonization rates of *Streptococcus pyogenes* and *Staphylococcus aureus* in the oropharynx of a young adult population. *CMI* **11**(2):153-155.

Martin J (2016). The *Streptococcus pyogenes* carrier state. In: Ferretti JJ, Stevens DL, Fischetti VA (eds). *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): University of Oklahoma Health Sciences Center.

Moloi HA (2015). Prevalence of group A β-hemolytic Streptococcal carriage in children in Africa: A systematic review. MSc Thesis (supervised by Dr Mark E. Engel and Ms Leila Abdullahi). Cape Town: University of Cape Town. https://open.uct.ac.za/bitstream/handle/11427/16604/thesis_hsf_2015_moloi_annesinah.pdf;sequence=1.

Sanyahumbi AS, Colquhoun S, Wyber R, Carapetis JR (2016). Global disease burden of group A *Streptococcus*. In: Ferretti JJ, Stevens DL, Fischetti VA (eds). *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations [Internet]. Oklahoma City (OK): University of Oklahoma Health Sciences Center.

Schwartz RH, Wientzen RL, Pedreira F, Feroli EJ, Mella GW, Guandolo VL (1981). Penicillin V for group A streptococcal pharyngotonsillitis. A randomized trial of seven vs ten days' therapy. *JAMA* **246**(16):1790-1795.

Sevinc I, Enöz M (2008). The prevalence of group A beta-hemolytic *Streptococcus* in healthy Turkish children in day-care centers in Ankara. *Chang Gung Med J* **31**(6):554-8.

Shaikh N, Leonard E, Martin JM (2010). Prevalence of streptococcal pharyngitis and streptococcal carriage in children: A meta-analysis. *Pediatrics* **126**(3):e557‑64.

Shulman ST (1994). Streptococcal pharyngitis: Diagnostic considerations. *Pediatr Infect Dis J* **13**(6):567-571.

Spellerberg B, Brandt C (2015). *Streptococcus*. In: Jorgensen JH, Pfaller MA, Carrol KC, Landry ML, Funke G, Richter SS, Warnock DW (eds). Manual of Clinical Microbiology. pp:383-402. Washington: ASM Press.

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Screening the cholinesterase inhibitory potential of some (1E, 4E)-1.5-diphenylpenta-1.4-dien-3-one derivatives

Acelya Mavideniz, Amirhossein Fallah, Foroogh Koshravi, Farimah Ahdno, Mehmet Arter, Tugba Ercetin, Mustafa Fethi Sahin, Hayrettin Ozan Gulcan*

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

α,β-unsaturated ketones are particularly important scaffolds to be utilized in diverse organic reactions including the synthesis of many heterocyclics. Regarding their reactivities as electrophiles, their nature to be utilized as drug candidates are quite limited, although there are natural molecules with diverse pharmacological activities whivh are known to have α,β-unsaturated functionalization. Within this preliminary and random drug-screening based medicinal chemistry study, some (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one derivatives were synthesized and their structures were identified employing chromatographic and spectral methods. The potential of the compounds to inhibit acetylcholinesterase and butyrylcholinesterase enzymes was measured employing modified Ellman's method. Although the compounds were not found to be potent inhibitors in comparison to current drugs, their activity spectra and selectivity properties displayed their availability to be utilized as important scaffolds for further design of similar α,β-unsaturated systems.

Keywords

Acetylcholinesterase, butyrylcholinesterase, α,β-unsaturated ketones.

Article History

Submitted: 13 June 2019 Accepted: 29 August 2019 Published Online: 11 September 2019

Article Info

***Corresponding author:** Ozan Gulcan, email: ozan.gulcan@emu.edu.tr

Research Article:
Volume: 2 Issue: 1 September 2019 Pages: 7-12

©Copyright 2019 by EMUJPharmSci – Available online at dergipark.org.tr/emujpharmsci.

INTRODUCTION

Alzheimer's disease (AD) is one of the life-threatening central nervous system diseases affecting ten millions of people worldwide (Selkoe 2001). The major symptoms of the disease include mainly the progressive cognitive decline, also referred to as dementia. Although dementia can appear throughout the scope of various disease states, AD related dementia is the major form (Whitehouse *et al.* 1982). The pathophysiology of the disease is quite complex involving diverse oxidative stress, and neurodegeneration related mechanisms (Kumar and Singh 2015).

So far, numerous scientific research studies have been conducted to discover the exact pathophysiology and epidemiology of AD. However, no single biochemical pathway has solely been attributed to be involved within the generation of the disease. It is known that cholinesterase system, particularly the action of acetylcholine on some muscarinic and nicotinic receptors, is an important tool of cognition, involving personal characteristics, recognition, learning, and daily activities (Ferreira-Vieira *et al.* 2016). Using this information, cholinesterase inhibition mechanism has been suggested to the clinic through the end of the last century to overcome with dementia related symptoms of AD. Indeed, acetylcholine hydrolyzing enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), have been targeted and clinically used current drugs have been obtained. Donepezil, rivastigmine, and galantamine are the currently used cholinesterase inhibitor drugs used for the treatment of cognition symptoms of AD (Deardorff WJ *et al.* 2015). Based on the pharmacokinetic and pharmacodynamic variances among these drugs, there has been a continuous interest on the discovery of novel cholinesterase inhibitor molecules. Regarding this point, within this preliminary random screening base study, we have synthesized some (1E, 4E)-1,5-diphenylpenta-1,4-dien-3-one derivatives and screened their potential to inhibit AChE and BuChE. Within the scope of Organic Chemistry II lectures in Eastern Mediterranean University, pharmacy students exploit from Aldol reaction to learn and practice the synthesis of (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one. Since the methodology employs the reaction between benzaldehyde and acetone, we have used several substituted benzaldehyde derivatives. The title compounds are shown in Figure 1.

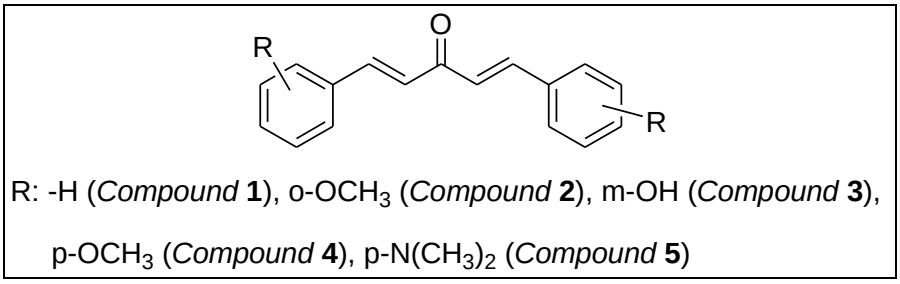


Figure 1: The title compounds synthesized.

MATERIALS AND METHODS

Benzaldehyde, o-anisaldehyde, p-anisaldehyde, 3-hydroxybenzaldehyde, 4-dimethylaminobenzaldehyde, acetone, sodium hydroxide and ethanol were obtained from Sigma Aldrich (CA, USA). Purities of the chemicals were more than 99% as stated on their labels. Therefore, no other purification was conducted on the reagents.

Synthesis of the title compounds

For a typical reaction, 62.5 mmol of sodium hydroxide was dissolved in water-ethanol solution (55:45) in a 100 ml reaction flask. 24.5 mmol of the benzaldehyde derivative and 12.9 mmol of acetone was added to the solution. The reaction was stirred at room temperature for 15 min. The precipitate formed was filtered off and washed with acidified aqueous.

Structure identification and characterization

The reactions were monitored employing Thin Layer Chromatography (TLC) (Alugram Xtra SIL G/UV_{254} 0,2 mm silica gel 60 with fluorescent indicator from Germany) with an n-hexane ; ethyl acetate (1:1) mobile phase. The infrared spectra of the compounds were obtained with a Shimadzu FT-IR Prestige Infrared Spectrophotometer. The ^{1}H-NMR and ^{13}CNMR spectra of the compounds were obtained using a Bruker 400 NMR Spectrophotometer. Trimethylsilane was used as internal standard and DMS-d6 as solvent.

(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (**compound 1**): Yield 85%, solid yellow crystals, mp 125°C (uncorrected data). IR, 3055 (ArC-H), 1648 (-C=O). ^{1}HNMR, 7.87 ppm (d, HC=CH-Ar), 7.81 ppm (dd, Ar-H, o), 7.48 ppm (dd, Ar-H, m), 7.42 ppm (s, Ar-H, p), 7.38 ppm (d, -CH=CH-Ar). ^{13}CNMR, 188ppm (C=O).

(1E,4E)-1,5-bis(2-methoxyphenyl)penta-1,4-dien-3-one (**compound 2**): Yield 87%, solid pale yellow crystals, mp 134°C (uncorrected data). IR, 3027 (ArC-H), 1668 (-C=O). ^{1}HNMR, 7.93 ppm (d, -CH=CH-Ar), 7.81 ppm (d, Ar-H, o), 7.42 ppm (t, Ar-H, p), 7.28 ppm (d, Ar-H, m), 7.09ppm (d, Ar-H, o′), 7.09ppm (d, -CH=CH-Ar). ^{13}CNMR, 188ppm (C=O).

(1E,4E)-1,5-bis(3-hydroxyphenyl)penta-1,4-dien-3-one (**compound 3**): Yield 89%, solid brown crystals, mp 136°C (uncorrected data). 3075 (ArC-H), 1620 (-C=O). ^{1}HNMR, 9.74 ppm (s, Ar-O<u>H</u>), 7.79 ppm (d, -CH=<u>CH</u>-Ar), 7.35 ppm (t, Ar-H, m), 7.31 ppm (d, Ar-H, o), 7.28 ppm (d, -<u>CH</u>=CH-Ar), 7.25 ppm (d, Ar-H, p), 6.95 ppm (d, Ar-H, o). ^{13}CNMR, 188ppm (C=O).

(1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (**compound 4**): Yield 81%, solid yellow crystals, mp 128°C (uncorrected data). 3033 (ArC-H), 1648 (-C=O). ^{1}HNMR, 7.73 ppm (d, -CH=<u>CH</u>-Ar), 7.68 ppm (d, Ar-H, o), 7.16 ppm (d, -<u>CH</u>=CH-Ar), 6.99 ppm (d, Ar-H, m), 3.79 ppm (s, Ar-O<u>CH_3</u>). ^{13}CNMR, 188ppm (C=O).

(1E,4E)-1,5-bis(4-(dimethylamino) phenyl)penta-1,4-dien-3-one (**compound 5**): Yield 85%, solid orange crystals, mp 141°C (uncorrected data). 3038 (ArC-H), 1634 (-C=O).). ^{1}HNMR, 9.69 ppm (s, N-H), 7.69 ppm (d, -CH=<u>CH</u>-Ar), 7.60 ppm (d, Ar-H, o), 7.05 ppm (d, -<u>CH</u>=CH-Ar), 6.74 ppm (d, Ar-H, m), 3.48 ppm (s, N-<u>CH_3</u>). ^{13}CNMR, 188ppm (C=O).

Determ*ination of AChE and BChE inhibitory activities*

The modified spectrophotometric method of Ellman was used to determine AChE and BuChE inhibitory activities of 5 compounds synthesized (Gulcan *et al.* 2014). The enzymes used for cholinesterase activity studies were, electric eel AChE (eeAChE) (Sigma) and equine BuChE (Sigma). 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 cholinesterase activity. 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 2 µl of sample solutions and 10 µl of AChE/BChE solution were added in a 96-well microplate. The reaction was then initiated with the addition of 10 µl of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (Varioskan Flash, Thermo Scientific, USA) and incubated for 15 min at 27°C. The measurements and calculations were evaluated by using SkanIt Software 2.4.5 RE for Varioskan Flash software. Percentage of inhibition of AChE and BuChE were determined by comparison of the rates of reaction of samples relative to blank sample (methanol) using the formula (E-S)/E x 100, where E is the activity of enzyme without the test sample and S is the activity of enzyme with the test sample. The experiments were

done in triplicate. Donepezil hydrochloride and rivastigmine were used as reference compound. The percent inhibition at 100 and 50 µM was obtained for each test compound with standard compounds.

RESULTS AND DISCUSSION

The general synthetic scheme of the title compounds is shown in the Figure 2. Employing the general synthetic scheme (1E, 4E)-1,5-diphenylpenta-1,4-dien-3-one derivatives were synthesized. Following the structure identification studies, the compounds were screened for their potential to inhibit AChE and BuChE enzymes. The results obtained are displayed the Table 1.

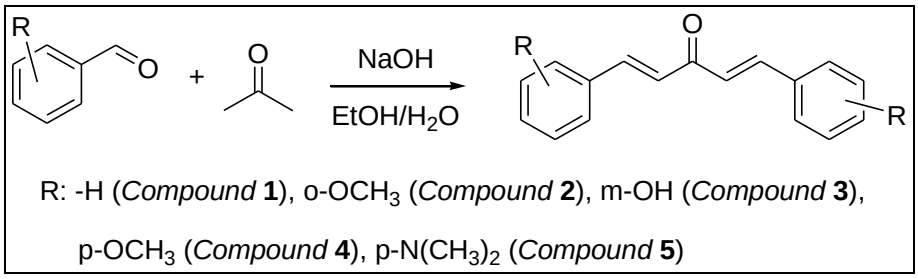


Figure 2: The general synthetic scheme and the title compounds.

Table 1: The potential the title compounds to inhibit AChE and BuChE enzymes.

Compounds	% Inhibition (AChE) 50µM	% Inhibition (AChE) 100µM	% Inhibition (BuChE) 50µM	% Inhibition (BuChE) 100µM
Compund 1	36.54 ± 0.33	54.2 ± 0.23	45.90 ± 0.29	58.6 ± 0.41
Compund 2	11.1 ± 0.04	25.6 ± 0.09	21.52 ± 0.04	36.85 ± 0.04
Compund 3	29.77 ± 0.36	45.04 ± 0.37	49.65 ± 0.31	68.11 ± 0.72
Compund 4	29.01 ± 0.12	44.37 ± 0.28	25.76 ± 0.13	39.37 ± 0.91
Compund 5	14.51 ± 0.07	24.75 ± 0.14	18.31 ± 0.08	39.66 ± 0.21
Rivastigmine	65.02 ± 0.11	76.12 ± 0.06	78.31 ± 0.03	83.61 ± 0.09
Donepezil	91.20 ± 0.17	94.19 ± 0.10	83.75 ± 0.04	88.22 ± 0.62

According to the results, the title compounds were not found to be superior to the currently used drugs donepezil and rivastigmine, which were already used as references in this study. However, each title compound displayed activity for both AChE and BuChE. Although it is not apparent for each title molecule, a tendency for more inhibition of BuChE particularly for compounds 2 , 3, and 5, was identified, it is noteworthy to state that it is very critical to identify IC_{50}s of the title compounds to

further proof this observation. Besides, the results were primitive to describe a structure activity relationship study. In other words, the (1E, 4E)-1,5-diphenylpenta-1,4-dien-3-one main moiety was obtained important for activity, but no net result was observed depending on the substitutions followed. From this point of view, the results of the study indicated that this scaffold stands a good candidate to design novel (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one derivative potent cholinesterase inhibitors. However, more data related to the number of more substitutions and concomitant IC_{50} are needed to explore both the molecule-receptor interactions and a concessive structure activity relationship studies.

ACKNOWLEDGEMENTS

This study was conducted employing the research facilities of Eastern Mediterranean University, Faculty of Pharmacy. It is important to note that the study published here belongs to the Graduation Thesis Projects of three EMU pharmacy students (Foroogh Koshravi, Farimah Ahdno, and Mehmet Arter) supervised by Assoc. Prof. H. Ozan Gulcan. The authors declare no conflict of interest. The title compounds are not original and only used for the thesis studies of the aforementioned students.

REFERENCES

Deardorff WJ, Feen E, Grossberg GT (2015). The use of cholinesterase inhibitors across all stages of Alzheimer's disease. *Drugs Aging* **32**(7): 537-547.

Ferreira-Vieira H, Guimaraes TM, Silva IR, Ribeiro F (2016). Alzheimer's disease: targeting the cholinergic system. *Cur Neuropharmaco* **14**(1): 101-115.

Gulcan HO, Unlu S, Esiringu I, Ercetin T, Sahin Y, Oz D, Sahin MF (2014). Design, synthesis and biological evaluation of novel 6H-benzo [c] chromen-6-one, and 7, 8, 9, 10-tetrahydro-benzo [c] chromen-6-one derivatives as potential cholinesterase inhibitors. *Bioorg Med Chem* **22**(19): 5141-5154.

Kumar A, Singh A (2015). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep* **67**(2): 195-203.

Selkoe DJ (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81**(2): 741-766.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**(4537): 1237-1239.

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Outer and inner morphological characteristics of *Saturea* and *Thymbra* taxa exported as Oregano from Turkey II

Narin Sadikoglu[1*], Neriman Ozhatay[2]

[1] Inönü University, Faculty of Pharmacy, Department of Pharmacognosy, Malatya, Turkey.

[2] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

Oregano, a collective name of a group of taxa that has been used as spice. Different genera and species of the family Lamiaceae that contain thymol and carvacrol are being used as oregano. In Turkey, there are 9 taxa that belong to 3 genera which are being exported as oregano. In the outer and inner morphological characteristics of the taxa *Satureja thymbra* L., *Satureja cuneifolia* Ten., *Thymbra capitata* (L.) Cav. and *Thymbra spicata* L. subsp. s*picata* which are exported as oregano have been reported. Distribution maps of the four taxa have been presented based on the examined specimens kept in the 15 herbaria. The specimens are listed in the appendix and local usage and vernacular names are shown as tables.

Keywords

Anatomy, morphology, oregano, *Satureja, Thymbra*, Turkey.

Article History

Submitted: 9 July 2019 Accepted: 30 July 2019 Published Online: 11 September 2019

Article Info

***Corresponding author:** Narin Sadikoglu email: narin.sadikoglu@inonu.edu.tr

Research Article:

Volume: 2 Issue: 1 September 2019 Pages: 13-32

©Copyright 2019 by EMUJPharmSci – Available online at dergipark.org.tr/emujpharmsci.

INTRODUCTION

Oregano is a collective name of a group of taxa in which different genera and species of the family *Lamiaceae* that contain thymol and carvacrol are present. These taxa are used as spice and known as "kekik" in Turkish and are very important exported plants in Turkey. Oregano has been used in Anatolia since the 7th century BC as a spice and for medical treatment (Sadikoglu and Ozhatay 2015). Various studies observed the position of these plants in nature and the place of oregano in the trade (Kirimer *et al*. 2003; Duzenli and Karaomeroglu 2003; Tumen *et al.* 2003; Gemici 2003). Oregano species are collected in Turkey and their trade is an important source of income.

In Turkey, 9 taxa are being exported as oregano. These are: *Origanum minutiflorum* O. Schwarz & P.H. Davis, *O. majorana* L., *O. onites* L., *O. syriacum* L. subsp. *bevanii* (Holmes) Greuter & Burdet, *O. vulgare* L. subsp. *hirtum* (Link) Ietsw., *Satureja thymbra* L., *S. cuneifolia* Ten., *Thymbra capitata* (L.) Cav. (Syn. *Coridothymus capitatus* (L.) Rchb.f.) and *T. spicata* L. subsp. *spicata*. The taxa that belong to the genus *Origanum* are round-leafed where as those that belong to *Satureja* and *Thymbra* are acute-leafed. The major genus *Origanum* taxa have recently been published (Sadikoglu and Ozhatay 2015). In this paper, outer and inner morphological characteristics of exported *Satureja* and *Thymbra* taxa have been presented together with their local name, usage and distribution in Turkey. *Coridothymus* was represented by only one taxon in Turkey. It was a monotypic genus but according to the latest systematic changes *Coridothymus capitatus* (L.) Rchb.f. is called as *Thymbra capitata* (L.) Cav. (Güner *et al.* 2012). Flavonoid analysis supports the close relationships of a taxon, such as the *Coridotyhmus capitatus* taxa, also known as *Thymus capitatus* (L.) Hoffmgg. & Link and *Thymbra capitata* (L.) Griseb. According to micro-morphological and caryological data, it has been suggested to be named as *Thymbra capitate*. The presence of flavonoid aglycones in this taxon is the same as those of other *Thymbra* species, but different from those of the *Thymus* species (Barberan and Gil 1992). According to another study that investigates difference between *Thymus* and *Thymbra* species, it was determined that the naming as *Thymbra capitata* was not accurate because of the convexity of the calyx and the absence of the 13-veins in the calyx (Tanker and Ilisulu 1981). In Turkey, the genus *Satureja* is represented by 15 taxa and 5 of them are endemic. The genus *Thymbra* is represented by 5 taxa and 1 of them is endemic (Guner *et al.* 2012). Endemic or rare taxa are also sold in the open markets for various usages.

MATERIALS AND METHODS

Flowering and fruiting specimens were collected in Adana, Antalya, Balıkesir, Burdur, Canakkale, Hatay, Isparta, Izmir, Kirklareli, Malatya, Mersin, Mugla, Van and Yalova provinces during June-September periods in 2001-2004. Specimens were identified by Narin Sadikoglu and kept in the Herbarium of Istanbul University Faculty of Pharmacy (ISTE).

ADA, AEF, ANK, EGE, ESSE, GAZI, HUB, ISTE, ISTF, IZEF, MARA, MARE, MUFE, VANF and INU herbaria were visited. The taxa that are exported and used as oregano in Turkey were investigated in detail. All examined specimens were presented in the appendix. Oregano specimens collected and fixed in 70% ethanol since 1996 were the material of the anatomical part which are housed in ISTE.

Morphological drawings were made by Olympus SZH10 stereomicroscope. Anatomical drawings were made by Leitz SM-LUX binocular microscope with Leitz Weitzler drawing tube. Photographs were taken by Olympus BH2 trinocular photomicroscope. Anatomical sections were taken by hand from the leaves of the plants. Leaves are cleared by using 15% sodium hypochlorite for 15 minutes. Sections were stained in 1% safranin and mounted in chloral hydrate: glycerol: water (8:2:1). For transverse sections, leaves were placed in Jeffrey's solution (10% aqueous nitric acid and 10% aqueous chromic acid [1:1]) for 1 week at room temperature until the epidermis began to separate (Berlyn and Miksch 1976). Segments of epidermis with the attached outermost cell layer of cortical parenchyma were collected, washed in water and the tissue is mounted.

Stomata amount per mm^2 were established and stomata index both upper and lower surfaces were calculated for using the formula of SI = (Stomata amount / Epiderm cell + Stomata amount) x 100. Averages of the counts were determined for 10 specimens obtained from 10 plants for 10 views of each. Palisade ratio and areoles per mm^2 were also established for the preparations of powdered oregano (Sener *et al.* 1985).

RESULTS

According to our observations, the exported *Satureja* and *Thymbra* taxa were distributed in the west, south and north of Turkey and they are used as spice, herbal tea and remedies. Local usages of each taxa are listed in the Table 1.

Table 1: Local names and usage of *Satureja* and *Thymbra* taxa that are exported as oregano.

Scientific name	**Local name**	**Local usage**
Satureja thymbra	Sahil sivrisi, kılıç kekiği, sivri kekik, taş kekiği	Spice
S.cuneifolia	Sivri kekik, kılıç kekik, yayla kekiği, dağ kekiği, aş kekiği, taş kekiği, kaya kekiği, çorba kekiği, yabani kekik	Oil, spice, herbal tea
Thymbra capitata	Timari, sivri kekik	Spice, herbal tea
T. spicata subsp. *spicata*	Zahter, sivri kekik, at kekiği, mor kekik, aş kekiği	Skin disorders, sedative, analgesic, cold, antitussive, antirheumatismal, hyperglisemia, antihypertansive, hypercholesterolemia, gastrointestinal disorders, fuel, spice, herbal tea

Outer morphological characteristics and distribution

1. *Satureja thymbra* L.

Type: Described from Crete (Hb. Linn. 723/2 photo!).

Main diagnostic morphological characteristics are:

Bracteoles ovate, acuminate-aristate, long-ciliate; leaves linear- to ovate-spathulate; calyx actinomorphic; corolla mauve or purple, 8-12 mm (Figure 1). The distrubution of *Satureja thymbra* is shown in Map 1.

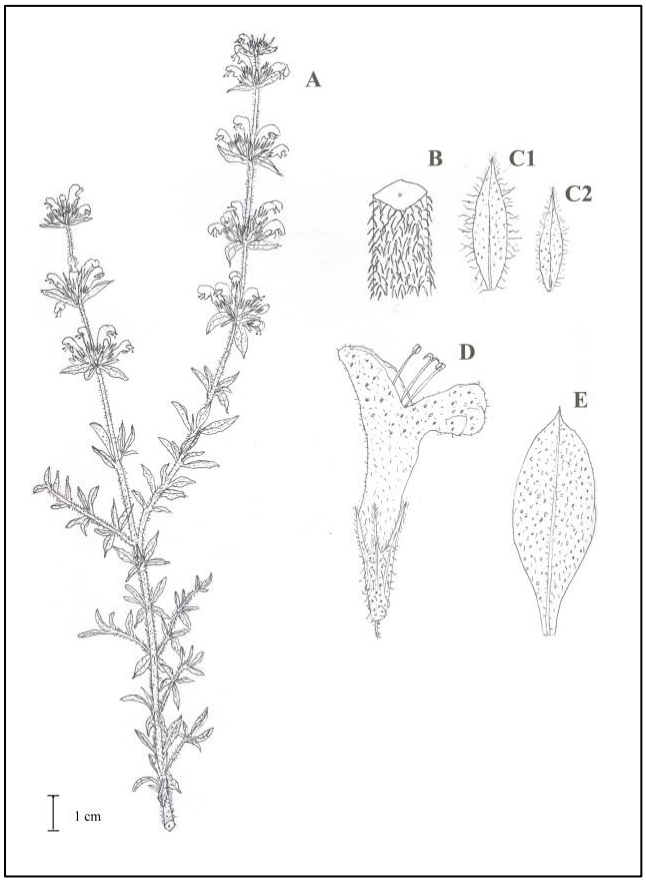
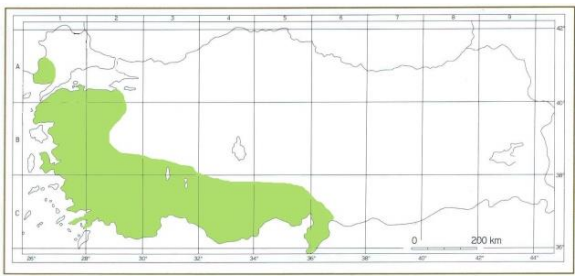


Figure 1: *Satureja thymbra* (ISTE 19137). A. general view, B. stem, C. bract, D. flower, E. leaf.



