Volume 3, Issue 2, December 2020
ISSN: 2619- 9645 (Print)
e-ISSN: 2667- 5722 (On-line, Pdf)

CUPMAP

Current Perspectives on Medicinal and Aromatic Plants
An International Scientific Journal
http://dergipark.gov.tr/cupmap - www.cupmap.org

Current Perspectives on Medicinal and Aromatic Plants

(CUPMAP)

Curr. Pers. MAPs

ISSN: 2619- 9645 (Print) **e-ISSN:** 2667-5722

This journal is international peer-reviewed and published two issues per year.

Corresponding Address
Current Perspectives on Medicinal and Aromatic Plants (CUPMAP),
Nazım Şekeroğlu
Kilis 7 Aralik University, Faculty of Agriculture, 79000, Kilis-Turkey
Phone: 0 348-814 26 66/7201/1820
Web: http://www.cupmap.org/
Contact: sekeroglunazim@gmail.com / editor@cupmap.org

Volume 3 **Issue 2** **December, 2020**

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

EXECUTIVE EDITORIAL BOARD

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

INTERNATIONAL ADVISORY BOARD

Current Perspectives on Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Current Perspectives on Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

JOURNAL INFORMATION

Journal Name	Current Perspectives on Medicinal and Aromatic Plants
Journal Abbreviation	*Curr. Pers. MAPs*
Scope & Subjects	Agriculture, Biology, Molecular Biology & Genetics, Chemistry, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition & Food Science, Pharmaceutical Sciences
ISSN	ISSN: 2619-9645 | e-ISSN: 2667-5722
Publisher	Nazım ŞEKEROĞLU
Language	English
Frequency	Biannually (June and December)
Type of Publication	Peer Review Double-Blinded
Publication Fee	Any Submission or Processing Charges
Access type	Open Access Policy
Manuscript Submission System	CUPMAP uses the submission system of TÜBİTAK-ULAKBİM Journal Park Open Journal Systems https://dergipark.org.tr/tr/pub/cupmap
License	Journal is licensed under a Creative Commons Attributions 4.0 International License
Legal Responsibility	Authors are responsible for content of articles that published in Journal.
Indexing and Abstracting	Google Scholar, Index Copernicus International, Worldcat, Elektronische Zeitschriftenbibliothek, Sobiad, Asos Index, Paperity Open Science Aggregated, Crossref, CiteFactor, International Scientific Indexing, Academic Journal Index, Publons
Address	Current Perspectives on Medicinal and Aromatic Plants (CUPMAP), Nazım Şekeroğlu Kilis 7 Aralik University, Faculty of Agriculture, 79000, Kilis-Turkey
Web	http://www.cupmap.org/
Contact	Phone: 0 348-814 26 66/7201/1820 E-mail:sekeroglunazim@gmail.com editor@cupmap.org

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)
ISSN: 2619- 9645 (Print) e-ISSN: 2667-5722

Current Perspectives on Medicinal and Aromatic Plants (CUPMAP) is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. This international scientific journal publishes research papers related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. CUPMAP publishes original research papers, applied studies, and review articles in MAPs science and technology. Special Issues devoted to important topics in the MAPs science and technology could also be published.

CUPMAP Journal publishes **Biannually** (on June and December) in both **print** and **on-line versions**. The publication language of the journal is **English**. Journal of CUPMAP welcomes article submissions and **does not charge any article submission or processing charges**.

Having well known board members distinguished scientists from different disciplines with huge experiences on MAPs all over the world, CUPMAP will be indexed in many databases after first issue. The goal of the journal is to be indexed in Thomson Routers in a short time.

CUPMAP is inviting papers for Volume 4 Issue 1, which is scheduled to be published on June, 2021. Last date of submission: June 15, 2021. However, an early submission will get preference in case of review and publication process. Please submit your manuscripts according to instructions for authors by the Journal online submission system.

Sincerely,
Prof. Dr. Nazım ŞEKEROĞLU
Editor-in-Chief
Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)
Contact: sekeroglunazim@gmail.com / editor@cupmap.org

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

AIM AND SCOPE

Current Perspectives on Medicinal and Aromatic Plants (CUPMAP) is an **open access**, double-blinded **peer-reviewed** and **refereed international** journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. CUPMAP Journal publishes **Biannually** (June and December). The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others that this has been appropriately cited or quoted. All submissions are screened by **iThenticate similarity** detection software and our maximum allowed score is **24%** for the document in which the References section truncated.

This international scientific journal publishes high-quality research articles related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on.

CUPMAP areas of interest include;

- Agricultural Practices of MAPs & NWFPs
- Aromatherapy & Phytotherapy & Phytochemistry
- Biodiversity
- Biology & Biochemistry & Biotechnology
- Botany & Ethnobotany & Ethnopharmacology
- Conservation, Management and Sustainable Uses of MAPs & NWFPs
- Essential Oils & Secondary Plant Metabolites
- Herbal & Traditional Medicines
- Industrial Processing Technologies of MAPs
- Legislations on MAPs & NWFPs
- Literature on MAPS
- Marketing of MAPs and Products
- Molecular Cancer Therapeutics
- Molecular Modeling and Simulations
- Natural Cosmetics
- Non-Governmental & Non-Profit Organizations (NGO & NPO) on MAPs
- Pharmacognosy & Phytopharmacology & Toxicology
- Standardization and Quality of MAP Products
- Traditional & Modern Herbal Products

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

OPEN ACCESS STATEMENT

Current Perspectives on Medicinal and Aromatic Plants (CUPMAP) is a fully open access journal, which means that all articles are **freely available** on the internet without charge to the user or his/her institution.

Except for commercial purposes, users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access. All the original articles and review papers published in CUPMAP journal are **free to access** immediately from the date of publication. All authors publishing with the CUPMAP accept these as the terms of publication. Copyright of the layout and design of 'Current Perspectives on Medicinal and Aromatic Plants' articles remains with the journal and cannot be used in other publications.

COPYRIGHT POLICY

Authors who publish with "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" agree to the following terms:

Authors transfer the copyright to Current Perspectives on Medicinal and Aromatic Plants (CUPMAP). Authors, however, upon the publication of their work, can republish it in another journal or book provided that they obtain the permission of the Journal. Moreover, they are to include the note indicating that their work has first been published in CUPMAP and the link to their work in the journal.

Authors, upon the publication of their work, can publish it in online environments and/or social networks (personal webpages, course content websites, Facebook, etc.). However, they also provide the link to the work as published in the journal.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

PUBLICATION CHARGES

No submission, processing, page, colored figures or publication charges are received from authors or elsewhere.

All the original articles and review papers published in CUPMAP journal are **free to access** immediately as early online and on the day of publication on the journal's website. **There is no article processing charges or submission fees for any submitted or accepted articles.**

PEER REVIEW PROCESS

Peer Reviewing Instructions for the "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" Journal

Introductions

The primary aims of peer review are to decide whether or not an article should be published (based on quality and relevance to the journal), and to improve the article before publication. All submissions first go through an internal peer review process: an assigned editor makes an initial decision to accept or to reject the manuscript (e.g. topic is outside the scope of the Journal, important flaws in scientific validity, etc). If the editor believes the article may be of interest, it is sent out for external peer review. The reviewers are selected by area of expertise (reviewers who grant high quality reviews within the requested time are preferred). The editorial board is frequently consulted. Once reviews are obtained, the editor makes a judgment considering the critiques and recommendations from reviewers, and other factors such as relevance to the Journal's aims and usefulness to clinicians or researchers.

Peer Reviewer Selection

Reviewers are selected according to their background and experience in some aspect of the subject. The most desirable reviewers identify the strengths and weaknesses of the submitted paper, and analyze it from different viewpoints. The peer reviewers are asked to read and analyze the assigned manuscript and provide a written opinion of its quality, novelty, relevance and suitability for publication in The "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" Journal. Peer reviewers also make suggestions to assist the authors in improving the article. Reviewers must not only analyze and comment on the paper, but also provide opinions about general concerns such as clarity and quality of the writing, validity of scientific approach, and whether the article provides new information.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Ethical Guidelines for Journal Peer Reviewers

When a selected individual accepts a peer reviewing assignment, the reviewer implicitly agrees to the ethical standards that are commonly accepted in biomedical publishing. Ethical guidelines for reviewers, authors, and editors are reported by the International Committee of Medical Journal Editors in the 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals' available from: www.icmje.org

Reviewers for the "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" Journal must agree to:

Produce as careful and objective a review as possible Respect the editor's deadline. Consider with an open mind innovations or approaches different from those of one's own.

Provide a balanced critique targeted not only to identify the strengths and weaknesses of the paper, but also to provide useful feedback to the authors to improve their manuscript, without being overly critical of minor points.

Avoid scientific misconduct such as the misappropriation of intellectual property.

Each manuscript should be treated as an extremely confidential document.

The privacy of the authors' ideas must always be guaranteed.

Direct comments about ethical concerns confidentially to the editors.

Contacting an author with questions about the manuscript is not allowed.

All critiques, including the latter, must be reported in the written critique.

Declare any conflict of interest (real or perceived) identified to the editor before the end of review. Not every potential conflict necessitates a rejection.

Reviewers are encouraged to discuss potential conflicts with the editors if they believe they can provide a fair review.

Reject an assignment if the following conflicts are present: Financial interests (e.g. paid consultancies, stock holdings), significant professional or personal relationships or rivalries, antipathy toward study question/approach, political or special interest affiliations (e.g. religious or deep convictions that conflict with the manuscript topic).

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Reviewer Guidelines

Potential reviewers are contacted by e-mail, which contains the manuscript title, abstract, and assignment deadline. The selected reviewer accepts or declines the assignment within 7 days. Failure to reply within the prescribed time will be treated as an implicit rejection. It is acceptable to propose an extended deadline when the given deadline (usually 4 weeks from the task acceptance date) cannot be met. The selected reviewers usually have extensive experience as faculty members, researchers, and published authors. Sometimes reviewers from other specific areas are selected. This selection is always well thought-out, and we encourage such potential reviewers to consider the assignment if they can make a contribution to some aspect of the work. The following points must be provided by the reviewers in the written response:

General Overview

Organized Critique

Assessment of Strengths and Weaknesses: the following should be evaluated: Literature review is up-to-date; Methods align with study purpose or research questions; Methods described in sufficient and appropriate detail; Research design or study approach is adequate; Approach to data analysis is appropriate; Thoughtful consideration given to the study limitations; Manuscript provides new information that is likely to be of interest to our readers.

Possible Improvements

Commonly Overlooked Areas: Reviewers should carefully note: title, abstract, tables and figures, references.

Editor's Final Decision

After the peer review process has ended and an adequate number of reviews has been received, the assigned editor makes the final decision about the manuscript (accept, invite a revision, or reject) based on a consideration of all the reviewer comments, general critique, and other external factors (e.g. the article is consistent with the Journal purpose, similar articles recently published, number of accepted articles awaiting publication, potential impact of the article, etc.). Editors may consult with each other when making the decision. A decision summarizing the opinions of editors and reviewers will be sent to the corresponding author.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

ETHIC RULES AND PLAGIARISM

Publishers Ethic Rules

"Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" is an international journal, which publishes at the highest scientific level on original research articles dealing with Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization. All authors submitting their works to "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" for publication as original articles attest that the submitted works represent their authors' contributions and have not been copied or plagiarized in whole or in part from other works. It is necessary to agree upon standards of expected ethical behavior for all parties involved in the act of publishing: the author, the journal editor, the peer reviewer and the publisher. "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" ethic statements are based on COPE's Best Practice Guidelines for Journal Editors.

Ethic Committee Report

The studies have to be prepared according to scientific rules and ethics. The results of any research (especially biology, veterinary, aquaculture and zootechnics) to be published as a manuscript need to have the report of a committee of ethic and that report's copy should be attached with the manuscript itself. Inclusion of the approval letter from the relevant Ethics Committee or Institution's Review Board regarding the research protocol and the rights of the subjects. The author or all the authors whose studies are to be published in the journal will own all kind of liabilities in terms of their manuscripts.

Publication Decisions

The editor is responsible for deciding which of the articles submitted to the "Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)" should be published. The editor may be guided by the policies of the CUPMAP's editorial board and constrained by

such legal requirements as shall then be in force regarding libel, copyright infringement and plagiarism. The editor may confer with other editors or reviewers in making this decision.

Fair Play

An editor at any time evaluate manuscripts for their intellectual content without regard to race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.

Confidentiality

The editor and any editorial staff must not disclose any information about a submitted manuscript to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.

Disclosure and Conflicts of Interest

Unpublished materials disclosed in a submitted manuscript must not be used in an editor's own research without the express written consent of the author.

Duties of Reviewers

Contribution to Editorial Decisions

Peer review assists the editor in making editorial decisions and through the editorial communications with the author may also assist the author in improving the paper.

Promptness

Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

Confidentiality

Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.

Standards of Objectivity

Reviews should be conducted objectively. Personal criticism of the author is inappropriate. Referees should express their views clearly with supporting arguments.

Acknowledgement of Sources

Reviewers should identify relevant published work that has not been cited by the authors. Any statement that an observation, derivation, or argument had been previously reported should be accompanied by the relevant citation. A reviewer should also call to the editor's attention any substantial similarity or overlap between the manuscript under consideration and any other published paper of which they have personal knowledge.

Disclosure and Conflict of Interest

Privileged information or ideas obtained through peer review must be kept confidential and not used for personal advantage. Reviewers should not consider manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or connections with any of the authors, companies, or institutions connected to the papers.

Duties of Authors

Reporting Standards

Authors of reports of original research should present an accurate account of the work performed as well as an objective discussion of its significance. Underlying data should be represented accurately in the paper. A paper should contain sufficient detail and references to permit others to replicate the work. Fraudulent or knowingly inaccurate statements constitute unethical behavior and are unacceptable.

Data Access and Retention

Authors are asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data (consistent with the ALPSP-STM Statement on Data and Databases), if practicable, and should in any event be prepared to retain such data for a reasonable time after publication.

Originality and Plagiarism

The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others that this has been appropriately cited or quoted. All submissions are screened by a similarity detection software. We are using iThenticate plagiarism solution and our maximum allowed score, for the document in which the References sections truncated, is 24%. Higher scores are not allowed and the

author will be given the plagiarism report and the manuscript will be returned to the authors.

Multiple, Redundant or Concurrent Publication

An author should not in general publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behavior and is unacceptable.

Acknowledgement of Sources

Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work.

Authorship of the Paper

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. All those who have made significant contributions should be listed as co-authors. Where there are others who have participated in certain substantive aspects of the research project, they should be acknowledged or listed as contributors. The corresponding author should ensure that all appropriate co-authors and no inappropriate co-authors are included on the paper, and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

Hazards and Human or Animal Subjects

If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript.

Disclosure and Conflicts of Interest

All authors should disclose in their manuscript any financial or other substantive conflict of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support for the project should be disclosed.

Fundamental Errors in Published Works

When an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

CUPMAP INSTRUCTIONS FOR THE AUTHORS

- Please read all of the following manuscript preparation instructions carefully.
- Manuscript should be written in **English.**
- Maximum length for articles is **15 pages**. Articles over 15 pages in length can only be considered on an exceptional basis.
- Manuscripts can only be submitted through the CUPMAP **online** manuscript submission and evaluation system https://dergipark.org.tr/tr/pub/cupmap
- The journal management requires that all authors register to **ORCID** at http://orcid.org/register and get a free ORCID ID.
- All submissions are screened by **iThenticate** similarity detection software and our maximum allowed score is **24%** for the document in which the References section truncated.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The manuscript should include an abstract with the following subheadings: "Introduction", "Materials and Methods", "Results and Discussion", and "Conclusion".

Short Communications: Short communication is for a concise to present scientific reports related to scope of the journal. Short communication is not intended to publish preliminary results, but if these results are of exceptional interest and are particularly topical and relevant will be considered for publication. It should include an abstract with the following subheadings: "Introduction", "Materials and Methods", "Results and Discussion", and "Conclusion".

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

CUPMAP STRUCTURE OF THE MANUSCRIPT

Font
Word document, Cambria, 12 point, single line space. Page margins are 2.5 for all sides.

Length
Maximum length for articles is 15 pages. Articles over 15 pages in length can only be considered on an exceptional basis.

Title
A concise title of the paper, avoid Abbreviations and formulae where possible.

- Use bold 14-point Cambria font. Use title uppercase, and make title in centered.
- Name(s) and SURNAME(s) of author(s) [Use centered, bold 12-point Cambria font, Use uppercase for surnames.]
- Affiliation(s) of author(s) [Use 10-point Cambria font.]
- E-mail address of the corresponding author [Use 10-point Cambria font.]
- ORCID(s) of author(s) [Use 10-point Cambria font.]

Abstract
Each article is to be preceded by a succinct abstract, of up to 250 words, that highlights the objectives, methods, results, and conclusions of the paper. The abstract should state briefly the purpose of the research, the principal results and major conclusions. The abstract body is typed in Cambria, 10 pt.

Key Words
Provide a maximum of 6 (six) key words or phrases in order of importance, separated by commas and typed in Cambria, 10 pt.

Headings
Use bold, uppercase, 12 Cambria font for headings.

Introduction
This should define the problem and, if possible, the frame of existing knowledge. Please ensure that people not working in that particular field will be able to understand the intention. The word length of the introduction should be 150 to 300 words.

Materials and Methods
Materials and methods should be clearly presented to allow the reproduction of the experiments.

Results and Discussion

A combined Results and Discussion section is often appropriate. Results should be clear and concise and give the significance of the results of the work. Data must not be repeated in figures and tables. Implications for further studies or application may be discussed.

Conclusion

A short Conclusions section should be added if results and discussion are combined.

Tables and Figures

- Tables should have a short descriptive title.
- The unit of measurement used in a table should be stated.
- Tables should be numbered consecutively.
- Figures should be prepared in GIF, TIFF, JPEG or PowerPoint.
- Tables and Figures should be appropriately cited in the manuscript.

Acknowledgements

Acknowledgements of financial support, advice or other kind of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organizations should be written in full.

Conflict of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

References

They should be grouped at the end of the paper in surname order of appearance. Abbreviated titles of periodicals are to be used according to Chemical or Biological Abstracts, but names of lesser-known journals should be typed in full.

References should be styled and punctuated according to the following examples:

Original Papers

Surname, N.N., and Surname, N.N., Year. The full title of the article. Journal Full Name, volume (issue), first and last page. DOI number

Book or Proceeding

Surname, N.N., and Surname, N.N., Year. Title of the contribution. In: Title of the book or proceeding. Volume (Publisher, City), pp. first and last page

Thesis

Surname, N.N., Year. Title of the thesis, University and Faculty, City. pages.

Current Perspectives on
Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

CURRENT PERSPECTIVES ON MEDICINAL AND AROMATIC PLANTS (CUPMAP)

An International Scientific Journal - ISSN: 2619- 9645 (Print)

Volume: 3, Issue: 2, December 2020

Current Perspectives on Medicinal and Aromatic Plants
Curr. Pers. MAPs

An International Peer Reviewed Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

---------- Kilis 7 Aralik University, Publishing House ----------
December, 2020 - KİLİS

Research article
Curr. Pers. MAPs, (2020); 3(2): 61-73

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Phytochemical Screening and Biological Activity Analysis of Some Selected Medicinal Plants of Ilam District of Nepal

Anju BASNET, Surya Kant KALAUNI*

Central Department of Chemistry, Tribhuvan University,
Postal code 44613, Kirtipur, Kathmandu, Nepal
*Corresponding author: skkaluni@gmail.com

https://doi.org/10.38093/cupmap.765409

Received : 08/07/2020
Accepted : 13/10/2020

Abstract

Nepal is considered as a natural storehouse of medicinal plants with aromatic and medicinal values, being a land of diverse topography with the different weather conditions. More than 75% of the total population of Nepal relies on the traditional medicines for their primary healthcare, and thus it is an important task to explore the chemical constituents and biological activities of plants grown in the local weather. So, we have selected ten medicinal plants as the experimental sample and extracted the contents in methanol by cold percolation method. The phytochemical screening revealed that glycosides, terpenoids, reducing sugars, polyphenols, and quinones were present in most of the plant extracts. In DPPH free radical scavenging test, the extracts of Nephrolepsis auriculata (46.22 µg/ml) and Rumex nepalensis (48.59 µg/ml) showed potent antioxidant activity. The extracts of N. auriculata and R. nepalensis contains the highest total phenolic and total flavonoid contents as 1076.73 & 964.56 mg/g GAE and 40.8 & 39.06 mg QE/g, respectively. The extract of Erythrina arborescens depicts significant antibacterial activity against the Gram-positive and Gram-negative bacteria. In brine shrimp lethality bioassay, the methanol extract of Zanthoxylum armatum (6.3 µg/mL) and Heracleum nepalense (88 µg/mL) shows considerable cytotoxicity as their LC50 values were found to be lower than 100 µg/mL. The results endorsed the notion behind the use of traditional medicinal plants to treat various diseases. So, these plant extracts could be used for isolating the bioactive compounds that could be used in the discovery process of noble drugs in the future.

Key Words: Medicinal plants, antioxidants, phenolic, flavonoids, antimicrobial

1. Introduction

Cradled on the laps of the central Himalayan range, Nepal is sandwiched between two Asian giants, China in the North and India on three sides; South, East, and West. Nepal is considered as a natural storehouse of medicinal plants representing distinct on the distribution of various species of plants with aromatic and medicinal values due to its geographical diversity, variations in topography, altitude, climatic gradients, and biological resources (Nepal: General Info). Nepal is ranked 9th among the Asian countries for its floral wealth. More than 9000 species of floral plants (Kunwar et al., 2013) are estimated in Nepal of which about 1600-1900 species are medicinal and aromatic plants commonly used for the medicinal purpose (Ghimire, 2008; Acharya, 2014). More than 75% of the total population of Nepal relies on traditional

medicine for their primary healthcare (Adhikary, 2011). Furthermore, 50 % of Nepal's rural family's livelihoods and income depends on the collecting and trading of medicinal plants (Kunwar et al., 2011). Medicinal plants being the most reliable source to acquire a variety of novel herbal drugs, scientists working on infectious diseases have great interest to derive biologically active compounds from natural resources. (Dhote, 2015).Medicinal plants are an excellent source of biologically active compounds known as phytochemicals such as; phenolic acids, polyphenols, and flavonoids, alkaloids, tannins which shows different biological activities such as antioxidant, antimicrobial, cytotoxicity, and antitumor activities (Saxena, 2013). Antioxidants are the substances that protect cells against the deleterious effects of the reactive oxygen species (Sen, 2010), which are highly reactive unstable ions (hydrogen peroxide, free radicals). Reactive oxygen species (ROS) are the weapons in causing various chronic and life-threatening diseases: cardiovascular diseases, inflammation, heart stroke, cancer, ageing processes (Oliveira, 2009; Acharya, 2014). The phytochemicals, mainly phenolic and flavonoid compounds are useful free radical scavengers (due to phenolic hydroxyl group), prevent the action of ROS to damage cells, and act as powerful antioxidants. (Shahidi, 2010; Saxena et al., 2012). About two-thirds of plant species worldwide have medicinal values, and most of all are potent antioxidants. A bioactive natural antioxidant, mainly of plant origin has fewer side effects, eco-friendly, and locally available. Therefore, evaluation of the antioxidant property of the plants used in herbal medicine is necessary that could lead to the discovery of natural antioxidants with pharmacological values. (Kasote et al., 2015; Jamuna, 2012).

Antimicrobial agents help in minimizing the global problem of infectious (bacterial, fungal, virus) diseases (Manandhar, 2019). However, the development of multidrug resistance (MDR) strain in pathogenic bacteria and the presence of various side effects of certain antibiotics has created a more significant threat to public health (Abdalla et al., 2007). Medicinal plants have been recognizing as vast potential sources for antimicrobial drugs (Cavalieri et al., 2005; Zaidan, 2005). Therefore, it is imperative to give continuous effort on systematic screening of antimicrobial plant extracts to find noble compounds with the substantial capacity to act against multi-resistant pathogenic bacteria and fungi and to understand better their properties, safety, and efficacy (Ullah, 2011).

People have been using plant and plant products for the healing of various diseases as medicine without knowing their chemical constituents and biological activities since prehistoric times (Yuan, 2016). Although most of the local communities of Nepal used traditional medicines to cure ailments, only limited research has been carried out to prove its use on a scientific basis. Thus, it essential to explore the chemical constituents and biological activities of plants and plant products, which influence human biochemistry in the medicinal plants for establishing a relation between traditional use and scientific meaning. In the present study, eight locally grown medicinal plants were chosen from Chamaita VDC of the Ilam district of Nepal based on traditional applications to assess their phytochemicals, antioxidant activity, and antibacterial potential.

2. Material and Method

2.1. Collection of plant materials

The fresh plant materials were collected in February 2016 from Chamaita VDC of the Ilam district based on their local medicinal uses and pharmacological importance as listed in Table 1. The taxonomic identification of plants was made by Prof. Dr Suresh Ghimire, Central Department of Botany, Tribhuvan University, Kirtipur.

2.2. Preparation of plant extracts

The collected fresh materials were washed under running tap water to remove the contaminations then clean parts were air-dried in the shade for 28 days at room temperature. The dried materials were ground to powder using the electric grinder and stored in separate clean plastic bags for further use. One hundred grams of powder of each plant were extracted by cold percolation method in methanol. The powder was kept into conical flasks, and 500 ml methanol poured into each of the flasks and soaked for 72 hours at room temperature and filtered through clean cotton. The filtrate thus obtained was concentrated by using Rotary Evaporator. Thus obtained methanol extracts were kept in a separate beaker for drying and then stored in cold place by wrapping with aluminum foil until further use.

2.3. Phytochemical screening

The phytochemicals present in the methanol plant extracts were screened by following the procedure described by Prof. I. Ciulei (1982). The major groups of natural constituents present in each plant extracts were analyzed by the color reaction using specific reagents.

2.4. Antioxidant Activity

The antioxidant activity of each plant extracts was assessed by using 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH•) scavenging method, as explained by Blois (1958) Ascorbic acid was used as a standard.

2.5. Total phenolic content

The total phenolic contents (TPC) of the plant extracts were studied by the Folin-Ciocalteu colorimetric method based on the oxidation-reduction reaction, as explained by Waterhouse (2001). Gallic acid is used as standard as it is less expensive and available in pure form.

2.6. Total flavonoid content

Total flavonoid contents (TFC) of the plant extracts were analyzed by aluminum chloride (AlCl3) colorimetric assay as described by Kalita, et al., (2013). Quercetin was used as a standard.

2.7. Antibacterial Activity

The antibacterial activity of the methanol extract of the plants was evaluated by the agar-well diffusion method. The potent antimicrobial plant extracts were determined by measuring the zone of inhibition (ZOI), according to Dingel et al. (1953).

2.8. Brine Shrimp Bioassay

The toxicity of the plant extracts was examined by brine shrimp bioassay by following the standard protocol given by Mayer et al., (1982). Bioactive compounds show toxicity towards brine shrimp larvae.

Table 1: List of plants selected for the study with their traditional uses and % yield in methanol

S.N	Scientific Name	Nepali Name	Used Part	Extraction Yield (%)	Altitude (m)	Traditional Uses
1.	*Erythrina arborescens* Roxb. (Leguminosae)	Phaledo	Plant stem	15.93	1500-3000	Tooth-earache, joint pain, Inflammations, fever, skin diseases (Baskar, 2010)
2.	*Heracleum nepalense* D. Don (Apiaceae)	Chimfing	Seeds	4.48	1800-3700 (Shrestha, 2019)	Tonic, Cold, cough, diarrhea, body ache, faint (Joshi, 2016)
3.	*Ligusticopsis* wallichiana DC. (Apiaceae)	Bhutkesh	Whole plant	30.41	2700-4800 (Shrestha, 2019)	Faints, diarrhea, vomiting, stomachache, cough, fever (Devkota, 2018)
4.	*Nephrolepis auriculata* L. (Nephrolepidaceae)	Paniamala	Whole plant	3.12	200-500	Cold, cough, fever, indigestion, liver and skin disorder (Rukmini, 2014)
5.	*Phytolacca acinosa* Roxb. (Phytolaccaceae)	Jaringo	Whole plant	8.88	500-3400 (Pliszko, 2018)	Indigestion, eye disorder, body ache (Parajuli, 2013)
6.	*Rubia cordifolia* L. (Rubiaceae)	Majhito	Plant body	11.46	1,200–2,100 (Shrestha, 2019)	Wounds, skin infections, leprosy, diarrhea, ulcers, snake bite (Meena, 2010)
7.	*Rumex nepalensis* Spreng. (Polygonaceae)	Halhale	Rhizome	12.39	1,200–4,200 (Shrestha, 2019)	Dysentery, body pain, colic ulcer, Jaundice, skin sores (Kumar et.al, 2011)
8.	*Zanthoxylum armatum* Linn. (Rutaceae)	Boke timor	Bark	13.39	1100-2500 (Shrestha, 2019)	Toothache, fever, stomach ache, low blood pressure (Kayat, 2016)

3. Results and Discussions

3.1. Phytochemical screening

The qualitative estimation of phytochemicals present in crude methanol extract of selected plant materials is shown in Table 2, which depicts that glycosides and terpenoids were present in all the extracts. Reducing sugars, polyphenols, and quinones were present on most of the plant extracts. Flavonoids and saponins were present in the extracts of R. cordifolia, R. nepalensis, and N. auriculata. Alkaloids were present only in Z. armatum and E. arborescens. The variation of the phytoconstituents of the same plant species (data present in the literature) may be due to altitude divergence of plants, environmental factors, extraction methods, sample collection time as well as chemical grades and laboratory setup.

Table 2: Phytochemical analysis of methanol plant extracts. Key: (+): Present (−): Absent

S.N.	Name of the Plants	Alk.	Coum.	Flav.	Gly.	Polyp.	Quin.	Red.S.	Sap.	Terp.
1.	*Erythrina arborescens*	+	-	-	+	-	+	+	-	+
2.	*Heracleum nepalense*	-	-	-	+	-	-	-	-	+
3.	*Ligusticopsis wallichiana*	-	-	-	+	+	+	+	-	+
4.	*Nephroleps auriculata*	-	+	+	+	+	-	+	+	+
5.	*Phytolocca acinosa*	-	-	-	+	-	+	+	-	+
6.	*Rubia cordifolia*	-	-	+	+	-	+	-	+	+
7.	*Rumex nepalensis*	-	+	+	+	+	+	+	+	+
8.	*Zanthoxylum armatum*	++	+	-	+	+	-	+	-	+

Key: Alk. = Alkaloids, Coum. = Coumarins, Flav. = Flavonoids, Polyp. = Polyphenols, Qui. = Quinones, Red. S = Reducing Sugar, Sap. = Saponins & Terp. = Terpenoids

3.2. Antioxidant activity

The antioxidant activity of methanol extracts of selected plant materials was studied by plotting percentage free radical scavenging versus concentration and 50% inhibitory concentration (IC50) values for each extract are calculated. Ascorbic acid was used as a standard solution. Figure 1A presents the dose-response curve of DPPH radical scavenging activity of plant extracts vs concentration, compared with Ascorbic Acid. The percentage of free radical scavenging is concomitantly increasing with the increase in the concentration of methanol extracts from 20 to 100 µg/ml. The concentration of the plant extracts required to inhibit 50% of DPPH activity (IC50) is shown in Figure 1B. The antioxidant potential is inversely proportional to the IC50 value, the lower the IC50 value, the higher the antioxidant potential, and vice versa. Among the studied plant extracts, the extracts of N. auriculata (46.22 µg/ml) and R. nepalensis (48.59 µg/ml) showed the highest antioxidant activity as their IC50 were lowest and closer to standard ascorbic acid (39.85 µg/ml). The result reflects that the plants studied above could be a good option in the field of medicine based on the antioxidant property.

3.3. Total phenolic and flavonoid contents

The total phenolic contents (TPC) for methanol extracts of selected plant materials were estimated by using the calibration curve and absorbance values (Figure 2a). The methanol extract of N. auriculata showed the highest and R. nepalensis showed the second-highest TPC as 1076.73 and 964.56 mg per gram Gallic acid equivalent (mg/g GAE) respectively. While the other plant extracts showed significantly lower value, as shown in Figure 3a. Similarly, total flavonoid contents (TFC) for methanol plant extracts were calculated by using the calibration curve and absorbance values (Figure 2b). The highest TFC was found in N. auriculata and R. nepalensis as 40.8 and 39.06 mg per gram quercetin equivalent (mg/g QE) respectively. Furthermore, the extract of L. wallichiana (35.63 mg/g QE) and E. arborescens (32.41 mg/g QE) also showed a significant amount of total flavonoid content (Figure 3b). The correlation of antioxidant potential (IC50 values) with total phenolic and flavonoid contents of the methanolic plant extracts are shown in Table 3. Phenolic compounds are potent chain-breaking antioxidant which scavenges free radicals due to their hydroxyl groups. Flavonoids are a group of

polyphenolic compounds, they possess a broad spectrum of chemical and biological activities, including scavenging of reactive oxygen species due to their phenolic hydroxyl groups and are effective antioxidants. The extracts of N. auriculata and R. nepalensis showed the most significant antioxidant potential, which also has the highest total phenolic and total flavonoid contents. These indicate that the medicinal plants with intense antioxidant activity also demonstrate high total phenolic and flavonoid contents.

3.4. Antibacterial Activity

Pathogenic bacteria cause many diseases to human beings and dysentery, tuberculosis, respiratory infections are the most common. Antimicrobial agents present in the plant extracts inhibit or kill the growth of such microorganism. The antimicrobial activity of the plant extracts is evaluated by calculating the zone of inhibition (ZOI); the area around the antimicrobial disk where there is no growth of microorganism takes place is called ZOI. The antibacterial activities of the methanol plant extracts were observed against Gram-negative bacteria (Escherichia coli ATCC 25922) and Gram-positive bacteria (Staphylococcus aureus ATCC 25923) which are shown in Table 4. The extract of E. arborescens showed the highest ZOI against both types of bacteria, which were 11 and 14 mm for Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) respectively. Similarly, the extract of R. nepalensis, N. auriculata and H. nepalense were found to be active for the inhibition of the growth of both strains of bacteria used. Moreover, the methanol extract of Z. armatum, L. wallichiana, and R. cordifolia were found to be resistant against Gram-positive strains only. The absence of ZOI in the extract of P. acinosa representing no antimicrobial activity. Out of the two strains, Gram-positive bacteria are inhibited more effectively than the Gram-negative bacteria. This fact can be described by the presence of a unique outer membrane that hinders the penetration of extract in the cell in Gram-negative bacteria which is lacking in Gram-positive bacteria (Biswas, 2013). These significant values of ZOI for the methanol extracts of E. arborescens, R. nepalensis, N. auriculata, and H. nepalense confirm their antimicrobial activity, i.e. the plants can be used to cure the diseases caused by E. coli and S. aureus bacteria.

3.5. Brine Shrimp Toxicity Assay

Brine shrimp lethality assay is a convenient approach for the estimation of the toxic potential of plants and their extracts being rapid, cheaper, and straightforward. Bioactive compounds show toxicity towards newly hatched Brine shrimp larvae (A. salina Leach). The le¬thal concentration of plant extracts that kills 50% of the exposed popula¬tion of A. salina (LC50) values in µg/ml is determined. The plant extracts having LC50 values below 1000 µg/ml are supposed to be pharmacologically active, and the extracts with LC50 values more than 1000 µg/ml are non-toxic ((Mayer et al., 1982). The results of the brine shrimp toxicity assay are shown in Table 5. The degree of lethality of the extracts was concentration-dependent, i.e. the maximum mortalities occurred at a concentration of 1000 µg/mL and least at the concentration of 10 µg/mL. The extract of Z. armatum (6.3 µg/mL) and H. nepalense (33.88 µg/mL) were found to be most cytotoxic against brine shrimps as their LC50 values were found to be lower than 100 µg/mL. Furthermore, the extracts of L. wallichiana (138.04 µg/mL) and P. acinosa (177.82µg/mL) also showed significant lethality to brine shrimp. The toxicity assay of the plant extracts correlates directly with cytotoxic and antitumor properties (Hamidi, 2014). This study gives the preliminary idea of the presence of cytotoxic and perhaps the potent antitumor constituents in the plants.

Table 3: Correlation of total phenolic contents, flavonoid contents and IC_{50} of methanol plant extracts

Plant extracts	Free radical scavenging IC_{50} (µg/ml)	Total phenolic contents (mg/g QE)	Total flavonoid contents (mg/g QE)
Erythrina arborescens	477.55	382.93	32.41
Heracleum nepalense	178.06	339.13	7.47
Ligusticopsis wallichiana	134.84	107.93	35.63
Nephroleps auriculata	**46.22**	**1076.73**	**40.8**
Phytolocca acinosa	226.35	92.28	7.68
Rubia cordifolia	357.9	128.48	8.52
Rumex nepalensis	**48.59**	**964.56**	**39.06**
Zanthoxylum armatum	117.04	291.95	10.86

Table 4: Antimicrobial screening results of methanol plant extracts

S.N	Plant Extract	Bacteria	ZOI (mm) of extracts at 100 mg/ml
1.	*Erythrina arborescens*	*E. coli*	**11**
		S. aureus	**14**
2.	*Heracleum nepalense*	*E. coli*	6
		S. aureus	8
3.	*Ligusticopsis wallichiana*	*E. coli*	-
		S. aureus	9
4.	*Nephroleps auriculata*	*E. coli*	4
		S. aureus	7
5.	*Phytolocca acinosa*	*E. coli*	-
		S. aureus	-
6.	*Rubia cordifolia*	*E. coli*	-
		S. aureus	8
7.	*Rumex nepalensis*	*E. coli*	4
		S. aureus	6
8.	*Zanthoxylum armatum*	*E. coli*	-
		S. aureus	4
	Ofloxacin (standard)	*E. coli*	14
		S. aureus	18

(-) = absence of antibacterial activity, ZOI = Zone of Inhibition, *E.coli:* Gram-negative bacteria, S.*aureus:* Gram-positive bacteria, Ofloxacin (antibiotic) as positive control

Table 5: Brine shrimp lethality assay of methanol plant extracts.

S.N	Plant extract	Concentration (µg/mL)	No. of subject	Average no. of survival out of 10	LC_{50} (µg/mL)
1.	*Erythrina arborescens*	10	10	10	912.01
		100	10	9.67	
		1000	10	4	
2.	*Heracleum nepalense*	10	10	2.33	**33.88**
		100	10	0.3	
		1000	10	0	
3.	*Ligusticopsis wallichiana*	10	10	9.33	**138.04**
		100	10	7.67	
		1000	10	0	
4.	*Nephrolepsis auriculata*	10	10	10	1.41×10^{16}
		100	10	9.67	
		1000	10	9.33	
5.	*Phytolocca acinosa*	10	10	9.97	**177.82**
		100	10	9	
		1000	10	0	
6.	*Rubia cordifolia*	10	10	10	489.78
		100	10	8.67	
		1000	10	3.33	
7.	*Rumex nepalensis*	10	10	9.67	16982.4
		100	10	9.33	
		1000	10	7.33	
8.	*Zanthoxylum armatum*	10	10	5	**6.31**
		100	10	1	
		1000	10	0	

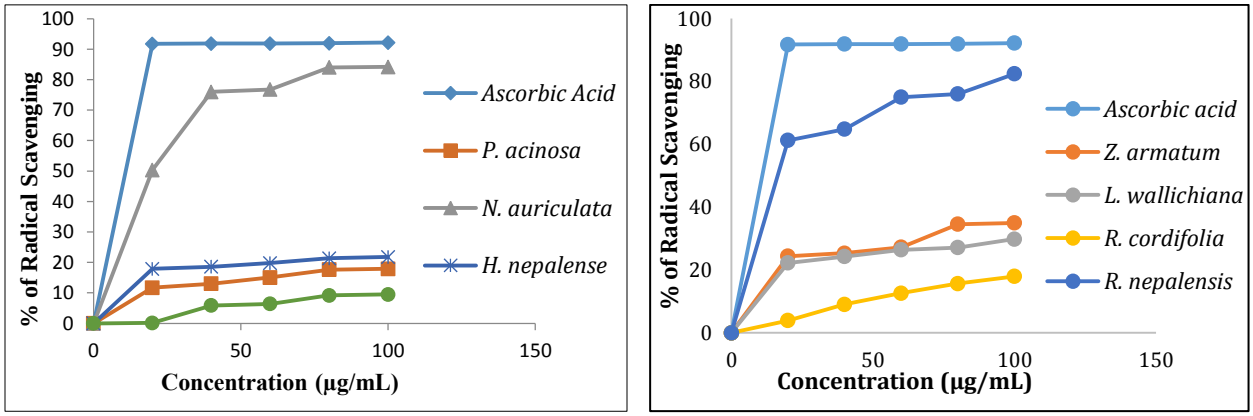


Figure 1A: Percentage scavenging of DPPH free radical by plant extracts vs Concentration (µg/mL)

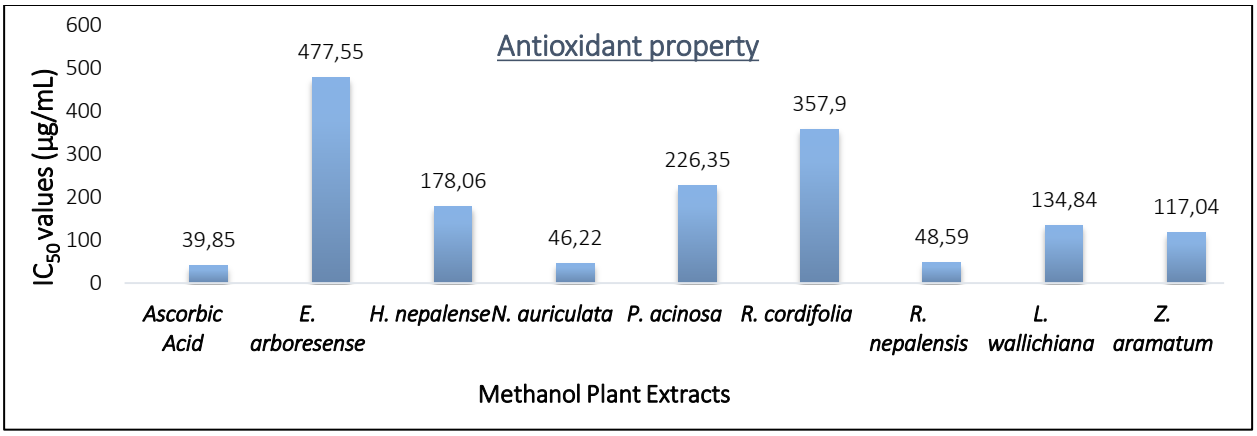


Figure 1B: IC_{50} values **(µg/mL)** for methanol plant extracts compared with ascorbic