Map 1: Distribution of *Satureja thymbra* in Turkey.

Flowering period: April-July

Habitat: Dry scrub, especially calcareous phrygana, sl.-400 m.

Distribution in Turkey: West and South Turkey (Map 1)

General distribution: Sardinia, Greece, Aegean, W. Syria, Cyprus, Cyrenaica.
East Mediterranean element.

2. *Saturea cuneifolia* Ten.

Type: Described from Italy (holo. NAP).

Main diagnostic morphological characteristics are:

Bracteoles linear, not ciliate; leaves cuneate towards base, broadest towards apex; calyx sub-bilabiate nearly to middle; corolla mostly white, 6-8 mm (Figure 2). The distrubution of *Satureja cuneifolia* is shown in Map 2.

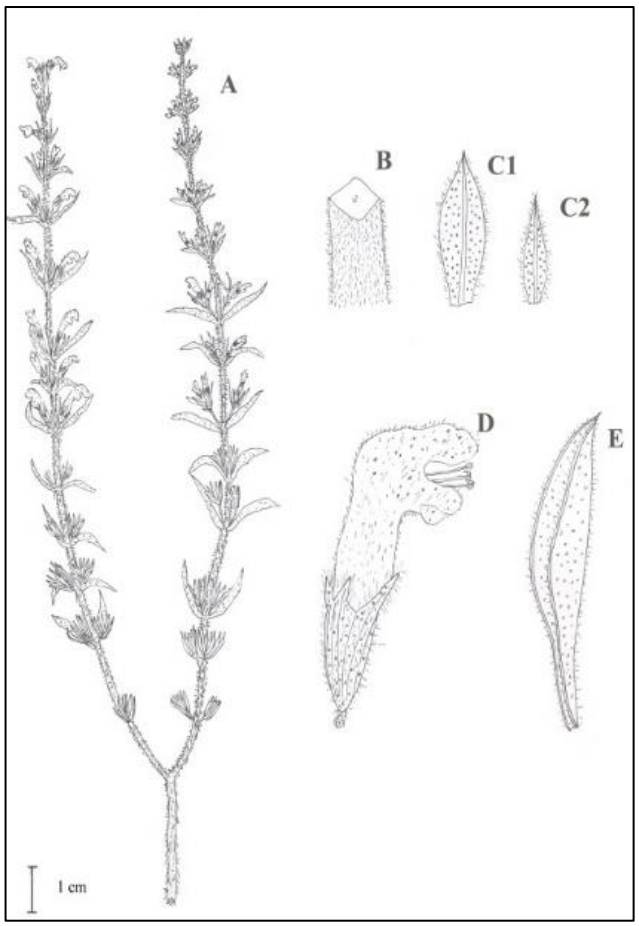
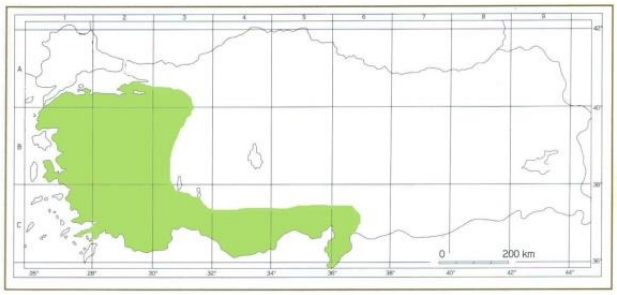


Figure 2: *Satureja cuneifolia* (ISTE 52634). A. general view, B. stem, C. bract, D. flower, E. leaf.



Map 2: Distribution of *Satureja cuneifolia* in Turkey.

Flowering period: July-August

Habitat: Rocky slopes, on schist and limestone, cliffs, 300-2000 m.

Distribution in Turkey: West and South Turkey (Map 2)

General distribution: Spain, Italy, Jugoslavia, Albania, Greece, Lebanon, N. Iraq, Turkey. Mediterranean element.

3. *Thymbra capitata* (L.) Cav. Syn. *Coridothymus capitatus* Rchb.f.

Type: Described from Baetica (Andalucia), Crete, Hispalis (Seville) and Greece (Hb. Linn. 723/11 photo!).

Main diagnostic morphological characteristics are:

Calyx tube dorsally compressed, with two ciliolate flanges. Inflorescence capitate; calyx 20-22-veined; leaves 4-10 mm, subtriquetrous (Figure 3). The distrubution of *Thymbra capitata* is shown in Map 3.

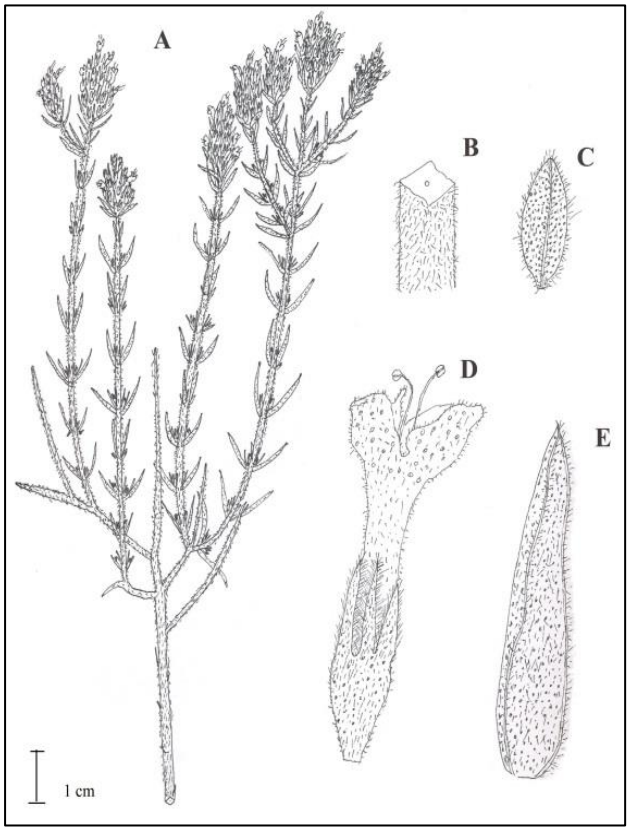
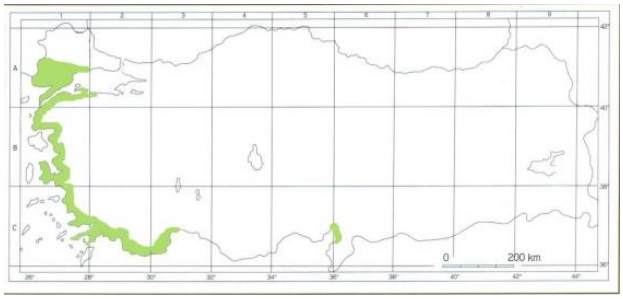


Figure 3: *Thymbra capitata* (L.) Cav. (ISTE 81671).
A. general view, B. stem, C. bract, D. flower, E. leaf.



Map 3: Distribution of *Thymbra capitata* in Turkey.

Flowering period: May-October

Habitat: Costal phrygana, open macchie, s.l.-1400 m.

Distribution in Turkey: West Turkey, South Anatolia (Map 3)

General distribution: Greece, Italy, Turkey.
Mediterranean element.

4. *Thymbra spicata* L. subsp. *spicata*

Type: Described from Macedonia: Libano (Hb. Linn. 724/1 photo!).

Main diagnostic morphological characteristics are:

Calyx tube dorsally compressed, with two ciliolate flanges. Inflorescence spicate; calyx 13-veined; leaves to 20 mm, conduplicate (Figure 4). The distrubution of *Thymbra spicata* is shown in Map 4.

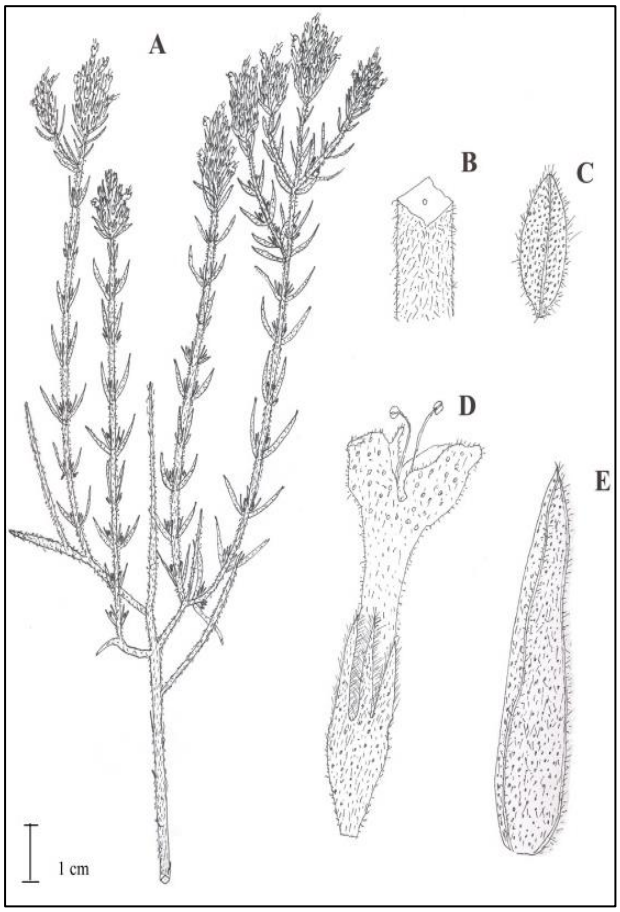
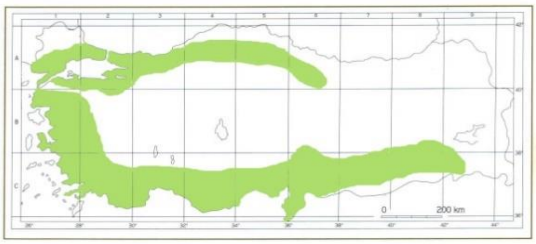


Figure 4: *Thymbra capitata* (L.) Cav. (ISTE 81671).
A. general view, B. stem, C. bract, D. flower, E. leaf.



Map 4: Distribution of *Thymbra spicata* subsp. *spicata* in Turkey.

Flowering period: June-July

Habitat: Dry often rocky places (usually calcareous), in scrub, phrygana, steppe, s.l.-1000 m.

Distribution in Turkey: North, West and South Turkey, SE Anatolia (Map 4)

General distribution: Greece, Aegean, Cyprus, W. Syria, N. Iraq.
East Mediterranean element.

Inner morphological characteristics of leaves

1. *Satureja thymbra* L.

On the superficial section, both upper and lower epidermal cells are straight. Hairs have cuticular patterns, 1-2 celled, contain crystals very densely. Labiatae type glandular hairs per cm^2 are 400-(630)-1200 on epidermis. Stomata occur on both upper and lower epidermis (amphistomatic) that are usually in same density. Stomata amount is established per mm^2 as 800-(1090)-1500 on the lower surface and as 500-(850)-1500 on the upper surface. On the cross-section, guard cells are on the same level with the epidermis cells (mesomorphic stomata). Subsidiary cells are 2. Stomata index is established 95.11 for the lower surface and 94.33 for the upper surface. Palisade ratio in powdered specimens is established 4.5, areoles per mm^2 is 7. Under the upper epidermis 4-5 layered collenchyma tissue is present (Figure 5).

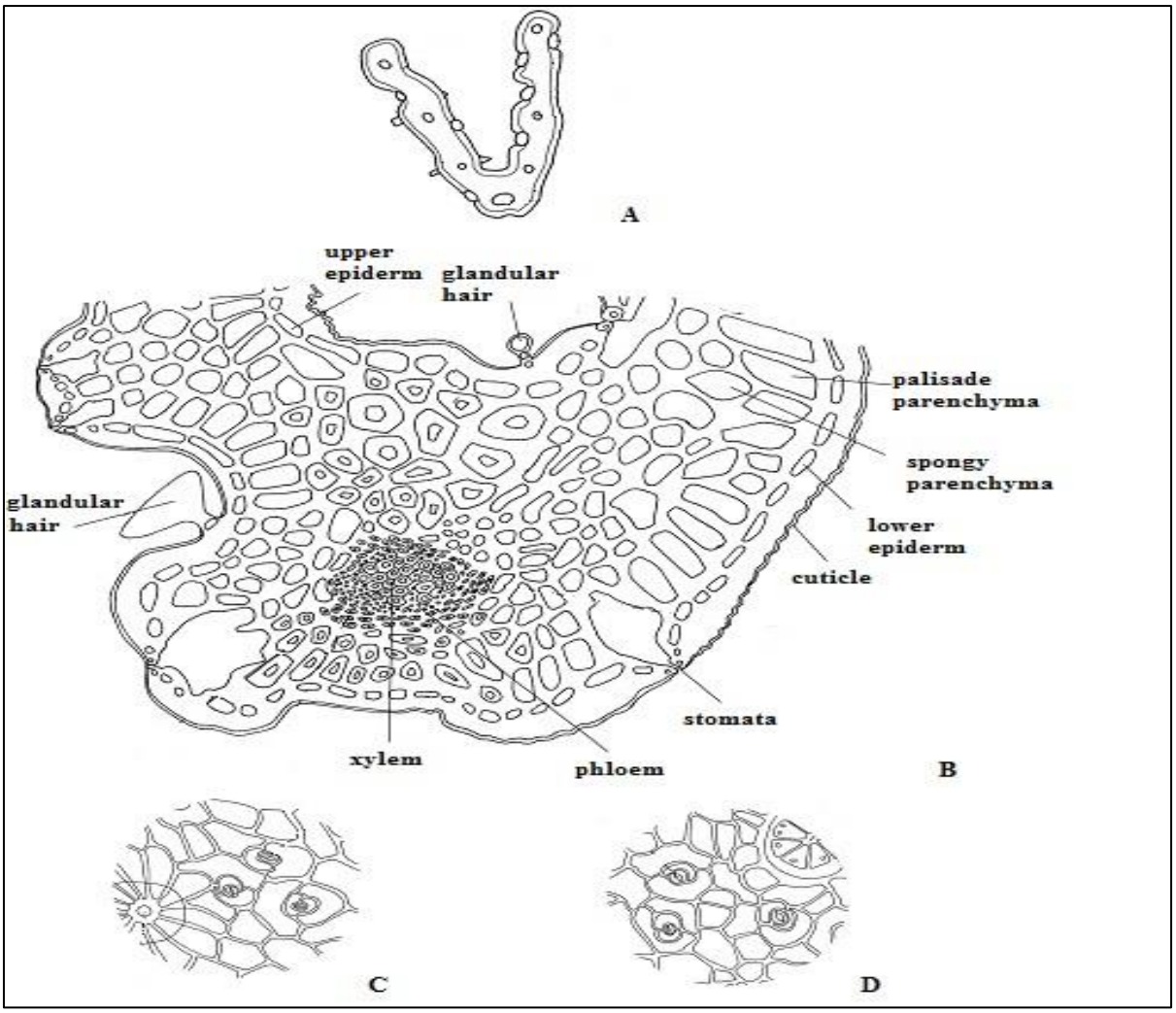


Figure 5: *Satureja thymbra* (Narin/kekik/051). A. Cross-section of leaf (x80), B. Middle vessel (x450), C. Lower surface of epiderm (x225), D. Upper surface of epiderm (x225).

2. *Satureja cuneifolia* Ten.

On the superficial section upper epidermal cells are straight, lower ones are slightly undulate. Hairs are 1-4 celled, contain crystals very densely. Labiatae type glandular hairs per cm^2 are 400-(710)-1000 on epidermis. Stomata occur on both upper and lower epidermis (amphistomatic) and are denser on the upper surface. Stomata amount is established per mm^2: 600-(1030)-1700 on the lower surface and, 1300-(2120)-3200 on the upper one. On the cross-section, guard cells are on the same level with the epidermis cells (mesomorphic stomata). Subsidiary cells are 2, rarely 3, very rarely 4. Stomata index is established 92.29 for the lower surface and as 96.14 for the uppers. Palisade ratio in powdered specimens is established as 3.35 and areoles per mm^2 is 5.17 (Figure 6).

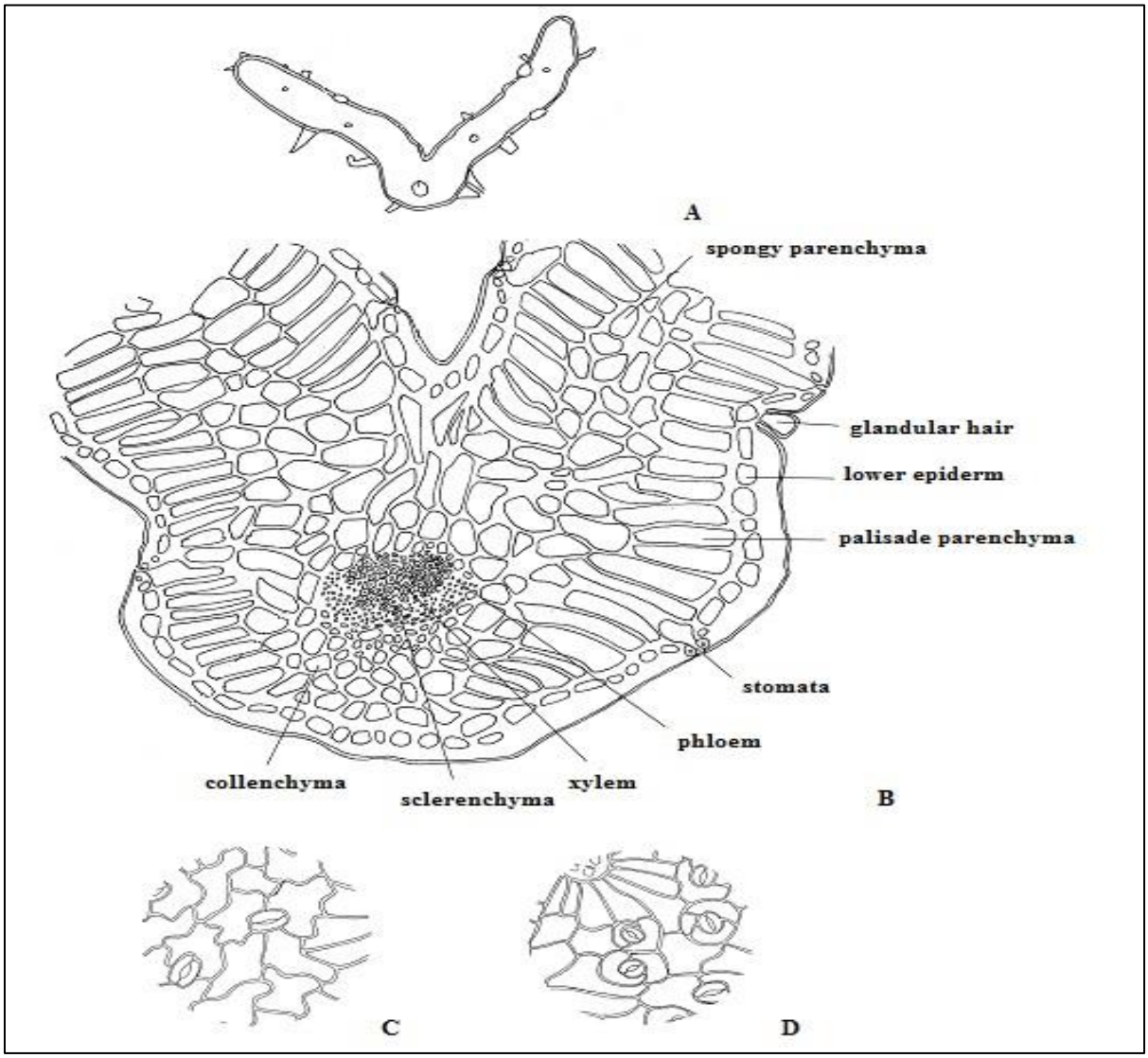


Figure 6: *Satureja cuneifolia* (Narin/kekik/051). A. Cross-section of leaf (x80), B. Middle vessel (x450), C. Lower surface of epiderm (x225), D. Upper surface of epiderm (x225).

3. *Thymbra capitata* (L.) Cav.

On the superficial section both upper and lower epidermal cells have straight walls. Epidermis cells of the upper surface are slightly bigger than those of the lower surface. Hairs have cuticular patterns, are 1-3 celled, and contain crystals very densely. Labiatae type glandular hairs per cm^2 are 400-(590)-1200 on epidermis. Stomata occur on both upper and lower epidermis (amphistomatic) and are denser on the upper surface. Stomata amount is established per mm^2 as 300-(1070)-1600 on the lower surface and as 600-(1230)-1700 on the upper surface. On the cross-section, guard cells are on the same level with the epidermis cells (mesomorphic stomata). Subsidiary cells are 2. Stomata index is established as 92.48 for the lower surface and 92.76 for the uppers. Palisade parenchyma is 1-layered and spongy parenchyma is 1-3 layered. Palisade ratio in powdered specimens is established as 3.8, areoles per mm^2 is 4.25. Below the lower and upper epidermis, 1-layered palisade parenchyma and 1-3 layered irregular parenchyma cells are present. A few collenchyma cells were observed on the lower surface (Figure 7).

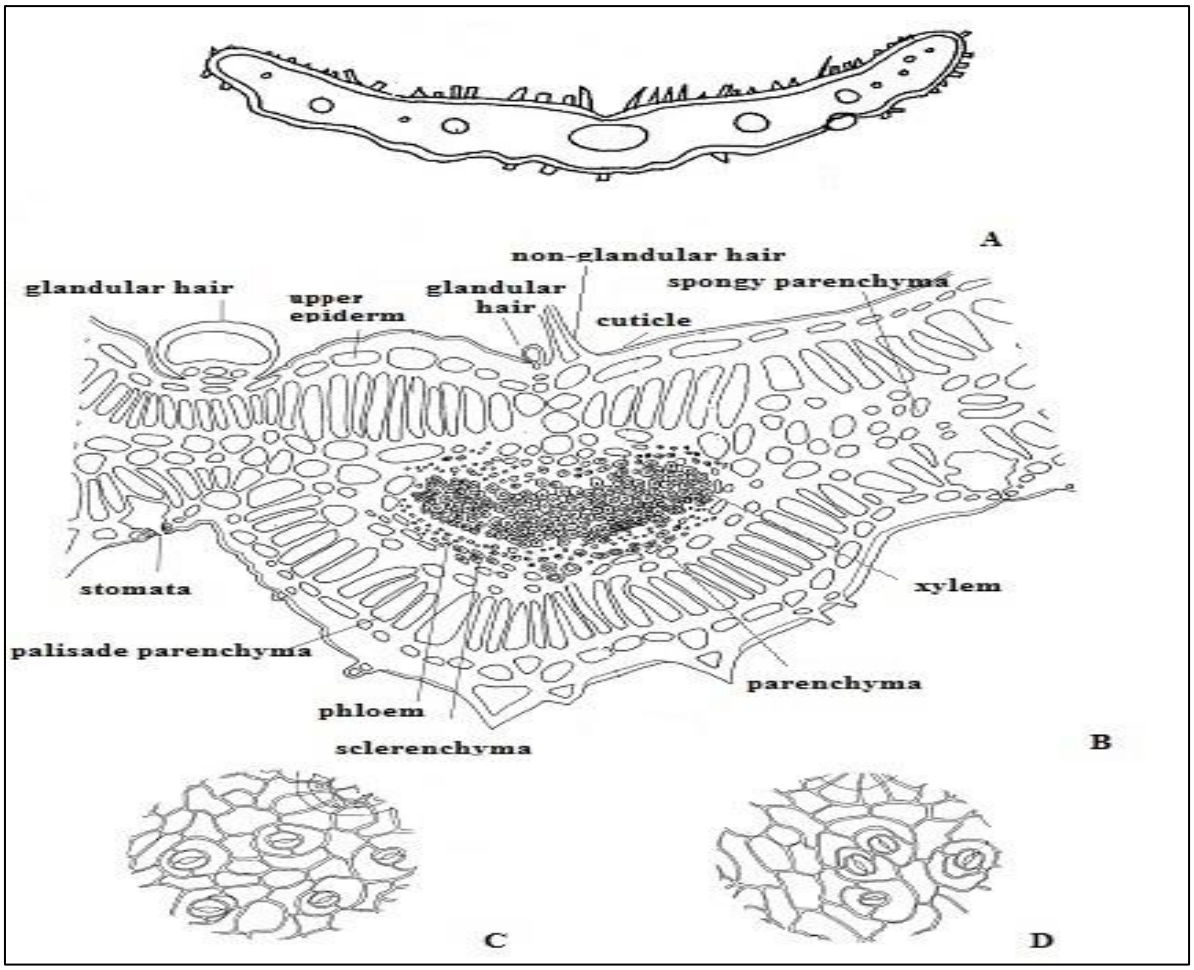


Figure 7: *Thymbra capitata* (Narin/kekik/097). A. Cross-section of leaf (x60), B. Middle vessel (x450), C. Lower surface of epiderm (x225), D. Upper surface of epiderm (x225).

4. *Thymbra spicata* L. subsp. *spicata*

On the superficial section, the walls of the upper epidermal cells are thicker than those of lower epidermal cells and both of them are straight. Epidermis cells of the upper surface are bigger than the lowers. Hairs have cuticular patterns, are 1-3 celled, and contain crystals very densely. Labiatae type glandular hairs per cm^2 are 1600-(2150)-2700 on epidermis. Stomata occur on both upper and lower epidermis (amphistomatic) that are denser on the upper surface. Stomata amount is established per mm^2 as 700-(1190)-1700 on the lower surface and, 400-(1180)-2100 on the upper one. On the cross-section, guard cells are upper than the epidermis cells (higromorphic stomata). Subsidiary cells are 2. Stomata space is evident. Stomata index is established as 95.35 for the lower surface and as 94.93 for the uppers. Palisade parenchyma is 1-layered, spongy parenchyma is 1-2 layered. Palisade ratio in powdered specimens is established as 4.8, areoles per mm^2 is 3.85. Below the lower and upper epidermis, 1-layered palisade parenchyma and 1-3 layered irregular parenchyma cells are present. Less sclerenchymatous tissue occur on the upper and lower of midrib. Tracheary elements and thin walled parenchyma cells between them are on the xylem (Figure 8).

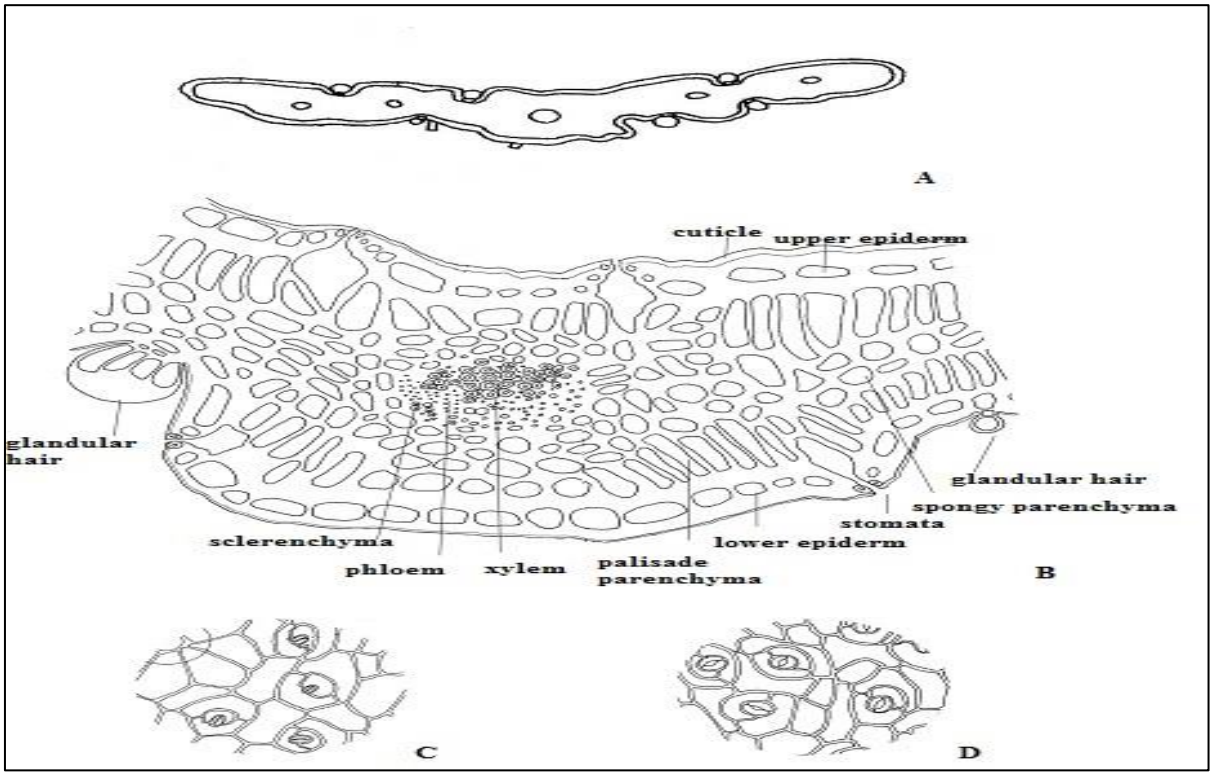


Figure 8: *Thymbra spicata* L. subsp. *spicata* (Narin/kekik/053). A. Cross-section of leaf (x75), B. Middle vessel (x450), C. Lower surface of epiderm (x225), D. Upper surface of epiderm (x225).

DISCUSSION

The findings of studied taxa are in compatible with the literature (Kaya *et al.* 1994; Tanker and Ilisulu 1981; Erken 2001). The *Thymbra spicata* subsp. *spicata* taxa collected from many populations were shown to reveal high morphological polymorphism. It is determined that these are not local. The differences of these taxa would be clarified by biosystematical investigations. Leaves are isobilateral. In all species lateral veins are not prominent. Anatomically, the lateral veins have the same structure with the midrib but the vascular bundles are reduced. *S. thymbra* has thick walls, 1-3 celled hairs and much prominent cuticular patterns. In *S. cuneifolia,* hairs are 1-3 celled and has thick walls, there are no cuticular patterns, and subsidiary cells are 2-3 or 4. In *T. capitate*, hairs are 1-3 celled and prickle hairs are dense and the outlines of the anticlinal walls are straight. Differences in morphological characteristics of examined *Satureja,* and *Thymbra* taxa are summarised in the Table 2. *T. spicata* subsp. *spicata* has sparsely hairs and straight anticlinal walls, subsidiary cells are smaller on the lower surface. *T. spicata* subsp. *spicata* is the species which has the densest glandular hairs and the sparsest non-glandular hairs.

T. capitata, has sparse glandular hairs. The glandular hairs are smallest in both *S. thymbra* and *T. capitata* species. *S. thymbra* has the densest cuticular patterns. *S. cuneifolia*, has the densest stomata amount on the upper surface. The crystals are densest in *T. spicata* subsp. *spicata* and *T. capitata* species. The differences of anatomical characteristics are summarised in the Table 3.

Table 2: Morphological differences of examined *Satureja* and *Thymbra* taxa.

	Satureja thymbra	***Satureja cuneifolia***	***Thymbra capitata***	***Thymbra spicata* subsp. *spicata***
Colour (dried and powdered)	 Brownish green	 Dark green	 Light green	 Green-purple
Hairs	Minutely recurved	Minutely recurved	Canescent	Minutely recurved on two opposite sides
Leaves	9-9.5×2.8-5.5 mm; cuneate-oblanceolate; apex acute, mucronate; entire, conduplicate; lateral veins not prominent	3-15×1-8 mm; cuneate-oblanceolate; apex acute, mucronate, subobtuse; entire, conduplicate; lateral veins not prominent	6-10×1-1.2 mm; triangular-linear; apex acute, entire, deltoid, lateral veins not prominent	3-15×1-3 mm; linear, linear-lanceolate; apex acute, entire, conduplicate; lateral veins not prominent
Inflorescence	Distant verticillate	Spicate	Capitate	Spicate
Bracts	Oblong-elliptic	Linear-lanceolate	Ovate	Elliptic-lanceolate
Calyx	Tubular-campanulate, 2-lipped	Tubular-turbinate, slightly 2-lipped	Dorsaly flattened, 2-lipped	Dorsaly compressed, 2-lipped

Table 3: Anatomical differences of leaves of *Satureja* and *Thymbra* taxa.

Characteristics	***Satureja thymbra***	***S.cuneifolia***	***Thymbra capitata***	***T.spicata* subsp. *spicata***
Palisade ratio	4.5	3.35	3.8	4.8
Areoles per mm^2	7	5.17	4.25	3.85
Stomata amount per mm^2 (L/U)*	1090/850	1030/2120	1070/1230	1190/1180
Stomata index (L/U)*	95.11/94.33	92.29/96.14	92.48/92.76	95.35/94.93
Glandular hairs (no stalk) per mm^2	630	710	590	2150
Subsidiary cells	2	2-4	2	2
Collenchyma (lower epidermis	2	2	Several cells one by one	-
Collenchyma (upper epidermis)	4-5	4-5	Several cells one by one	-
Palisade parenchyma	2-layered	2	1	1
Spongy parenchyma	1-2	1-2	1-2	1-2
Hairs	1-2	1-4	1-3	1-3
Glandular hairs	Head 1, stalk 1-celled	Head 1, stalk 1-celled	Head 1, stalk 1-celled	Head 1, stalk 1-celled
Sclerenchyma	1	2-3	1	1
Collenchyma (corner)	8	5-7	5-6	6-7
Collenchyma	1-3	1-3	2-3	2-3
Floem	3-5	8	5-6	3-4
Cuticle (L/U)*	Smooth, slightly undulate / smooth	Slightly undulate / smooth	Smooth / smooth	Smooth / smooth
Epidermis cells (L/U)*	Big / small	Big / small	Small / big	Small / big

***** (L/U) : Lower/upper

ACKNOWLEDGEMENTS

This study was supported by The Research Fund of Istanbul University (Project number: T-1115). The authors are grateful to the staff of ADA, AEF, ANK, EGE, ESSE, GAZI, HUB, ISTE, ISTF, IZEF, MARA, MARE, MUFE, VANF and Herbarium of Inönü University herbaria. We are also grateful to Prof. Dr. T. Ekim, Prof. Dr. N. Kırımer, Prof. Dr. B. Yıldız and MSc. M. Öztekin for supplying literature, MSc. M. Keskin and P. Sadıkoğlu for their kindly help. We thank for their help in field studies to: İ. Nacakçılar, H. M. Ar, A. Datumani, F. Erbaş, Ş. Türüdü, H. Çankırı, H. Kahraman, E. Sözen, F. Yeşil, E. Çınkır, A. Samray and A. Gülbaba from Forestry Administration, A.B. Tınmaz, M. Doğan, M. Atmaca, U. Sakallı, S. Doğan and A. Gökdemir from Agricultural Administration, B. Saruhan (Tamsan), A. Aji (Figsan), E.R. Roditi, Timtaş, E. Pariente (Demar), M. Yakıcı (Ardıç Tarım), F. F. Türkmen (Türkmen Tarım), A. İnan (İnan Tarım), S. Özdemir (Özdrog Yağ, Tic. San.), M. Kara, N. Şentürk, M. Kayacan and M. & M. Berber.

REFERENCES

Barberan FAT, Gil MI (1992). Chemistry and natural distribution of flavonoids in the Labiatae. In: Harley RM, Reynolds T. (eds). *Advances in Labiatae Sciences*, p. 299-305 The Royal Botanic Gardens Kew, Richmond, Surrey, UK.

Berlyn GP, Miksche JP (1976). Botanical microtechnique and cytochemistry, Iowa State University Press, USA.

Duzenli A, Karaomerlioglu D (2003). Ticareti yapılan *Thymbra spicata* var. *spicata* ve *Laurus nobilis* L.'in Güney Anadoludaki durumu (TBAG-DPT. Ç. Sek/11 Proje No: 101T014), Adana.

Erken S (2001). Morphological and anatomical studies on *Thymbra spicata* L. *Acta Pharmaceutica Turcica*; XLII(3,4):189-193.

Gemici Y (2003). Ticareti yapılan *Salvia* L. ve *Coridothymus* Reichb. fil turlerinin dogadaki durumu (TBAG-DPT. C. Sek Proje No: 101T01).

Guner A (2012). Aslan S, Ekim T, Vural M, Babac MT (2012). Turkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gokyigit Botanik Bahcesi ve Flora arastırmaları Dernegi Yayını İstanbul.

Kaya A, Koca F, Başer KHC, Tümen G (1994). *Satureja cuneifolia* turu uzerinde morfolojik, anatomik, palinolojik calısmalar, p. 208-216. In: *XII. Ulusal Biyoloji Kongresi (Edirne, 6-8 Temmuz 1994) Bildirileri*. Edirne.

Kırımer N, Boydağ I, Sargın N, Arslandere O (2003). Ticareti yapılan *Origanum* turlerinin dogadaki durumu (TBAG-DPT. Ç. Sek/10 Proje No: 101T012), Eskişehir.

Sadikoglu N, Ozhatay N (2015). Morphological characteristics of exported taxa as oregano from Turkey I: *Origanum*. *J. Fac. Pharm. İstanbul* **45**:87-126.

Sener B, Tosun F, Kusmenoglu S, Ergun F, Turkoz S, Toker G, Baykal T, Bingol F, Temizer H, Mutlugil A, Basgul M (1985). Drogların Morfolojik, Anatomik ve Kimyasal Analiz Ornekleri. Seldem Ofset Ankara.

Tanker M, Ilisulu F (1981). Turkiye'de kekik olarak kullanılan bitkilerden *Thymus capitatus* (L.) Hoffm. et Link. *Ankara Ecz Fak Mec* **11**:127-135.

Tumen G, Satıl F, Dirmenci T, Oztekin M (2003). Ticareti yapılan *Satureja* L. turlerinin dogadaki durumu (TBAG-DPT. Ç. Sek/12 Proje No: 101T011), Balıkesir.

Appendix

Examined specimens;

***Satureja thymbra* L.**

A1(E) Edirne: Kaya kö., Kınak mevkii, 26.v.1996, G. Tümen (ESSE 12805)! **B1 Balıkesir:** Edremit-Avcılar kö, 250 m, 9.v.1966, (EGE 5291)! Alibey adası, Alibey Tepe batısı, 180 m, 26.v.1997, K. Alpınar (ISTE 74190)! **İzmir:** Makilik, Sığacık, Seferihisar, 15.v.1993, E. Saver (IZEF 1150)! d. Seferhisar, Sığacık-Teos, 15.v.1993, N. Zeybek (IZEF 1233)! Bergama, 26.vı.1977, İ. Akbulut (IZEF 2172)! Kemalpaşa, Kızılüzüm kö., 200 m, 27.v.1996, E. Saver (IZEF 4061)! Seferihisar-Terkos, ca. 0-50 m, 16.v.1986, (EGE 21800)! Çimentaş, 15.ıv.1966, (EGE 5085)! Hills N of Bornova, 2.v.1969, (EGE 7884)! Hills NE of Bornova, 15.v.1969, (EGE 4305)! Çeşme, 7.ıv.1968, (EGE 3336)! Balıklıova-Mordoğan, 5.ı.1966, (EGE 5431)! **Manisa:** Üçpınar-Gediz, Muhtarlık özel alanı, 06.v.1995, U. Zeybek (IZEF 3296)! Soma, orta istasyondan 200 m yukarısı, ca. 400 m, 12.v.1977, (EGE 26032)! **C1 Aydın:** Ortaklar-Çamlık, 300 m, 12.v.1967, (EGE 6359)! Kuşadası yolu üzeri, 6.xı.1967, (EGE 4690)! Kuşadası, Kalamaki deresi, c. 200 m, 12.v.1968, (EGE 6109)! Paşa yaylası, c. 250 m, 27.ıx.1965, (EGE 6051)! Samsundağı, above Güzelçamlı, 29.v.1969, (EGE 4407)! Akköy, Söke-Didim, Yenihisar'a 5 km kala, 3.ıv.1995, (EGE 18996)! Germencik-Ortaklar kasabası, Öğretmen Lisesi çevresi, 27.v.1993, G. Tümen (ESSE 10479, 10487)! Kuşadası-Davutlar Milli parkı, kanyan bölgesi, 13.v.1995, N. Öztürk (ESSE 11701)! Kuşadası yolu, Kuşadası yakını, kayalık sırtlar, 9.ıv.1971, A. ve T. Baytop (ISTE 19137)! Didim yolu, Didim'e 10 km kala, maki arası, 10.ıv.1971, A. ve T. Baytop (ISTE 19192)! Dilek Yarımadası, 100 m, 11.vı.1982, M. Miski, E. Bütün (ISTE 48944)! **İzmir:** d. Selçuk, 3-4 km N Yoncaköy, 250 m, 26.v.1993, U. Zeybek (IZEF 1219)! Samsun D., Güzel çamlı üstleri, 520 m, 5.vıı.1989, E. Tuzlacı (MARE 1970)! **Muğla:** Bodrum-Mumcular, 01.ı.1950, M. Polat (IZEF 1757)! Datça, 19.vıı.1966, (EGE 6034)! Bodrum, Gölköy yakını, Cennet koyu çevresi, E. Tuzlacı (MARE 8540)! Yalıkavak-Bodrum yolu 3. km, yol kenarı, 10.v.1995, N. Öztürk (ESSE 11699)! Datça, Bozdağ (Kocadağ), Mesudiye kö. üstleri, 600 m, 3.vıı.1983, E. Tuzlacı (ISTE 51504)! Datça- Marmaris, Datça'dan 13 km, Gebekum mevkii, d.s. kumullarda, 5.vıı.1983, E. Tuzlacı (ISTE 51589)! Datça, Kocadağ kuzey etekleri, Karaköy yakını, d.s., 12.v.1984, E. Tuzlacı (ISTE 53318)! Datça-Knidos, Datça'dan 10 km, kireçtaşlı sırtlar ve tepelerin rutubetli kuzey yüzü, 5.ıv.1995, A.J. Byfield, D. Pearman B 1501 (ISTE 68850)! **C2 Antalya:** Kaş 5 km, 26.vıı.1960, Khan et all. 188 (ANK)! **Burdur:** Dirmil-Fethiye, 51 km S Dirmil, 1000 m, 20.vı.1981, Max Nydegger 16330 (HUB 22691)! Kaş, 22.v.1967, T. Baytop (ISTE 12280)! Kaş Fethiye yolu, Fethiye'ye 90 km, 22.ıv.1978, A. ve T. Baytop (ISTE 39063)! Kalkan Kaş yolu, Kalkan yakını, Kalkan'a 3 km, 120 m, 5.v.1980, A. ve T. Baytop, A. Attila, N. Sütlüpınar (ISTE 44163)! **Muğla:** d. Marmaris, 6 km W Reşadiye, 15.v.1997, E. Saver, N. Zeybek (IZEF 4929)! Fethiye, Kemer bucağı, Dereçatı kö., Bayaslar mevkii, c. 300 m, 15.vı.1967, (EGE 5812)! Fethiye, Bayaslar-Dereçatı, 15.vı.1967, (EGE 4913)! Fethiye, Hisarönü kö., Belceğiz mevkii, 250 m, 11.v.1967, (EGE 4959, 4935)! Fethiye, Kemer-Taşocağı, 10.vıı.1966, (EGE 6073, 6061, 6004)! Köyceğiz, Ekincik-Marina, 20 m, kızılçam ormanı, metamorfik kalkerli arazi, 17.ıv.1991, A. Güner 8785, M. Vural, H. Duman, AA. Dönmez, B. Mutlu (HUB 2293)! Köyceğiz, Yangı kö., Yangı de., 100-200 m, sarp ve derin vadi, kalkerli kayalıklar, 18.ıv.1991, A. Güner 8888, M. Vural, H. Duman, AA. Dönmez, B. Mutlu (HUB 2292)! Marmaris, M. Milli Parkı, 150 m, serpentin, 29.vı.1997, H. Şağban 1868 (HUB 22381)! Marmaris-Datça yolu, yamaçlar, 16.v.1987, K.H.C. Başer (ESSE 8183)! Gökova inişi, yol kenarı, yamaçlar, 15.v.1987, K.H.C. Başer (ESSE 7809)! Fethiye, Akbel, Çöğmen kö., 15.xı.1992, G. Tümen (ESSE 9984)! Fethiye, 10.vı.1990, G. Tümen (ESSE 8949)! Marmaris-Datça, Çubucak orman kampı çevresi, vıii.1992, G. Tümen (ESSE 9820)! Fethiye, Söğütlüdere kö., 29.viii.1995, G. Tümen (ESSE 12250)! Muğla, 23.vıı.1949, T. Baytop (ISTE 2429, 2435)! Marmaris'e 30 km, yol kenarı, 320 m, 20.vı.1980, N. ve E. Özhatay, E. Tuzlacı (ISTE 44877)! Sandras da., Akköprü orman işletmesi-Armutveren, 700 m, N. ve E. Özhatay (ISTE 44932)! Fethiye, Kemer yakını, ulualan ağaçlandırma sahası, 400 m, 1.vıı.1983, E. Tuzlacı (ISTE 51462)! Fethiye, Baba da., batı etekleri, Gıdırak, Belceğiz arasındaki yamaçlar, 30 m, E. Tuzlacı (ISTE 53130)! Köyceğiz, Sandras da., Ağla sırtları, orman altı, 900 m, 22.vı.1980, N. ve E. Özhatay (MUFE 4302)! **C3 Antalya:** Antalya, 140 m, 14.v.1971, R. Çetik 3760 (ANK)! Maki zonu, 2.ıv.1984, Y. Akman 13638 (ANK)! Antalya'nın 12 km güneybatısı, Karadağ, 40 m, 22.ıv.1954, Nijhoffet et all. 612 (ANK)! Antalya, Plaj ve kayalar, H. Birand 17 (ANK)! Alanya-Manavgat, 23.vı.1977, İ. Akbulut (IZEF 2178)! Kemer, Faselis koyu ve çevresi, 0-150 m, 23.vı.1978, H. Peşmen 4036, B. Yıldız, Ş. Kaplan (HUB 22690)! Kemer, Beldibi köyü üstü, kalkerli derin vadi, 30-100 m, 20.vıı.1978, H. Peşmen 3897, A. Güner (HUB 22687)! Side çevresi, 5.vı.1970, A. Pamukçuoğlu, Quezel (HUB 22688)! Kemer yolu, karayolu tüneli çevresi, masif kalker kayalığı, 23.iıı.1978, H. Peşmen 3617, B. Yıldız (HUB 22689)! Antalya-Kurşunlu şelalesi, Barış parkı, 2.vı.1989, K.H.C. Başer, N. Kurtar (ESSE 8764)! Antalya-Muğla karayolu, Tekirova mevkii, 1.vı.1989, K.H.C. Başer, N. Kurtar (ESSE 8763)! Antalya-Kemer karayolu 15 km, 14.ıv.1991, K.H.C. Başer, N. Öztürk (ESSE 9378)! Antalya- Konaklı kö., 1991, K.H.C. Başer (ESSE 9697)! Alanya, Konaklı kö., vıii.1991, G. Tümen (ESSE 9735)! *ibid.*, 19.ıx.1992, G. Tümen (ESSE 10497)! *ibid.*, 20.viii.1994, K.H.C. Başer (ESSE 11857)! Hisarçanlı TV kulesi, 21.vıı.1989, K.H.C. Başer, N. Kurtar (ESSE 10123)! Alanya, Altes, 18.ıx.1995, K.H.C. Başer (ESSE 11038)! Manavgat-Alanya, Çavuşköyü

yol ayrımı, kumluk yer, 28.v.1966, A. ve T. Baytop, B. Çubukçu (ISTE 9789)! Konyaaltı, 12 Eylül ormanı, 20 m, 15.vı.1983, H. ve G. Çakırer (ISTE 50913)! **Burdur:** pazardan satın alındı, 8.vıı.1986, A. ve C. Mat (ISTE 56962)! **C4 Antalya:** Gazipaşa, 29.ıv.1996, G. Tümen (ESSE 12251)! Alanya, 7.vııı.2000, *s.n.* (ESSE 14025)! Alanya kalesi içi, 21.ıv.1974, G. Dökmeci, E. Tuzlacı, Y. Doğantan (ISTE 27515)! Güzelbağ-Gündoğmuş, 520 m, 5.vııı.1980, E. Tuzlacı, B. Çubukçu, A. Meriçli (ISTE 45678)! **Mersin:** Anamur, Gazipaşa, 15.vı.1976, Y. Akman-Quézel 7677 (ANK)! Anamur, Kaledıran kö., 300-400 m, 15.vııı.1984, U. Temel (MARE 4561)! Gülnar çevresi, 10.vı.1995, G. Tümen (ESSE 11941)! *ibid.*, 5.v.1995 (ESSE 11486)! Mut çevresi, v.1996, G. Tümen (ESSE 12118, 12175)! Anamur-Silifke, Silifke'ye 88 km, deniz kıyısındaki yamaçlar, 20 m, 6.vııı.1980, E. Tuzlacı, A. Meriçli (ISTE 45697)! **C4/5 Mersin:** Silifke, Göksu deltası, Kum M.-Akgöl, 0-5 m, 30.ıv.1993, Düzenli, Çakan, Türkmen (ADA 2777!, 4408)! **C5 Adana:** Obaçayı vadisi, vııı.1995, N. Mumcuoğlu (ESSE 11974)! **Mersin:** Bayburun, Harabe, *Pinus brutia* ormanı, 750 m, 27.v.1970, T. Uslu 160 (ANK)! Mersin Gözne yolu, Buluklu kö. üstü, 420 m, 5.vı.1981, E. Tuzlacı (ISTE 46364)! **C6 Hatay:** Amik Gölü, 150 m, 25.ıv.1957, Davis et Hedge 27133 (ANK)! Antakya, Arsuz, 21.ıv.1981, M. Miski (ISTE 46262)! Antakya, Habibneccar da., 28.vı.2004, N. Sadıkoğlu (ISTE 81695)! Belen Y., Sirken