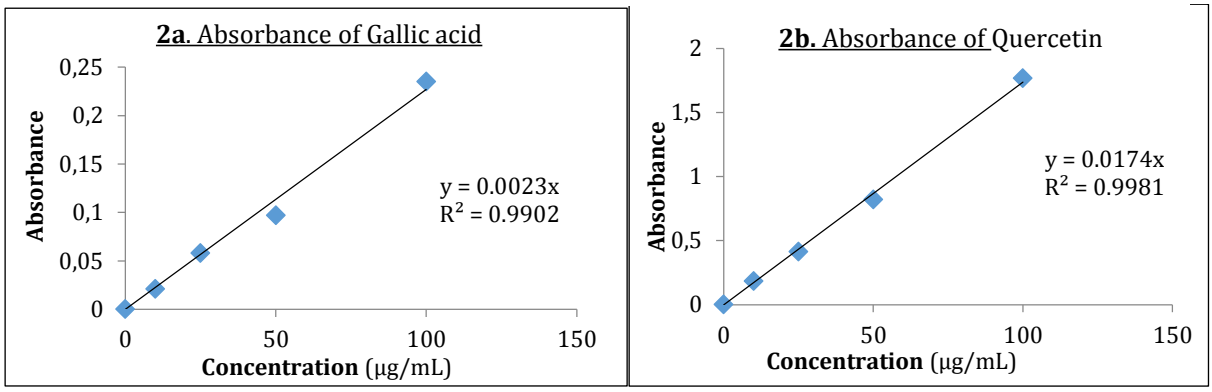


Figure 2: Calibration curve for total phenolic and total flavonoid content determination

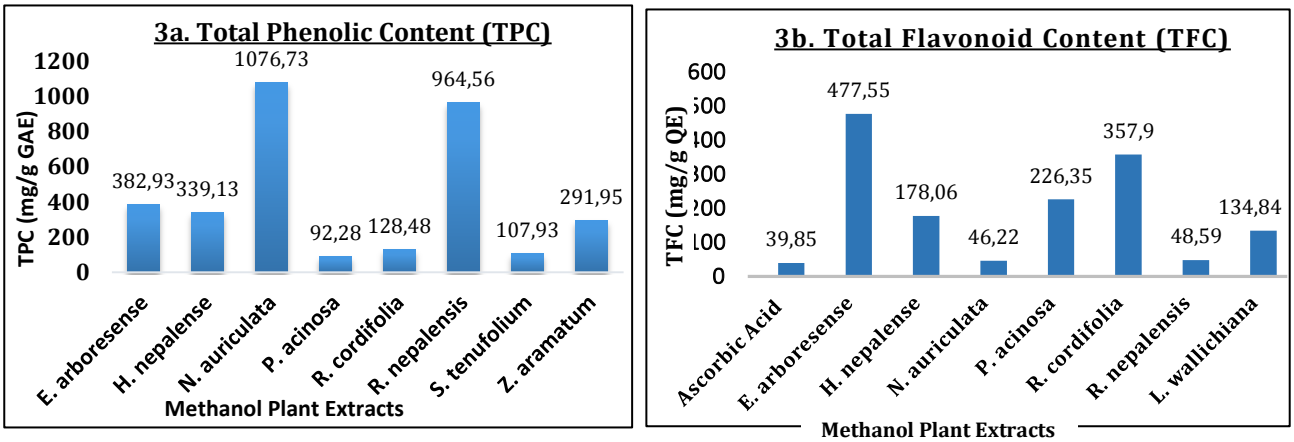


Figure 3: Total phenolic content (TPC) and total flavonoid content (TFC) of methanol plant extracts

4. Conclusion

The phytochemical screening of methanol extract of the plants divulged the presence of essential phytoconstituents; flavonoids, alkaloids, glycosides, saponins, terpenoids, reducing sugars, polyphenols, and quinones. The IC50 values of the methanol extract of N. auriculata and R. nepalensis were found to be nearly equal to the standard ascorbic acid indicating these plants are the potent source of antioxidant. Furthermore, the extracts of N. auriculata and R. nepalensis also showed the highest total phenolic and total flavonoid contents among the selected plant materials. The mechanism of antioxidant activity of the plant extracts may be due to the presence of phytochemicals, flavonoids, and polyphenols. As the phenolic and flavonoid compounds are known to minimize the risk of oxidative stress-induced diseases, the methanol extracts of these plants could be used to develop different types of medicine that may prevent the cell damage (antioxidant).

The antibacterial activity of the plant extracts was evaluated by calculating the zone of inhibition (ZOI). The extract of E. arborescens (11 & 14 mm), H. nepalense (6 & 8 mm), N. auriculata (4 & 7 mm), and R. nepalensis (4 & 6 mm) showed significant antibacterial activity against both Gram-negative bacteria (E. coli) and Gram-positive bacteria (S. aureus) respectively. The significant values of ZOI for the methanol extracts of the plants indicating they can be used to cure the diseases caused by Escherichia coli and Staphylococcus aureus bacteria. The brine shrimp bioassay of the plant extracts showed that the extract of Z. armatum (6.31 µg/mL), H. nepalense (33.88 µg/mL), L. wallichiana (138.04 µg/mL) and P. acinosa (177.82 µg/mL) were most cytotoxic against brine shrimps and may contain pharmacologically active compounds as its LC50 value are significantly less than 1000 µg/mL.

The present work has demonstrated that the methanolic extracts of plants possess promising antioxidant, antibacterial, and cytotoxic potential, thereby endorsed the notion behind the traditional use of the medicinal plants to treat different diseases. However, further, in vitro and in vivo studies as well as isolation and characterization of the active constituents responsible for the observed biological activities are needed to conduct in order to understand the exact mechanisms of such actions.

Acknowledgements

The authors are grateful to the University Grant Commission (UGC), Bhaktapur, Nepal for financial support, Central department of Chemistry, Tribhuvan University, Kathmandu for providing laboratory facility, and NIST College, Khusibu, Kathmandu for providing laboratory to conduct antibacterial assay test.

Conflicts of Interest

There is no conflict of interest to disclose.

References

1. Abdalla, A. E. M., 2007. Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chemistry, 103(4), 1141–1152. DOI: 10.1016/j.foodchem.2006.10.026
2. Acharya, S. R., et al. 2014. Antioxidant and Antimicrobial Properties of Leaves of Lyonia ovalifolia Wallich. International Journal of Pharmaceutical & Biological Archives, 5(4), 76 -81.
3. Adhikary, P., et al. 2011. Phytochemical Screening and Anti-Microbial Properties of Medicinal Plants of Dhunkharka Community, Kavrepalanchowk, Nepal. International Journal of Pharmaceutical & Biological Archives, 2(6), 1663-1667.
4. Baskar, N., Parimala, D. B., & Mohan, K. R., 2010. Anti-Cancer Activity of Methanol Extract of Root Bark of Erythrina variegata Linn. International Journal of Toxicological and Pharmacological Research, 2(2).

5. Biswas, B., et al. 2013. Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria. International Journal of Microbiology. DOI: 10.1155/2013/746165

6. Blois, M. S., 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 181:1199-1200.

7. Cavalieri, S. J., Rankin, I. D., et al. 2005. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology, Washington, DC.

8. Ciulei, I., 1982. Practical Manuals on the Industrial Utilization of Medicinal and Aromatic Plants: Methodology for analysis of vegetable drugs. University of Bucarest, 1-10.

9. Devkota, H. P., Adhikari, B., Watanabe, T. & Yahara, S., 2018. Nonvolatile Chemical Constituents from the Leaves of Ligusticopsis wallichiana (DC.) Pimenov & Kljuykov and their Free Radical-Scavenging Activity. Journal of Analytical Methods in Chemistry, 1-8. DOI:10.1155/2018/1794650.

10. Dingel, J., Reid, W. W., & Solomons, G. L., 1953. The enzymic degradation of pectin and other polysaccharides. II-Application of the 'Cup-plate' assay to the estimation of enzymes. Journal of the Science of Food and Agriculture, 4(3):149-155.

11. Dhote, K., Dhote, V., & Khatri, K., 2015. Phytochemical Screening and Pharmacological Activity in Punica granatum. Asian Journal of Pharmaceutical Education and Research, 4(4), 69-80.

12. Ghimire, S. K., Sapkota, I. B., Oli, B. R., & Parajuli, R., 2008. Non-Timber Forest Products of Nepal Himalaya; Database of Some Important Species Found in the Mountain Protected Areas and Surrounding Regions. WWF Nepal, Kathmandu, Nepal. NTFP of Nepal Himalaya.

13. Gonçalves, S., Moreira, E., Grosso, C., Andrade, P. B., Valentão, P., Romano, A., 2017. Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. Journal of Food Science and Technology, 54(1):219-227. DOI: 10.1007/s13197-016-2453-z

14. Hamidi, M. R., Jovanova, B., Panovska, T. K., 2014. Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.). Macedonian pharmaceutical bulletin, 60 (1) 9 – 18.

15. Jamuna, S., Paulsamy S., & Karthika, K., 2012. Screening of in vitro antioxidant activity of methanolic leaf and root extracts of Hypochaeris radicata L. (Asteraceae). Journal of Applied Pharmaceutical Science, 02 (07), 149-154.

16. Joshi, R. K., 2016. Chemical constituents of leaf essential oils of Heracleum candicans Wall. ex DC from western Himalaya of Uttarakhand, India. American Journal of Essential Oils and Natural Products, 4(2), 01-04.

17. Kalita, P., Tapan, K. B., Pal, T. K., Kalita, R., 2013. Estimation of Total Flavonoids content and Anti-oxidant activities of Methanolic Whole plant extract of Biophytum Sensitivum Linn. Journal of Drug Delivery and Therapeutics, 3(4), 33-37. DOI: 10.22270/jddt.v3i4.546

18. Kasote, D. M., Katyare, S. S., Hegde, M. V., and Bae, H., 2015. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. International Journal of Biological. Science, 11(8), 982-991. DOI: 10.7150/ijbs.12096

19. Kumar, S., Joseph, L., George, M., & Bharti, V., 2011. Antimicrobial Activity of Methanolic Extract of Rumex Nepalensis Leaves. International Journal

of Pharmacy and Pharmaceutical Sciences, 3(4), 240-242.

20. Kunwar, R. M., et al. 2011. Medicinal and Aromatic Plants Network (MAPs-Net) Nepal: An open access digital database. Banko Janakari, 21(1) DOI: 10.3126/banko.v21i1.9064
21. Kunwar, et al. 2013. Medicinal plants, traditional medicine, markets and management in far-west Nepal. Journal of Ethnobiology and Ethnomedicine, 9:24.
22. Manandhar, S., Luitel, S., & Dahal, R. K., 2019. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine, 1-5. DOI: 10.1155/2019/1895340
23. Meena, A. K., Bhavana, P., Panda, P., Sannd, R., and Rao, M. M., 2010. A review on Rubia cordifolia: Its phyto constituents and therapeutic uses. Drug Invention Today, 2(5), 244-246.
24. Meyer, B. N., et al. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 45(5), 31-34. DOI: 10.1055/s-2007-971236
25. Nepal: General Info, Federation of Nepalese Chambers of Commerce and Industry (FNCCI).
26. Oliveira de, A. C., et al. 2009. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chemistry, 115(2), 469–475. DOI: 10.1016/j.foodchem.2008.12.045
27. Parajuli, R. R., 2013. Study on Local Uses of Medicinal Plants in Nayabazar, Pyang and Jamuna VDCs of Ilam District. Nepal Journal of Science and Technology, 14(1), 57-66. DOI: 10.3126/njst.v14i1.8923
28. Parajuli, S., et al. 2012. Antioxidant Activity, Total Phenol and Flavonoid Contents in Some Selected Medicinal Plants of Nepal. Journal of Health and Allied Sciences, 2(1), 27-31.
29. Pliszko, A. & Klich, S., 2018. A new record of Phytolacca acinosa (Phytolaccaceae) in Poland. Acta Mus. Siles. Sci. Natur., 67: 181-183. DOI: 10.2478/cszma-2018-0013
30. Rukmini, K., & Suvarnalatha, P. D., 2014. GC-MS Analysis and Phytochemical Screening of a rare Pteridophyte Nephrolepis Cardifolia (L.) Presl. from Tirumala Hills. International Journal of Pharma Research & Review, 3(12), 13-19.
31. Saxena, M., et al. 2012. Flavonoids and phenolic acids as antioxidants in plants and human health. International Journal of Pharmaceutical Sciences Review and Research, 16(2):130-134.
32. Saxena, M., Saxena, J., 2013. Phytochemistry of Medicinal Plants. Journal of pharmacognosy and Phytochemistry, 1(6), 168-182
33. Sen, S., et al. 2010. Free Radicals, Antioxidants, Disease and Phytomedicines: Current Status and future Prospect. International Journal of Pharmaceutical Sciences Review and Research, 3(1), 91-100.
34. Shahidi, F, Zhong, Y., 2010. Novel antioxidants in food quality preservation and health promotion. European Journal of Lipid Science and Technology. 112(9), 930-940. DOI: 10.1002/ejlt.201000044
35. Shrestha, B. P., et al. 2019. Biodiversity Profile of the API Nampa Conservation Area, Darchula. Department of National Parks and Wildlife Conservation, Kathmandu, Nepal, 1-108.
36. Ullah, S., et al. 2011. Antibacterial activity of some selected plants of Swat valley. Bioscience Research, 8(1), 15-18.

37. Waterhouse, A. L., 2003. Determination of Total Phenolics. Current Protocols in Food Analytical Chemistry. John Wiley & Sons, New York. DOI: 10.1002/0471142913.fai0101s06

38. Yuan, H., Ma, Q., Li Y., & Piao, G., 2016. The Traditional Medicine and Modern Medicine from Natural Products. Molecules, 21(5): 559. DOI: 10.3390/molecules21050559

39. Zaidan, M. R. S., et al. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine, 22(2), 165–170.

Research article
Curr. Pers. MAPs, (2020); 3(2): 74-86

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Taxonomic Investigation of Medicinal and Aromatic Plants with Natural Growing Characteristics in Kastamonu (Hanönü) Region

Murat ÖZOCAK[1]*, Erkan ÜNALAN[2]

[1]Ministry of Agricultural and Forestry, District Agriculture and Forestry Directorate of Kastamonu- Hanönü Postal code: 37100, Kastamonu, Turkey
*Corresponding author: murat.ozocak@outlook.com
[2]Mınıstry of Agrıcultural and Forestry, District Agrıculture and Forestry Directorate of Kastamonu- Hanönü, Postal code: 37100, Kastamonu, Turkey
E-mail: mai_turk37@hotmail.com

https://doi.org/10.38093/cupmap.765409

Received : 19/05/2020
Accepted : 19/11/2020

Abstract

Our country has a wide flora in terms of growing and general distribution of medicinal and aromatic plants. The wide spread of these plants and the work in this field are increasing day by day. Based on this situation, a taxonomic study of 33 medical and aromatic plants showing natural distribution and playing a big role in terms of human health has been carried out in Hanönü district of Kastamonu region, which has a botanical wide flora. Images of all the identified species were taken in the growing region. Along with the pictures of the plants detected, the names of local usage and scientific Latin names and the places where they grow in the region are briefly explained. Recommendations were made to raise awareness of the consumption of the identified species, and to prevent unnecessary consumption and extinction. Beside, it is recommended to increase the scientific research on the subjects such as cultivation, drying parameters and storage of medicinal and aromatic plants starting from the Kastamonu region.

Key Words: Aromatic plants, Botanical, Kastamonu, Medicinal Plants, Flora, Taxonomical Investigation

1. Introductıon

Humanity has been benefiting from plants for a therapeutic purpose since its existence. In the current situation, although the use continues, the majority of researches on medicinal and aromatic plants show the prevalence of these plants. Our country has a rich plant resource in terms of climate conditions. With the latest diagnoses, it has been determined that more than 11.000 plant taxa grow in Turkey, and approximately 3035 of these taxa are endemic (endemism rate 31.12%)(Gürbüz, 1999; Güner, 2012; Öztürk et al.)

Medicinal and aromatic plants used in a wide range. Although it does not contain a wide range of areas at present, you can choose to group the plants according to their families beneficial parts and content-based active ingredients (Ceylan, 1995; Altay et al., 2015a,b). Due to the ecological richness of our country, a large number of plants that can be used for medicinal and

aromatic purposes can be grown. (Karahan et al., 2020). Some plants are collected from nature and some are provided by agricultural production. laurel plants collected from nature, mahleb, lime blossom, sage, rosemary, juniper and licorice are examples shells. Those cultivated can be cumin, anise, thyme, fenugreek, fennel, mint and coriander (Kızıl et al., 2010).

In a study conducted on medicinal and aromatic plants in Kastamonu region, the awareness of naturally growing medicinal and aromatic plants in Taşköprü district was investigated by the local people. In the study, questions were asked to the people of the region about 26 medicinal and aromatic plants. As a result of the research, it was determined that most garlic, blackberry, nettle, rosehip, wild pear, linden and thyme plants are known and used in the region. The least known plants were found to be ragweed, yarrow and laden (Öztürk et. al. 2017)

In the study conducted by Yaman (2001) in which medicinal and aromatic plants that grow naturally in Kastamonu province and generate income for the region were evaluated, some plants that stand out economically were determined. According to the research, St. John's Wort, rosehip, salep, lamb belly mushroom, yellow mushroom, bear mushroom came to the fore in terms of income and natural growing conditions.

Within the scope of the research, the taxonomic examination of the medicinal and aromatic plants, which have become a very important sector in the world, under the conditions of Kastamonu (Hanönü), which has rich ecological features, is aimed. For this purpose, field studies were carried out in the region and all of the found species were recorded. The locations of each plant determined in the region are also recorded, and a study that will shed light on the medicinal and aromatic plants that are becoming more and more important every day.

2. Material and Method

The material of the research forms medicinal and aromatic plant species grow naturally in Hanönü of Turkey's Kastamonu province. Kastamonu Turkey's Black Sea Region in the northern part of our country is indigenous. Sinop, Çorum, Çankırı, Karabük and Bartın are the provinces and they have a coast to the Black Sea in the north.

The center of the city is in the city of Kastamonu with the same name. 74,6% of the face measurement of Kastamonu province consists of mountainous and woodland, 21,6% plateau and 3,8% plains. In general, there are large areas suitable for agriculture. Small plains, which take an important place in the valleys, are treated as important areas where such operations are carried out. The district of Alan Hanönü, which is a branch of Kızılırmak, is located in Taşköprü district, Sinop province Boyabat district, which is connected to many towns until 1988.

Figure 1. Kastamonu (Hanönü) Region (Anonymus, 2020).

For the method to be followed in the research, a literature review related to flora studies related to Kastamonu (Hanönü) has been made, and no comprehensive study

has been found regarding the region, especially in terms of medicinal and aromatic plants. In this respect, the way to be followed as a research method has been completely formed by land formation. During the studies conducted in the district center and villages, continuous meetings were held with the people of the region, and subjects such as the local user names of the medicinal and aromatic plants grown in the region from the past to the present day and the regions where they were grown and recorded.

3. Results and Discussıon

Land works in the region were carried out until July 2020, throughout 2019. 33 medical and aromatic plant taxa were determined in the region, which was carried out by the Agricultural Engineer Murat ÖZOCAK and Agricultural Engineer Erkan ÜNALAN. The scientific names and families of the taxa in accordance with the systematic index obtained as a result of the local names of all the plants found, for what purpose and which regions are used, as well as the researches performed by Davis (1965; 1967; 1970; 1972; 1975; 1978; 1982; 1984; 1985). It indicated.

The families and the names of the medicinal and aromatic plants in the field studies conducted in the scope of the research, their location in the region, their local names from the past and their Scientific names are briefly explained.

As a result of the field studies in the region, a total of 33 plants that can be used for medicinal and aromatic purposes were determined. All information about the detected plants are presented in Table.1 and tried to be explained under subtitles.

Anatolian Sage
(*Salvia anatolica* Hamzaoglu & A.Duran)
Salvia anatolica Hamzaoglu & A.Duran , which is known as Anatolian sage and belongs to *Lamiaceae* family, is grown in many places in the region. It was discovered in the Yozlu area of the Vakıf District in the research area. 623 m from the sea Anatolian Sage, located at a height of 79 km from the city center.

(Murat Özocak/Erkan Ünalan- 07.06.2020)

Figure 2. *Salvia anatolica* Hamzaoglu & A.Duran

Basil
(*Ocimum basilicum* var. *album* (L.) Benth.)
Belonging to the *Lamiaceae* family, basil is also known as basil in the region. It was discovered in the Karabük Neighborhood of Aşağı Çakırçay Village in the research area. location 914 m from the sea. and 81 km away from the city center.

(Murat Özocak/Erkan Ünalan- 12.06.2020)

Figure 3. *Ocimum basilicum* var. *album* (L.) Benth.