***Satureja cuneifolia* Ten.**

A1(E) Çanakkale: Lapseki, Eçialan kö. civarı, 22.vııı.1995, G. Tümen (ESSE 12029)! Gökçeada, Araz tepesi doğusu, ca. 300 m, 28.vıı.1975, (EGE 15921)! Gökçeada, Demirkaya te., ca. 672 m, 10.vııı.1976, (EGE 15923)! Gökçeada, Ulukaya te., ca. 630 m, 21.v.1975, (EGE 15922)! Gökçeada, Rıhı tepesi doğusu, ca. 350 m, 11.vııı.1976, (EGE 15924)! **A2 Bilecik:** Dingler, (ANK)! **A4 Zonguldak:** Safranbolu, 29.vııı.1995, G. Tümen (ESSE 11675)! **A5 Amasya:** kayalıklar, 1.vııı.1994, N. Ermin (ESSE 10666)! **B1 Balıkesir:** Balıkesir-Kazdağ, Babadağ yöresi, Kapıkule zirve, 17.vıı.1991, G. Tümen, H. Çakır (ESSE 10440)! Ayvalık yolu üzerindeki denize bakan yamaçlar, 10.vııı.1989, G. Tümen (ESSE 8461)! Edremit, Ayvacık yolu üzerinde, Küçükkuyu'ya 10 km, 100-150 m, 5.vııı.1989, G. Tümen (ISTE 62546)! **Çanakkale:** Balıkesir-Çanakkale, Ayvacık'a varmadan, karayolları dinlenme tesisi, 50-100 m, kayalık yamaçlar, 28.ıx.1995, G. Tümen (ESSE 12021)! Küçükkuyu, Subaşı-Ayı kayası mevkii, 24.ıx.2001, N. Sadıkoğlu (ISTE 81653)! **İzmir:** Ödemiş, Bozdağ, 1050 m, 24.ııı.1962, K. Karamanoğlu 890 (ANK)! Ödemiş-Bozdağ, 5.x.1992, G. Tümen (ESSE 10112)! Ödemiş, Birgi, G. Tümen (ESSE 10081)! Ödemiş, Bozdağ, vı.1993, G. Tümen (ESSE 10203)! *ibid.*, 1991, G. Tümen (ESSE 9559)! *ibid.*, vıı.1994, G. Tümen (ESSE 10992)! *ibid.*, 20.vııı.1995, G. Tümen (ESSE 11670)! Bozdağ, Büyükçavdar Y. sırtları, 1400 m, 9.x.1980, A. Baytop (ISTE 45905)! **Manisa:** Alaşehir-Bozdağ, Azıtepe kö., 20.vııı.1993, G. Tümen (ESSE 10170)! **B2 İzmir:** Bozdağ, Yayla, ca. 1100 m, 05.x.1994, E. Saver, U. Zeybek (IZEF 3058)! Kiraz, Küçük Menderes kenarı, x.1992, G. Tümen (ESSE 9867)! Kiraz çevresi, 25.ıx.1995, G. Tümen (ESSE 11656)! Kiraz-Küçükmenderes ırmağı çevresi, 320 m, 21.ıx.1995, F. Yılmaz (ESSE 11672)! Ödemiş, Gölcük, 21.x.1980, E. Tuzlacı, N. Sütlüpınar, A. Meriçli, Y. Kalav (ISTE 45944)! **Denizli:** Çivril-Akdağ, 6.x.1983, (EGE 25943)! **Manisa:** Alaşehir, Azıtepe kö., Bozdağ, 20.ıx.1994 (ESSE 10954)! Kula, Azıtepe, 20.vıı.1995, İ. Çınar, G. Tümen (ESSE 12129)! Salihli, Bozdağ, 2.x.1997, G. Tümen (ESSE 12474)! **B3 Eskişehir:** Eskişehir-Seyitgazi yolu 10-15 km, sol taraftaki araziden 1 km, 1972, K.H.C. Başer, (ESSE 179)! Kanlıpınar göleti, kayalıklar, 6.ıx.1991, K.H.C. Başer (ESSE 9591)! Mayıslar-Eskişehir 2 km, 12.vı.1991, K.H.C. Başer, A. Kaya (ESSE 8452)! Dağküplü-Mayıslar, 9 km, 14.vı.1994, K.H.C. Başer, A. Kaya (ESSE 11147)! Mayıslar yakını, vıı.1992, G. Tümen (ESSE 10031)! Mayıslar-Dağküplü 1 km, 28.v.1994, G. Tümen (ESSE 10481)! Şöförler çeşmesinden sonra 8. km, kayalıklar, 20.vıı.1991, A. Kaya, N. Ermin, T. Özek (ESSE 9200)! Gökçekaya barajı, vıı.1994 (ESSE 10726)! Gökçekaya barajı, Hidrofilik santralinin çevresi, Doğu Savak bölgesi, 25.ıx.1998, N. Tabanca, A. Altıntaş (ESSE 12754)! Kanlıpınar göleti, 6.ıx.1991, K.H.C. Başer, A. Kaya (ISTE 63602)! **C1 Muğla:** Bodrum, Bitez kö. çevresi, d.s. 6.x.2002, E. Tuzlacı (MARE 8545)! *ibid.*, bahçelerin üzerindeki sırtlarda, 13.xı.1976, E. Tuzlacı (ISTE 36290)! **C2 Antalya:** Altes bahçesinden, vıı.1995, N. Mumcuoğlu (ESSE 11973)! **Aydın:** Karacasu, Türer Tarım Orman Ürünleri İthalat İhracat Sanayi, 30.vıı.2000 (ESSE 13287)! **Denizli:** Bozdağ, 14.7.1947, P.H. Davis 13333 (ANK)! Babadağ-Başaran yaylası, 1200 m, 7.ıx.1994, (EGE 18904)! Babadağ, 900-1000 m, 23.vıı.1950, (EGE 27252)! Babadağ yaylasından kooperatif evlerinin başlangıcı, 26.ıx.1997, G. Tümen (ESSE 12484)! Olukbaşı-Geyran Y., Koyunini de. üstü, 1540 m, 11.vıı.1999, K.H.C. Başer 1618, H. Duman, A. Altıntaş (ESSE 12859)! Babadağ, Türer Tarım Orman Ürünleri İthalat İhracat Sanayi, 30.vıı.2000 (ESSE 13290)! Babadağ, 900-1000 m, 23.vıı.1950, P.H. Davis 18425 (ISTE 52321)! **Muğla:** Fethiye, Kemer, Çayan kö., Gavır mevkii, x.1993, G. Tümen (ESSE 10466)! Ortaca, Çövenli Y., dağlık yerler, vıı.1997, G. Tümen (ESSE 12790)! **C3 Antalya:** Kemer, 2000-2200 m, 10.vıı.1947, P.H. Davis 14190 (ANK)! Kemer, Tahtalı dağ, Çukur Y.-Ağla Y., kalkerli kuzey yamaç, *Cedrus libani* ormanı, 1100-1650 m, 23.vıı.1978, H. Peşmen-A. Güner, P.H. Davis 4074 (ANK)! *ibid.*, H. Peşmen 4100, A. Güner (HUB 22657)! *ibid.*, H. Peşmen 4074, A. Güner (HUB 22660)! Elmalı civarı, Kayalar, 1968, Quézel et all. 29 (ANK)! Elmalı, Beydağ, 2000 m, 28.vıı.1960, Khan et all. 288 (ANK)! Göynük, 50 m, 6.vıı.1949, P.H. Davis 15026 (ANK)! Han Boğazı, 2.ıx.1947, P.H. Davis 14722 (ANK)! Tahtalı Da., W Yukarı Beycik, 2000-2350, 1.vıı.1984, (EGE 31175)! Çalbalı Da., SE Bakırlı Dağ, 1550-1650 m, 18.vıı.1984, (EGE 31174)! Kemer, Beycik kö., Tahtalı Da., ca. 1250-2100 m, 26.vıı.1980, (EGE 32352)! Kemer, Yaylabuz dere-Çukur Y. *Cedrus libani* ormanı, 1200-1500 m, 28.vıı.1979, H. Peşmen 4448, A. Güner, Ş. Kaplan

(HUB 22661, ISTE 52634)! Kemer, Beycik kö. üstü, kalkerli kayalık arazi, *P. brutia-C. libani* ormanı ve alpinik step, 800-1900 m, 19.vıı.1978, H. Peşmen 3891, A. Güner (HUB 22652)! Antalya-Burdur karayolu, 17.ıx.1994, K.H.C. Başer (ESSE 11035)! Akseki, Sadıklar kö., 14.vıı.1974, A. Alpaslan (ISTE 30778)! Çalbalı da., Fesleğen Y. yakını, 1850 m, 4.vııı.1980, N. Özhatay, E. Tuzlacı, B. Çubukçu, A. Meriçli (ISTE 45631)! **Burdur:** 11.vı.1993, G. Tümen (ESSE 10082)! **Isparta:** Eğridir, Barla Dağı, 2000 m, 1.vııı.1960, Khan et all. 396 (ANK)! Ş. Karaağaç, Kızıldağ Milli Parkı, Kızıldağ kuzey yamacı, 1300 m, 24.vıı.1994, B. Mutlu 1078 (HUB 22662)! Eğirdir, Anamas, Yaka kö., Kapız de., kalkerli sap ve derin vadi, 1250-1450 m, 5.vııı.1974, H. Peşmen, A. Güner 1881 (HUB 22653)! Sütçüler, Çandır-Akçay, yangın gözetleme kulesi civarı, vıı.1995, K.H.C. Başer, H. Duman, A. Altıntaş (ESSE 11830)! Sütçüler, Çandır, 1800 m, ıx.1999, S. Oflaz (ESSE 13357)! Sütçüler, Darıbükü, N37°34' E31°11', 12.vııı.2001, N. Sadıkoğlu (ISTE 81648)! **C4 Antalya:** Alanya, Gönül dere, 1000 m, K. Karamanoğlu, 27.vııı.1947, P.H. Davis 14293 (ANK)! Gazipaşa, Sugözü kö., Maha Y., 450 m, 5.vııı.1983, H. Sümbül 2382 (HUB 22655)! Alanya, Köprübaşı mevkii, Arpalık Y., kuzey sırtları, 1600-2000 m, 10.vııı.1994, H. Duman (ESSE 10731)! Alanya, Gökbel yolu, Bucak Çökelek Y., Alanya'dan 32. km, 1330 m, 17.vıı.1995, K.H.C. Başer 1172, H. Duman, A. Altıntaş (ESSE 11506)! **Karaman:** Ermenek, 1300 m, 10.vııı.1947, P.H. Davis 16156 (ANK)! Ermenek, Göktepe nahiyesi, Dumlugöze (Muzvadi) kö., Aşakbel tepesi, 1900 m, 13.ıx.1983, H. Sümbül 2405 (HUB 22656)! Ermenek, 1300-1400 m, 13.vııı.1949, P.H. Davis 16156 (ISTE 52320)! Ermenek Yellibel yolu, Tekeçatı, yol kenarı, 26.vıı.1991, F. ve A.H. Meriçli (ISTE 63770)! **Konya:** Bozkır, Dikilitaş Y., R. Çetik-T. Ekim-E. Yurdakulol, 6.vııı.1967, Huber-Morath 12 (ANK)! Bozkır, 1000 m, 7.ıx.1969, P.H. Davis 16610 (ANK)! Bozkır, Yılanlı kaya mevkii, kaya üzeri, 1550 m, 11.vıı.1989, H. Sümbül 3389 (HUB 22650)! **Mersin:** Anamur, Hamitseydi Boğazı, Ermenek, 1500-1700 m, P.H. Davis 16241 (ANK)! Gülnar-Silifke, 1000 m, 28.ıx.1994, kayalıklar, M. Vural 7236, M. Koyuncu, M. Ekici (HUB 24382)! Gülnar çevresinden, vıı.1991, G. Tümen (ESSE 9558)! Silifke-Ardıçkuyusu Y., vııı.1992, G. Tümen (ESSE 10027)! Gülnar çevresi, 20.vııı.1995, G. Tümen (ESSE 12116)! Silifke, Sarıaydın kö., 1750 m, 4.v.1995, T. Baytop (ISTE 71374)! **C5 Adana:** Dildil Dağ, Başkonur Y., Hüseyin oluk çeşmesi, 1800 m, 27.vııı.1949, P.H. Davis 16400 (ANK)! Bahçe (Amanus), Dildil dağ between Başkonus Y. and Hüseyin Oluk Y., 1800 m, 27.vııı.1949, P.H. Davis 16400 (ISTE 52319)! **Konya:** Ereğli, Aydos Da., Delimahmutlu-Çakıllar, meşe ormanı, kalker anakaya, 1600 m, 29.vııı.1977, S. Erik 2652 (HUB)! Ereğli, Aydos Da., Delimahmutlu, otlak tepe, kalker anakaya, bozkır, 1600 m, 19.vııı.1978, S. Erik 3054 (HUB 22658)! Ereğli, Aydos Da., Kayasaray, mermer anakaya, 1700 m, 15.vıı.1977, S. Erik 2589 (HUB 22659)! Halkapınar-İvriz, Karacaser dağı yamacı, 1200 m, 13.vıı.1998, Y. Yaman (MUFE 115)! **Mersin:** Mersin-Aslanköy Y., vııı.1990, G. Tümen (ESSE 9557)! Silifke-Sulama Y., Karadedeli kö., G. Tümen (ESSE 10174)! Erdemli, Büyüksorgun Ziyaret da., 950 m, yamaçlar, 8.vı.1996, G. Tümen (ESSE 12249)! Bolkar da., Aslanköy, Kazangöl pınarı, kayaların üstünde, 1600 m, 12.vııı.1976, K. Alpınar (ISTE 35832)! **C6 Hatay:** Altınözü, Yanıkpınar kö., Kayacık mevkii, kayaların üzerinden, 22.x.1995, B. İshakoğlu (ESSE 12028)! Hassa, taşlık arazi, 21.x.1996, G. Tümen (ESSE 12271)!

***Thymbra capitata* (L.) Cav.**

A1(E) Çanakkale: Eceabat-Abide yolu, 17.vı.1986, N. Zeybek (IZEF 2284)! Bozcaada, Sulubahçe güneyi, ca. 25 m, 7.vııı.1976, (EGE 20491)! Bozcaada, Göztepe batısı, çayır, ca. 80 m, 14.vı.1976, (EGE 20487)! Eceabat, Kabatepe sırtları, 50 m, 24.ıx.1984, A. Çırpıcı, T. Ekim, H. Malyer (ESSE 7516)! Eceabat, Kilitbahir, Havuzlar, Şarlayandere yolu, 14 m, 24.ıx.1984, A. Çırpıcı, H. Malyer (ESSE 8044)! Eceabat-Kabatepe mevkii, Saroz körfezi, yamaçlar, 16.vıı.1991, G. Tümen (ESSE 10009)! Gelibolu-Burhanlı köyünün hemen üstü, denize bakan yamaçlar, 29.vııı.1991, G. Tümen (ESSE 10023)! Eceabat-Gelibolu yolu, 10.vıı.1960, A. Baytop (ISTE 6091)! Gelibolu-Eceabat, Gelibolu'dan 25 km ileride, Galata ovası, sırtlarda, 29.vıı.1971, A. Baytop (ISTE 20729)! İmroz adası, Dereköy, 19.vıı. 1974, Y. Saviç (ISTE 30519)! Eceabat, Şeddülbahir civarı, 21.vıı.1975, N. ve E. Özhatay (ISTE 32999)! **Tekirdağ:** Gelibolu, Buharlı köyü 10 km sonra, maki, K. Karamanoğlu, 617 (ANK)! **B1 Balıkesir:** Marmara adası, vııı.1991, G. Tümen (ESSE 9818)! Marmara adası-Çınarlı kö., koylardan biri, 25.vııı.1991, G. Tümen (ESSE 10219)! **Çanakkale:** Bozcaada, batı burnu, adanın en batı ucu, c. 30 m, açık kireçli kayalıkların bulunduğu tepeler, 28.vııı.1994, A.J. Byfield, S. Atay B 1271 (ISTE 67526)! **İzmir:** Urla-Kalabak, 25.vııı.1977, İ. Akbulut (IZEF 339)! Urla İskelesi, 12.vı.1977, İ. Akbulut (IZEF 357)! Bornova, Sabuncubeli, 150 m, 11.vıı.1982, N. Zeybek (IZEF 665)! Bornova, askeriye çıkışı, 24.vı.1977, İ. Akbulut (IZEF 2176)! Bornova-Papaz tepesi, 20.x.1995, E. Saver (IZEF 3638)! Hills NE of Bornova, 1.vıı.1969, (EGE 8094)! Eski İzmir'in çıkışı, çatalkayaya doğru, ca. 250 m, 28.vıı.1982, (EGE 26499)! Çeşme, 1.x.1967, (EGE 4614)! Çeşme, Çiftlik kö., 10.vı.1971, (EGE 7612)! Karaburun, Saip dağ, 4.vııı.1965, (EGE 6052)! Mordoğan, Ziraat Fak. Kampı üstü, kalkerli toprak, c. 100 m, 6.vııı.1978, Ş. Yıldırımlı 1055 (HUB 22961)! Çeşme, Askeri lojmanların arkası, 100 m, 28.vıı.1997, B. Mutlu 1917 (HUB 22964)! Mordoğan, 28.vıı.1995, S., M. ve H. Alan (ESSE 11845)! Çeşme, Dalyan, 24.vıı.1997, G. Tümen (ESSE 12810)! Seferihisar, 11.x.2000, (ESSE 13298)! Çeşme, Çiftlik kö. ilerisi, deniz kenarı, 1.vıı.1969, T. Avcıgil (ISTE 15929)! **C1 Aydın:** Kuşadası-Aydın yolu, İ. 18.vı.1977, İ. Akbulut (IZEF 381)! Kuşadası, 21.vı.1964, (EGE 2328!, 2332!, 8721)! Didim-Söke, c. 10 m, 5.vııı.1984, B. Dinçtürk 1006 (HUB 22950)! Germencik, 10.vıı.1993, G. Tümen (ESSE 10098)! Kuşadası, 25.vı.1996, G. Tümen (ESSE 12182)! **İzmir:** Selçuk, Pamucak beli, 27.vıı.1965, (EGE 6060)! Selçuk, Meryemana, 15.vıı.1966, (EGE 5349)!

Muğla: Datça, Karaköy civarı, Maki açıklıklarında, 200 m, 14.vıii.1985, M. Demirörs 2058 (ANK)! Datça, 19.vıı.1996, (EGE 5342)! Datça, (EGE 5978)! Bodrum, Geriş kö. güneyindeki yamaçlar, 250 m, 14.vı.2001, E. Tuzlacı (MARE 6793)! Bodrum, Bitez kö. kuzeyindeki dağ yamaçları, 100 m, 15.vı.2001, E. Tuzlacı (MARE 6813)! Bodrum: Bodrum-Çamlık kö., 280 m, 5.v.2002, E. Tuzlacı (MARE 7536)! Datça, Gebekum, 6.ıx.2001, E. Tuzlacı (MARE 7157)! Bodrum, Kadı kalesi çevresi, 20.vıı.1994, G. Tümen (ESSE 10975)! Datça, Bozdağ (Kocadağ), Mesudiye kö. üstleri, 600 m, 3.vıı.1983, E. Tuzlacı (ISTE 51503)! Datça-Marmaris, Datça'dan 13 km, Gebekum mevkii, kumullarda, d.s., 5.vıı.1983, E. Tuzlacı (ISTE 51590)! Bodrum, Bitez kö. çevresi, 30 m, 7.vıı.1983, E. Tuzlacı (ISTE 51609)! Datça, Kocadağ kuzey etekleri, Karaköy yakını, d.s., 12.v.1984, E. Tuzlacı (ISTE 53290)! Bodrum, Gölköy, Eski Gölköy Lisesi sırtları, 11.vı.2003, N. Sadıkoğlu (ISTE 81672)! **C2 Antalya:** Elmalı-Akdağ yolu, 18.vı.1968, A. Pamukçuoğlu, Quezel (HUB 22962)! Kaş-Fethiye karayolu 32. km, 150 m, yol kenarı, yamaçlar, 20.vı.1995, K.H.C. Başer 1039, H. Duman, A. Altıntaş (ESSE 11359)! **Muğla:** Marmaris-Datça, 17.vıı.1960, Khan et all, 79 (ANK)! Köyceğiz, 03.vıı.1977, İ. Akbulut (IZEF 2171)! Marmaris, Hisarönü kö., 10.xı.1984, N.Zeybek (IZEF 2912)! Köyceğiz, Dalyan, Sülüngür gölü, 27.vıı.1981, (EGE 32468)! Fethiye-Tersakan, 9.vıı.1966, (EGE 5443)! *ibid.,* (EGE 5981)! Marmaris, Pamucum orman dinlenme kampı yakını, yamaçlar, G. Tümen (ESSE 9816)! Marmaris-Kadıkale, vıii.1993, G. Tümen (ESSE 10202)! Marmaris'e 30 km, 320 m, yol kenarı, 20.vı.1980, E. Tuzlacı, N. ve E. Özhatay (ISTE 44877a)! **C3 Antalya:** Kemer-Göynük, 5 km, kumul, 29.vıı.1980, H. Peşmen 4915 (HUB 22963)! Beycik kö., İkiağızlar mevki, 31.v.1990, K.H.C. Başer (ESSE 8786)! Konyaaltı, 10 m, kumullar, N. Özhatay, E. Tuzlacı, B. Çubukçu, A. Meriçli (ISTE 45665a)! **C6 Hatay:** İskenderun, Soğukoluk, Maki çalılığı boşluklarında, 25.vı.1944, B. Kasaplıgil 69 Kew (ANK)! İskenderun, Soğukoluk, Maki zonu, 350 m, 25.vı.1967, Y. Akman 7722 (ANK)!

Thymbra spicata* L. subsp. *spicata

A1(A) Balıkesir: Gönen, Taşocağı sırtları, 40 m, 30.vı.1996, P. Eryaşar (MARE 5107)! **Çanakkale:** Ezine, Pazarköy, 150 m, 25.vı.2002, G. Emre (MARE 8392)! Ezine, Akköy, 170 m, 13.ıv.2002, G. Emre (MARE 8253)! **A1(E) Çanakkale:** Gelibolu-Eceabat, Eceabat'a 10 km kala, 9.xı.1968, A. Baytop, B. Çubukçu (ISTE 14713)! Havuzlu-Behramlı, 9.xı.1968, A. Baytop, B. Çubukçu (ISTE 14732)! **Tekirdağ:** Kumbağ sahil yolu, 13.vıii.1983, N. Zeybek (IZEF 2972)! Tekirdağ-Barbaros, 25.iii.1968, A. Baytop, G. Atila (ISTE 12432)! Ganosdağ, Gaziköye inerken, 100 m, 14.vıı.1968, A. Baytop (ISTE 13577a)! Keşan-Malkara, il hududu, 1.vıii.1971, A. Baytop (ISTE 20858)! Tekirdağ-Kumbağ yolu, Barbaros'a 1 km kala, çeşme üstündeki sırtlarda, 16.vıı.1974, N. ve E. Özhatay (ISTE 30432)! Karıştıran-Şarköy, Çınarlıdere kö. ayrımı, 19.vıı.1992, E. Akalın (ISTE 64511)! Merkez, Dedecik kö. çıkışı, yol kenarları, 30.vıı.1994, E. Akalın (ISTE 67409)! **A2(A) Bursa:** İznik yol ayırımı, maki, 50 m, E. Yurdakulol-M. Kılınç-M. Aydoğdu (ANK)! Bursa'nın 25 km kuzeyi, Mudanya'nın 5 km batısı, ca. 20 m, 21.vı.1973, (EGE 15463)! Mudanya'nın 5 km batısı, Mudanya-Zeytinbağı (Tirilye), 20 m, 21.vı.1973, (EGE 23422)! **İstanbul:** Şile, Akçakese kö., 80 m, 30.v.1996, E.T. Fenercioğlu (MARE 4835)! *ibid.,* 5.vı.1995, E.T. Fenercioğlu (MARE 4677)! Şile, İmrenli kö., 30 m, 8.vı.1995, E.T. Fenercioğlu (MARE 4665)! **A2(E) İstanbul:** Küçükçekmece gölünün kuzey ucu-Hoşdere, kuru tepeler, 17.vıı.1969, A. Baytop (ISTE 15686)! Büyük Halkalı köyünden dönüşte, 3.vıı.1970, N. Özocak, E. Özhatay (ISTE 18148)! Halkalı batısındaki kireçli yamaçlar, 5.vıı.1976, E. Tuzlacı (ISTE 40293)! İzmir'den getirilen ve Maltepe'de bahçede yetiştirilen numunelerden, 25.vı.1967, A. ve T. Baytop (ISTE 12060)! **A3 Adapazarı:** Pamukova'dan Sapanca'ya doğru dört yol ayrımı, ca. 40 m, 31.vıı.1984 (EGE 17883)! Geyve-İznik, Osmaneli yol ayrımından 5-6 km, Mekece kö.-İznik, 20.vı.190, E. Tuzlacı, M. Öksüz, F. Hırlak (MARE 2607)! **Bolu:** Abant gö. civarı, vıı.1956, Ş. Tercan (ISTE 4637)! **A4 Karabük:** Kum ocakları karşısı, 700 m, 21.vı.1985, M. Demirörs 1558 (ANK)! **Kastamonu:** Araç, *P.nigra,* 900 m, 24.vıı.1981, M. Demirörs 414 (ANK)! **A5 Amasya:** Amasya civarı, 13.vıı.1956, T. Baytop (ISTE 4648)! **A6 Tokat:** Reşadiye, 10 km batısı, Kelkit vadisi, Taşlı yerler, 10.vıı.1955, R. Çetik 1047/548 (ANK)! **B1 Balıkesir:** Akçay-Edremit, ıx.1986, K. Sır (MARE 678)! **Çanakkale:** Çanakkale-Ezine'nin 4 km kuzeyi, 8.vıii.78, 160 m, (VANF 664)!, **İzmir:** İzmir, 8.vı.1931 Krause 337 (ANK)! 12.vı.1935, Gassner 70 (ANK)! Çeşme, 16.vı.1976, F. Şekerci (IZEF 323)! Urla İskelesi, 12.vı.1977, İ. Akbulut (IZEF 327)! Gümüldür-Karacadağ, Değirmendere, ca. 100 m, 16.vı.1986, (EGE 21792)! Bornova, Hacılar kırı, Naldöken'in kuzeydoğusu, 25 m, 1.vı.1967, (EGE 6369)! Hills NE of Bornova, 1.vıı.1969, (EGE 8089)! *ibid.*, 26.v.1969, (EGE 4210)! Ödemiş, Gölcük-Bozdağ, 4.vıı.1966, (EGE 6033)! İnciraltı-Urla, 20.ıx.1962, (EGE 873)! Selçuk, Meryemana, 18.vı.1971, A. Pamukçuoğlu (HUB 22971)! Kemalpaşa, piknik yeri, Nif D. Etekleri, 280 m, 23.vı.1990, E. Tuzlacı (MARE 2747)! İzmir Çeşme yolu, İçmeler'e varmadan, 21.iii.1967, A. ve T. Baytop (ISTE 10690)! **Manisa:** Bozdağ, 16.vıii.1984, Ş. Yıldırımlı 7486 (HUB 22972)! Akhisar, Yel değirmeni, H. Bağda 460 (ANK)! Akhisar, Yel değirmeni, 9.vı.1942, H. Bağda (ISTE 1362)! Manisa da., 3.v.1961, T. Baytop (ISTE 6415)! **B6 Adana:** Saimbeyli, Himmetli köyü güneyi, Mermer kayalık, 900 m, 20.vı.1978, T. Ekim 3513 (ANK)! Sasak, Hacin, Manissadjian 1012 (ANK)! **B8** Diyarbakır: Hani-Dicle, Hani'den 14 km, 900 m, kıraç yerler, killi topraklar, 11.vı.1979, E. Tuzlacı, M. Saraçoğlu (ISTE 42188)! **Siirt:** Silvan-Kurtalan, 1000 m, 24.vı.1954, Davis 22176 (ANK)! **B8/9 Siirt:** Şirvan, Cevizlik Siirt yol ayrımı çevresi, bozuk meşelik, kuru yamaçlar, kalkerli arazi, 1000 m, 13.ıv.1980, A. Güner 2349, M. Koyuncu (HUB 22974)! **C1 Aydın:** Kuşadası, 250 m, 18.vı.1977, İ. Akbulut (IZEF 341)! Kuşadası, 01.ı.1950, M. Polat (IZEF 1759)! Söke-Kuşadası, ca. 300 m, 21.vı.1977, İ. Akbulut (IZEF 2165)! Kuşadası, 7.xı.1967, (EGE 4692)!

C2 Antalya: Kaş, Elmalı, 27.vıı.1960, Khan et all 226 (ANK)! Fethiye-Kemer, 02.vıı.1977, (IZEF 344)! Kumluca, Mavikent, Yenice kö., 8.ıv.1998, B. Sadak (MARE 5813)! **Aydın:** Sultanhisar, 01.ı.1950, M. Polat (IZEF 1769)! **Denizli:** Tavas'dan 10 km sonra, Kızılçam açıklıklarında, 9.vıı.1962, 840 (ANK)! Tavas-Denizli, Tavas'tan 7 km, yol kenarındaki killi yamaçlar, 1100 m, 7.vıı.1989, E. Tuzlacı (MARE 2019)! Denizli, 7.vıı.1905, St. Lager (ISTE 1361)! Honaz da., Karatepe kö. üstü, 730 m, orman yolu kenarı, 1.vıı.1972, E. Tuzlacı (ISTE 22899)! Denizli Kazıkbeli yolu, Karatepe Gökkaya ağaçlandırma sahası, 750 m, 7.vı.1973, A. Baytop, E. Tuzlacı (ISTE 25505)! Honaz'ın 4-5 km kuzeydoğusu, 500 m, dere kenarı, 13.vı.1973, E. Tuzlacı (ISTE 25917)! Denizli kuzeyi 4 km kala (Aydın yönünden), 8.vıı.1973, E. Tuzlacı (ISTE 26267)! **Muğla:** Fethiye, S of Kestep, Kaş, 11.vı.1969, (EGE 7815)! Fethiye, Kemerbucağı, Dereçatı kö., Bayaslar mevkii, c. 300 m, 15.vı.1967, (EGE 5800)! Fethiye, Bayaslar, Dereçatı, 15.vı.1967, (EGE 4914)! Muğla, 23.vııı. 1949, T. Baytop 1363 (HUB 22976)! Köyceğiz çevresi, 60 m, yol kenarı, 22.v.1991, A. Güner 9250, M. Vural, H. Şağban (HUB 2966)! Köyceğiz, Çandır kö., 360 m, kızılçam ormanı, 19.vı.1991, A. Güner 9516, M. Vural, H. Duman, A. Dönmez, H. Şağban (HUB 22965)! Muğla, 23.vııı.1949, T. Baytop (ISTE 1363)! Fethiye, Baba da. yolu, Fethiye'ye 4 km, kaya üstü, 120 m, 1.vıı.1983, E. Tuzlacı (ISTE 51428)! Fethiye-Kemer, Kemer yakını, 100 m, 1.vıı.1983, E. Tuzlacı (ISTE 51430)! Muğla-Tavas, 2-3 km from Muğla, 150 m, D. 35506 (ISTE 51819)! **C3 Afyon:** Başmakçı, 27.vı.1995, U. Zeybek (IZEF 3417)! **Antalya:** Alanya, Davis 14486 (ANK)! Göynük, 50 m, Davis 15032 (ANK)! Kemer, Kumluca, marnlı topraklar, 250 m, 26.v.1984,Y. Akman 13643 (ANK)! Lara, 29.vı.1938, Gassner 1173 (ANK)! Kargı çayı, 24.vııı.1947, Davis 14417 (ANK)! Alanya, Alarahan, 23.ıv.1978 (EGE 15295)! Alanya'nın 5 km batısı, 80 m, 17.vı.1966, (EGE 6379)! Side çevresi, 5.vı.1970, A. Pamukçuoğlu-Quezel (HUB 22970)! Akseki, 18.vıı.1967, H. Küçük (ISTE 12006)! Çakırlar, Akdamlar kö. üstleri, Fesleğen Y. yolu, 200 m, 4.vııı.1980, N. Özhatay, E. Tuzlacı, B. Çubukçu, A. Meriçli (ISTE 45662)! Manavgat üstleri, Beşkonak-Olukköprü, 100 m, 3.vıı.1982, G. Çakırer, A. Öztekin (ISTE 49125)! Manavgat'tan 21 km ileride, Oymapınar barajı civarı, 100 m, 19.vı.1983, H. ve G. Çakırer (ISTE 51086)! Finike-Elmalı yolu, Arif köyüne gelmeden Aygır suyu mevkii, Arycandra Lokantası civarı yamaçlar, 13.vııı.1990, A.H. ve F. Meriçli (ISTE 62458)! **Isparta:** Sütçüler, Darıbükü, N37°34' E31°11', 12.vııı.2001, N. Sadıkoğlu (ISTE 81647)! **C4 Antalya:** Gazipaşa, Sugözü kö., *P. brutia* ormanı, 1600 m, 12.vıı.1982, H. Sümbül 2983 (HUB 22967)! Kumluca, Andrasan koyu kuzeyi, serpentin arazi, *P. brutia* ormanı, 0-120 m, 8.vı.1979, H. Peşmen 4384 et Güner (HUB 22969)! Alanya-Gazipaşa 35. km, 9.vııı.1965, N. ve M. Tanker, E. Sezik (ISTE 8280)! Alanya, Emirgan civarı, yol kenarları, 21.vııı.1965, E. Sezik, M. Tanker (ISTE 8316)! Alanya, Deretürkenaz yolu, Bektaş çeşmesi, 325 m, 24.ıı.1966, A. ve T. Baytop, N. Tanker, E. Sezik (ISTE 8529)! Alanya kalesi, 26.v.1966, A. ve T. Baytop, B. Çubukçu (ISTE 9670)! Güzelbağ-Gündoğmuş, 520 m, 5.vııı.1980, E. Tuzlacı, B. Çubukçu, A. Meriçli (ISTE 45675)! **Karaman:** Ermenek, Kazancı kasabası civarı, 650-850 m, 21.vı.1984, H. Sümbül 2983 (ANK)! Ermenek-Kazancı kasabası civarı, 650-850 m, 21.vı.1984, H. Sümbül 2983 (HUB 22968)! Bucakkışla, Kuru dere içleri, 450 m, 30.v.1979, M. Vural 1795 (ANK)! **Mersin:** Mut-Silifke, Göksu nehrinin vadisi, vadinin sırtlarında kumlu topraklar, vı.1994, A.J. Byfield B 1212 (ISTE 67467)! Anamur-Silifke, Anamur'un 40 km doğusu, 210 m, 23.ıv.1985, M. Nydegger (ISTE 74762)! **C5 Adana:** Karsantı, Ortaca köprüsü civarı, 1000 m, 26.vı.1973, E. Yurdakulol 1542 (ANK)! Süphan de., Belen köy, 900-1000 m, 2.vıı.1952, Davis 19570 (ANK)! Feke Bakırdağ arasındaki orman yolu, Feke'den 15 km (Feke'nin kuzeybatısı), 820 m, maki arası, 31.vıı.1979, E. Tuzlacı, M. Saraçoğlu (ISTE 43388)! Kozan, Akçalı Orman İşletmesi, istif sahası civarı, 16.vıı.1985, İ. Saracoğlu, T. Ersöz 3010 (ISTE 55628)! **Hatay:** Tekepınar aşağısı, Musa da. etekleri, 330 m, maki arası, 17.vı.1979, E. Tuzlacı, M. Saraçoğlu (ISTE 42305)! **Mersin:** Bahçe, Paslıkale, *P.brutia* ormanı, 450 m, 22.vı.1972, T. Uslu (ANK)! Mersin Gözne yolu, Buluklu kö. üstü, 420 m, 5.vı.1981, E. Tuzlacı (ISTE 46365)! Gülnar-Aydıncık, Gülnar'a 15 km, Ezkiyörük kö. çevresi, 850 m, 15.vı.1981, E. Tuzlacı (ISTE 46509)! **Niğde:** Ulukışla, Aydos Da., Ali hoca yakınları, kalkerli yamaç, 1200 m, 28.vıı.1977, S. Erik 2989 (HUB 22973)! **C6 Gaziantep:** Besni, v.1936, Gleisberg 110 (ANK)! **Hatay:** Hatay-Hassa, L. Behçet *s.n.* (VANF)! Harbiye'nin güneyi, kurak, 18.x.97, 100-130 m, L. Behçet 9 (VANF)! Antakya, Habibineccar da., Taş ocağı yanı, 24.vı.1974, A.H. Meriçli (ISTE 29970)! Altınözü, 3.vıı.1975, M. Miski (ISTE 32979)! Belen sırtları, 20.vııı.1976, M. Miski (ISTE 35959)! Reyhanlı, Harran (Kavalcık) kö., Suriye sınırı bölgesindeki ekilmemiş kıraç bölgeler, 26.v.1977, E. Tuzlacı (ISTE 37181)! Antakya yakınları, 25.vı.1982, M. Miski (ISTE 49934)! İskenderun, pazardan satın alındı, 6.ıv.1989, T. Baytop (ISTE 60150)! Antakya, Habibneccar da., 28.vı.2004, N. Sadıkoğlu (ISTE 81696)! Yayladağ, Sebenoba kö, tarla kenarı, 29.vı.2004, N. Sadıkoğlu (ISTE 81648)! **Maraş:** Maraş Göksun yolu, Ceyhan köprüsü çevresi, 700 m, 13.vı.1981, E. Tuzlacı (ISTE 46494)! **Osmaniye:** Sorkun yaylası, Amanos da., 400 m, 21.vı.1967, Y. Akman 7721 (ANK)! **C7 Adıyaman:** Kahta-Sincik, Cendere köprüsünün 1 km ilerisi, Kahta'ya 22 km, 680 m, killi tepeler, 15.vı.1979, E. Tuzlacı, M. Saraçoğlu (ISTE 42247)! **C8 Mardin:** Mardin-Deyrulzafaran kilisesi, kilise yakınındaki kıraç yamaçlar, 880 m, 9.vı.1979, E. Tuzlacı, M. Saraçoğlu (ISTE 42127)! **C9 Siirt:** Eruh-Şırnak, Eruh'a 12 km, Yanılmazı kö. yakınları, 1200 m, 18.vıı.1981, E. Tuzlacı (ISTE 47387)!

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Adsorption of iron, lead, paracetamol, imipramine on natural polymers

Gonul Sahin*, Sonia Sanajou, Reihaneh Behnoush, Ehsan Bahramzadeh

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

Poisoning results from many reasons such as misuse or overdose of drugs. Suicidal and murder purposes are mostly severe, serious and life-threatening cases which require immediate intervention and treatment. Among lifesaving methods external and/or internal decontamination is the most important. Internal decontamination (gastrointestinal) is an effective process for intoxication control that can be done by adsorptive materials. Activated charcoal is used as unique local antidote for adsorption of causative agent. Considering their significance and effectiveness, adsorptive materials are necessary to be developed. In the present study, starch and naturally extracted pectin from citrus, in the presence of trace amount of potassium per sulphate as initiator, were thermally grafted to chitosan to form natural, inert, and highly adsorptive polymeric surfaces. This polymer is convenient for biomedical purposes. Upon drying at 37°C for 48 hours, thermally cross-linked products were obtained. FTIR, UV-Visible spectrophotometer and SEM analyses were applied in order to characterize the products. To evaluate the adsorption potency of new adsorptive material, lead and iron which cause common poisoning were applied on the polymers. The results showed that adsorption degree of lead and iron were maximum 50% and 30% respectively. Desorption amounts can be a sign of adsorption potency. In this study, paracetamol and imipramine, which are commonly used drugs that can and caused intoxication in case they are misused or use for suicidal purpose were applied onto two polymers which contain pectin desorption amount for two drugs were determined. SEM pictures taken before and after blood/polymer contact didn't reveal any significant blood component attachment on the chitosan-*graft*- (starch; pectin) film surface. Indicating no hemocompatibility.

Keywords

Chitosan, decontamination, hemocompatibility, imipramine, iron, lead, paracetamol, pectin, starch.

Article History

Submitted: 24 June 2019 Accepted: 30 July 2019 Published Online: 11 September 2019

Article Info

***Corresponding author:** Gonul Sahin, email: gonul.sahin@emu.edu.tr

Research Article:
Volume: 2 Issue: 1 September 2019 Pages: 33-54

©Copyright 2019 by EMUJPharmSci – Available online at dergipark.org.tr/emujpharmsci.

INTRODUCTION

Intentional and unintentional exposure to various types of drug, chemicals, residuals, wasting materials, herbal products, food contaminants and environmental pollutants at high doses via many ways are common reasons for health problems especially in human (Crowl and Louvar 2001). As a result, many different type poisonings can be seen. Intoxication approaches that consist of airway, breathing, circulation and decontamination (ABCD) are lifesaving and important.

Decontamination has both external and internal types. Internal decontamination of gastrointestinal system is very important in poisoning. For this purpose, some processes such as stomach washing and emesis are applied. Furthermore, adsorption of causative substance would be useful and effective.

Many drugs, chemicals and causative agents are easily adsorbed to local antidote at considerable amount. On the other hand, some others don't show affinity to local antidote. One of the best-known adsorptive materials is activated charcoal that has a wide range of adsorptive potency to different materials and drugs. Development of such adsorptive material with higher affinity will be very useful.

For this reason, in the present study some polymers were prepared as new adsorptive materials. Heavy metals like lead (Pb), essential metal iron (Fe), paracetamol as analgesics and a tricyclic antidepressant, imipramine, were analyzed to evaluate their adsorptive degree to these new polymers.

Although some metals are essential for the body heavy metals which cause toxic effects in human can be very harmful (Geiger and Cooper 2010). Lead exposure happens through air and food in almost the same amounts. More than 50% of lead productions are produced from petrol. Industrial lead exposure happens in mines and smelters, together with lead painted metal repairing, and in battery plants. Glass industries are responsible for low or moderate exposure. High levels of air discharges may lead to pollution in areas near lead mines and smelters. Soil and water can be contaminated by airborne lead deposition which may lead to human exposure via food chain.

Lungs absorb up to 50% of inhaled inorganic lead. Adults absorb 10–15% of the lead inside food, while children's gastrointestinal tract may absorb up to 50%. Lead is bound to erythrocytes in blood, and has a slow elimination rate via urine. Lead accumulates in the skeletal system and has a slow releasing rate from different tissues and structures. The half-life of lead is about 20–30 years and 1 month in skeleton and blood, respectively. Organic lead compounds can enter the body and cell membranes (Järup 2003). Tetramethyl and tetraethyl lead have

good skin penetration. These compounds also have the ability to cross the blood-brain-barrier in adults. That's why organic lead compounds may cause acute poisoning and encephalopathy in adults in consequence. Blood-brain-barrier permits the entrance of organic lead in adults. In babies the crossing ratio to brain is higher than adults. The high gastrointestinal uptake and the penetrable blood-brain-barrier make children good candidates for brain damage related with lead exposure. Headache, irritability, abdominal pain, proximal renal tubular damage and other different nervous system related symptoms are the symptoms of acute lead poisoning. Sleeplessness and restlessness are the most important symptoms of lead encephalopathy. Behavioral disturbances and learning and concentration difficulties are the results of lead poisoning in children (Järup 2003). Patients with lead encephalopathy may experience acute psychosis, confusion and reduction in consciousness. People who have been under lead exposure for a long time may undergo memory deterioration, prolonged reaction time and reduction in understanding ability. Individuals whose blood lead levels are under 3 μmol/l may express peripheral nervous symptoms with decreased nerve transference velocity and dermal responsiveness. If severe neuropathy occurs, the lesion may stay for life long. In less severe circumstances, the most noticeable sign of lead poisoning is hemoglobin synthesis disturbance, and long-term lead exposure may lead to anemia and kidney damage (Järup 2003). Inorganic lead shows toxicity in nephrons because of its high affinity to accumulate in proximal tubule. Kidney damage can occur as result of long-term lead exposure. According to a recent study including Egyptian policemen, NAG excretion was directly shown to be related with the duration of lead exposure (Gang *et al*. 1988).

Iron in trace amounts is essential element for body. Iron overdose may cause serious poisoning in which symptoms usually appear within 6 hours after poisoning. Iron poisoning occur at 5 stages which may result in life threating circumstances such as destructive damages to gastrointestinal (GI) mucosa, hemorrhagic gastritis, considerable fluid loss, bleeding and shock (Baranwal and Singhi 2003).

Moreover, drugs which are used at normal therapeutic doses for diagnosis, therapy and poisoning treatment, can also lead to poisoning (Prescon 1983).

Normally paracetamol (acetaminophen) is known as a safe drug but since it's an OTC drug which is consumed in large amount by adults and children, its toxicity which may cause acute centrilobular hepatic necrosis is common. Paracetamol poisoning has no specific or early signs and symptoms and doesn't lead to impaired consciousness.

Acute renal failure which is an uncommon complication may also occur (Prescon 1983; Haddah *et al.* 1983).

Imipramine is an antidepressant medication that is a cheap and accessible. Therefore, its accidental consumption or use for suicidal purposes is common. The toxicity signs include, slow breath, low blood pressure, rapid heartbeats and disturbance of electrocardiograph (Brush and Aaron 2007). An adsorptive blood compatible polymer must be inert, non-reactive, form's strong bonds and possess high affinity to agents. The polymer-agent complex should be easily excreted by feces (Bahramzadeh *et al.* 2019).

MATERIALS AND METHODS

Following materials, instruments and methods were used to prepare and characterize the polymers: Chitosan medium molecular weight (450 kDa) with degree of deacetylation of 85% (Sigma-Aldrich), corn starch, lemon extracted from citrus fruit potassium per sulfate (KPS) (EDH Chemicals LTD), (Titrachem), acetic acid (Sigma-Aldrich), acetone (Tekkim Kimya San), ethanol (Selim ve Oglu Ltd), hydrochloric acid (Merck) without any purification, lead two oxide (Sigma-Aldrich), iron three chloride (Sigma-Aldrich), dithizone (Sigma-Aldrich), salicylic acid (Sigma-Aldrich), paracetamol tablet (Minoset), imipramine tablet (Novartis 25 mg) and methanol (Sigma-Aldrich). The products were characterized by FTIR (Perkin Elmer, Spectrum Two Spectrometer), UV-visible spectrophotometer (Perkin Elmer) and scanning electron microscope (SEM) (LEO 1450 VP Scanning Electron Microscope). SEM analysis was carried out in Ferdowsi University, Mashhad, Iran.

Preparation of new adsorptive material

Pectin extraction

25 g of lemon was weighted and transferred in a 250 ml beaker. 100 ml of water and 1.5 ml of 3 N sulphuric acid (H_2SO_4) solution were added. The mixture was heated by magnetic stirrer. Temperature of the mixture was controlled by a thermometer and heated at 85-90 °C for 30 minutes. Following heating, the aqueous part was filtered through a cotton swab of cotton wool. Cotton was squeezed with the help of baguette. The remaining crusts were further extracted for 15 minutes under the same conditions. The filtrates were combined. The filtrate was transferred to a 500 ml sieve and 96° ethanol was added until the alcohol grade became 55°. The precipitated pectin was filtered under Buchner funnel under a slight vacuum. The precipitate was completely transferred to a funnel and washed firstly with 25 mL of 96° alcohol and then continued washing, for two times with 15 mL of acetone.

Preparation of films

Specific amount of starch and pectin were dissolved in 15 mL of chitosan solution (1% w/v solution in 1% v/v acetic acid solution) at room temperature, as shown in Table 1. The mixture was transferred to a petri dish, and a film layer was formed due to thermal crosslinking, following the evaporation of solvent at 37°C for 48 hours. Dried samples were taken and impurities were cleaned off the films by immersing in water. Grafted products were named as chitosan-*graft*-(starch; pectin).

Under similar experimental conditions, in the absence of pectin, a mixture of chitosan/starch solution was also prepared in a form of film.

Table 1: Synthesis of chitosan-graft-starch and chitosan-graft-(starch; pectin) films*.

Film	Pectin (ml)	Starch (g)
F1	0	0.2
F2	2	0.2
F3	5	0.2

*15 mL of 1% v/v acetic solution, 0.15 g chitosan, 0.075 g KPS, 37°C, 48 hours.

Swelling kinetics

From each of dry thermally cross-linked sample film samples, 0.01 g was taken, soaked in water. The weight was recorded in every 30 minutes (leaking was avoided). Swelling percentage was calculated with respect to the following equation (Bahramzadeh *et al.* 2019):

$$\text{Swelling \%} = \frac{Ws-Wd}{Wd} \times 100 \text{ ; eq. (1)}$$

where Ws (g) and Wd (g) stands for the weights of swelled and dry hydrogels, respectively.

In-vitro platelet adhesion analyses

Films were covered by human fresh blood obtained from healthy donors, washed by ultra-pure water and dried to examine contact properties by SEM (Caner *et al.* 1998).

Lead adsorption by chitosan-*graft*-(starch; pectin) films

Films (0.05 g) were covered by 10 ml Pb^{2+} solution at 125, 250 and 500 ppm concentrations at 1, 2, 3 and 24 hour time intervals. 2 ml Pb^{2+} solution from each test tube was taken and mixed with 1 ml alcohol dithizone solution. Resultant absorbance values was recorded, at room temperature at 472 nm wavelength. Triplicated measurement was applied for each sample and the average value was recorded. Percent removal of Pb^{2+} was calculated according to following equation:

$$\frac{(Ai-Af)}{Ai} \times 100 \text{ ; eq. (2)}$$

where Ai is initial absorbance and Af shows final absorbance.

Fe^{3+} adsorption by chitosan-*graft*- (starch; pectin) films

0.01 g of the film was added to 4 different test tubes and covered by 4 ml Fe^{3+} solution at the concentrations of 125, 250, 500, 900 ppm and incubated for 1, 2, 3, 4 hours. From each of the test tubes, 0.5 ml Fe^{3+} solution was drown and mixed with 0.5 ml of 5-sulfosalicylic acid dehydrate, (10% w/v), and the volume was completed upto 4 ml using pH = 1 buffer solution. Absorbance was recorded by visible spectrophotometry at 505 nm. Finally, percent removal of Fe^{3+} was calculated according to equation 2.

Paracetamol loading into chitosan-*graft*-(starch; pectin) films

0.01 g of the film was added to 4 different test tubes and covered by 4 ml paracetamol solution at the concentrations of 3.75, 7.5 and 15 ppm and remained for 48 hours. After 48 hours, the films were taken from solution and dried. Afterwards, they were placed in a test tube and 4 ml distilled water was added to release the adsorbed paracetamol. Desorption results were monitored by UV-Visible spectrometer at 242 nm in 5 hours with 1 hour intervals.

Imipramine loading into chitosan-*graft*-(starch; pectin) films

0.01g of the film was added to 4 different test tubes and covered by 4 ml imipramine dissolved in methanol at the concentrations of 0.077, 0.155, 0.31 ppm. After 48 hours, the films were taken and placed in a test tube covered with 4 ml methanol and the desorption results were taken by visible spectrometer at 251 nm.

RESULTS

Figure 1 shows photograph of Chitosan-*graft*- (starch; Pectin) films.

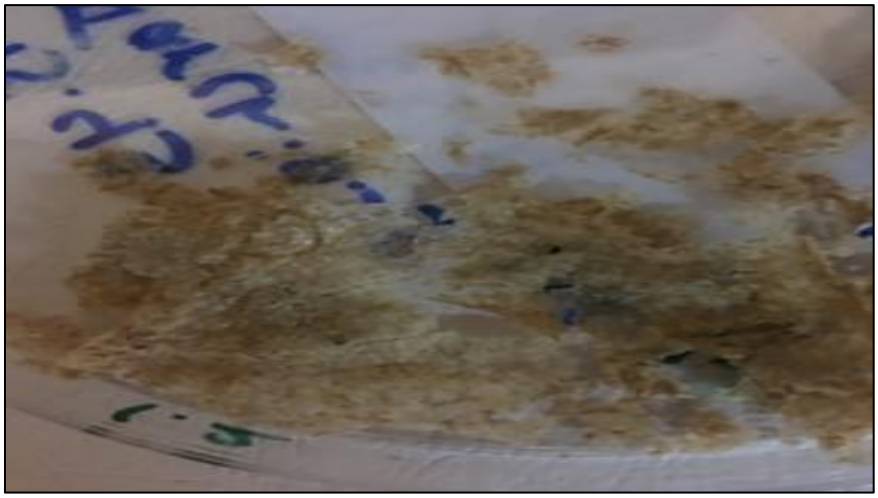


Figure 1: *Chitosan-graft- (starch; Pectin) films.*

Table 2 shows at (37℃) the percent of grafting increases while the amount of pectin increased.