Table.1. Medicinal and aromatic plants detected in Kastamonu (Hanönü) Region

Plant name	Famıly	District / Village	Altitude (m.)	Location (Distance to the city center (km)	Geography Coordinates
Anatolian Sage *(Salvia anatolica* Hamzaoglu & A.Duran)	*Lamiaceae*	Vakıf/Yozlu located	623	79	34°-26'-14,86'' / 41°-37'-40,42''
Basil (*Ocimum basilicum* var. *album* (L.) Benth.)	*Lamiaceae*	Aşağıçakırçay/ Karabük located	914	81	34°-33'- 39,09''/ 41°-38'-26,65''
Belladonna (*Atropa belladonna* L.)	*Solanaceae*	Hanönü/Donaşar located	472	72	34°-27'-33,92'' / 41°-37'-25,11''
Bitter melon (Ecballium elaterium (L.) A.Rich)	*Cucurbitaceae*	Yenice located	438	85	34°-32'-59,14'' / 41°-36'-56,37''
Blackthorn *(Paliurus spina-christi* Mill.)	*Rhamnaceae*	Kavak/Sinözü located	929	81	34°-28'-3,17'' / 41°-39'-29,07''
Burdock (*Arctium lappa* subsp. *platylepis* (Boiss. & Balansa) Arènes)	*Compositae*	Bölükyazı/Çay located	972	61	34°-25'-48,34'' /41°-39'-25,64''
Buttercup (*Ranunculus ficaria* var. *bulbifera* Albert)	*Ranunculaceae*	Sirke/Düzen located	546	57	34°-22'-20,30'' / 41°-37'-48,69''
Caper (*Capparis spinosa* subsp. *aegyptia* (Lam.) Kit Tan & Runemark)	*Capparaceae*	Küreçayı/ Kıraç located	605	80	34°-24'-21,08'' / 41°-38'-16,73''
Charlock *(Sinapis arvensis* L.)	*Brassicaceae*	Bölükyazı/ Yozlu located	987	65	34°-25'-54,49'' / 41°-38'-3,10''
Dandelion (*Taraxacum officinale* (L.) Weber ex F.H.Wigg.)	*Asteraceae*	Gökçeağaç/Karaağaçaltı	448	76	34°-30'-27,29'' / 41°-37'-48,53''
Dead nettle *(Urtica dioica* L.)	*Urticaceae*	Hocavakıf/Karaoluk located	643	83	34°-23'-52,80'' / 41°-40'-17,02''
Elderberry (*Sambucus adnata* Wall. ex DC.)	*Adoxaceae*	Hanönü/Bürnük located	423	79	34°-27'-9,22'' / 41°-42'-45,23''
False hemp (*Cannabina laevis* Moench)	*Datiscaceae*	Hanönü/Göller located	436	78	34°-27'-28,94'' / 41°-37'-32,92''
Fire thorn *(Pyracantha angustifolia* (Franch.) C.K.Schneid.)	*Rosaceae*	Bölükyazı/Kadıağa located	975	59	34°-24'-35,07'' / 41°-41'-30,22''
Horsetail (*Equisetum arvense* L.)	Equisetaceae	Hocavakıf/Öküzaltı located	642	83	34°-22'-38,06'' / 41°-41'-51,23''
Immortelle (*Helichrysum angustifolium* (Lam.) DC.)	Asteraceae	Yukarıçakırçay/ Yayundurun located	820	89	34°-34'-34,66''/ 41°-40'-14,52''
Linden *(Tilia amurensis* Rupr.)	*Malvaceae*	Kavak/Bürnük located	958	83	34°-26'-50,66''/ 41°-42'-25,09''
Lion's Claw (*Allcemilla vulgaris* L.)	Rosaceae	Yeniköy/Kiremitlik located	1021	85	34°-24'-41,35''/ 41°-33'-3,02''
Marigold (*Calendula officinalis* L.)	*Asteraceae*	Central vıllages of Hanonu	442 -501	75	34°-28'-1,81'' / 41°-37'-37,16''
Meadow triangular *(Trifolium pratense* L.)	*Leguminosae*	Kayabaşı/ Melleşler located	1096	84	34°-18'-26,80'' / 41°-42'-14,93''
Medicinal chamomile *(Matricaria chamomilla* L.)	*Composita*	Kavak/ Soyuk located	945	86	34°-27'-56,96'' / 41°-41'-53,14''
Mint (*Mentha × piperita* L.)	*Lamiaceae*	Hanönü/Karayaprak located	463	79	34°-28'-21,09''/ 41°-37'-33,00''
Mistletoe *(Viscum album* L.)	*Santalaceae*	Bölükyazı/ Abazoğlu located	972	66	34°-23'-59,88'' / 41°-41'-51,56''
Orchis *(Orchis morio* L.)	Orchidaceae	Yeniköy/Yörükören located	1003	81	34°-24'-42,38''/ 41°-32'-52,31''
Plantago *(Plantago afra* L.)	*Plantaginaceae*	Aşağıçakırçay located	912	80	34°-34'-51,21''/ 41°-37'-50,92''
Prunella plum *(Prunus domestica* L.)	*Rosaceae*	Akçasu/Çevrik located	479	65	34°-21'-42,04'' / 41°-35'-53,48''
Rose (*Rosa × damascena* Herrm.)	*Rosaceae*	Hanönü/Donaşar located	429	78	34°-27'-48,70''/ 41°-37'-29,62''
Rosemary (*Rosmarinus eriocalyx* Jord. & Fourr.)	*Lamiaceae*	Yenice/Köyiçi located	433	81	34°-32'-59,26''/ 41°-36'-58,46''
Snake pillow (*Dracunculus vulgaris* Schott)	*Araceae*	Halkabük/Göçebe located	462	84	34°-30'-48,66'' / 41°-37'-9,06''
St. John'sWort *(Hypericum perforatum* L.)	*Hypericaceae*	Yeniköy/Kiremitlik located	1003	85	34°-25'-1,29'' / 41°-33'-8,36''
Milk Thistle *(Silybum marianum* (L.) Gaertn.)	*Compositae*	Hocavakıf/ Ayşeoğlu located	645	85	34°-23'-13,92''/ 41°-41'-16,27''
Thyme *(Thymus plasonii* Adamovic)	*Lamiaceae*	Yukarıçakırçay/ Yayundurun located	819	89	34°-34'-37,46''/ 41°-40'-21,20''
Toothpic kweed *(Amni visnaga* L.)	*Apiaceae*	Vakıfgeymene/Yazı located	556	81	34°-25'-45,26'' / 41°-37'-0,58''

Belladonna
(*Atropa belladonna* L.)
From the *Solanaceae* family, the beautician grass was observed on the barren and stony grounds in the research area. These areas are Donaşar Mevkii parts of Hanönü District. the area where it is located is 472 m from the sea. and 72 km from the city center. Away.


(Murat Özocak/Erkan Ünalan- 13.09.2019)

Figure 4. *Atropa belladonna* L.

Bitter melon
(*Ecballium elaterium* (L.) A.Rich)
It belongs to the *Cucurbitaceae* family and is known by names such as bitter melons, cırlatan, donkey cucumber. It has been observed that it grows in Yenice Village settlement area and its surroundings in the research area. 85 km from the city center. The bitter melon which is determined to be located at a distance of 438 m from the sea. It is located at a height.


(Murat Özocak/Erkan Ünalan- 21.08.2019)

Figure.5. *Ecballium elaterium* (L.) A.Rich

Blackthorn
(Paliurus spina christi Mill.)
Blackthorn, a member of the Rhamnaceae family, is also known as Shrub thorn and barrack thorn. Within the scope of the research, it has been determined that it is present in almost all villages and located in Sinözü District of Kavak village. Sinözü area, where the blackhead is located, is 929 m. and 81 km from the city center away.


(Murat Özocak/Erkan Ünalan- 16.10.2019)

Figure 6. *Paliurus spina-christi* Mill.

Burdock
(*Arctium lappa* subsp. *platylepis* (Boiss.& Balansa) Arènes)
Burdock, a family of Asteraceae, has local uses called pitrak. It was determined that it grows naturally in Çay Mahallesi, in the borders of Bölükyazı Village. 972 m from the sea. Dulavrat grass is located at a height of 61 km from the city center away.


(Murat Özocak/Erkan Ünalan- 09.10.2019)

Figure 7. Arctium lappa subsp. platylepis (Boiss. & Balansa) Arènes

Buttercup
(*Ranunculus ficaria* var. *bulbifera* Albert)
Ranunculaceae family is also known as a buttercup. It has been identified in almost every region in the region and it has been observed that it grows in all villages and gardens of Hanönü district. average 546 m. It has been observed that it grows at altitude levels, 57 km from the city center. It was determined at a distance.

(Murat Özocak/Erkan Ünalan- 23.07.2019)

Figure 8. *Ranunculus ficaria* var. *bulbifera* Albert

Caper

(*Capparis spinosa* subsp. *aegyptia* (Lam.) Kit Tan & Runemark)

Caper, a member of the Capparaceae family, is also referred to as the Gebre in the region. Within the scope of the research, it has been determined that it is present in Kıraç District of Küreçayı Village. 605 m above sea level. The current location of the capers found at a height is 80 km from the city center.

(Murat Özocak/Erkan Ünalan- 24.07.2020)

Figure 9. *Capparis spinosa* subsp. *aegyptia* (Lam.) Kit Tan & Runemark

Charlock

(*Sinapis arvensis* L.)

From the Brassicaceae family, it grows frequently in the mustard region, especially in the Yozlu Region within the boundaries of the village of Bölükyazı. The height information determined was 987 m. and the province is 67 km. is for data.

(Murat Özocak/Erkan Ünalan- 11.08.2020)

Figure 10. *Sinapis arvensis* L.

Dandelion

(*Taraxacum officinale* (L.) Weber ex F.H. Wigg.)

Dandelion plant, belonging to the *Asteraceae* family, was found in the Karağaçaltı locality of Gökçeağaç village in the research area.

(Murat Özocak/Erkan Ünalan- 19.08.2019)

Figure 11. *Taraxacum officinale* (L.) Weber ex F.H.Wigg.

Determined area is 448 m. It has a height from the sea and is 76 km away from the city center.

Dead Nettle

(*Urtica dioica* L.)

Dead Nettle, a member of the Urticaceae family, was discovered in the Karaoluk neighborhood of the Hocavakıf Village in the research area. the height of 643 m from the sea. Urtica dioica L. is 83 km from the city center.

(Murat Özocak/Erkan Ünalan- 29.07.2019)

Figure 12. *Urtica dioica* L.

Elderberry
(*Sambucus adnata* Wall. ex DC.)
The elderberry, also known as Yivdin, is from the Adoxaceae family. It was determined that it grows in Karayaprak District of Hanönü Merkez District in the research area. 423 m from the sea and the province is 79 km.


(Murat Özocak/Erkan Ünalan- 22.09.2019)

Figure 13. *Sambucus adnata* Wall. ex DC.

False hemp
(*Cannabina laevis* Moench)
It belongs to Datiscaceae family and grows naturally in Gölle Mevkii in Hanönü Central District under false hessian region conditions. False hemp research area 436 m. detected in height. The detected area is 78 km from the city center.


(Murat Özocak/Erkan Ünalan- 26.07.2020)

Figure 14. *Cannabina laevis* Moench

Fire thorn
(*Pyracantha angustifolia* (Franch.) C.K.Schneid.)
It belongs to the Rosaceae family and is known as the fire thorn or dog apple among the people. It has sweet fruits and can be used for medicinal and aromatic purposes. It was determined that it is located within the boundaries of the village of Bölükyazı in the research area. It is also observed that it is located in the natural environment in the Kadıağa Farm Area. Fire thorn research area 975 m. detected in height. The detected area is 59 km from the city center.


(Murat Özocak/Erkan Ünalan- 25.07.2020)

Figure 15. *Pyracantha angustifolia* (Franch.) C.K.Schneid.

Horsetail
(*Equisetum arvense* L.)
Horsetail plant is belongs to the family Equisetaceae. It was determined that it grows within the boundaries of Öküzaltı District of Hocavakıf Village in the research area. the height of the determined areas from the sea is 642 m. and the distance to the city center was determined as 83 km.


(Murat Özocak/Erkan Ünalan- 27.09.2019)

Figure 16. *Equisetum arvense* L.

Immortelle Herb
(*Helichrysum angustifolium* (Lam.) DC.)
The immortal herb from the Asteraceae family is also known as the golden herb. It has been determined that it is present in the Yayundurun Site of Yukaricakircay Village in the research area. Sea height of the designated location is 820 m. It is 89 km away from the city center.

(Murat Özocak/Erkan Ünalan- 10.08.2020)

Figure 17. *Helichrysum angustifolium* (Lam.) DC.

Linden
(Tilia amurensis Rupr.)
It is determined that linden tree, which is a family of Malvaceae, is common in Bürnük neighborhood of Kavak Village in the researched region. The altitude of the determined area is 958 m. It is 83 km away from the city center.

(Murat Özocak/Erkan Ünalan- 12.07.2019)

Figure 18. Linden *Tilia amurensis* Rupr.

Lion's Claw
(Allcemilla vulgaris L.)
The lion claw, which belongs to the Rosaceae family, is also known in the region as lion oil, hazelnut and falcon

(Murat Özocak/Erkan Ünalan- 26.08.2019)

Figure 19. *Allcemilla vulgaris* L.

It has been determined that the lion claw plant grows frequently in the Kiremitlik region of the Yeniköy Village. Lion's claw research area 1021 m. detected in height. The detected area is 85 km from the city center.

Marigold
(*Calendula officinalis* L.)
The calendula plant, also known as nocturnal or orange daffodil, belongs to the Asteraceae family. It grows in the garden of Hanönü District Agriculture and Forestry Directorate. Although its height from the sea varies between 442-501 m, it is 75 km away from the city center.

(Murat Özocak/Erkan Ünalan- 05.07.2020)

Figure 20. *Calendula officinalis* L.

Meadow triangular
(*Trifolium pratense* L.)
The meadow, which belongs to the Gramineae family, is also known as triple red clover. It has been determined that it is common in the research area of Kayabaşı Village, Melleşler. Meadow triangular research area 1096 m. detected in height. The detected area is 84 km from the city center.

(Murat Özocak/Erkan Ünalan- 19.08.2019)

Figure 21. *Trifolium pratense* L.

Medicinal Chamomile
(*Matricaria chamomilla* L.)
Medicinal chamomile is a family of Asteraceae, also referred to as authentic chamomile.

(Murat Özocak/Erkan Ünalan- 20.08.2019)

Figure 22. *Matricaria chamomilla* L.

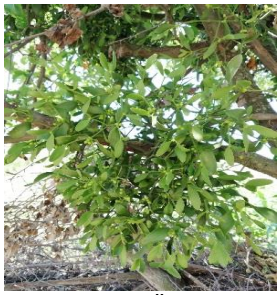
Within the scope of the research, it has been determined that it grows in Soyuk District of Kavak Village. Its location is 945 m from the sea and its distance to the city center is 86 km.

Mint
(*Mentha × piperita* L.)
Mint from the Lamiaceae family is popularly known as mint and garden mint. It is frequently encountered in the research area in the Karayaprak District of the Hanönü Merkez District. Altitude information is 463 m and its distance to the center is 79 km.

(Murat Özocak/Erkan Ünalan- 09.08.2020)

Figure 23. *Mentha × piperita* L.

Mistletoe
(*Viscum album* L.)
Mistletoe from the Santalaceae family is also known as moth and moth. It has been determined that it grows in the Abazoğlu Neighborhood of Baöükyazı Village in the research area.



(Murat Özocak/Erkan Ünalan- 27.08.2019)

Figure 24. *Viscum album* L.

Orchis
(*Orchis morio* L.)
Orchis, which belongs to Orchidaceae family, grows in the borders of Yuruköy Village, Yurukoy Quarter in the research area. The height from the sea was determined as 1003 m. The distance to the city center was determined as 81 km.

(Murat Özocak/Erkan Ünalan- 22.07.2020)

Figure 25. *Orchis morio* L.

Plantago
(*Plantago afra* L.)
Although it is known as plantain or herb grass belonging to the family of Plantaginaceae, it is known as boiled grass, wound grass, vascular grass in the region.

(Murat Özocak/Erkan Ünalan- 22.07.2020)

Figure 26. *Plantago afra* L.

It has been found abundantly in the research area of the Lower Çakırçay Village settlement area and its surroundings. Sea height of the designated location is 912 m. It is 80 km away from the city center.

Prunella Plum
(*Prunus domestica* L.)
The plum, which belongs to the Rosaceae family, known as alaik or uryani, is grown especially in the Kastamonu region and its fruit has a positive effect against digestive system disorders. Their location in the research area is in the Çevrik Mevkii region within the borders of Akçasu Village.


(Murat Özocak/Erkan Ünalan- 28.08.2019)

Figure 27. *Prunus domestica* L.

Rose
(*Rosa × damascena* Herrm.)
The rose, which belongs to the Rosaceace family, is 429 m above sea level in the research area. It was determined in height. It is 78 km. grows at a distance. It grows intensely in natural conditions in Hanönü Donaşar location.


(Murat Özocak/Erkan Ünalan- 10.08.2020)

Figure 28. *Rosa × damascena* Herrm.

Rosemary
(*Rosmarinus eriocalyx* Jord. & Fourr.)
Rosemary, also known as bird grass in the region, belongs to the Lamiaceae family. It grows abundantly in the village area of Yenice Village in the research area. The height from the sea was determined as 423m and the distance to the city center was determined as 81 km.


(Murat Özocak/Erkan Ünalan- 25.07.2020)

Figure 29. *Rosmarinus eriocalyx* Jord. & Fourr.

Snake Pillow
(*Dracunculus vulgaris* Schott)
Snake pillow (Beech beet), which belongs to the Araceae family and is known in the region with names such as snake pillow, elephant ear, and shaggy, is known for its good skin psoriasis. It was discovered in the research area around the Göçebe area, within the borders of Halkabük Village.


(Murat Özocak/Erkan Ünalan- 10.08.2020)

Figure 30. *Dracunculus vulgaris* Schott

St. John's Wort
(*Hypericum perforatum* L.)
St. John's Wort, also known as Hypericaceae family, is also known as sword grass and yeast. In the research area, its groove was

determined in the Kiremitlik area within the borders of Yeniköy Village. The altitude information is 1003 m and the distance of the determined region to the city center is determined as 85 km.

(Murat Özocak/Erkan Ünalan- 21.07.2019)

Figure 31. *Hypericum perforatum* L.

Milk Thistle
(*Silybum marianum* (L.) Gaertn.)
Thistle, also known as the Virgin Mary's thorn in the region, is a member of the Compositae family. It was discovered at the Ayşeoğlu Site within the boundaries of the Hocavakıf Village in the research area. It is 645 m high from the sea and it was located at a location 85 km from the city center.

(Murat Özocak/Erkan Ünalan- 12.07.2020)

Figure 32. *Silybum marianum* (L.)Gaertn.

Thyme
(*Thymus plasonii* Adamovic)
Thyme, which belongs to Lamiaceae family, is frequently consumed in the region. It has been observed that it is present at the Yayundurun location within the borders of the Yukari Çakırçay Village in the research area. altitude information 819 m. and the provincial distance was determined as 89 km.

(Murat Özocak/Erkan Ünalan- 28.08.2019)

Figure 33. *Thymus plasonii* Adamovic

Toothpic Kweed
(*Amni visnaga* L.)
The apricot of the Apiaceae family is also known as toothpick in the region. It has been determined that there are plenty in the district of Vakıfgeymene Mahallesi Yazi. The height of the determined area from the sea is 556 m and its distance to the city center is 81 km.

(Murat Özocak/Erkan Ünalan- 09.08.2020)

Figure 34. *Amni visnaga* L.

4. Conclusıon

The findings obtained from the research results made in the region of Kastamonu (Hanönü), which includes the natural growing conditions of many plants in terms of vegetation and climate characteristics, are listed below.

As a result of the field studies, 33 medicinal and aromatic plants were identified. People living in the district center and villages, especially those who have been in the region for a long time, know these plants. However, there was no activity in the form of

collecting from nature or agricultural production.

Although not very much, unconscious removal or damage of these plants has been observed in some regions. There are many medicinal and aromatic plant taxa in the region. A new job opportunity can be easily created by gathering them for commercial purposes or switching to agricultural production. Supporting subjects such as research and R&D in the region may encourage production and collection. It is possible to establish agricultural structures integrated with the studies related to drying and storage of these plants.

As a result, Kastamonu (Hanönü) region is one of our regions where there are many medicinal and aromatic plants thanks to its suitable climate and vegetation characteristics. Along with these advantages, it has been observed that it is possible to make it better with research and technical activities to be carried out on the subject.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Altay, V., Karahan, F., Sarcan, Y.B. & İlçim, A. 2015a. An ethnobotanical research on wild plants sold in Kırıkhan district (Hatay/Turkey) herbalists and local markets. Biological Diversity and Conservation, 8/2: 81-91.
2. Altay, V., Keskin, M., Karahan, F. (2015b). An assessment of the plant biodiversity of Mustafa Kemal University Tayfur Sokmen campus (Hatay-Turkey) for the view of human health. International Journal of Scientific and Technological Research, 1(2): 83-103
3. Anonymous., 2020. Kastamonu location, Erişim tarihi: 02.03.2020. https://tr.wikipedia.org/wiki/Kastamonu_(il)#/media/Dosya:Kastamonu_districts.png
4. Ceylan, A., 1995. Tıbbi Bitkiler I. E.Ü. Ziraat Fakültesi Yayınları III. Basım No:312. Bornova/İzmir
5. Davis, P.H., 1965. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 1., Edinburgh.
6. Davis, P.H., 1967. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 2., Edinburgh.
7. Davis, P.H., 1970. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 3., Edinburgh.
8. Davis, P.H., 1972. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 4., Edinburgh.
9. Davis, P.H., 1975. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 5., Edinburgh.
10. Davis, P.H., 1978. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 6., Edinburgh.
11. Davis, P.H., 1982. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 7., Edinburgh.
12. Davis, P.H., 1984. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 8., Edinburgh.
13. Davis, P.H., 1985. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Vol. 9., Edinburgh.
14. Gürbüz, B., 1999. Çok yıllık tıbbi ve aromatik bitkiler. Türk-Koop. Ekin Dergisi, Yıl: 3/7,Ankara, (s:83-87).
15. Güner A, Aslan S, Ekim T, Vural M, Babaç MT (2012) Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, Istanbul.
16. Karahan, F., Ozyigit, I. I., Saracoglu, I. A., Yalcin, I. E., Ozyigit, A. H., & Ilcim, A. (2020). Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern Mediterranean region of Turkey. Biological Trace Element Research, 197:316–329.