Table 2: Synthesis of Chitosan-graft- (starch; pectin) Films.

Sample ID	Starch (g)	Pectin (g)	Grafting % 37℃
S1	0.2	0	0
S2	0.2	2	23
S3	0.2	5	43

Swelling behavior of chitosan-graft-polyHEAA and chitosan-graft-(polyHEAA;MBA) films

Figure 2A and 2B show the swelling behavior of chitosan-*graft*- (starch; pectin) films with different amount of pectin. They both showed maximum 70% swelling but at different time intervals. S3, standing for a compound with higher amount of pectin, marked maximum swelling after 30 minutes whereas S2, representing the same compound containing less amount of pectin showed maximum swelling after 90 minutes. They both increased the biodegradability time because samples lasted for a longer period. This promises the natural modification for drug loading and adsorbing toxins. On the other hand, S1 was degraded far faster and less controllable.

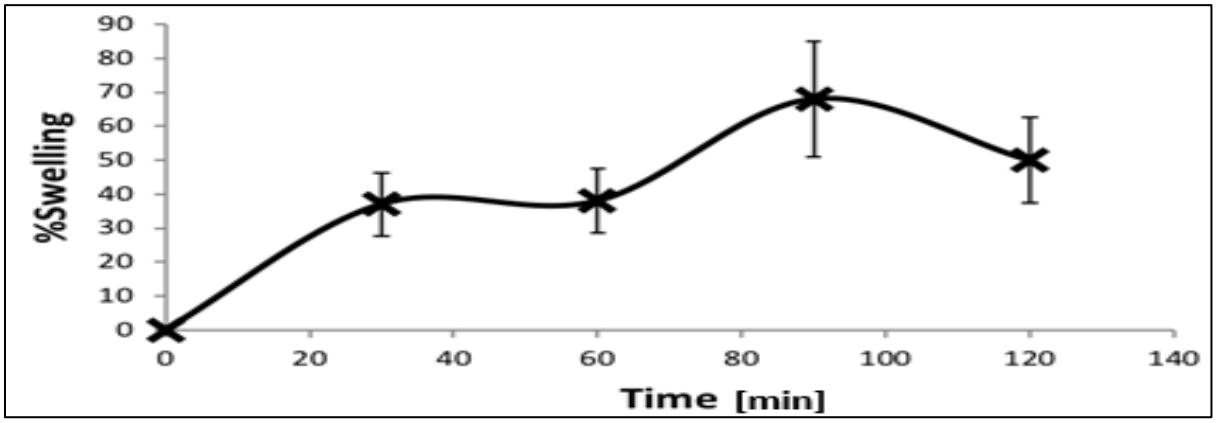


Figure 2A: Swelling behavior of S2 (Chitosan-graft-(starch; 2g Pectin) films at pH=7.4 in 2 hours within 30 min interval.

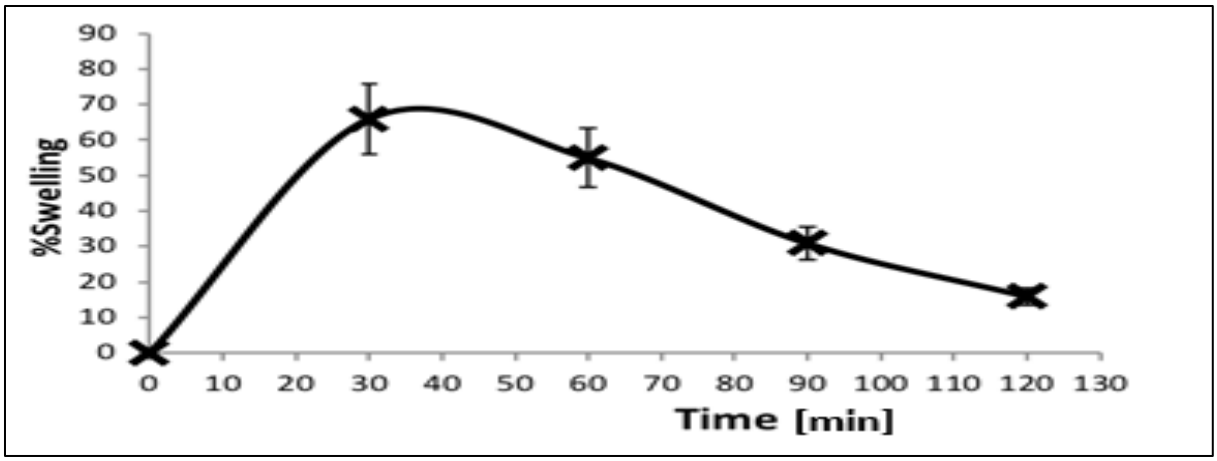


Figure 2B: Swelling behavior of S3 chitosan-graft-(starch; 5 g pectin) films at pH=7.4 in 2 hours within 30 min interval.

FT-IR Analysis

Films were characterized by FTIR spectrometer to assess modifications. Figure 3a shows major functional groups for chitosan where broad band after 3000 cm^{-1} represents H-bonding and 2 picks on 1551 and 1649 cm^{-1} stands for C-O and amide functional groups, respectively. Moreover, 2 picks at 2884 and 2974 cm^{-1} show C-H stretching. However, a pick at about 1370 cm^{-1} appeared when the polysaccharide chains were grafted onto the chitosan backbone which were concluded as C-H bond. In the FTIR spectrum of chitosan-starch shown in Figure 3b, amide band at 1649 cm^{-1}, C–H bending vibrations in the 1400–1500 cm^{-1} region, $–CH_3$ bending at 1380 cm^{-1}, C-H stretching at 2884 and 2974 cm^{-1} and O-H stretching at 3339 cm^{-1} were recorded.

When it comes to the pectin containing films, (Figure 3c and 3d), all the previous spectra were similar except between 1630 $cm^{-1}$and 1747cm^{-1}. Moreover, an additional signal appeared which became more intense once the pectin amount had been increasing (Figure 3d).

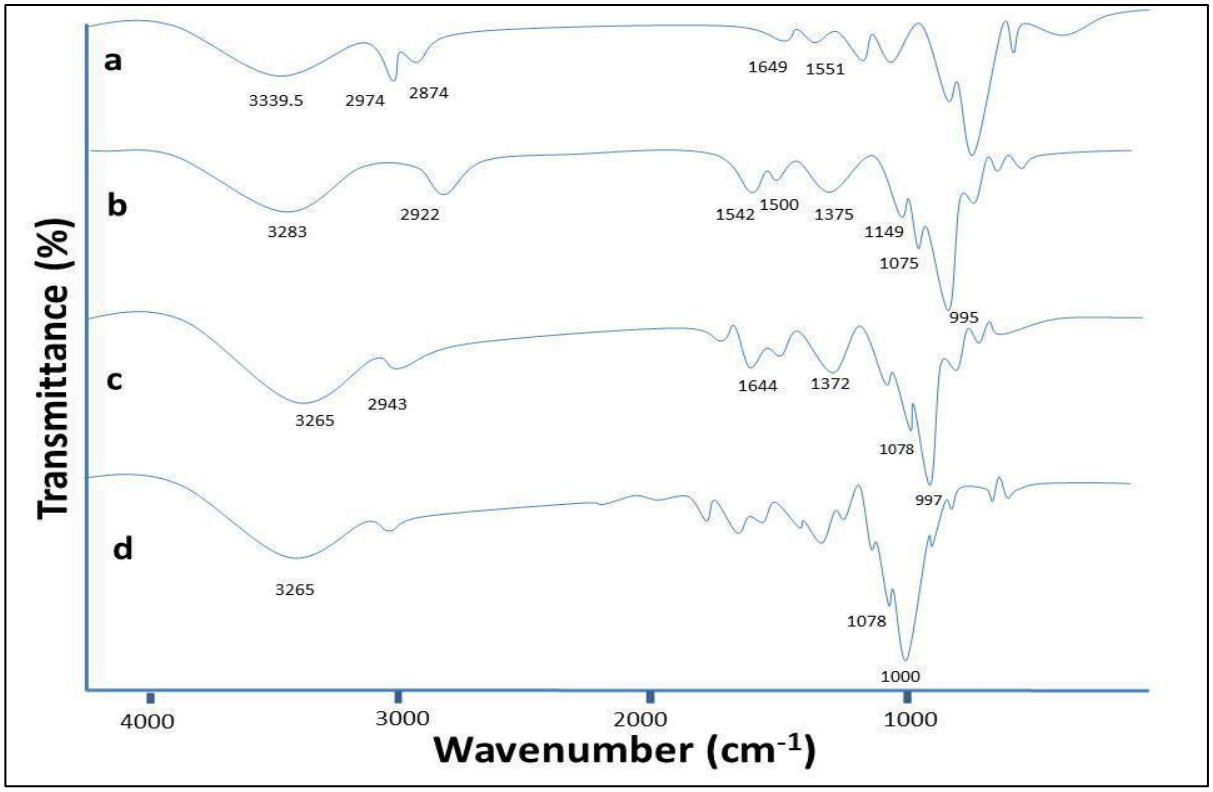


Figure 3: The FTIR spectrum of a. chitosan b. S1 (chitosan grafted starch) c. S2 (chitosan-graft-(starch; 2 g Pectin) films d. S3 (chitosan-graft-(starch; 5 g pectin) films.

SEM analysis for chitosan-graft-(starch; pectin)

The surface morphologies of grafted products were examined using SEM pictures as shown in Figure 4A, 4B and 4C. SEM images of samples exhibit more spherical structures on the surface and therefore wider surface area and more adsorption potency due to excessive exposed active sites, in the presence of pectin in samples S2 and S3. On the other hand, less fine spherical structures were detected in S1 where starch was grafted on to chitosan. In brief, addition of pectin showed a positive modification role when its entire surface area was taken into account.

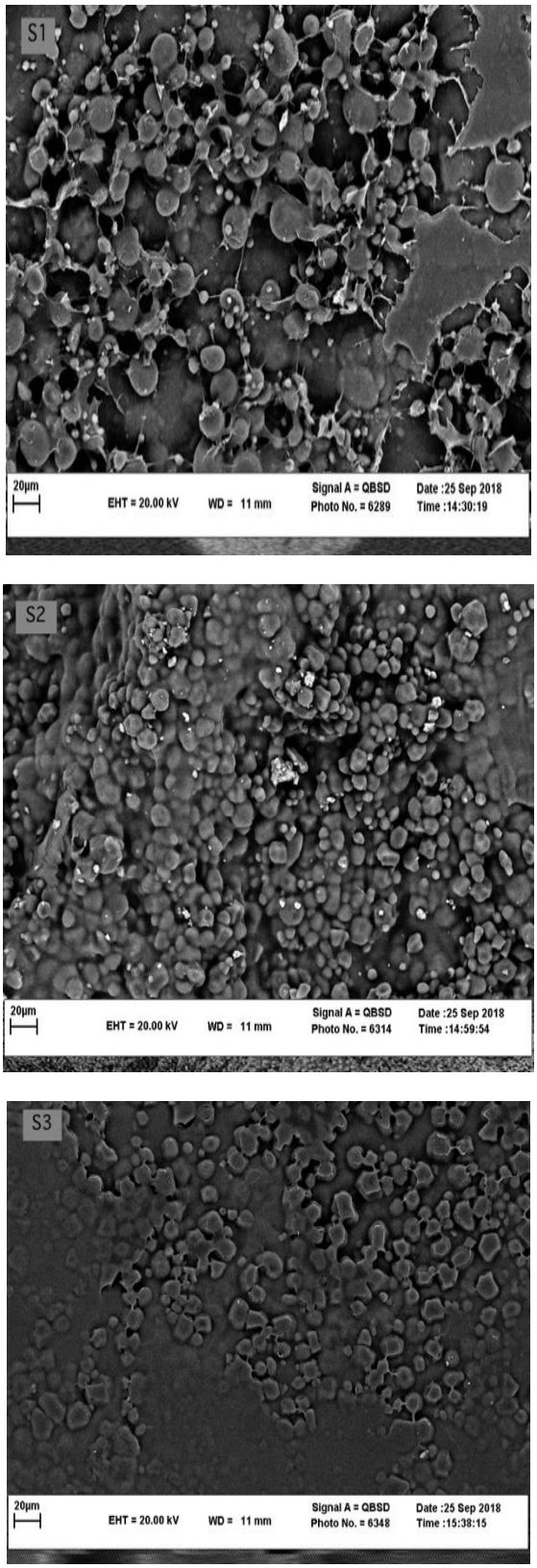


Figure 4A: SEM pictures (200 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (2 g) (S2), and starch (0.2 g) chitosan-graft-pectin (5 g) (S3),

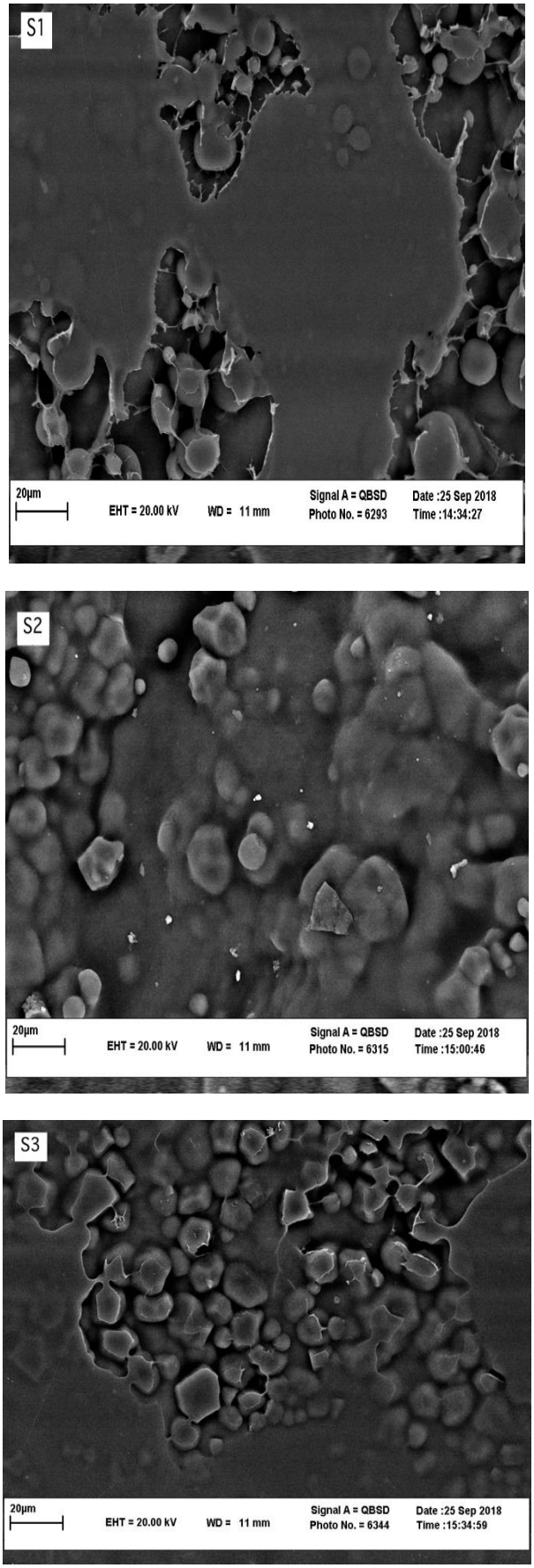


Figure 4B: SEM pictures (2000 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (2 g) (S2), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

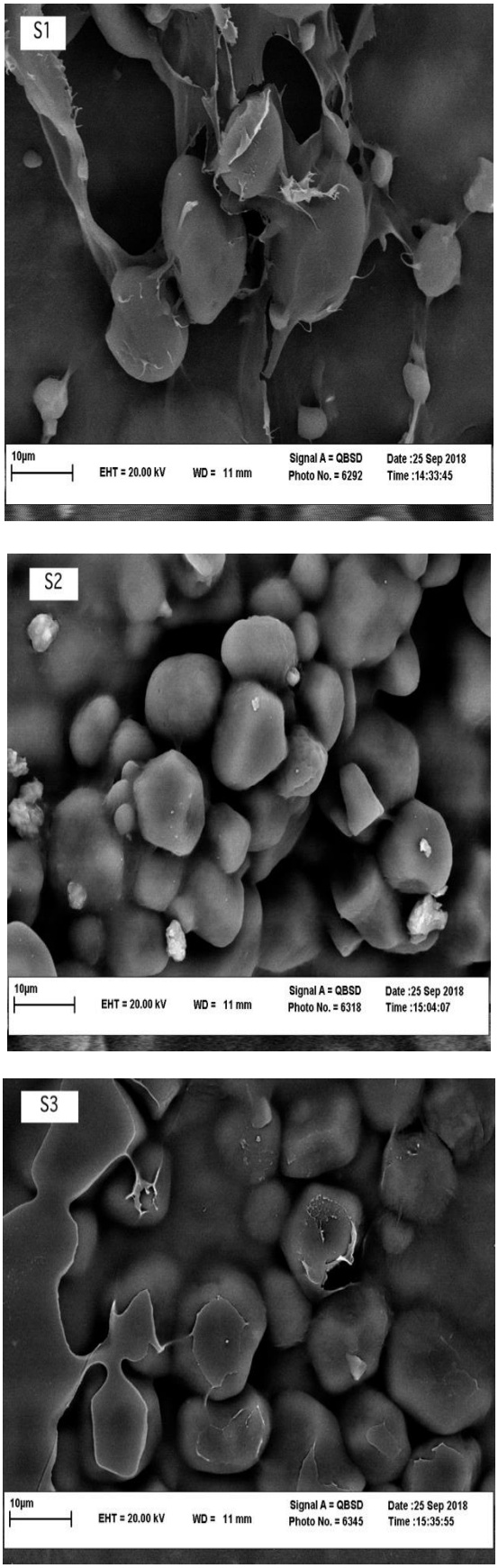


Figure 4C: SEM pictures (5000 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (2 g) (S2), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

In vitro platelet adhesion

Figure 5 shows the SEM images for films when they come in contact with blood in vitro conditions. The surface of polymers does not exhibit any notable different texture. Any sign of blood coagulation is not detectable before and after blood contact. However; denser matrix, as a result of physical blood stream, seems to be loaded in pores of films.

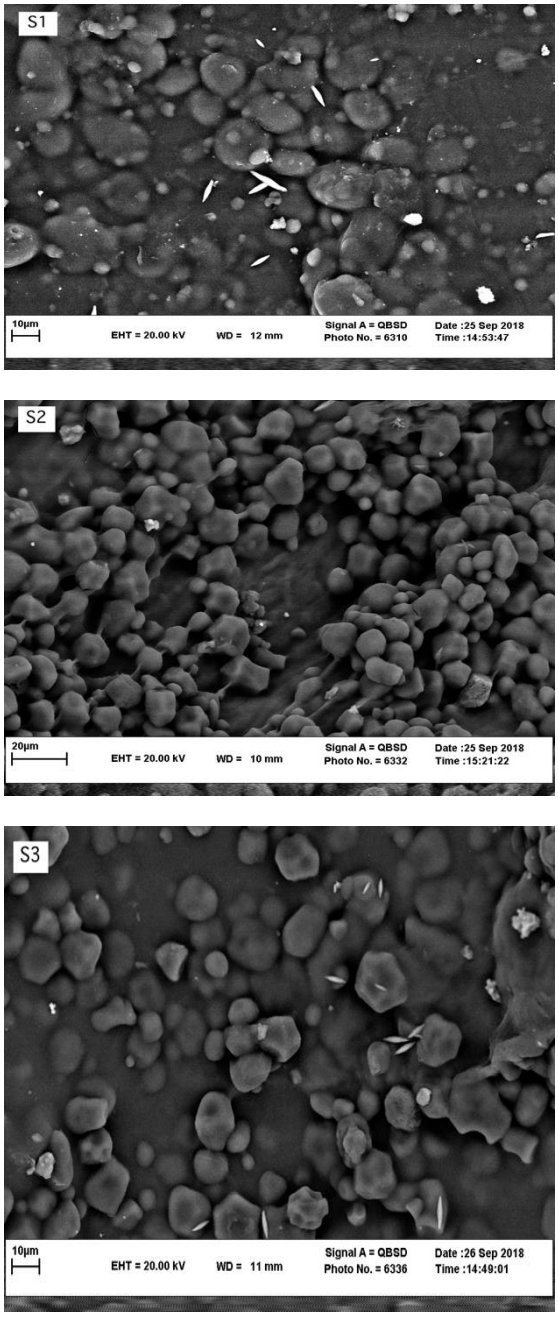


Figure 5: SEM picture after blood contact (2000 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (2 g) (S2), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

Lead adsorption on chitosan-*graft*- (starch; pectin) films spectrometric adsorption analysis of lead on chitosan-*graft*- (starch; pectin) films

Tables 3A, 3B and 3C show lead removal percentage from lead solutions at various concentrations.

The results show that as the amount of pectin increases, higher amounts of lead were adsorbed by the films. This evidence was generally and specifically confirmed by SEM. The SEM pictures are exhibited in Figure 4A, 4B and 4C. Despite some fluctuations at low concentration, S3 was adsorbed well whereas at high concentration S2 was dominating adsorbent. In order to monitor lead adsorbtion, SEM pictures were taken. As a result, lead was adsorbed more on the surface of chitosan-*graft*-starch films when compared to chitosan-*graft*-(starch; pectin) sample that showed intensive adsorption. This could be the result of higher lead adsorption potency of pectin containing films.

Table 3A: Pb^{2+} removal at 125 ppm solution.

Removal % (125 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	0	1,531729	1,531729
2	32,82276	43,32604	55,57987
3	21,00656	27,78993	41,57549
24	17,72429	23,63239	38,0744

Table 3B: Pb removal at 250 ppm solution.

Removal % (250 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	2,534113	0,584795	2,534113
2	23,97661	20,07797	22,02729
3	37,4269	27,87524	38,79142
24	-6,23782	26,90058	13,84016

Table 3C: Pb removal at 500 ppm solution.

Removal % (500 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	7,749077	9,409594	8,671587
2	9,225092	10,88561	8,671587
3	11,43911	12,36162	9,594096
24	28,78229	31,18081	27,12177

Results about lead adsorption on chitosan-*graft*- (starch; pectin) films

Figure 6A, 6B and 6C exhibit lead adsorption on low pectin and high pectin polymers in different magnifications.

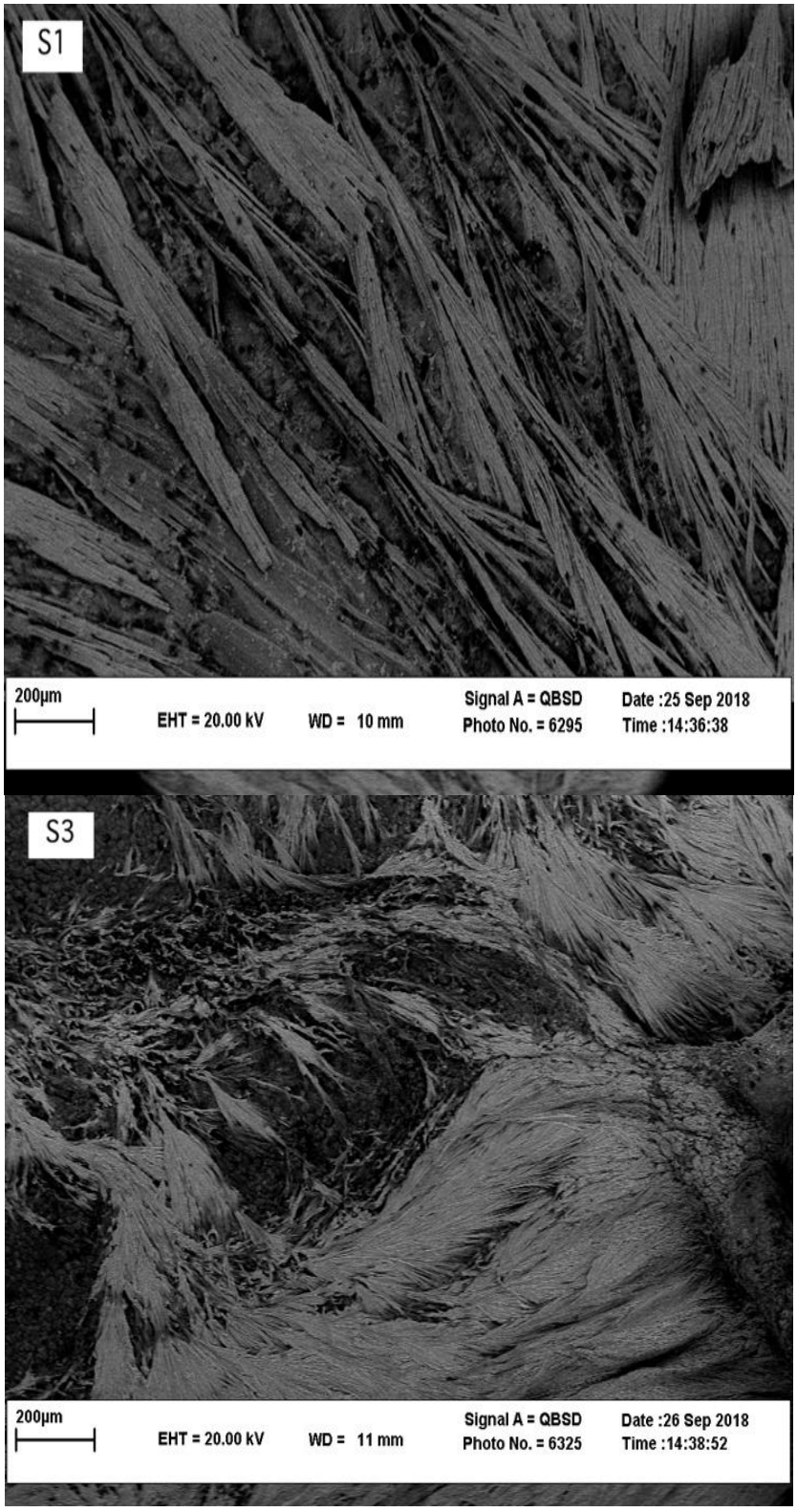


Figure 6A: SEM pictures of lead adsorption (200 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

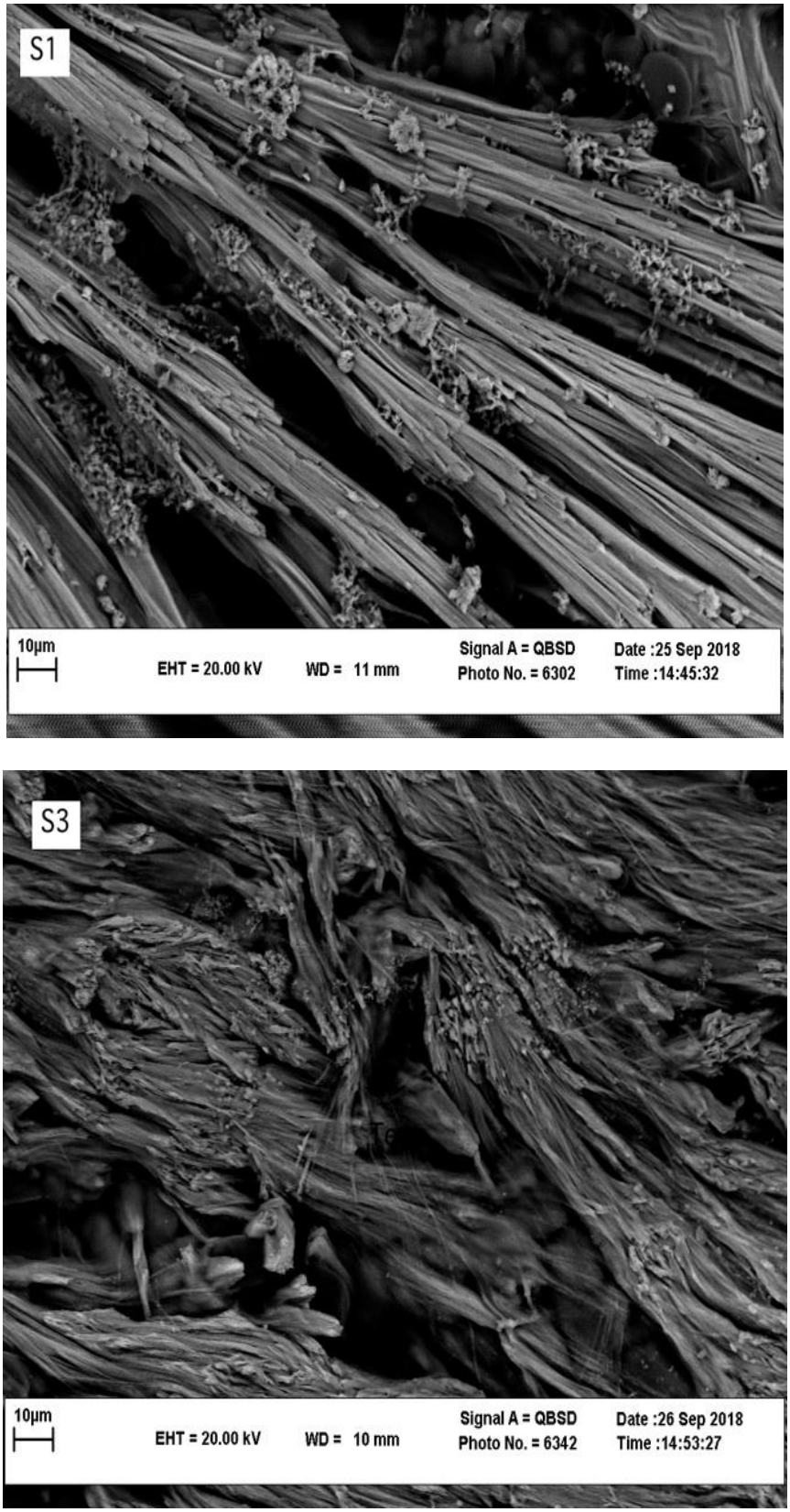


Figure 6B: SEM pictures of lead adsorption (2000 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

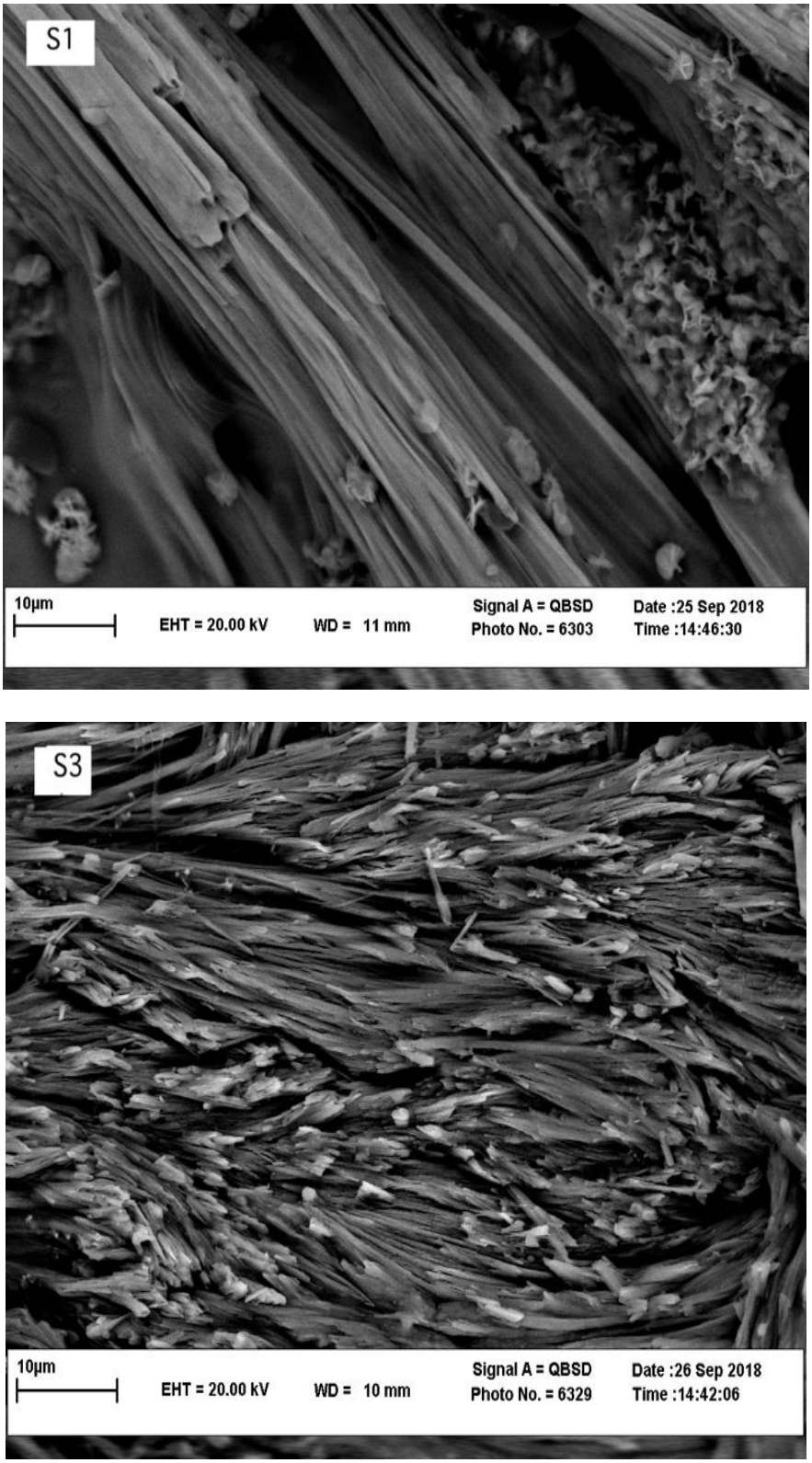


Figure 6C: SEM pictures of lead adsorption (5000 times magnified) of starch (0.2 g) chitosan (S1), starch (0.2 g) chitosan-graft-pectin (5 g) (S3).

Iron (Fe^{3+}) adsorption

Table 4A, 4B, 4C and 4D show iron adsorption at different concentrations. Except 125 ppm which low amounts of pectin showed higher adsorption potency in comparison with S1 (no pectin), at other concentrations all samples show some degree of adsorption but generally S1 was the best adsorbent.

Table 4A: Fe removal at 125 ppm solution.

Removal % (125 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	9,558824	25	15,44118
2	-1,47059	37,5	13,97059
3	8,088235	18,38235	15,44118
4	14,70588	25,73529	18,38235

Table 4B: Fe removal at 250 ppm solution.

Removal % (250 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	28,48665	32,64095	16,32047
2	29,67359	34,7181	28,18991
3	16,32047	13,05638	6,824926
4	33,53116	23,1454	16,91395

Table 4C: Fe removal at 500 ppm solution.

Removal % (500 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	4,761905	11,72161	10,80586
2	22,71062	14,46886	16,66667
3	12,08791	13,00366	10,25641
4	29,30403	31,68498	27,65568

Table 4D: Fe removal at 900 ppm solution.

Removal% (900 ppm)			
Time (h)	S1	S2	S3
0	0	0	0
1	4,761905	11,72161	10,80586
2	22,71062	14,46886	16,66667
3	12,08791	13,00366	10,25641
4	29,30403	31,68498	27,65568

Drug loading

Paracetamol

Table 5A, 5B and 5C show drug loading results after 2 hours of contact time. chitosan-graft- (pectin; starch) films released more paracetamol than chitosan-graft-starch films in certain time intervals which means pectin containing films have higher desorption potency and indirectly can be a sign of higher adsorption potency.

Table 5A: Desorption of sample placed at 3.75 ppm paracetamol solution.

Time (h)	S1	S2	S3
1	0.124	0.105	0.124
2	0.147	0.155	0.168
3	0.156	0.179	0.198
4	0.185	0.178	0.202
5	0.182	0.190	0.219

*Initial: 0.233

Table 5B: Desorption of sample placed at 7.5 ppm paracetamol solution.

Time (h)	S1	S2	S3
1	0.138	0.107	0.116
2	0.154	0.155	0.163
3	0.175	0.182	0.190
4	0.183	0.180	0.230
5	0.186	0.187	0.211

*Initial: 0.464

Table 5C: Desorption of sample placed at 15 ppm paracetamol solution.

Time (h)	S1	S2	S3
1	0.127	0.109	0.108
2	0.156	0.158	0.157
3	0.178	0.187	0.186
4	0.174	0.192	0.238
5	0.190	0.225	0.224

*Initial: 0.233

Imipramine

Table 6A, 6B and 6C shows drug loading results after 2 hours of contact time. Generally, imipramine loaded films follow the same patterns of paracetamol loaded films. This shows that pectin containing films have higher desorption potency for imipramine and indirectly can be a sign of higher adsorption potency.

Table 6A: Desorption of sample placed at 0.31 ppm imipramine solution.

Time (h)	S1	S2	S3
1	0.188	0.153	0.152
2	0.189	0.169	0.198
3	0.197	0.155	0.206
4	0.206	0.169	0.225
5	0.217	0.183	0.207

*Initial: 0.956

Table 6B: Desorption of sample placed at 0.155 ppm imipramine solution.

Time (h)	S1	S2	S3
1	0.164	0.162	0.166
2	0.157	0.193	0.156
3	0.155	0.181	0.186
4	0.176	0.184	0.197
5	0.190	0.212	0.203

*Initial: 0.538

Table 6C: Desorption of sample placed at 0.077 ppm imipramine solution.

Time (h)	S1	S2	S3
1	0.146	0.149	0.164
2	0.171	0.180	0.168
3	0.155	0.181	0.186
4	0.165	0.175	0.179
5	0.166	0.197	0.197

*Initial: 0.357

DISCUSSION

Poisoning cases are very common due to improper drug usage all around the world. These types of cases require immediate curative application and treatment. Among these, decontaminations is very important. For this purpose, adsorbant material like activated charcoal as a local antidote is used. In previous studies, the polymer without pectin was prepared (Hasipoglu *et al.* 2005; Caner *et al.* 2007; Yilmaz *et al*. 2007; Adali and Yilmaz 2009; Yilmaz *et al.* 2016). However, in the present study almost the same polymer with high and low amount of pectin were assayed. The present polymer (thermally grafted natural chitosan-(starch; pectin) showed promising adsorptive potency with respect to natural adsorbent advantages for Pb^{+2}, Fe^{3+} removal and also for paracetamol, imipramine drug loading properties. Pectin grafted polymeric film shows up to 50% Pb^{+2} and up to 34% Fe^{3+} adsorption potency. It also shows up to 46% and 36% better releasing property for paracetamol and imipramine, respectively, after 48 hours of drug loading which indirectly can be a sign of better absorbance capacity.

According to the results of present study, the grafted polymer may be a good candidate as a local antidote for internal decontamination in the treatment of drug and metal poisoning due to it's natural, blood compatible, cost-effective, high chelating and sustained released nature.

Internal decontamination is a very important and effective process for intoxication control that can be done by adsorptive materials.

It should be non-thrombogenic and non-hemolytic which can be found in pectin and starch. Since chitosan is known for its biocompatibility, pectin for its being adoptive, environmentally friendly and having controlled release and starch for its having hydrogen accepting and polymeric properties, the material has got the potential to improve the surface and bulk properties of chitosan as a biomaterial. As synthesis of chitosan-graft starch pectin copolymers have not been reported in the literature before, this study aimed to find out if natural thermal grafted polymers have the adsorptive and drug loading properties. If so, to identify the optimum process conditions, and to characterize the physicochemical characteristics of the products. Although the adsorbent properties of pectin had been studied before, it has not been investigated in terms of it hemocompatibility as an adsorptive matrix grafted to chitosan and starch. The polymer may be applicable for soil, water, air decontamination purposes and worths the further studies.

REFERENCES

Adali T, Yilmaz E (2009). Synthesis, characterization and biocompatibility studies on chitosan-graft-poly (EGDMA). *Carbohydr. Polym.* **77**:136–141.

Bahramzadeh E, Yilmaz E, Adali T (2019). "Chitosan-graft-poly (N-hydroxy ethyl acrylamide) copolymers: Synthesis, characterization and preliminary blood compatibility in vitro." *Int J Biol Macromol* **123**: 1257-1266.

Baranwal AK, Singhi SC (2003). Acute iron poisoning: management guidelines. *Indian Pediatr* **40**(6): 534-540.

Brush DE, Aaron CK (2007). *Tricyclic and other cyclic antidepressants. In: Shannon MW, Borron SW, Burns MJ, eds. Haddad and Winchester's Clinical Management of Poisoning and Drug Overdose*. 4th ed. Philadelphia, Pa: Saunders Elsevier.

Caner H, Hasipoglu H, Yilmaz O, Yilmaz E (1998). Graft copolymerization of 4- vinylpyridine on to chitosan-1, by ceric ion initiation. *Eur. Polym*. J. **34**: 493–497.

Caner H, Yilmaz E, Yilmaz O (2007). Synthesis, characterization and antibacterial activity of poly (N-vinylimidazole) grafted chitosan. *Carbohydr. Polym*. **69**:318–325

Crowl DA, Louvar JF (2001). Chemical process safety: fundamentals with applications Pearson Education.

Geiger A, Cooper J (2010). Overview of airborne metals regulations, exposure limits, health effects, and contemporary research. Environmental Protection Agency, Air Quality: Washington, DC, USA.

Haddad LM, Shannon MW, Winchester JF (1983). Clinical Management of Poisoning and Drug Overdose. 3rd Edition.

Hasipoglu HN, Yilmaz E, Yilmaz O, Caner H (2005). Preparation and characterization of maleic acid grafted chitosan. *Int. J. Polym Anal Charact* **10:** 313–327.

Järup L (2003). Hazards of heavy metal contamination. *Br Med Bull* **68(1)**:167-182.

Prescott LF (1983). Paracetamol overdosage. Pharmacological considerations and clinical managemente. *Drugs* **25**(3): 290-314.

Yilmaz E, Adali T, Yilmaz O, Bengisu M (2007). Grafting of poly (triethylene glycol dimethacrylate) onto chitosan by ceric ion initiation. *React* Funct Polym **67:**10–18.

Yilmaz E, Yalinca Z, Yahya K, Sirotina U (2016). pH responsive graft copolymers of chitosan. *Int J Biol Macromol* **90**:68–74.

Review EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Analgesic nephropathy

Gonul Sahin[1*], Sonia Sanajou[1,2], Hananeh Kordbacheh[1]

[1]Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey.

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs), which are easily accessible, inexpensive and have many therapeutic effects, are used frequently and in large quantities by every age group of patients. NSAIDs reversibly inhibit cyclooxygenase enzymes at various degrees and they have the same action and toxicity mechanism. These groups of drugs can cause damage in many organs when there used at high dose and for a long-time period. Analgesic nephropathy is one of the prominent side effects of NSAIDs which damage kidneys. The present review focused on renal toxic mechanisms induced by NSAIDs. The geriatric group which is the most vulnerable group to misusage of NSAIDs should be well informed and monitored by healthcare professionals to decrease the risk of adverse effects related to NSAIDs.

Keywords

Analgesic, analgesic nephropathy, cortical tubulointerstitial nephritis, non-steroidal anti-inflammatory drugs, renal papillary necrosis.

Article History

Submitted: 24 June 2019 Accepted: 30 July 2019 Published Online: 11 September 2019

Article Info

***Corresponding author:** Gonul Sahin, email: gonul.sahin@emu.edu.tr

Research Article:
Volume: 2 Issue: 1 September 2019 Pages: 55-67

©Copyright 2019 by EMUJPharmSci – Available online at dergipark.org.tr/emujpharmsci.

INTRODUCTION

Analgesic nephropathy is a kind of chronic renal incompetency which is caused by long term usage of one or more analgesic for medication. The specific medication and the dose interval required have not been fully understood (De Broe 1998). Analgesic nephropathy can be considered as one of the causes of end-stage renal failure (De Broe 1998; Chang *et al.* 2008). These patients generally had been taken analgesics for months or years because of chronic pain like headaches or backaches (Gault and Wilson 1978). Its pathological signs; include atrophic kidneys, renal papillary calcification, and irregular renal contour.

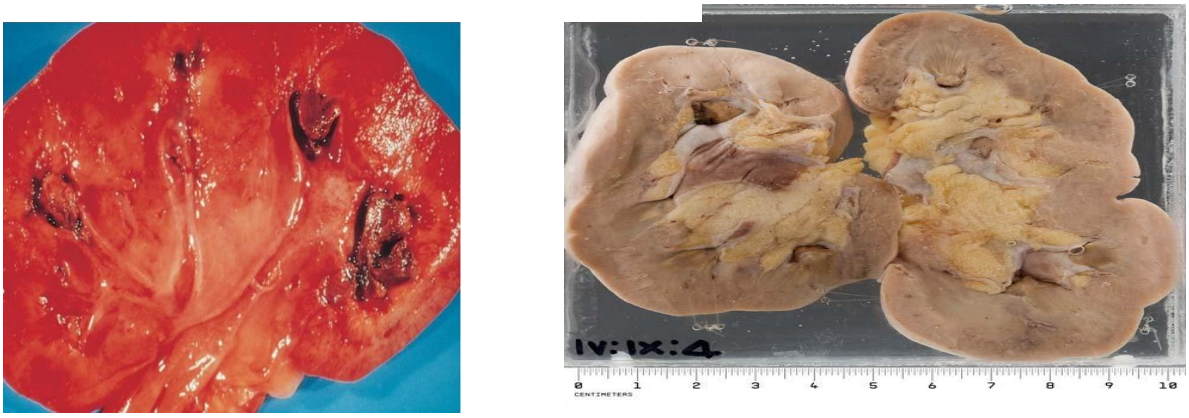


Figure 1: Pathological signs of analgesic nephropathy (Pinter *et al*. 2004; Noels *et al.* 1995; De Broe 2009).

A relation between non-steroidal anti-inflammatory agents (NSAIDs) and chronic kidney disease has long been under investigation. NSAIDs are the most commonly used medicines in the treatment of pains, inflammations and fever. NSAIDs are accessible, cheap, and can be sold with or without prescription (Buer 2014).

Chronic pain is a treatable condition that at any one point in time affects 20%–46% of community-dwelling older adults and 28%–73% of residents in aged-care facilities. A number of practice guidelines and literature reviews related with to the management of chronic pain in the elderly patients suggest paracetamol as the first-line management option (Abdulla *et al.* 2013). Several case-control studies have reported associations between chronic renal failure and other analgesic preparations, including aspirin, antipyretics, and NSAIDs in combination with caffeine, codeine, and/or barbiturates (De Broe 2009). It has been proposed that paracetamol, not phenacetin, accumulated in the renal papilla, and in animal experiments phenacetin appeared to be less nephrotoxic than the other analgesics (Bluemle 1969).

In the 1950s, researchers in Sweden and Switzerland illustrated that renal papillary necrosis developed from long term ingestion

of large number of phenacetins containing analgesic mixtures. During the 1960s and 1970s, phenacetin was singled out as the nephrotoxic agent in the analgesic mixtures, leading to its ban in several countries. Thereafter, the incidence has declined markedly. The nephrotoxicity of phenacetin is dose dependent. An intake of 6–8 tablets per day for a period of 6–8 years lead to the development of AN (Nanra *et al*. 1987).

Continuous and persistent consumption of NSAIDs cause many adverse effects in the body. Especially, prolonged use can cause damage to the kidneys and lead to the development of nephropathy. The major aim of present study was to review nephrotoxicity mechanisms induced by NSAIDs. For this purpose, many literatures were reviewed and under the light of these literatures the mechanisms of analgesic nephropathy were evaluated in detail.

Therapeutic effect mechanism of NSAIDs

NSAIDs reversibly inhibit cyclooxygenase (COX) enzymes at various degrees. Prostaglandins are responsible in functional regulation of several organs such as the gastrointestinal tract (maintaining GI mucosal integrity), increase renal blood flow, promote blood clotting by activating platelets, and also affect kidney function (Hawkey 2001). However, excessive number of prostaglandins are responsible for enhancing fever, inflammation and pain. Therefore, inhibiting prostaglandin production can cause adverse effects even in the therapeutic range of NSAIDs usage. There are two types of isoenzymes, COX-1 and COX-2, which were identified in early 1990s and known to be involved in production of prostaglandin. The main role of COX-1 is to produce prostaglandins and COX-2 become induced in response to inflammation (Hilário *et al.* 2006). Figure 2 shows the arachidonic acid pathway. The location of isomerases of the prostaglandins have be shown in Figure 3.

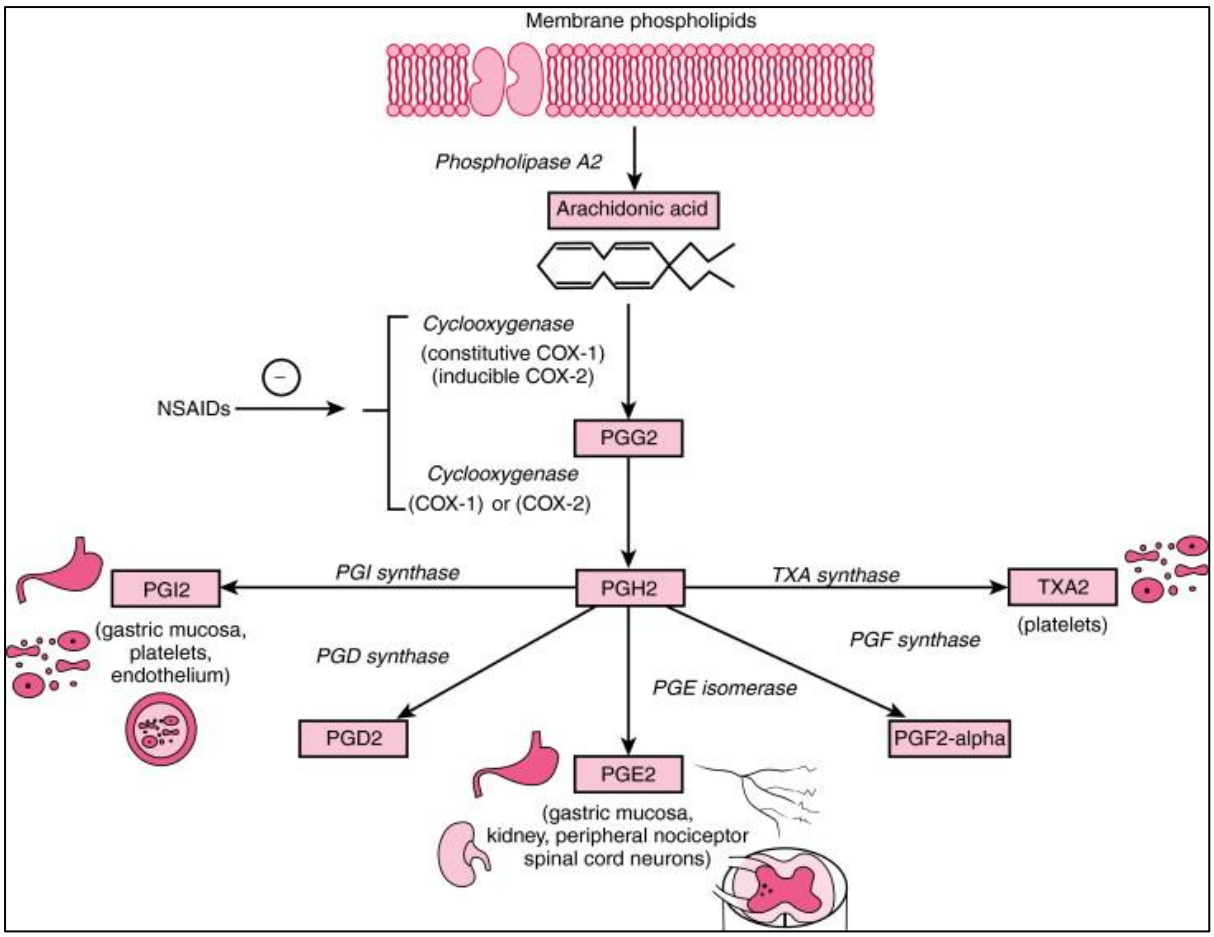


Figure 2: Arachidonic acid pathway (Kawahara *et al.* 2015)

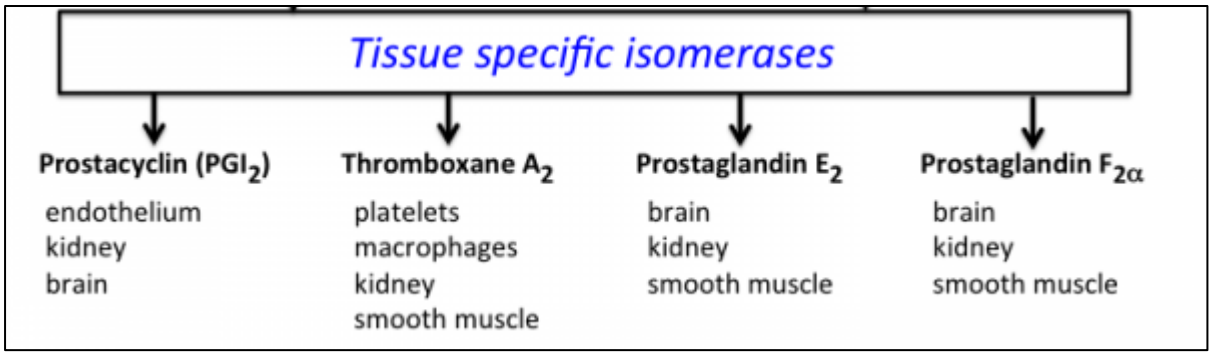


Figure 3: Prostaglandin isomerases specific to each tissue (Kawahara *et al.* 2015).