17. Kızıl, S., Bayram, E., Kirici, S., Tansi, S., 2010. Tıbbi Ve Aromatik Bitkiler Üretiminin Arttırılması Olanakları. TMMOB, Ziraat Mühendisleri Odası 7. Teknik Kongresi, 11 Ocak 2010, At Ankara, Turkey, Volume: I
18. Ozturk, M., Altay, V., Latıff, A., Salman, T. and Choudhry I. (2018). A Comparative Analysis of the Medicinal Pteridophytes in Turkey, Pakistan, and Malaysia. In Plant and Human Health; Ozturk, M., Hakeem, K. R., Eds.; Springer: Cham, 2018; Vol. 1, pp. 349-390.
19. Öztürk, A., Seki N., Yiğit N., 2017. Ethnobotanical features of some plants used in Taşköprü (Kastamonu/Turkey) region. Biological Diversity and Conservation. 10/3, 136-141.
20. Yaman, K., 2001. Kastamonu İlinde Doğal Olarak Yetişen Başlıca Tıbbi ve Aromatik Bitkilerin Toplama, İşleme ve Pazarlama Maliyetleri ve Söz Konusu Ürünlerin İl Ekonomisine Katkıları. Yüksek Lisans Tezi. Ankara Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Anabilim Dalı, Ankara.81pp.

Research article
Curr. Pers. MAPs, (2020); 3(2): 87-96

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Amelioration of External Surgical Wound by Topical Application of the Aqueous Formulation of Neem Leaves (*Azadirachta indica*) in Caprine Model

Ajmary SULTANA[1], Marzia RAHMAN[2], Md. Mahmudul ALAM[1*]

[1]Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh
Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh
Postal code 2202, Mymensingh, Bangladesh
*Corresponding author: mahmud.dso@bau.edu.bd

https://doi.org/10.38093/cupmap.828751
Received : 20/11/2020
Accepted : 05/12/2020

Abstract

The study was conducted to evaluate the healing potentials of Neem leaf on surgical wounds. A total of sixteen surgical wounds were studied under two groups; Neem (group A) and Normal saline (group B, control). Wound morphology, histopathology, and bacteriological examinations were carried out to assess wound healing potentials of this plant leaves. We have found a remarkably lower swollen area (3.91±0.10mm), suture line levation (2.64±0.19mm) and length of the wound (15.78±0.19mm) in the treatment group than the control (0.36±0.12mm, 3.59±0.12mm, and 17.11±0.08mm respectively). The mean healing period Neem treated wound was significantly ($P<0.05$) lower (12.33±0.42 days) than that of control (18.67±0.33 days). Histopathological study revealed the presence of substantial inflammation with fibroblastic proliferation in samples collected at day 3 in the control group whereas these features were distinctly reduced in the treatment group. There was a distinct thickening of the keratinized layer of the epidermis in Neem treated wound on day 21. In bacteriological study, huge bacterial colonies were found on day 3 in the control group whereas this was markedly reduced in number in the wound of Group-A. Thus, the present study supports the scientific rationale for the use of Neem leaf in the management of wounds.

Key Words: Surgical wound, Neem leaf, Histopathology, Bacteriology, Amelioration

1. Introduction

Wound healing is a complex and dynamic process of several overlapping events following injury, including coagulation, leukocyte infiltration, matrix deposition, epithelialization, and resolution of inflammation with the formation of a mature scar (Sorg et al. 2017). It is dependent on several cell types and mediators that interact in a highly sophisticated temporal sequence. Human and animal being susceptible to bacterial, fungal, and viral infection, skin infection, and tropical wounds require special attention to minimize secondary complications (Mummed et al. 2018). Wounds are considered as one of the major problems in developing countries resulting in severe complications in many cases that lead to high cost for therapy (Alam et al., 2007; Miah et al., 2017; Rassel et al., 2020). Despite advances in controlling

the infection of surgical wounds, bacterial wound contamination is still remaining the most common postoperative complication (Rivandi et al. 2012). The presence of infection in wounds, in addition to interference with the healing process can be resulted in increasing the duration of wound repair, therapeutic period, costs, and even morbidity and mortality rate. Various bacterial species isolated from wounds which can seriously delay the wound healing process by disrupting the normal clotting mechanisms and promoting disordered leukocyte function and poor-quality granulation tissue formation, reduce tensile strength of connective tissue, and impair epithelization (Annan and Houghton 2008; Juyena et al., 2013; Tan et al., 2020).

Nowadays, excessive and inappropriate use of antimicrobial drugs has developed the resistant bacteria and difficulty in the management of infected wounds, so consideration to new antibacterial agents and the least adverse effects seems necessary. Medicinal plants are effective in the treatment of infectious diseases and infections of various types of external wounds and have been used for these purposes in humans and different species of animals (Alam et al. 2005; Tamanna et al. 2020). Many plants are known for related pharmaceutical activities. Among them Azadirachta indica, a member of the Meliaceae family, commonly known as Neem has long been recognized as an excellent therapeutic tool. The Neem leaves contain a mixture of compounds like ascorbic acid, various amino acids, nimbanene, 6-desacetylnimbinene, n-hexacosanol, nimbiol, nimbandiol, nimbolide and several other types of ingredients (Subapriya et al. 2006). Besides this Neem leaves also contain carbohydrates, protein, minerals, calcium, phosphorus, carotene, etc. Neem leaves also contain glutamic acid, tyrosine, aspartic acid, alanine, proline, glutamine, and cystine like amino acids, and several fatty acids (Hossain et al. 2013). Neem leaves have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycemic, anti-ulcer, anti-malarial, antifungal, antibacterial, antiviral, anti-oxidant, anti-mutagenic, and anti-carcinogenic properties (Alzohairy 2016).

Bangladesh is rich in medicinal plants and Neem is a very well-known and one of the most versatile medicinal plants in the country having a wide spectrum of biological activity as mentioned earlier. Regarding the above mentioned and easy accessibility, we investigated the effect of an aqueous paste of Neem Leaves on postsurgical wound healing in the goat model.

2. Material and Method

2.1. Plant materials and preparation of extract

The branches of Neem plant (*Azadirachta indica*) were collected from the vicinity of Bangladesh Agricultural University (BAU) campus with the verbal permission of the authority. The leaves were separated from the branches, properly cleaned with distilled water, kept in a blender, and blended with some sterile distilled water to make a paste. The paste was kept in a sterile plastic container and stored at 4°C until further use. It was allowed to warm up to room temperature before applying to the wounds.

2.2. Experimental animals

Four apparently healthy goats were purchased from the local market with body weight ranged from 8-10 kg were purchased and used for this experiment. They were kept in the animal shed of the Veterinary Teaching Hospital of BAU, Mymensingh. The animals were kept under standard clinical conditions and veterinary supervision with no restrictions on food and water. Before the study the goats were kept in quarantine for two weeks and vaccinated against PPR (P.P.R Vaccine®, LRI, Dhaka, Bangladesh) and dewormed with Albendazole @15mg/kg body weight. All the animal experiments were carried out in accordance with the

guidelines and approval of the Animal Ethics and Experimentation Committee (AEEC, Permission number: AEEC/DSO-BAU/02/2017) of the Department of Surgery and Obstetrics, BAU, Mymensingh.

2.3. Wounding of animals

To ensure the animals' health, a clinical examination was performed. All surgical interventions were conducted under sterile conditions. After proper restraint and infiltration analgesia with 2% Lidocaine HCl (Jasocaine®, Jayson Pharmaceuticals Ltd., Dhaka, Bangladesh), 20cm long full-thickness cutaneous wounds were made on either side of the vertebral column. Four wounds were prepared in each goat, two on either side of the vertebral column. Goats were divided into two groups. Group-A: Fresh Neem leaf paste was applied locally once daily to eight wounds made in two animals. The animals were maintained carefully to avoid contamination and interference with the healing of wounds. Group-B: Animals of this group were used as control where the wounds were received topically normal saline only (Table 1).

All the wounds were closed using nylon with a simple interrupted pattern. Antibiotic, antihistaminic, and anti-inflammatory drugs were avoided to mitigate their effects on the healing process. Follow-up information was obtained from the day of surgical operation (day 1) up to the end of the experiment (day 21).

2.4. Evaluation of wound healing activity

Morphological characters such as swelling of the wound area, the elevation of the suture line from the skin surface, length of wound area were recorded to determine the healing of the wounds. After surgery the elevation of the suture line was recorded up to 7th days of surgery. Slide Calipers was used to measure the swelling area (mm), the elevation of suture line (mm), and length of suture line (mm) of the wounds on the day-1 (D1), day- 3 (D3), day- 7 (D7), day- 14 (D14), and day- 21 (D21) post wounding. Wounds were closely monitored daily to observe any complications such as swelling, wound dehiscence, suture abscess, local infection, and exudation. The progress of healing in animals of each group was recorded daily. Healing score was categorized as (a) excellent- no inflammation, no exudation, no infection, no dehiscence, gradual decrease of the width of a wound area, (b) good- minimum inflammation with minimum exudation, no dehiscence, gradual decrease of the width of a wound area, and (c) poor- marked inflammation, presence of infection, and exudation.

2.5. Bacteriological study

2.5.1. Sample collection

For the bacteriological study, wound swabs were collected aseptically by using sterile cotton buds from all groups on day 1 and day 3. Cotton buds were moistened first with normal saline solution and applied to the closed wounds by circling to collect the swab samples. Then the cotton buds were quickly transferred into screwed capped test tubes containing nutrient broth.

2.5.2. Culture and staining of bacteriological samples

Culture and staining of collected bacteriological samples were done as the procedure described by Jaman et al. (2018).

2.5.3. Histopathological study

The biopsies were collected from the wound areas of each experimental animal on 3rd, 7th, and 21st days after the creation of wounds using standard surgical procedures. The biopsy tissues contained dermis and epidermis were fixed in 10% neutral buffered formalin for 48 hours for histopathological study.

Table 1. The experimental protocol to study the efficacy of Neem leaf paste in the treatment of artificially produced surgical wounds

Groups	Material used	Form of materials	No. Of animals	No. Of wounds
Group A	Neem	Aqueous paste	2	4 in each animal
Group B	Control (NS)	0.85% NaCl in distilled water	2	4 in each animal

Table 2. The effects of aqueous paste of Neem leaf (Group A) and Normal saline (Group B) on various features of wound healing in goats.

Groups	Area of swelling of wounds (mm)	Elevation of suture line (mm)	Length of wounds (mm)	Healing time (days)
Group A	3.91± 0.10^{a}	2.64± 0.19^{a}	15.78± 0.19^{a}	12.33± 0.42^{a}
Group B	5.36± 0.12^{b}	3.59± 0.12^{b}	17.11± 0.08^{b}	18.67± 0.33^{b}

Values with the different superscript letter in the same column indicate significance ($p<0.05$) Mean± SEM

Slides were prepared and stained in the histopathology lab of the Department of Surgery and Obstetrics, BAU according to the method described by Ashraf et al. (2019). Finally, photomicrography of stained slide was performed.

2.6. Statistical analysis

All the data were expressed as Mean ± SEM (Standard Error of Mean). To compare data among groups, one-way ANOVA (Analysis of variance) factor one analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22.0. Probability $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Morphological change

The wound healing activities of Neem paste are shown in Table 2. The swelling of the wound edges was observed in both the groups of animals. Treatment with aqueous Neem paste (Group-A) and Normal Saline (Group-B, control) resulted in the swelling of wounds at 3.91±0.10 mm and 5.36± 0.12 mm respectively and this difference was significant ($P<0.05$). The elevation of a suture line also differed significantly ($P<0.05$) among the groups. The elevation of the suture line was higher in saline-treated wounds (3.59±0.12 mm) than those treated with Neem paste (2.64±0.19 mm). The higher swelling and elevation of the suture line indicate presence of more inflammation in the wounds of the control group. Treatment with aqueous Neem paste resulted in a prompt reduction of the length of wounds (15.78±0.19 mm) on the other hand it was much higher in the control group (17.11±0.08 mm). The mean healing period was significantly ($P<0.05$) lower in Neem treated wounds (12.33±0.42 days) than those treated with normal saline (18.67±0.33 days). The wound morphology in response to treatment on day 1, day 7, and day 14 are depicted in Figure 1, Figure 2, and Figure 3 respectively.

3.2. Histopathological examination

Day 3: The inflammatory lesions in the regenerating tissues were evaluated based on the infiltration of reactive cells including macrophages, lymphocytes, and neutrophils. Reactive cells decreased gradually in wounds treated with an aqueous paste of Neem.

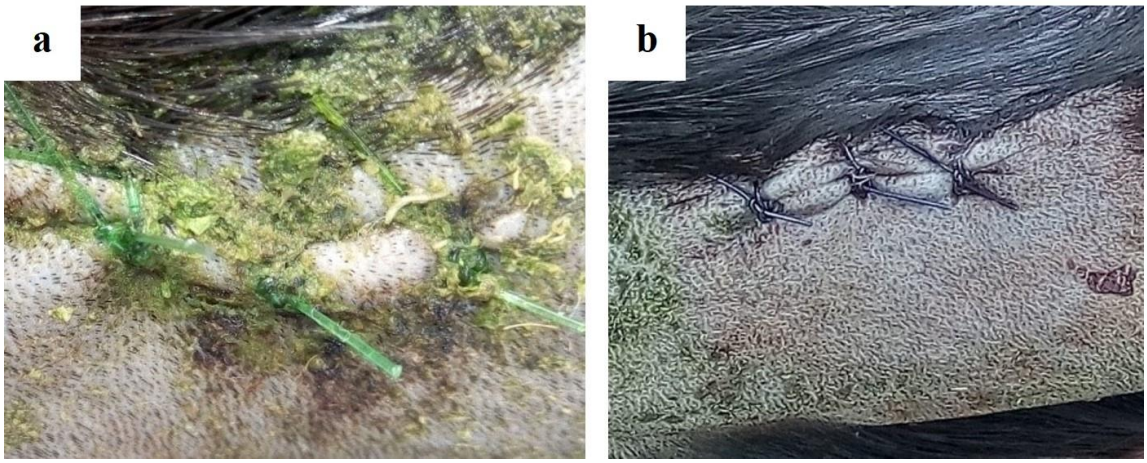


Figure 1. Gross observation of wounds on day 1 treated with (a) Neem, (b) Normal saline (control)

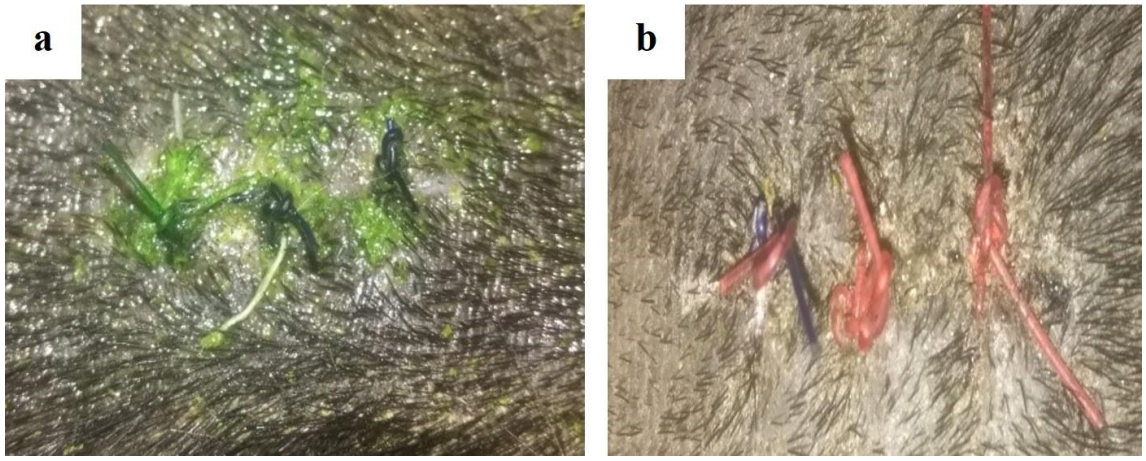


Figure 2. Gross morphology of wounds on day 7 treated with (a) Neem, (b) Normal saline (control)

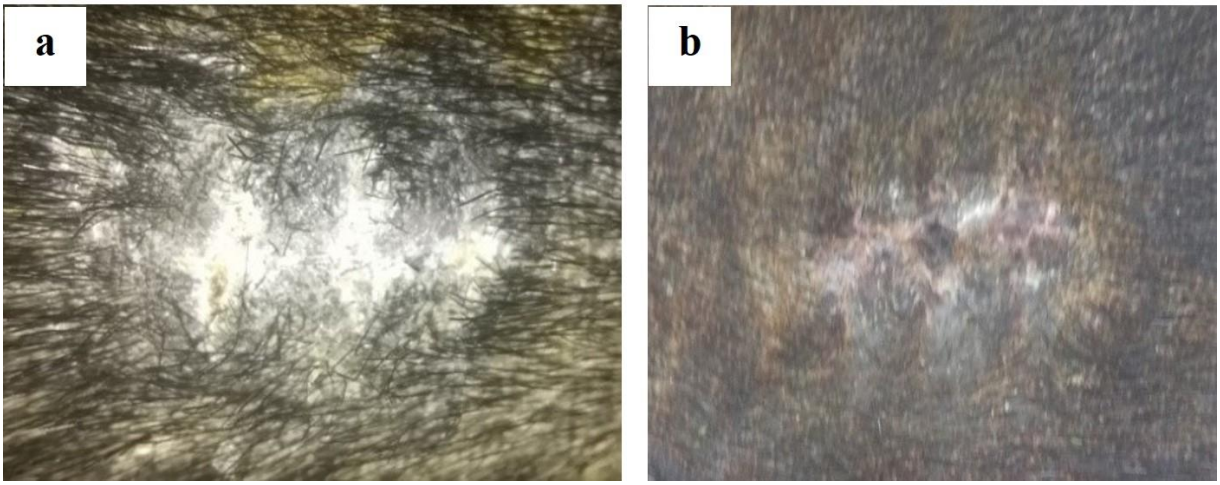


Figure 3. Wound healing on day 21 after treatment with (a) Neem, (b) Normal saline (control)

Moreover, the proliferation of fibrous connective tissue was notably observed in wounds of group A on day 3 (D3) (Figure 4a) while huge inflammation and fibroplasia were encountered along with widespread hemorrhages and congestion in the group B (Figure 4b).

Day 7: Fibroblastic cells were more pronounced than other inflammatory cells and much of the debris and hemorrhages were disappeared and formation of thin keratin layer in group-A (Figure 5a). However, reactive cells are seen in wounds treated with normal saline till day 7 with less fibroblastic proliferation in group-B (Figure 5b).

Day 21: There was the least degree of inflammation in both groups on day 21. Marked thickening of the keratinized layer of the epidermis in wound received aqueous Neem paste (Figure 6a) while a thin keratinized layer of the epidermis in the control group (Figure 6b).

3.3. Bacteriological study

Wound swab samples were spread on the Plate Count Agar (PCA) plates after dilution and incubated overnight at 37°C temperature. The next day cultural characteristics and growth of bacteria were observed. Swab samples collected on day 3 post wounding have demonstrated that wound treated with Neem paste has a remarkably lower colony than those treated with normal saline only (Figure 7a, 7b). Gram's staining confirmed the presence of Staphylococcus spp. characterized by Gram-positive, spherical-shaped, and clustered form bacteria (Figure 7c). Wound healing involves growth factors, cytokines, extracellular matrix (ECM), and relevant enzymes along with the differentiated cells that modify molecular components of the matrix. In developing countries like Bangladesh, the wound is one of the most concerning fact in humans and livestock especially in food and zoo animals (Hoda et al., 2018; Talukder et al. 2018; Sarker et al. 2020). Our study evaluated the efficacy of the aqueous paste of Neem leaves on the healing of surgical wounds in the goat model. Results from the study showed that the mean value of the swelling area, elevation of the suture line, and length of wound was significantly lower in Neem treated wounds than those treated with normal saline (Table 2). This might be because of the presence of active compounds like alkaloids, flavonoids, phenolic compounds, steroids, carotenoids, ketones, and azadirachtin in neem leaves and also the inflammatory response as a foreign body reaction due to traumatic tissue handling and suture placement (Biswas et al. 2002). Furthermore, Neem oil contains fatty acids and maintains the skin's elasticity by building up collagens and provides a moist and soft texture to the skin (Raina et al. 2008). Similarly, average healing time for group A was 12 days while for the control group it was 18 days (Table 2) which is also similar to our previous study with other herbal plants having wound healing effects (Alam et al. 2005; Tamanna et al. 2020). In terms of histopathology, the degree of inflammation and proliferation of blood vessels in the regenerating tissue was higher in the control group at day 3 (Figure 4b) of post wounding compared to Neem treated wounds (Figure 4a). The first inflammatory cells to appear during healing are the neutrophils, which presumably control the microbial growth and sepsis (Sorg et al. 2017). Neutrophilic infiltration in healed tissue was not observed in the present study. This may be because the tissues were collected on day 3 post-operation when the neutrophilic infiltration was replaced by macrophages and lymphocytes. The highest number of monocyte and lymphocyte on day 3 (Figure 4b) of the experiment were seen in the control group compared to group A (Figure 4a). The lymphocyte and macrophage were found to disappear from the Neem treated group on day 7 post wounding (Figure 5a).

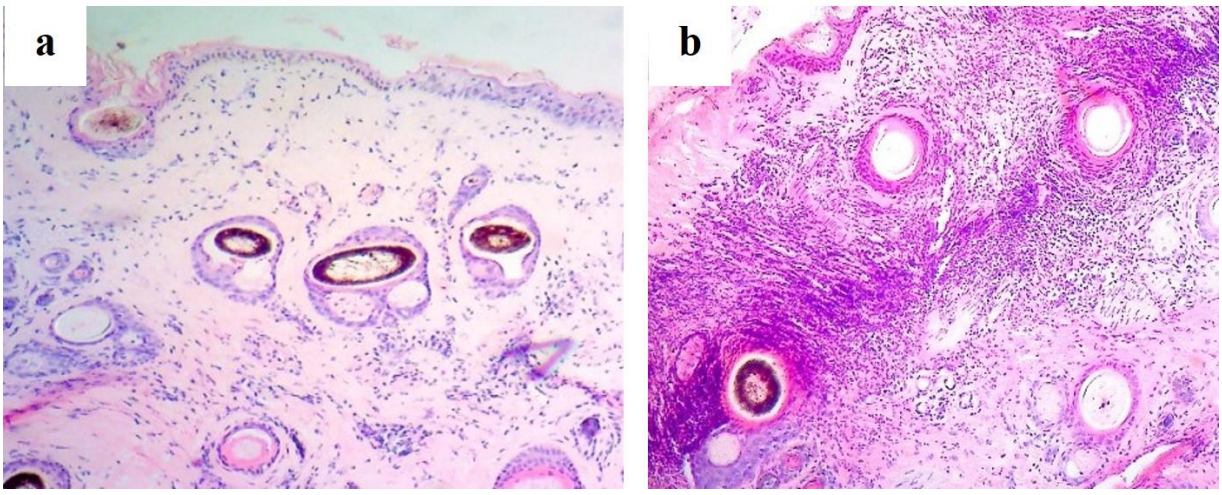


Figure 4. Status of inflammation in the wounds of two groups. (a) Less infiltration of inflammatory cells with marked fibroblastic proliferation in group A, (b) huge inflammation and fibroplasia less intensively found in group B on day 3

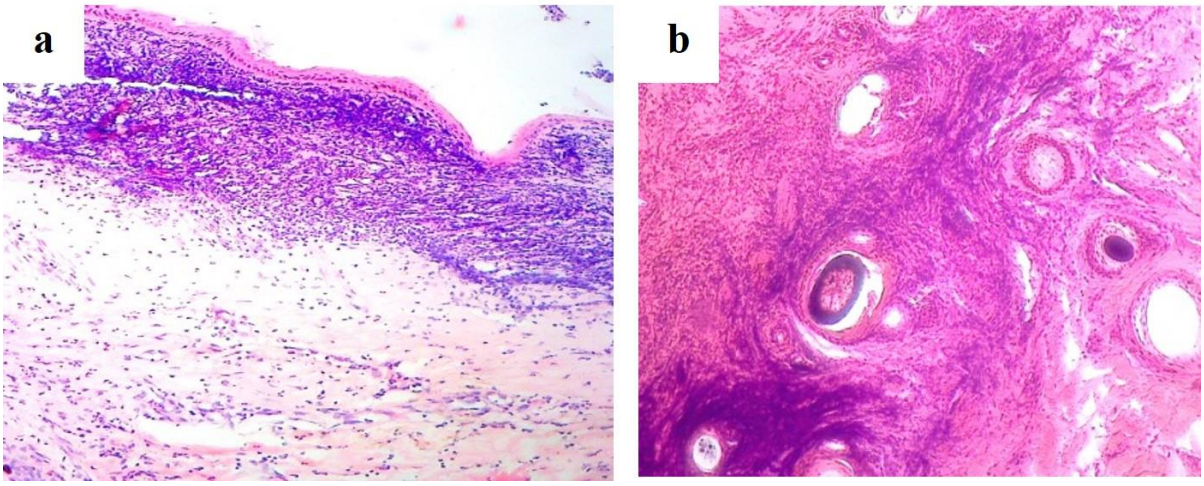


Figure 5. Histological features on day 7. (a) Fibroblastic cells were remarkable than other inflammatory cells in group-A, (b) fibroblastic proliferation with minimal infiltration inflammatory cells in group-B

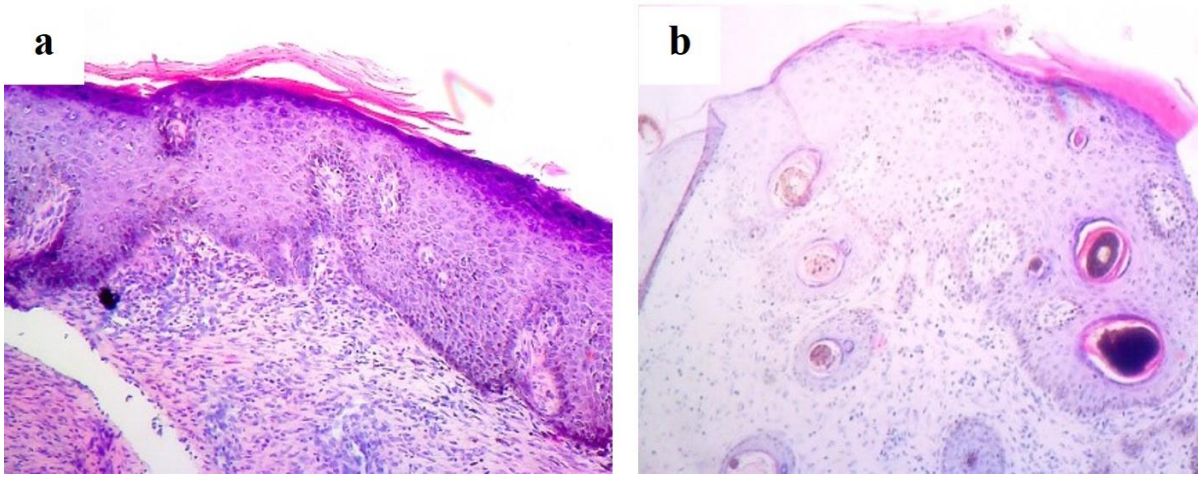


Figure 6. Keratinization of wounds on day 21. (a) Marked thickening of the keratinized layer of the epidermis in group- A (b) thin keratinized layer of the epidermis was observed in group-B

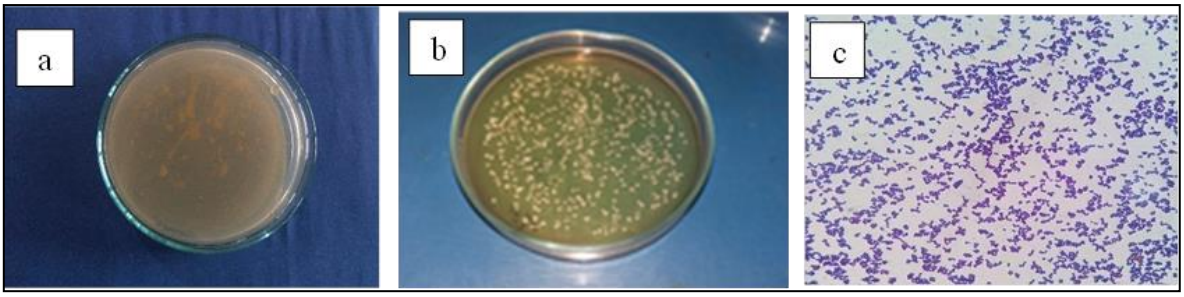


Figure 7. Presence of bacterial colony in primary culture of Mannitol salt agar observed in samples collected from wound treated with (a) Neem leaf paste and (b) normal saline (c) Staining characteristics of isolated bacteria. The bacteria were arranged in grape like structure with spherical shaped indicating Staphylococcus spp.

There was a marked thickening of the keratinized layer of the epidermis in aqueous Neem extract-treated group while a thin keratinized layer in the control group on day 21 post-operation (Figure 6a and Figure 6b). This might be because of the anti-inflammatory properties of Neem which act as effective as cortisone acetate and also helps to accelerate wound healing (Raina et al. 2008). The mean healing period was significantly lower in Neem treated wounds than those of control, which has a clear message that Neem may accelerate the healing of wounds.

Bacteriological studies revealed a remarkably lower number of bacterial colonies in Group-A than that of Group-B. Gram's staining confirmed the presence of Staphylococcus spp. (Figure 7c). The near absence of bacterial colony might be the effects of the antibacterial activity of Neem which has already reported by Patel et al. (2009) against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.

4. Conclusion

We have shown that the aqueous paste of A. indica accelerates wound healing in goats. Further investigation is necessary with these findings at subcellular levels including gene studies which could lead to other beneficial effects.

Ackoledgements

We thank Director, Veterinary Teaching Hospital for providing animal keeping facilities.

Conflict of Interest

The authors do not have any conflict of interest.

References

1. Alam, M.M., Islam, S.A., Hashim, M.A. and Saha, D., 2007. A comparison of the effect of medicinal plant extract and antibiotic on wound healing with changes of blood values in Black Bengal goat. Journal of Bangladesh Society for Agricultural Science and Technology, 4: 141-144.
2. Alam, M.M., Islam, S.A., Mohammed, Y., Juyena, N.S., and Hashim, M.A., 2005. Comparative efficacy of two medicinal plant extracts and an antibiotic on wound healing. Pakistan Journal of Biological Sciences, 8: 740-743. DOI: http://dx.doi.org/10.3923/pjbs.2005.740.743.
3. Alzohairy, M.A., 2016. Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complementary and Alternative Medicine, (11):1-11. DOI: https://doi.org/10.1155/2016/7382506
4. Annan, K. and Houghton, P.J., 2008. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq. and Gossypium arboreum L., wound-healing plants of Ghana. Journal of Ethnopharmacology, 119: 141-144. DOI: https://doi.org/10.1016/j.jep.2008.06.017
5. Ashraf, M.B., Akter, M.A., Saha, M., Mishra, P., Hoda, N., and Alam, M.M., 2019. Clinicopathological evaluation on capture myopathy due to chemical immobilization in spotted deer. Turkish Journal of Veterinary Research, 3: 81-84.
6. Biswas, K., Chattopadhyay I, Banerjee RK, and Bandyopadhyay U. 2002. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, 82, 1336-1345.
7. Hoda N, Talukder, Z, Mishra, P., Jaman, K., Alam, M.M., 2018. Occurrence of surgical affections in zoo herbivores: a retrospective study. Research in Agriculture, Livestock and Fisheries, 5(2): 209-214. DOI: https://doi.org/10.3329/ralf.v5i2.38110
8. Hossain, M.A., Al-Toubi, W.A.S., Weli, A.M., Al-Riyami, Q.A. and Al-Sabahi, J.N. 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science, 7: 181-188. DOI: https://doi.org/10.1016/j.jtusci.2013.05.003
9. Jaman, M.M., Mishra, P., Rahman, M. and Alam, M.M., 2018. Clinical and laboratory investigation on the recurrence of the umbilical hernia after herniorrhaphy in bovine calves. Journal of the Bangladesh Agricultural University, 16: 464-470. DOI. https://doi.org/10.3329/jbau.v16i3.39418
10. Juyena, N.S., Tapon, M.A.H., Ferdousy, R.N., Paul, S. and Alam, M.M., 2013. A retrospective study on the occurrence of myiasis in ruminant. Progressive Agriculture 24(1 & 2): 101-106. DOI: https://doi.org/10.3329/pa.v24i1-2.19110
11. Miah, M.A.H., Hasan, M., Sarker, Y.A., Alam, M.M., Juyena, N.S., 2017. Clinical evaluation of ethanolic extract of curcumin (Curcuma longa) on wound healing in Black Bengal goats. Journal of Advanced Veterinary and Animal Research 4 (2): 181-186. DOI. http://doi.org/10.5455/javar.2017.d209
12. Mummed, B., Abraha, A., Feyera, T., Nigusse, A., and Assefa, S., 2018. In vitro antibacterial activity of selected medicinal plants in the traditional treatment of skin and wound infections in eastern Ethiopia. BioMed Research International, https://doi.org/10.1155/2018/1862401
13. Patel, J.D., Shrivastava, A.K. and Kumar, V., 2009. Evaluation of some medicinal plants used in traditional wound healing preparations for antibacterial property against some pathogenic bacteria. Journal of Clinical Immunology and Immunopathology Research, 1: 007-012.
14. Raina, R., Prawez, S., Verma, P.K. and Pankaj, N.K., 2008. Medicinal plants and their role in wound healing. Vet Scan 3: 1-7.
15. Rassel, M.G.R., Mishra, P., Rahman, M. and Alam, M.M. 2020. Exploring bacterial pathogens and risk factors associated with the occurrence of navel ill in calves. Journal of Istanbul Veterinary Sciences, 4(2): 37-42. DOI: http://dx.doi.org/10.30704/http-www-jivs-net.722788
16. Rivandi, M., Emami, M.R., Rad, M., Kazemi Mehrjerdi, H., Azizzadeh, M. and Ghasemi S. 2012. Bacteriological Evaluation of Aloe vera L. Fresh Gel on Experimental Infected Full-Thickness Open Wounds Induced with Staphylococcus aureus in Dogs. Iranian Journal of Veterinary Surgery 7: 75-84.
17. Sarker, D., Akter, M.A., Rahman, M.S., Yesmin, N. and Alam, M.M., 2020. Clinicopathological consequences of urinary retention due to urolithiasis in indigenous goats. PSM Veterinary Research 5(2): 28-37.
18. Sorg, H., Tilkorn, D.J., Hager, S., Hauser, J. and Mirastschijski, U., 2017. Skin wound healing: an update on the current knowledge and concepts. European Surgical Research 58: 81-94. DOI: https://doi.org/10.1159/000454919
19. Subapriya, R., Kumaraguruparan, R. and Nagini, S., 2006. Expression of PCNA, cytokeratin, Bcl-2 and p53 during chemoprevention of hamster buccal

pouch carcinogenesis by ethanolic neem (Azadirachta indica) leaf extract. Clinical biochemistry 39: 1080-1087. DOI: https://doi.org/10.1016/j.clinbiochem.2006.06.013

20. Talukder, Z., Hoda, N., Jha, P.K., Mishra. P., Momy, M.K. and Alam, M.M., 2018. A retrospective study on the occurrence of surgical affection in zoo carnivores of the national zoological garden at Dhaka, Bangladesh. Research in Agriculture, Livestock and Fisheries, 5(3): 335-339. DOI. https://doi.org/10.3329/ralf.v5i3.39582
21. Tamanna, S.J., Shihab, M.M., Akter, M.A., Rahman, M. and Alam, M.M., 2020. Therapeutic potentialities of green tea (Camellia sinensis) and aloe vera (Aloe barbadensis) on Staphylococcus aureus induced septic wound in goats. Journal of Bangladesh Agricultural University 18: 105-110. DOI: https://doi.org/10.5455/JBAU.94746
22. Tan, M.A., Akter, M.A., Rahman, M.S., Rahman, M. and Alam, M.M. 2020. Standardization of surgical site preparation with various formulations of Povidone Iodine, Chlorhexidine, and Chlorxylenol in goat model. Journal of Innovative Sciences. 6(1): 34-40. DOI: http://dx.doi.org/10.17582/journal.jis/2020/6.1.34.40

Research article
Curr. Pers. MAPs, (2020); 3(2): 97-103

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Comparative Cytotoxic Effects of the Hydrosols of some Ethnobotanic Plants

Ela Nur ŞİMŞEK SEZER[1*], Meryem BOZKURT[1], Eray TULUKÇU[2], Tuna UYSAL[1]

[1]Selçuk University, Science Faculty, Biology Department, Konya, TURKEY
[2]Selçuk University, Çumra Vocational High School, Medicinal Plant Program, Konya, TURKEY
*Corresponding author : simsekelaa@gmail.com

https://doi.org/10.38093/cupmap.765089

Received : 06/07/2020
Accepted : 08/12/2020

Abstract

Hydrosols are aqueous solutions and by-products of the essential oil distillation procedure. They have many medicinal benefits because contain the water-soluble components, vitamins and minerals of the plant that has been distilled. On the other hand, colorectal cancer (CRC) is the third most common cancer in both men and women worldwide and is the main cause of death in gastrointestinal cancer. Hence in this study, the cytotoxic effects of hydrosols obtained from five different ethnobotanical plants (*Melissa officinalis* L., *Achillea teretifolia* Willd, *Achillea aleppica* subsp. *zederbaueri* (Hayek) Hub.-Mor., *Origanum onites* and *Salvia fruticosa* Mill have been investigated on the colorectal cancer cell line. For the determination of the hydrosols cytotoxic effect MTT assay was used. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is used to determine the cytotoxic or cytostatic effects of plant extracts, potential medicinal agents and toxic materials. Based on the results of the cytotoxicity assay, the most effective extract was obtained from *Origanum onites* and the next effective extract from *Melissa officinalis*. On the other hand, both *Achillea* extracts showed low cytotoxic activity. Consequently, this study provides some criticism on the potentials of some plants traditionally used as an anticancer agent, and also shows that there is more need to investigate the cause of cell death and active substances.

Key Words: Cytotoxicity, Hydrosols, Lemon balm, Turkish oregano, CRC.

1. Introduction

Ethnobotanical researches contribute significantly to the scientific evaluation of plants with their content, which has been acquired through trial and error and reflects valuable information that has been passed down from generation to generation as a result of a long period of time (Kendir & Güvenç 2010). Our country, which has a rich cultural heritage, also has an extensive ethnobotanical information treasure. The use of plants as complementary medicine for cancer, especially in difficult cancer types, has recently increased.

Cancer is an important public health problem and causes serious pain and economic losses worldwide. Colorectal cancer is the third most common cancer in the world. Therefore, more research is needed to develop safe products for the prevention and treatment of all human cancers. Approximately 60% of the drugs currently used for cancer treatment have been isolated from natural products (Gordaliza, 2009), and the plant kingdom

has been the primary source. Wild sources of medicinal plants have been used by humans for centuries in traditional healing systems. Indigenous people have adopted different forms of application and use to take advantage of this natural resource (Adnan et al., 2014).

Melissa officinalis L. (Lamiaceae) is one of the most used medicinal plants in Europe and the Mediterranean region, as a herbal tea for their aromatic, digestive and antispasmodic properties in nervous disturbance of sleep and functional gastrointestinal disorders (Bisset, 1994). *Achillea* L. (civanperçemi; Turkish name) is a medicinal plant genus which has been used since ancient times. It possesses diversity all around the world. The majority of the *Achillea* species are important native economic plants of Anatolia. In Turkey, herbal teas prepared from some *Achillea* species are used in folk medicine for abdominal pain, diarrhea, and flatulence as well as a diuretic and emmenagog. Some investigations about anti-oxidant and cytotoxic effects of Achillea species have been performed. The extracts or essential oils of *Achillea wilhelmsii* C. Koch, *A. micrantha* Willd., *A. millefolium* L., *A. pannonica* Scheele and *A. fragrantissima* (Forssk.) Sch. Bip. has been reported to be natural antioxidant sources (Souri et al., 2004; Nickavar et al., 2006; Wojdylo et al., 2007; Bozin et al., 2008; Tarawneh et al., 2010). Also, *Achillea millefolium* L., *A. clavennae* L., *A. talagonica* Bioss., *A. wilhelmsii* C. Koch and *A. fragrantissima* are important species with cytotoxic effects (Sathiyamoorthy et al., 1999; Trifunovic et al., 2006; Saeidnia et al., 2009; Ali et al., 2011; Li et al. 2011; Bali et al. 2015; Amini Navaie et al., 2015; Köngül et al., 2017). *Origanum onites* L., known as Turkish oregano, is a traditional and medicinal herb. Infusions obtained from aerial parts are effective in the treatment of several gastrointestinal diseases, influenza, bronchitis, hypertension, diabetes, high cholesterol, stomach disorders and leukaemia (Sivas and Tomsuk 2011; Sargın et al., 2013; Gürdal & Kültür 2013). *Salvia fruticosa*, insufficiently studied for various activities, is a Mediterranean medicinal and aromatic herb. It is known for its antioxidant, antimicrobial and antiproliferative activities (Alimpic et al., 2015; Altay & Bozoğlu 2017). Essential oils are usually extracted from plants by steam distillation, where an aqueous phase called hydrosol is obtained. Unlike essential oils, hydrosol studies are limited, despite the interest of the food, cosmetic and phytotherapeutic industries to find a natural protective alternative (Tornuk et al., 2011; Hamedi et al., 2017). Hydrosol is highly valuable as it contains all the water-soluble substances of the plant.

The present study was designed to investigate cytotoxic effects of the hydrosols of five ethno-botanic plants (*Melissa officinalis* L., *Achillea teretifolia* Willd, *Achillea aleppica* subsp. *zederbaueri* (Hayek) Hub.-Mor., *Origanum onites* and *Salvia fruticosa* Mill.) on the colorectal cell line.

2. Material and Method

2.1. Plant material

In our study, investigated plant samples were provided from Eray TULUKÇU. Plant samples were grown at Selcuk University, Çumra Vocational School, Medical and Aromatic Plants Department. Hydrosol preparations were performed the protocol of Linskens (1997). The hydrosols were kept in refrigerated at 4°C until they are used. The extracts coded as *Melissa officinalis* (MOE) *Achillea teretifolia* (ATE), *Achillea aleppica* subsp. *zederbaueri* (AAE), *Origanum onites* (ORE) and *Salvia fruticosa* (SFE), respectively.

2.2. Cell lines

Human colon adenocarcinoma cells, DLD1 was obtained from ATCC and they were cultured in 10% (v/v) heat-inactivated fetal

bovine serum (FBS), 1 % (v/v) penicillin-streptomycin supplemented RPMI 1640 medium. Cells were incubated at 37° C under 5% CO_2 conditions. After reaching 70% confluency, the cells were trypsinized, counted and transferred in 96-well plates.

2.3. Cytotoxicity assays

The cytotoxic activity of the hydrosols was tested on the DLD1 cell line. Hydrosols were prepared in six different dilutions (v/v) and were applied to the cells. Different amounts of the hydrosol were prepared for cell treatment by diluting the stock with medium only. Then the plates were incubated for 24 - 48 hours. The cell proliferation assay was carried out via MTT (5mg/ml). The optical density of the plates was measured using the Elisa microplate reader at 540 nm. Each experiment was performed three times and the mean values were taken into consideration.