Many of the NSAIDs act nonspecifically on COX but more recently developed NSAIDs have been reported to act more specifically on the COX-2 isoenzyme, with the aim to decrease fever, pain and inflammatory response whereas reducing associated gastrointestinal and renal side-effects relating to COX-1 inhibition. According to recent studies, COX-2 selective agents such as Rofecoxib and Celecoxib can promote thrombosis and substantially increase the risk of heart attack (Serhan and Levy 2003). However, the pattern of toxicity is the same with COX-2 selective and COX non-

selective (e.g. aspirin) NSAIDs at overdose cases. These medicines act by binding to the active sites of COX and preventing the catalysis of arachidonic acid (AA) to prostaglandins therefore exerting analgesic, antipyretic, and anti-inflammatory properties (Hunter *et al*. 2011). The chronic use of NSAIDs at therapeutic doses is generally safe in patients with normal physiology and without any underlying problem. Toxicity associated with NSAIDs is result of excessive inhibition of COX-1 and eventually reduction in prostaglandin synthesis. Therefore, this condition can trigger organ damage such as gastrointestinal, renal and central nervous systems (CNS) as adverse effects both in therapeutic use and in acute overdose (Hunter *et al.* 2011).

Nephrotoxicity mechanisms of NSAIDs

NSAID-induced renal adverse effects are rare, sometimes temporary and usually reversible at the time of drug withdrawal. The occurrence rate and the seriousness of the renal effects raise in patients with risk determinants such as those who has diabetes, heart failure, cirrhosis, renal dysfunction, users of diuretics and in the elderly subjects. Unwanted effects extend from electrolyte retention and reduce glomerular filtration (by means of inhibiting vasodilator prostaglandins) to nephritic syndrome and chronic renal failure. While acetaminophen and aspirin may develop chronic interstitial nephritis, remaining NSAIDs may generate acute interstitial nephritis, altered intraglomerular hemodynamics, chronic interstitial nephritis and glomerulonephritis. The correlation has been found between high plasma concentration and the renal adverse effect of NSAIDs (Emkey *et al.* 1982). Early diagnosis is important because chronic interstitial nephritis has been known to progress to end-stage renal disease (Harirforoosh and Jamali 2009).

In cases of severe toxicity, detoxification and compensatory mechanisms are insufficient and an increase in rate of kidney insufficiency can be encountered. It has been reported that over 300 chemical substances including NSAIDs cause kidney damage and the incidence of drug-induced nephrotoxicity is as high as 66 % in elderly patients (Murray *et al.* 1971).

Nephropathy can be the result of oxidative stress, impaired membrane integrity, direct toxicity because of chromium or cadmium accumulation. While the different parts of nephron may be the targets of nephrotoxins, the proximal tubule is the most common target site of nephrotoxins, including the drugs (Murray *et al.* 1971).

Glomerular filtration is reduced because of the increase in the amount of tubular cell death. If this happens too many nephrons, the total glomerular filtration rate would

decrease and tubular cell loss leads to the abrasion of the basolateral membrane which would prevent clearance of compounds that are required to be removed from the body by urine. This may lead to acute renal failure within a few days. Severe DNA damage can also cause cell death. If DNA damage that is not very severe is repaired improperly, the remaining DNA lesions may lead to the formation of renal cancer over the years. Long-term treatment with opioid analgesics may not cause direct damage to the kidney. However, it may induce the infiltration of immunocytes to kidneys over a long time. While these immunocytes do not directly produce acute nephritis, the frequent use of these agents may gradually reduce renal function (Schrier 2013).

On the other hand, kidney has a great regeneration ability. Even if a large part of the proximal tubular cells is lost, the cells multiply within 1-3 days after the initial injury. The new cells are flat and within a few days they will differentiate into unique proximal tubule cell (Schrier 2013).

NSAIDs cause nephrotoxicity that can present as various renal syndromes such as acute kidney injury, nephritic syndrome, interstitial nephritis, and chronic renal failure (Figure 4). The common link among these various syndromes is the disruption of PG synthesis. The PGI2 has predominant vascular actions in the form of renal vasodilation. PGE2 has renal tubular action in the form of inhibition of salt and water reabsorption, especially in the thick ascending limbs of loop of Henle and collecting ducts (Schrier 2013).

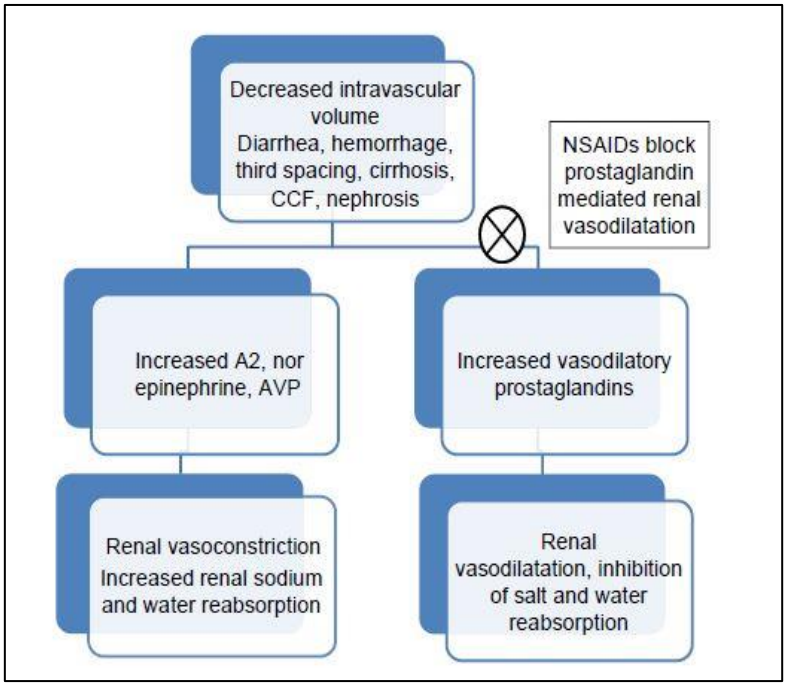


Figure 4: Mechanism of renal toxicity of NSAIDs (Schrier 2013).

These biological actions of the autacoids on the kidney are prominent in the setting of a decreased effective arterial blood volume during which there is an increase in the circulating levels of angiotensin II (AII), arginine vasopressin (AVP) and catecholamines. PGs, once released, act to counterbalance the effect of the abovementioned hormones by causing renal vasodilation and inhibition of salt and water reabsorption. Therefore, the inhibition of PG synthesis by NSAIDs (COX inhibitors) leads to the unopposed action of AII, AVP, and catecholamines, resulting in enhanced renal vasoconstriction and salt and water reabsorption. As the renal medulla is dependent on the production of PGs for its blood flow, the inhibition of PG synthesis by NSAIDs leads to medullary ischemia and papillary necrosis (Schrier 2013).

In order to comprehend the renal effects of NSAIDs, a brief knowledge of the physiologic function of eicosanoids in the kidney is essential. A composite term, eicosanoid, is the outcome of arachidonate or other polyunsaturated fatty acid metabolism and covers prostaglandins, prostacyclin, thromboxane, epoxides and lipoxygenase products. COX isoforms are responsible for this conversion and they are inhibited irreversibly by aspirin and reversibly by the remaining NSAIDs (Schrier 2013). There are four main areas within the kidney that are rich in the concentrations of both isoforms of COX; the afferent and efferent arterioles, interstitial cells of renal medulla, the glomerulus and medullary collecting ducts. Eventual products of the conversion are further metabolized to PGI_2, PGE_{2alpha}, PGD_2 and TXA_2. This suggests that each cell which has cyclooxygenase enzyme can create all types of eicosanoids, however one or two are predominant depending on the situation. Accordingly, arterioles produce PGI_2, glomeruli produce PGI_2, PGE_2 and TXA_2, and both interstitial cells and collecting duct cells produce PGE2. Both TXA_2 and PGH_2 have practically same actions and they activate a mutual receptor (Schrier 2013). Their binding ends up with phospholipase C stimulation, creation of inositol diacylglycerol and triphosphate, and super elevation of free cytosolic calcium derivatives from intra- and extracellular origins. Subsequently, smooth-muscle contraction and renin release inhibition are generated. In contrast, PGI_2, causes vasodilation and renin release stimulation by activating adenyl cyclase and raising intracellular cyclic adenosine monophosphate (Lote *et al.* 1989).

Phospholipase A_2 which is found in renal arterioles and is activated by circulating vasoconstrictors like angiotensin II and norepinephrine, leads to production of PGI_2, that adjusts renal vasoconstriction. The lipoxygenase juxtaglomerular specialized

smooth muscle cells release rennin when they are exposed to PGI_2. Glomerular originated synthesis of PGI_2, PGE_2 and TXA_2 are also stimulated by angiotensin II too (Schrier 2013). These three affect glomerular size: While PGI_2 and PGE_2 expands the glomerulus, TXA_2 leads to the contraction. They also affect ultrafiltration coefficient. TXA_2 which is formed in the glomerulus narrows the downstream efferent arteriole, intensifying the resistance and managing glomerular capillary pressure. Interstitial cells of deep renal medulla generate high amounts of PGE_2 when subjected to angiotensin II or vasopressin. Moreover, an increase in renal artery perfusion pressure provokes the increase of PGE_2 production. PGE_2 reduces sodium reabsorption in Henle's loop, hence undermining sodium retention of angiotensin II and subscribing to pressure natriuresis. Ultimately, when collecting duct cells are exposed to vasopressin, they produce PGE_2 and other eicosanoids (Fischer and Weber 1984).

To summarize, products of COX are formed in both renal cortex and medulla and the rate of their generation increases in response to particular stimuli. Once they are released, they affect renal hemodynamics, renin release, sodium and water excretion, erythropoietin production and aids natriuresis and diuresis (Fischer and Weber 1984).

Effects of NSAIDs on renal hemodynamics

There is a relationship between the level of plasma renin activity and the decline in renal blood flow after COX inhibition. As the pretreatment of plasma renin activity increases, the renal blood flow decreases after NSAIDs (Rossat *et al.* 1999).

In addition to the activation of the renin angiotensin system, other risk factors for NSAID-induced reduction of GFR may exist. Renal glomerular diseases such as; renal systemic lupus erythematosus and nephrotoxic serum nephritis are among these factors. Renal insufficiency caused by excision of renal mass is not affected by NSAIDs. Immune-induced glomerulopathy triggers vasodilator eicosanoid production, which manage GFR. It decreases if glomerular COX is inhibited. However, GFR is not reduce by NSAIDs in the absence of active glomerulonephritis because of not increasing vasodilator eicosanoids (Rossat *et al.* 1999).

Renal artery stenosis increases NSAID caused nephrotoxicity whereas, renin angiotensin system may trigger and support GFR in the stenotic kidney. However, their impacts in the no stenotic kidney is less presumable. NSAIDs could reduce contralateral blood flow because of lessen vasodilator eicosanoid formation or raise it by decreased renin release. So that, bilateral renal artery disease or renal artery stenosis in

a kidney might be a risk determinant for NSAID-induced renal failure (Rossat *et al*. 1999).

Progressive age is a dependent risk factor for NSAID-induced renal dysfunction. Prevalence of the activation of renin-angiotensin system by various factors increases in elderly people. These various factors include; congestive heart failure, diuretic usage, decreased thirst mechanism or atherosclerotic major renal vascular disease (Rossat *et al.* 1999).

To sum up, NSAIDs have little effect on renal blood flow of GFR in normal kidney. However, when the kidney is overexposed to vasoconstrictor stress, eicosanoid production is increased and this plays an important role in maintaining GFR. Similar case occurs during active glomerular inflammation and in the case of renal artery stenosis. Indomethacin can produce profound and prolonged depression of GFR (Loudon *et al.* 1997).

Effects of NSAID on renin release

There are two clinically convenient consequences of decreased renin release: Reduction of blood pressure and hyperkalemia. In many conditions, it is reported that NSAIDs either do not affect or nebulously increase the blood pressure. Nevertheless, since blood pressure and very high rennin plasma activity are dependent to each other, NSAIDs might reduce blood pressure indeed. The renal baroreceptor mechanism that leads to increased renin synthesis and release when renal perfusion pressure is deducted is enhanced by PGI_2 synthesis. Therefore, blood pressure reduce can be ensured by reducing renin by inhibiting COX. Another common and life-threatening condition is hyperkalemia (Harris 2003). Prolonged NSAID treatment increases serum potassium levels respectably that peaks at 3-7 days of treatment. Levels of potassium return to normal in 1 or 2 days after withdrawal of NSAIDs. Another risk factor for NSAID-induced hyperkalemia is diabetes mellitus where; there is a deficiency of insulin mediated cellular potassium reuptake and angiotensin-converting enzyme inhibitor treatment which reduces angiotensin II levels. As a result, NSAIDs continuously decrease basal and stimulated plasma renin activity and plasma aldosterone. In normal functioning kidneys, NSAIDs cause remarkable increase in serum potassium. In the case of renal insufficiency or renal insufficiency additional to diabetes mellitus, the extent of hyperkalemia turn out lethal conditions (Stichtenoth *et al.* 1998).

NSAIDs and sodium and water metabolism

Inhibition of renal PG synthesis can affect sodium excretion by various mechanisms which are shown in Table 1 (Hao *et al.* 2000). PGs have both direct and indirect effects on sodium excretion. Direct impacts

are supposedly restricted to the distal nephron, likely to the collecting duct epithelia. Indirect effects, running through hemodynamic and Starling forces, alterations in medullary interstitial pressure or salt content, varied concentrations of other determinants such as; angiotensin II and vasopressin may define the whole outcomes of NSAIDs on sodium metabolism. Depending on the potency of NSAID used, sodium excretion would increase, remain unaffected or decrease. In a research, it was reported that; 75 mg single dose of indomethacin lowered urine PGE_2 by 65% and urinary sodium from 200 to 125 mmol/day after 24 hours in high sodium diet and from 43 to 21 mmol/day in low sodium diet. In prolonged studies, indomethacin was reported to cause weight gain by 1 and 2% averagely but rarely up to 5%. Formed fluid retention is in charge of suppressing basal plasma renin activity. In patients with inherent renal disease or disorders where circulating vasoconstrictors are increased, NSAIDs withdrawal lead to fluid retention. Also, reductions in renal blood flow and GFR reduces the filtered load sodium. Water retention generating hyponatremia is occasionally an adverse effect of NSAIDs. However unexpectedly; PGE_2 inhibits the effect of vasopressin on the collecting duct and NSAIDs may increase the discharge of vasopressin during the volume contraction stimulus. Furthermore, decreasing medullary blood flow may lead to raised osmolality in the medulla and increased water reabsorption which can cause promoted water reabsorption from the collecting duct when administered NSAIDs actually causes serious hyponatremia. A study which was performed among normal subjects showed that 75 mg indomethacin for a week and for 42 days do not cause any change on serum sodium levels whereas, patients who were previously exposed to hyponatremia or those with severe congestive heart failure, cirrhosis with ascites, or who were taking diuretics or with nephrotic syndrome might possibly develop severe hyponatremia by the over usage of NSAIDs (Waddington *et al.* 2014).

Table 1: Possible effects of cyclooxygenase inhibition on sodium excretion.

Effect	Mechanism	Effect on sodium excretion
Renal blood flow	Proximal convoluted tubule reabsorption	decrease
Rennin	The ascending loop of Henle reabsorption	increase
Interstitial pressure	The ascending loop of Henle reabsorption	decrease
Medullary blood flow	Addition of Na to tubular fluid	increase
PGE effect on collecting tubules	Collecting duct reabsorption	decrease
Natriuretic effect of AVR	The ascending loop of Henle reabsorption	decrease

Effects of NSAIDs on other renal functions

As mentioned before, there is a direct relation between erythropoietin synthesis, PGE_2 synthesis and renal hypoxia. Hypoxia reduces adenosine triphosphate (ATP) which possibly prevent reacylation of arachidonic acid, subsequently allowing it to be more metabolized by COX, leading an increase in the production of PGE_2. PGE_2 increases cAMP by increasing the activity of adenyl cyclase and activates the phosphorylation of protein kinases (PKs). Transcription of erythropoietin gene can elevate during all stages. It is therefore probable that NSAID-induced deterioration of circulating erythropoietin may lead to anemia usually observed in patients having these drugs (Borda 1992).

DISCUSSION

This present review focused on renal toxic mechanisms induced by NSAIDs. NSAIDs induced nephrotoxic mechanisms including, effects of NSAIDs on renal hemodynamics, renin release and sodium and water metabolism were clarified. The use of NSAID for a long period of time and in large quantities results in the formation of renal papillary necrosis and interstitial nephritis. This pathological condition is called analgesic nephropathy. This review will raise awareness of the patients on the severity of the problem and the importance of reasonable and safe usage of NSAIDs. Adverse drug reactions of NSAIDs especially on kidney should never be underestimated for protection from serious irreversible risks.

Pharmacists and physicians should sufficiently be informed and be aware of the seriousness of analgesic nephropathy in order to avoid the unnecessary usage of NSAIDs, both with prescriptions and without prescription. The geriatric population is the most vulnerable group to toxic effects of NSAIDs. These people feel more pain and in order to reduce the pain they take NSAIDs in high doses for a long period of time. Moreover, geriatric individuals use many different drugs which

can show interactions with NSAIDs. Overall, this population should be well informed and monitored by healthcare professionals in order to decrease the risk of adverse related to NSAIDs.

REFERENCES

Abdulla A, Adams N, Bone M, Elliott AM, Gaffin J, Jones D, Knaggs R, Martin D, Sampson L, Schofield P, British Geriatric Society (2013). Guidance on the management of pain in older people. *Age Ageing* **42**:1–57.

American Geriatrics Society Panel on Pharmacological Management of Persistent Pain in Older Persons (2009). Pharmacological management of persistent pain in older persons. *J Am Geriatr Soc* **57**(8):1331–1346.

Bluemle LW Jr, Goldberg M (1969). Renal accumulation of salicylate and phenacetin: possible mechanisms in the nephropathy of analgesic abuse. *J Clin Invest* **47**(11):2507–2514.

Borda IT, Koff RS (1992). In: NSAIDs : A profile of adverse effects. Philadelphia: Hanley & Belfus;. pp. 240-256.

Buer JK. (2014). Origins and impact of the term 'NSAID'. *Inflammopharmacology* **22**(5):263–267.

Chang S, Mathew T, McDonald S (2008). Analgesic nephrop- athy and renal replacement therapy in Australia: Trends, comorbidities and outcomes. *Clin J Am Soc Nephrol* **3**:768–776.

Cove-Smith JR (1981). Analgesic nephropathy in the United Kingdom: incidence, clinical features and pathogenesis. *J Clin Pathol* **34**:1255–1260.

DeWitt DL, Meade EA, Smith WL (1993). PGH synthase isoenzyme selectivity: The potential for safer nonsteroidal anti-inflammatory drugs. *Am J Med* **95**(2):40–44.

De Broe M, Elseviers M (2009). Over the counter analgesic use. *J Am Soc Nephrol* **20**:2098–2103.

De Broe Marc E, Elseviers Momique M (1998). Analgesic nephropathy. *N Engl J Med* **338**:446–452.

Dunn MJ, Scharschmidt L, Zambraski E (1984). Mechanisms of the nephrotoxicity of non-steroidal anti-inflammatory drugs. Archives of Toxicology. Supplement. *Arch Toxicol Suppl* **7**:328–337.

Emkey RD, Mills JA (1982). Aspirin and Analgesic Nephropathy. *JAMA* **247**(1):55.

Fischer S, Weber PC (1984). Prostaglandin l3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature* **307**:165-8.

Gault M, Wilson D (1978). Analgesic nephropathy in Canada: Clinical syndrome, management and outcome. *Kidney Int* **13**:58–63.

Hao CM, Yull F, Blackwell T, Kömhoff M, Davis LS, Breyer MD (2000). Dehydration activates an NF-kappaB-driven, COX2-dependent survival mechanism in renal medullary interstitial cells. *J Clin Invest* **106**:973–982.

Harris RC (2003). Interactions between COX-2 and the renin-angiotensin system in the kidney. *Acta Physiol Scand* **177**:423-427.

Harirforoosh S, Jamali F (2009). Renal adverse effects of nonsteroidal anti-inflammatory drugs. *Expert Opin Drug Saf* **8**(6):669–681.

Hanna VS, Hafez EAA (2018). Synopsis of arachidonic acid metabolism: A review. *J Adv Res* **11**:23–32.

Hawkey CJ (2001). COX-1 and COX-2 inhibitors. *Best Pract Res Clin Gastroenterol* **15**(5), 801–820.

Hilário MOE, Terreri MT, Len CA (2006). Nonsteroidal anti-inflammatory drugs: cyclooxygenase 2 inhibitors. *J Pediatr (Rio J)* **82**(8):206–212.

Hunter LJ, Wood DM, Dargan PI (2011). The patterns of toxicity and management of acute nonsteroidal anti-inflammatory drug (NSAID) overdose. *Open Access Emerg Med* **6**(3):39-48.

Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y (2015). Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochim Biophys Acta* **4**:414–421.

Lote CJ, Haylor J (1989). Eicosanoids in renal function. *Prostaglandins Leukot Essent Fatty Acids* **36**(4):203–217.

Loudon JM, Bromidge SM, Brown F, Clark MS, Hatcher JP, Hawkins J, Patrono C (1997). SB 202026: a novel muscarinic partial agonist with functional selectivity for M1 receptors. *J Pharmacol Exp Ther* **283**(3):1059–1068.

Murray RM, Lawson DH, Linton AL (1971). Analgesic nephropathy: clinical syndrome and prognosis. *Br Med J* **1**(5747):479–482.

Nanra RS, Stuart-Taylor J, de Leon AH, White KH (1978). Analgesic nephropathy: etiology, clinical syndrome, and clinicopathologic correlations in Australia. *Kidney Int* **13**(1):79-92.

Noels L, Elseviers M, De Broe M. Impact of legislative measures on the sales of analgesics and the subse- quent prevalence of analgesic nephropathy: A com- parative study in France, Sweden and Belgium. *Nephrol Dial Transplant* **10**:167–174.

Palaniyappan L, Insole L, Ferrier N (2009). Combining antidepressants: a review of evidence. *Advances in Psychiatric Treatment* **15**(2): 90–99.

Pintér I, Mátyus J, Czégány Z, Harsányi J, Homoki M, Kassai M, Kiss E, Kiss I, Ladányi E, Locsey L, Major L, Misz M, Nagy L, Polner K, Rédl J, Solt I, Tichy B, Török M, Varga G, Wagner G, Wórum I, Zsoldos B, Pótó L, Dérczy K, Wittmann I, Nagy J (2004). Analgesic nephrop- athy in Hungary: The HANS study. *Nephrol Dial Transplant* **19**:840–843.

Rossat J, Maillard M, Nussberger J, Brunner HR, Burnier M (1999). Renal effects of selective cyclooxygenase-2 inhibition in normotensive salt-depleted subjects. *Clin Pharmacol Ther* **66**:76–84.

Sakai M, Kakutani S, Horikawa C, Tokuda H, Kawashima H, Shibata H, Okubo H, Sasaki S (2012). Arachidonic acid and cancer risk: a systematic review of observational studies. *BMC Cancer* **12**:606.

Serhan CN, Levy B (2003). Success of prostaglandin E2 in structure-function is a challenge for structure-based therapeutics. *Proc Natl Acad Sci USA* **100**(15):8609–8611.

Schrier RW, Coffman TM, Falk RJ, Molitoris BA, Neilson EG (2013). Schrier's Diseases of the Kidney. 9th ed, Wolters Kluwer, Baltimore.

Stichtenoth DO, Wagner B, Frolich JC (1998). Effect of selective inhibition of the inducible cyclooxygenase on renin release in healthy volunteers. *J Investig Med* **46**:290-296.

Waddington F, Naunton M, Thomas J (2014). Paracetamol and analgesic nephropathy: Are you kidneying me?. *Int Med Case Rep J* **8**:1–5.

Wlodawer P, Samuelsson B (1973). On the organization and mechanism of prostaglandin synthetase. *J Biol Chem* **248**(16):5673–5678.

Zarghi A, Arfaei S (2011). Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships. Iran J Pharm Res IJPR **10**(4):655–683.