2.4. Statistical Analysis

For statistical analysis of the data, multiple comparisons were made using one-way variance analysis (ANOVA) followed by Dunnett's test for post hoc analysis. The differences in $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

3. Results and Discussion

As a result of the link between man and plant that has been going on for centuries, the ethnobotanical discipline, which is considered important all over the world, was born. Ethnobotanical research contributes to the scientific evaluation of plants by providing valuable information obtained through experimentation and passed down from generation to generation. The use of medicinal plants for thousands of years has played a major role in the emergence of ethnobotany for the treatment of various diseases such as cancer. By the World Health Organization (WHO) data (WHO, 2001), 80% of the world population prefer herbal medicines in the treatment of various diseases (Chikezie & Ojiako, 2015; Msomi and Simelane, 2018; Nguyen et al., 2020; Khan et al., 2020). With this situation, ethnobotanical knowledge transferred from the early ages comes to the fore and the use of medicinal plants becomes important (Bozyel & Merdamert, 2018). Turkey, which has hosted many civilizations, constitutes a rich cultural heritage and rich research environment in terms of ethnobotanical studies. Medicinal plants are vital sources of readily accessible remedy used in the countryside healthcare system. Since cancer is a leading cause of death, various researches are carried out in this field every day worldwide. These studies usually involve investigating the effects of biologically active substances on cancer cells and are often derived from plants (Mukherjee et al. 2001). There is a great need to examine the sources of safe and inexhaustible natural substances. It is also important to understand the mechanisms of anticancer agents for future application in cancer treatment (Half et al. 2009). Increasing evidence suggests that extracts and/or active ingredients from plants are effective against cancer by preventing carcinogenesis and tumour progression or by killing cancer cells (Demir et al., 2020).

Although the cytotoxic effects of the plants preferred in our study have been previously examined in the several cell lines such as colon, breast, prostate and liver cell lines. As far as we know our study is a first in terms of the cell line used in our study. This is the first study to reveal the cytotoxic effect of hydrosols from various ethnobotanical plants on colorectal cancer cell line DLD1. According to the cytotoxicity test for cells treated by *Melissa officinalis, Achillea teretifolia, Achillea aleppica* subsp *zederbaueri, Origanum onites* and *Salvia fruticosa* aqueous extracts, our results show that the cytotoxicity for the DLD1 cell line

between the applied dose ranges. From the results of MTT, the most effective extract is *Origanum onites*. On the other hand, two Achillea extracts exhibited less cytotoxic effects than others in the doses and time intervals administered. The concentration of 50% cellular cytotoxicity of extracts on cancer cells (IC_{50}) was based on 48-hour absorbance values (Chen et al., 2009). The IC_{50} dose is approximately 25% for *Melissa officinalis* (MOE), 25% for *Origanum onites* (ORE) and 50% for *Salvia fruticosa* (SFE). The cytotoxicity graphs of 24-48h treatments were given Figure 1 and Figure 2. Different solvents and extraction methods can be tried to determine the cytotoxic effects of *Achillea* extracts.

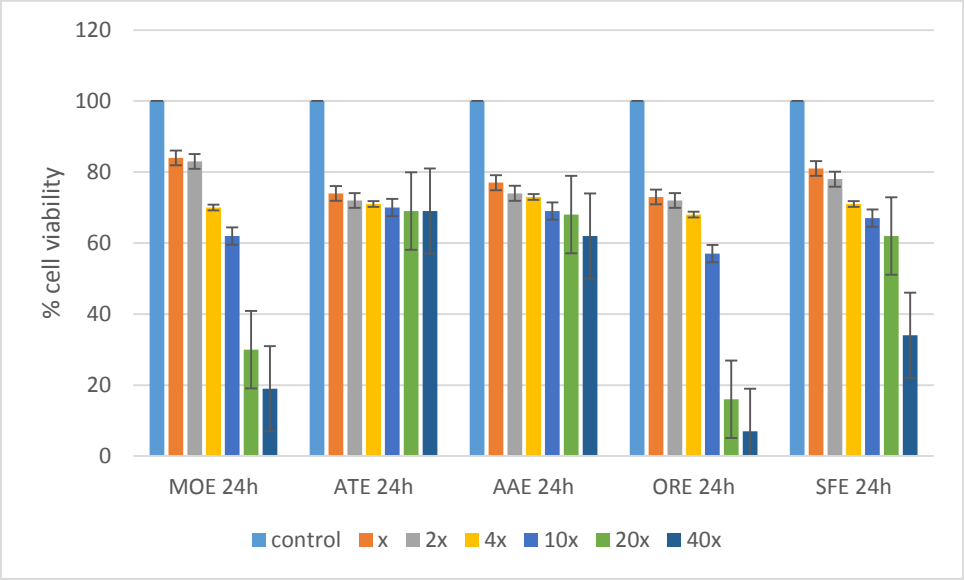


Fig.1. Graph of MTT assay after 24h treatment (40x: direct, 20x: ½ (v/v), 10x: ¼ (v/v), 4x: 1/10 (v/v) 2x:1/20(v/v) x: 1/40 (v/v))

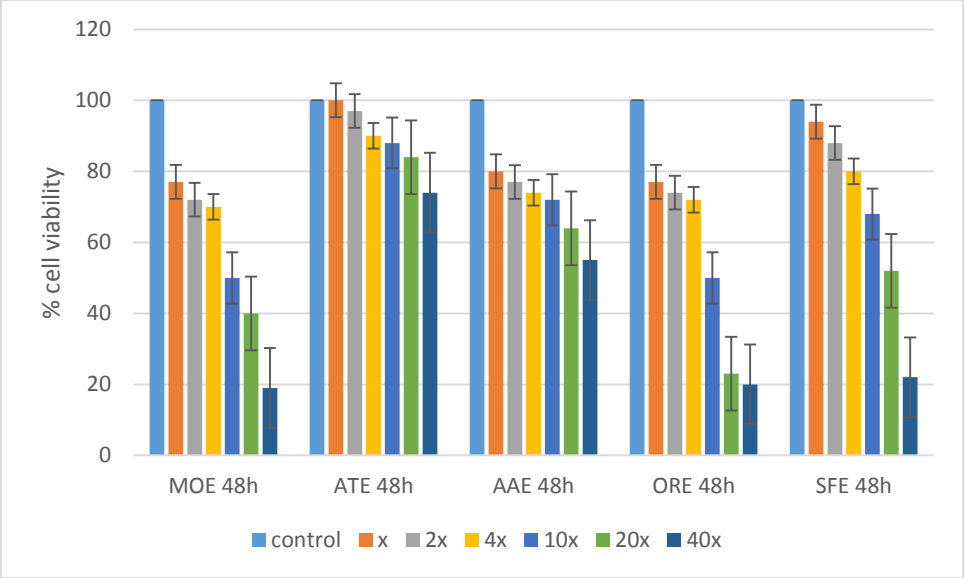


Fig.2. Graph of MTT assay after 48h treatment (40x: 1, 20x: ½ (v/v), 10x: ¼ (v/v), 4x: 1/10 (v/v) 2x:1/20(v/v) x: 1/40 (v/v))

Colorectal cancer is the second most frequently diagnosed in women, the third in men and fourth cancer worldwide (Jemal et al., 2011). Colorectal cancer, which is reported to cause death in approximately 60% of cases, is common in developed countries (Ferlay et al., 2015; Merika et al., 2015). Herbal extracts or preparations inhibit colorectal cancer cells by stimulating apoptosis or autophagy, inducing the cell cycle and triggering signal pathways. There are many studies on the cytotoxic and apoptotic effects of plants used in our study on various colon cancer cell lines, but as far as we know, no previous studies have been performed on the DLD1 cell line. The ethanolic and aqueous extracts from *Melissa officinalis* have been reported to have cytotoxic effects on human colon cancer cell line HCT-116 (Encalada et al., 2011). The hydroalcoholic extract from *Melissa officinalis* has been reported to inhibit apoptosis and cell proliferation in HT29 and T84 colon cancer cells (Weidner et al., 2015). In another study, it was reported that essential oil of *Origanum* and main component planned carvacrol has cytotoxic and apoptotic activity on Hep-G2 cells and can be evaluated as potential anticancer agents (Sivas and Tomsuk 2011). Xavier et al. (2009) have been reported that *Salvia fruticosa* and *Salvia officinalis* water extracts and their main phenolic compound rosmarinic acid has antiproliferative and pro-apoptotic effects on two human colon cancer cell lines, HCT15 and CO115.

4. Conclusion

In conclusion, this study gives some criticism on the potentials of some plants traditionally used in the field of pharmacology as an anticancer agent. This is a preliminary study and, in the future, it is aimed to find out the molecular mechanisms that cause cell death. Additionally, it is planned to prepare various extracts by using new solvents and different extraction techniques with the plants used in the study and investigate their cytotoxic potential.

Acknowledgments

-

Conflict of Interest

No conflict of interest was reported by the authors.

References

1. Adnan, M., Ullah, I., Tariq, A., Murad, W., Azizullah, A., Khan, A., Ali, N., 2014. Ethnomedicine use in the war affected region of Northwest Pakistan. J Ethnobiol Ethnomed 10: 16.
2. Ali, N., Shah, S.W., Shah, I., Ahmed, G., Ghias, M., Khan, I., 2011. Cytotoxic and anthelmintic potential of crude saponins isolated from Achillea wilhelmsii C. Koch and Teucrium stocksianum Boiss. BMC Complement Altern Med. 11:106.
3. Alimpic, A. Z., Kotur, N., Stanković, B., Marin, P. D., Matevski, V., Al Sheef, N., & Duletić-Laušević, S. 2015. The in vitro antioxidative and cytotoxic effects of selected Salvia species water extracts. Journal of Applied Botany and Food Quality, 88(1).
4. Altay, A., & Bozoğlu, F., 2017. Salvia fruticosa modulates mRNA expressions and activity levels of xenobiotic metabolizing CYP1A2, CYP2E1, NQO1, GPx, and GST enzymes in human colorectal adenocarcinoma HT-29 cells. Nutrition and cancer, 69(6), 892-903.
5. Amini Navaie, B., Kavoosian, S., Fattahi, S., Hajian-Tilaki, K., Asouri, M., Bishekolaie, R., & Akhavan-Niaki, H., 2015. Antioxidant and cytotoxic effect of aqueous and hydroalcoholic extracts of the Achillea millefolium L. on MCF-7 breast cancer cell line. *International Biological and Biomedical Journal*, *1*(3), 119-125.
6. Bisset, N.G., 1994. Herbal Drugs and Phytopharmaceuticals: A handbook for practice scientific basis (1st ed.). Stuttgart, Germany: Medpharm GmbH Scientifiv Publisher.
7. Bozin, B., Mimica-Dukic, N., Bogavac, M., Suvajdzic, L., Simin, N., Samojlik, I., & Couladis, M., 2008. Chemical composition, antioxidant and antibacterial properties of Achillea collina Becker ex Heimerl sl and A. pannonica Scheele essential oils. Molecules, 13(9), 2058-2068.
8. Bozyel, M. E., & Merdamert, E., 2018. Antiurolithiatic activity of medicinal plants in turkey. Science, ecology and engineering research in the globalizing world, 152.
9. Chikezie, P. C., & Ojiako, O. A., 2015. Herbal medicine: yesterday, today and tomorrow. Alternative & Integrative Medicine.

10. Demir, S., Demir, E. A., & Aliyazıcıoğlu, Y., 2020. Selective Cytotoxic Effect of Astaxanthin on Human Lung and Colon Cancer Cells. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(6), 1489-1494.
11. Bali, E. B., Açık, L., Elçi, P., Sarper, M., Avcu, F., & Vural, M., 2015. In vitro anti-oxidant, cytotoxic and pro-apoptotic effects of Achillea teretifolia Willd extracts on human prostate cancer cell lines. Pharmacognosy magazine, 11(Suppl 2), S308.
12. Encalada, M. A., Hoyos, K. M., Rehecho, S., Berasategi, I., de Ciriano, M. G. Í., Ansorena, D., ... & Calvo, M. I. 2011. Anti-proliferative effect of *Melissa officinalis* on human colon cancer cell line. Plant Foods for Human Nutrition, 66(4), 328-334.
13. Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M., 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer, 127(12), 2893-2917.
14. Gordaliza M., 2009. Natural products as leads to anticancer drugs. Clin Transl Oncol 9:767-776.
15. Gürdal, B., & Kültür, Ş., 2013. An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). Journal of Ethnopharmacology, 146(1), 113-126.
16. Half, E., Arber, N., 2009. Colon cancer: Preventive agents and the present status of chemoprevention. Exp. Opin. Pharmacother. 10: 211–219.
17. Hamedi, A., Afifi, M., & Etemadfard, H., 2017. Investigating chemical composition and indications of hydrosol soft drinks (aromatic waters) used in Persian folk medicine for women's hormonal and reproductive health conditions. Journal of evidence-based complementary & alternative medicine, 22(4), 824-839.
18. Jemal, A., Bray, F., Center, M.M., Ward, E., Forman, D., 2011. Global cancer statistics. Cancer J Clin. 61(2): 69-90.
19. Kendir, G., & Güvenç, A. 2010. Etnobotanik ve Türkiye'de yapılmış etnobotanik çalışmalara genel bir bakış. Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi, (1), 49-80.
20. Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K., 2020. Anticancer Plants: A Review of the Active Phytochemicals, Applications in Animal Models, and Regulatory Aspects. Biomolecules, 10(1), 47.
21. Köngül, E., Taş, Ö., Paşayeva, L., & Karatoprak, G. Ş., 2017. Analysis of the Cytotoxic Effects of Achillea millefolium L. Extracts on MCF7 Cell Line. Multidisciplinary Digital Publishing Institute Proceedings, 1(10), 1077.
22. Li, Y., Zhang, M. L., Cong, B., Wang, S. M., Dong, M., Sauriol, F., ... & Kiyota, H., 2011. Achillinin A, a cytotoxic guaianolide from the flower of Yarrow, Achillea millefolium. Bioscience, biotechnology, and biochemistry, 1107062554-1107062554.
23. Linskens, H. F., & Jackson, J. F. (Eds.). 1997. Plant volatile analysis. Berlin: Springer.
24. Merika, E., Saif, M. W., Katz, A., Syrigos, C., & Morse, M. 2010. Colon cancer vaccines: an update. In vivo, 24(5), 607-628.
25. Msomi, N. Z., & Simelane, M. B., 2018. Herbal Medicine. In Herbal Medicine. IntechOpen.
26. Mukherjee, A. K., Basu, S., Sarkar, N., & Ghosh, A. C., 2001. Advances in cancer therapy with plant based natural products. Current medicinal chemistry, 8(12), 1467-1486.
27. Nguyen, N. H., Ta, Q. T. H., Pham, Q. T., Luong, T. N. H., Phung, V. T., Duong, T. H., & Vo, V. G. 2020. Anticancer activity of novel plant extracts and compounds from adenosma bracteosum (bonati) in human lung and liver cancer cells. Molecules, 25(12), 2912.
28. Nickavar, B., Kamalinejad, M., Haj-Yahya, M., & Shafaghi, B., 2006. Comparison of the free radical scavenging activity of six Iranian Achillea. species. Pharmaceutical biology, 44(3), 208-212.
29. Saeidnia, S., Moradi-Afrapoli, F., Gohari, A.R., Malmir, M., 2009. Cytotoxic flavonoid from Achillea talagonica Bioss. J Med Plants Res 8: 52 6.
30. Sathiyamoorthy, P., Lugasi-Evgi, H., Schlesinger, P., Kedar, I., Gopas, J., Pollack, Y., & Golan-Goldhirsh, A., 1999. Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products. Pharmaceutical biology, 37(3), 188-195.
31. Shinwari, Z.K., 2010. Medicinal plants research in Pakistan. J Med Plants Res 4: 161–176.
32. Sivas, H., & Tomsuk, Ö., 2011. Antiproliferative and apoptotic effects of the essential oil of *Origanum onites* and carvacrol on Hep-G2 cells. Anadolu Unıversıty Journal of Scıence And Technology, 1: (2) 171-180.
33. Souri, E., Amin, G., Dehmobed-Sharifabadi, A., Nazifi, A., & Farsam, H., 2010. Antioxidative activity of sixty plants from Iran. Iranian Journal of Pharmaceutical Research, (1), 55-59.
34. Tarawneh, K.A., Irshaid, F., Jaran, A.S., Ezealarab. M., Khleifat, K.M., 2010. Evaluation of anti-bacterial and anti-oxidant activities of methanolic extracts of some medicinal plants in northern part of Jordan. J Biol Sci 10: 325 32.
35. Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O., & Yetim, H., 2011. Efficacy of various plant hydrosols as natural food sanitizers in reducing Escherichia coli O157: H7 and Salmonella Typhimurium on fresh cut carrots and apples. International Journal of Food Microbiology, 148(1), 30-35.
36. Trifunovic, S., Vajs, V., Juranić, Z., Žižak, Ž., Tešević, V., Macura, S., & Milosavljević, S., 2006. Cytotoxic constituents of Achillea clavennae from Montenegro. Phytochemistry, 67(9), 887-893.

37. Weidner, C., Rousseau, M., Plauth, A., Wowro, S. J., Fischer, C., Abdel-Aziz, H., & Sauer, S., 2015. *Melissa officinalis* extract induces apoptosis and inhibits proliferation in colon cancer cells through formation of reactive oxygen species. Phytomedicine, 22(2), 262-270.
38. Wojdylo, A., Oszmianski, J., Czemerys, R., 2007. Anti-oxidant activity and phenolic compounds in 32 selected herbs. Food Chem 105:940 9.
39. World Health Organization. World Health Organization. [Online].; 2010. Available from: http://www.who.int/cancer/detection/colorectalcancer/en/
40. Xavier, C. P., Lima, C. F., Fernandes-Ferreira, M., & Pereira-Wilson, C. 2009. *Salvia fruticosa*, Salvia officinalis, and rosmarinic acid induce apoptosis and inhibit proliferation of human colorectal cell lines: the role in MAPK/ERK pathway. Nutrition and cancer, 61(4), 564-571.

Review
Curr. Pers. MAPs, (2020); 3(2): 104-112

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Poisonous Plants for Cats and Dogs Kept in House 1: *Dieffenbachia* spp., *Melia azedarach*, *Ricinus communis*, *Euphorbia pulcherrima*, *Narcissus* spp.

Ali BİLGİLİ[1*], Başak HANEDAN[2], Muhammet Haydar UYSAL[3]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ankara, 06110, Ankara, TURKEY
*Corresponding author: abilgili@gmail.com
[2]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ataturk, 25240, Erzurum, TURKEY, E-mail: fbhanedan@gmail.com
[3]Department of Pharmacology and Toxicology, Health Sciences Institute, Ankara University, 06110, Ankara, TURKEY, E-mail:uysalhaydar@hotmail.com

https://doi.org/10.38093/cupmap.828624

Received : 20/11/2020
Accepted : 11/12/2020

Abstract

Poisonous plants are responsible for many poisoning events. These plants are commonly grown in homes and gardens and are eaten by dogs and cats. In this context, it was dealt with poisonous effects of especially *Dieffenbachia* spp., chinaberry tree (*Melia azedarach*), castor bean (*Ricinus communis*), poinsettia (*Euphorbia pulcherrima*) and daffodil (*Narcissus* spp.) from plants leading to poisoning, grown commonly in parks, gardens, and homes, in our country. The plant species leading to poisoning for both dogs and cats were presented in table. In addition, comprehensive information was presented on their adverse effects in different organ and tissues occurring in the result that they are eaten by dogs and cats, their clinical signs, and their treatments. The concise knowledge was given on the required measures for preventing poisoning of animals kept in home with these plants in also our country, as in world countries.

Key Words: Dogs, cats, poisonous plants

1. Introduction

Severe poisonings occur in the result that dogs and cats eat these plants with increase of import hall plants and ornamental plants, and together with widespread landscape design of house, office, and garden, and increased domestic cat and dog population also in our country, as all over the world. In USA, it is reported that 12% of poisonings of cats and dogs kept in house are caused by plants (Mahdi and van der Merwe, 2013). Indoor plants as a part of trade sector are used commonly for esthetic and functional features in living spaces. As a result that poisonous plants are present commonly in places such as apartment, house, office, and shopping center, pet animals chew or eat these plants and intoxications occur (Tiwari and Sinha, 2010).

Dogs and cats can show behaviour to put them into their mouths and to chew leafs, stems, flowerings and seeds of plant due to various reasons, especially getting bored in restraint area when the owner is not in the house, curiosity, and dentition in young

animals (Botha and Penrith, 2009; Tiwari and Sinha, 2010).

Plant eating, especially grass eating, is commonly appeared in all domestic cats and dogs, and to vomit when they feel ill themselves (to recover the gastric problems or gastrointestinal parasites) or because of dietary deficiencies or as general behavior, they eat plants. In this condition, it is appeared in not only dogs kept in house and in but also wolves and other wild dog species (Sueda et al., 2008). Tendencies of cats to eat plants are less than those of dogs (Sueda et al., 2008). In a study made by Sueda et al. (2008), it has been reported that 79% of well-kept healthy dogs eat grass, eating plant in domestic dogs is a behaviour commonly seen, and also that dogs are appeared normal before eating plant and 87% of dogs do not vomit after eating plant.

Poisonous plants include primary substances (carbohydrate, organic acids, and amino acids) required to be able to maintain main metabolisms, and secondary substances (alkaloids, glycosides, oxalates, resinous compounds, tannins, and volatile oils) generated as byproducts during main metabolisms. Major parts of secondary substances have toxic effects to humans and animals (Yılmaz et al., 2006). Toxic properties of some of these plants commonly used in landscaping of indoor and outdoor places are not known. To be eaten by cat, dog and other domestic animals can cause from simple clinical signs such as vomiting, diarrhea, dermal problems to negative effects such as deaths. Severity of poisoning with plants can vary according to the factors related to plant (species, region, consumed amount, parts of plant, its growth stage, etc.) and animal species (age, health condition, etc.) (Anadon et al., 2012). The toxicity of plant varies according to the season, climate, soil, and geographical area (Panter et al., 2013). In addition, sensitivity to poisonous plant may show variations specific to species or among individuals of same species (Anadon et al., 2012). For these reasons, plants to be used in the landscaping should not be considered with only ecological, economics and esthetic features. Thus, toxicity features of indoor and outdoor ornamental plants located in living places should be also known.

Generally poisoning of dogs and cats kept in house by plants occurs via eating plant, exposure to plant, photosensitivity, flavoring and odour with by-products, and mechanical damage. In poisoning of dogs and cats with plants, factors belonging to plant consist of soil, climate, growth stage, parts of plant, and health of plant. Animal factors consist of natural immunity, alimentary system, acquired immunity, breed, age, nutrition, gender, and dependency conditions.

By dealing with toxicities of several indoor and outdoor ornamental plants involved in all society and shared in living places and their adverse effets to dogs and cats in this study, uses of plants were aimed to take attention. In dogs and cats, some of the most plants leading to poisoning were presented in Table 1.

2. Poisonous plants

2.1. *Dieffenbachia* spp.

Characteristics and resources: They are of the most dangerous and wide plant groups comprising many toxic components. These plants can reach a height of up to 2 m and of 1-1.5 m in an appropriate environment in house. This plant is perennial and evergreen. It is grown as ornamental plant in house due to easy maintenance (Cortinovis and Caloni, 2013; Akça et al., 2014).

Toxic components: The plant contains saponins, alkaloids, cyanogenic glycosides, proteoliytic enzymes, calcium oxalate crystals and oxalic acid. The plant sap is poisonous. The main cause of poisoning is calcium oxalate crystals, oxalic acid, and protease irritating mucosa and releasing histamin. The stems and leaves of dumb cane like other members of the Araceae

family contain clusters of long and needle-like calcium oxalate crystals called raphides. Raphides are packed in specialized cells called idioblasts and released outside the cell if the plant is damaged (e.g., chewing, rubbing or cutting off the leaves). If raphides are embedded in mucosa, they entail extensive irritation and inflammation (Knight, 2007).

Toxicity: Poisoning in dogs generally occurs via ingestion of the plant or exposure to the eye. All parts of the plant especially stem and leaves are poisonous.

Clinical signs: Dogs and cats are the most affected animals. In the result of the chewing of stem or leaves of dumb cane, effects from mild local irritation to death may occur (Cortinovis and Caloni, 2017; Akça et al., 2014). Contents of the plant cause local irritations via inflammatory reaction. Edema in respiratory tract and obstructions may occur. After chewing the plant, clinical signs such as pain in the mouth, moderate swelling in the mouth and throat, hypertrophy in the tongue, edema and ulceration in the mouth mucosa, hypersalivation, dyspnea, odynophagia, difficulty chewing, hoarse voice, and vomiting occur (Knight, 2007). In severe cases, animals may die with dyspnea due to upper respiratory obstruction (Loretti et al., 2003). Cases mostly show mild clinical signs and may recover completely. Contact of the plant sap to the eye can lead to edema in the eye lids, inflammation in the cornea and the conjunctiva (Anadon et al., 2012), and temporary blindness from several hours to several days. Dumb cane with contents of calcium oxalate crystals can cause irritation in the gastrointestinal tract, acute hypocalcemia, acute tubular necrosis in the kidney, and multiple organ failure (Anon, 2007). Information has been obtained that child, at a 1 year and 11 months of age, chews dumb cane when presented to the hospital due to erythema and swelling in the lip (Akça, 2014). In dogs and cats, after exposure to the plant, gastric ulceration and obstruction in the respiratory tract have been reported (Müller et al., 1998; Peterson et al., 2009). Diagnosis is made by information that the owner gives and clinical signs.

Treatment: Treatment must be supportive including fluid therapy, and oxygen treatment. Measures should be taken for mouth mucosa and tongue swelling when ingested in excess amount of plant. Prognosis is usually good (Peterson et al., 2009).

2.2. Chinaberry tree (*Melia azedarach*)

Characteristics and Resources: *Melia azedarach* (*M. australis*, *M. japonia*, *M. sempervivens*) are a plant of Meliaceae family, cultivated as ornamental plant all over the world, rapid grown up, and fall leaf in winter (Mendez et al., 2002; Tiwari and Sinha, 2010; Cortinovis and Caloni, 2017). Fruits are yellow when they are ripe (Cortinovis and Caloni, 2017). It is used for anthelmintic, tonic, antipyretic, antifungal and also alleviating of leprosy, eczema, and asthma (Phua et al., 2008). In southern Europe, it is grown as an ornamental tree along streets, and roadsides and around houses.

Toxic components: Tetranortriterpenes are important toxic components (Tiwari and Sinha, 2010). Toxic components of tree are tetranortriterpene neurotoxins of the cytotoxic limonoid class known as meliatoxins A1, A2, B1, and B2 in high level in fruits. These toxic components affect as enterotoxins and neurotoxins (Oelrichs et al., 1983). Alkaloids, flavonoids, limonoids, steroids and triterpenoids are also isolated from leaves and barks of tree (Ge et al., 2016; Pan et al., 2014). Other potentially toxic components comprise azadarin (alkaloid), meliotannic acid, benzoic acid, and resins such as azaridine, parisine, and

margosinine (Plumlee, 2003). Action mechanism of meliatoxins is not well known. The toxicity of tree and its fruits has been demonstrated to vary due to environmental conditions according to growing area and stage of growth (Knight, 2007; Ferreiro et al., 2010).

Table 1. Some important household plants leading to poisoning in dogs and cats

***Dieffenbachia* spp.**	***Taxus baccata***
***Lilium* spp.**	***Hamerocallis fulva***
Aloe vera	***Cannabis* spp.**
African violet	***Brunfelsia* spp.**
Begonia	***Melia azedarach***
Montera deliciosa	*Digitalis* spp.
Euphorbia pulcherrima	*Ilex aquifolium*
Ricinus communis	*Convallaria majalis*
***Narcissus* spp.**	*Laburnum* spp.
Cyclamen spp.	*Cassia* spp.
Azalea spp.	*Hyacinthus orientalis*
Nerium oleander	*Euphorbia* spp.
Spathiphyllum spp.	*Nicotiana tabacum*
***Lonicera* spp.**	*Cestrum* spp.
***Vitis* spp.**	Araceae spp.
***Allium* spp.**	

Toxicity: Poisoning related to chinaberry tree has been reported in horses, cattle, sheep, goats, pigs, dogs, rabbits, rats, guine pigs, and poultry (Cooper, 2007; Tiwari and Sinha, 2010; Cortinovis and Caloni, 2017). It is known by veterinary surgeons that chinaberry tree is toxic (Cortinovis and Caloni, 2013). Its fruits are highly toxic. Other leaves, stems, flowers are mildy toxic. Poisoning events are usually occurred in dogs. All parts of tree are toxic but poisoning usually occurs after fruits are ingested. Five or six fruits (less than 100 g) at 0.6 g/kg can cause poisoning (nausea, spasm and death) in small dogs.

Clinical signs: Clinical signs in domestic animals quickly occur and usually start several hours after ingestion (usually within 2-4 hours), and may be dominated as gastrointestinal and nervous system signs (Botha and Penrith, 2009; Ferreiro et al., 2010). Clinical signs related to gastrointestinal tract include anorexia, nausea, vomiting, constipation, diarrhea (frequently bloody), gastric pain, and colic. Clinical signs related to nervous system include excitement or weakness, convulsions, ataxia, paresis, and coma (Botha and Penrith, 2009; Ferreiro et al., 2010). Other signs are depression,

bradycardia or cardiac arrest, collapse of circulatory system, congestion in the lungs, and dyspnea (Hare et al., 1998; Cortinovis and Caloni, 2017; Tiwari and Sinha, 2010). Death may be occurred 1 day after ingestion (Tiwari and Sinha, 2010). Clinical signs in humans are nausea, vomiting, diarrhea, thirst, perspiring, grinding of the teeth, drowsy, and colvulsions. Diagnosis is made by information that the owner gives and with clinical signs.

Treatment: Treatment is supportive care including stabilization of vital signs, maintaining a patent airway, oxygen supplementation, maintaining cardiovascular function, fluid and electrolyte administration, removing gastrointestinal plant materials via medical or operation, alleviating abdominal pain, and controlling nervous system signs (Poppenga, 2017).

Dog, Teckel breed, 4 years old, 8 kg weight, has been presented to the animal hospital tremors, and moderate limp in the hind leg. In clinical signs, abdominal pain has been determined and radiological examination has showed foreing radiodense bodies. Oral paraffin oil (6 ml q 8 h for 2 days) and carprofen (4 mg/kg daily and orally) have been administrated. After 2 days, increase in abdominal pain, depression, ataxia in the hind legs has been more evident. In the enterotomy, seeds of chinaberry tree (*Melia azedarach*) have been evacuated. Ringer's lactate fluid to treat dehydration, dexamethazone (0.1 mg/kg/gün) to alleviate pain and inflammation, phosphor and vitamin to stimulate nervous system (Catosal, 0.5 ml/ q 24 h, i.m., only once) have been administrated. Patient has fully recovered. Ataxia in the hind legs has been recovered after two months (Ferreiro et al., 2010).

2.3. Castor Oil Plant (*Ricinus communis*)

Characteristics and resources: Castor oil plant is a flowering plant of Euphorbiaceae family, widespread grown all over the world, especially in tropical and temperate regions (Cortinovis and Caloni, 2017). This plant is grown widespread in Western and South Anatolia regions of our country. Flowers are head of the trunk and fruits are spiny. All parts of the plant, especially seeds, are toxic and rich in oil and ricin (Cortinovis and Caloni, 2017).

Toxic components: The compound responsible for poisoning is toxalbumin ricin with two A and B chains that are bound with disulfide bond. B chain is bound to the proteins containing galactoside on the cell surfaces and facilitates the entry of A chain to the cell cytosol, where ribosomes are rendered inactive and thus, protein synthesis is inhibited (Lord et al., 1994). Toxalbumin in the shell of fruits is a water soluble glycoprotein and is one of the most poisonous compounds among the compounds with plant origin. Besides toxic ricin, the plant includes piperidine alkaloid ricinine, and affects nerve receptors.

Toxicity: All animal species are highly sensitive to the toxic effects of ricin (Worbs et al., 2011), and this plant should be paid attention (Bradberry et al., 2003). This plant is also toxic in horses, usually in dogs, and ricin is one of the most toxic compounds among plant originated compounds. This plant contains ricin and piperidine alkaloid ricinine. Ricinine compound can cause neuromuscular weakness as a result of the effect on nerve receptors (Bailey, 2013).

Clinical signs: Poisoning with castor oil plant is usually associated with ingestion of seeds (Bailey, 2013). Signs occur with gastrointestinal inflammation and bleeding in the result of damage of cells of alimentary tract and severe irritation. In the event of poisoning of dogs, about 9% have died or euthanasia has been made. Clinical signs occur longer time or 8 h after ingestion of this plant and nausea, severe vomiting,

anorexia, thirst, irritation of gastrointestanal system, abdomianl pain, bloody diarrhea, and tenesmus with rectal bleeding, fluid loss, and severe inflammation in the gastrointestinal system during postmortem period are usually seen. Weight loss, tremors, convulsions, dispnea, opisthotonus, coma and death occur. Increase in bleeding time, hypoproteinemia, and cyanosis occur (Albretsen et al., 2000; Anadon et al., 2012; Doan, 2004). In addition, convulsions and tachycardia may be seen up to death time (Audi et al., 2005; Soto-Blanco et al., 2002).

Treatment: Induction of emesis can be helpful early after ingestion of castor bean with use of hydrogen peroxide 3%, 1 to 5 ml/kg. In addition, activated charcoal and cathartic (magnesium sulphate 5-25 g or sorbitol 3 ml/kg) can be given unless animal has diarrhea. Gastrointestinal protectants such as sucralfate should be used as needed. Fluid therapy with balanced electrolyte solutions is given and diazepam can be given for seizures. After vomiting is controlled, soft, bland diet is given for one to four days (Poppenga, 2017; Albretsen et al., 2000).

2.4. Poinsettia (*Euphorbia pulcherrima*)

Characteristics and resources: Poinsettia (*Euphorbia* spp.) in Euphorbiaceae family is a small shrub widely cultivated in tropical regions. This plant grows up to 30-40 cm. It is widely used as indoor ornamental plant and using of it as ornamental plants in parks and along streetsides increases day by day because of its beatiful view (Cortinovis and Caloni, 2017).

Toxic components: Poinsettia has milky sap. Toxic ingredients present in milky sap are responsible from irritation and this sap contains diterpenoid euphorbol esters and steroids with saponin-like properties (Botha and Pentrith, 2009; Gwaltney-Brant, 2013).

Toxicity: Many cases have been reported to have no clinical signs when this plant is ingested. Intoxications occur when the plant produce red bracts. All parts of the plant have toxic effects and have moderate toxic effects in dogs and cats (Anon, 2020).

Clinical signs: Poisoning by poinsettia in dogs and cats may occur. After ingestion of the plant, licking the lip, irritation of the face, mouth, lips and nare, hypersalivation, vomiting and rarely diarrhea in dogs and cats are seen (Anon, 2020; Botha and Penrith, 2009; Campbell and Chapman, 2000). Contact to the skin results in irritation, erthema and itching of the skin. Contact of the sap to the eye may cause redness of the eye, conjunctivitis and lacrimation (Gwaltney-Brant, 2013). Death has occurred with severe gastrointestinal disorder and fever when large amounts of plant are ingested. Other signs are vomiting, hypersalivation, diarrhea, abdominal distention, and fever.

Treatment: Medical treatment is based on the exposure amount of toxic components. Treatment may include gastrointestinal decontamination, fluid therapy, and antihistaminic administration (Anon, 2020).

2.5. Daffodil (*Narcissus* spp.)

Characteristics and resources: This plant that blooms in the spring is perennial and is grown from a bulb of daffodil plant. It is known that ingestion of daffodil bulbs in Amarylidaceae family causes poisoning. It leads to persistent intoxication in humans and animals (Kretzing et al., 2011). Their stems contain clear viscous sap. It has small fruits, filled with black seeds, and wrapped with green capsule (Campbell and Chapman, 2000).

Toxic components: The parts of the plant related to poisoning contain alkaloids and glycosides as toxic components. These components are present in high concentrations in the bulb of the plant. Alkaloids responsible from the toxic effects

are narcissine, narciclasine, galanthamine, and lycorine in high amounts. They contain calcium oxalate crystals (Severino, 2009; Kızıl and Çiftçi, 2018). Glycosides of this plant include scillitoxin. Lycorine and other alkaloids cause irritation, emesis and purgative effects and calcium oxalate crystals cause mechanical irritation (Campbell and Chapman, 2000).

Toxicity: Poisoning cases related to daffodil generally result from ingestion of the plant and drinking water in the plant container (Severino, 2009). In dogs, poisoning cases frequently occur because they chew and consume (Campbell and Chapman, 2000; Saxon-Bury, 2004). Several plant bulbs (15 g plant bulb) is fatal to a small animal kept in home. Severe vomiting and weakness have occurred in a Cocker Spaniel breed dog 1 h after ingesting 12 the head of plant flower and then dog has recovered without any treatment. Within 3 h of ingestion of unknown amounts of plant bulb, in a Golden Retriever dog, 8 months old, weakness, mucosal paleness, tachycardia, decrease in body temperature, hyperglycemia and vomiting have occurred. This dog has recovered with fluid, dexamethazone, insulin, adrenaline and diazepam administration. In 6 years old cross-bred dog, daffodil toxicity has occurred due to ingestion of bulbs. This dog had showed severe abdominal pain, hypersalivation, pale mucous membranes, and diarrhea. Serum biochemistry analysis has revealed increased urea and creatinine. The animal has been euthanased at the owner's request (Campbell and Chapman, 2000).

An adult cat, 2 years old, has been presented to the animal hospital due to weakness and vomiting after ingestion of dried daffodil stems (*Narcissus* spp.). Hypotension, hypothermia, bradycardia, and fluid loss have been determined. Complete blood count had normal and increase in urea and glucose, decrease in sodium, potassium and chloride concentrations in serum biochemical examination has been determined (Saxon-Buri, 2004).

In London, Veterinary Poisons Information Service has examined cases related to daffodil ingestion. It was reported that of 4 poisoning cases, 1 case died and 1 case was euthanized. Important clinical signs are vomiting, diarrhea, weakness, depression, hypothermia, hypotension, bradycardia, abdominal pain, hyperglycemia, and fluid (Campbell and Chapman, 2008). In humans, clinical signs are nausea, vomiting, diarrhea, and vertigo. Irritation and skin inflammation occurs in people collecting daffodil due to skin contact of daffodil. Recent studies have demonstrated that oxalate crystals in the plant stems cause skin lesions (Chiu et al., 1992).

Clinical signs: Clinical signs are vomiting, diarrhea, abdominal pain, anorexia, hypersalivation, and restlessness (Fitzgerald, 2010). Ingestion of high amounts may cause ataxia, lethargy, hypothermia, bradycardia, hypotension, and depression. About 15 g plant bulb can cause death in dogs (Severino, 2009). Clinical signs occur within 15 min to 24 h. In the event of ingestion of high amounts, severe ataxia, collapse, hypothermia, arrythmia, severe abdominal pain, hyperglycemia, fluid loss, tremors, convulsions occur. In the case of contact with the plant sap, skin itching and eczema occur. Diagnosis is made based on that the owner gives information and clinical signs (Campbell and Chapman, 2000).

Treatment: Supportive care is required including stabilization of vital signs, maintaining a patent airway, maintaining cardiovascular function, fluid and electrolyte administration, gastrointestinal decontamination, alleviating abdominal pain, and controlling nervous system signs (Poppenga, 2017). If animal ingests daffodil and does not vomit, then emesis can be induced. If persistent emesis occurs, then

antiemetic treatment is given. A dog has been treated using fluid, dexamethazone, insulin, adrenaline and diazepam (Campbell and Chapman, 2000). A cat has been treated using fluid therapy, atropin, dexamethazone, and wrapping to the warm towel (Saxon-Buri, 2004).

3. Conclusions and proposals

Considering toxicity features of the plants while in areas around parks and gardens, and houses and yards, landscaping will be performed, in order to inform the user, labels identifying severity of toxicity of the plant, its effects, its parts should be attacted on plants in plantation, nursery gardens, flower shops, and other selling places, and this condition should be necessary. The owners of animals, local authorities, landscape architects, other related occupation groups and their associations, public and private enterprises, and veterinary surgeons should recognize these plants and know prevention measures to them. In addition, veterinary surgeons should know treatments choices in the case of animal poisoning with these poisonous plants.

In the case of poisoning with plants, recognition of the plant, identification of toxic components, and diagnosis of affected system are highly beneficial for treatment. In the event of plant poisonings of dogs and cats, if no antidote, supportive care is necessary by identifying treatment regime with considering toxicokinetics and toxicodynamics of toxic components. In addition, increase of physical activity and increase of time of playing game, decrease of boring, observing them and spending more time with them may decrease tendencies to plant eating (Fitzgerald, 2010).

Acknowledgments

-

Conflict of Interest

No conflict of interest was reported by the authors.

References

1. Albretsen, J.C., Gwaltney-Brant, S.M., and Khan, S.A., 2000. Evaluation of castor bean toxicosis in dogs: 98 cases. Journal of the American Animal Hospital Association, 36 (3), 229-233. DOI number: 10.5326/15473317-36-3-229
2. Akça, H., Polat, E., Tuygun, N., Gürcan Kaya, N., ve Demir Karacan, C., 2014. Evdeki Tehlike: Difenbahya. Journal of Academic Emergency Medicine Case Reports/Akademik Acil Tıp Olgu Sunumları Dergisi, 5 (4), 107-109. DOI number: 10.5152/jaemcr.2014.47568
3. Anadón, A., Martínez-Larrañaga, M.R., and Castellano, V., 2012. Poisonous plants of Europe. In: Gupta RC, editor. Veterinary Toxicology: Basic and Clinical Principles. 2nd ed. San Diego: Elsevier.
4. Anon, 2007. T.C. Sağlık Bakanlığı, Refik saydam Hıfzıssıhha Merkezi Başkanlığı, Hıfzıssıhha Mektebi Müdürlüğü, Birinci Basamağa Yönelik Zehirlenmeler Tanı ve Tedavi Rehberleri, Bitkilerle Zehirlenmeler, 712, 171-3.
5. Anon, 2020. "Poinsettia poisoning in dogs". https://wagwalking.com/condition/poinsettia-poisoning. Access Date: 04.02.2020.
6. Audi, J., Belson, M., Patel, M., Schier, J., and Osterloh, J., 2005. Ricin poisoning: A comprehensive review. Journal of the American Medical Association, 294 (18), 2342-2351. DOI number: 10.1001/jama.294.18.2342
7. Bradberry, S.M., Dickers, K.J., Rice, P., Griffiths, G.D., and Vale, J.A., 2003. Ricin poisoning. Toxicological Reviews, 22 (1), 65-70. DOI number: 10.2165/00139709-200322010-00007
8. Bailey, Jr EM., 2013. Ricin. In: Petersen ME, Talcott PA, editors. Small Animal Toxicology. 3rd ed. St. Louis: Saunders.
9. Botha, C.J., and Penrith, M.L., 2009. Potential plant poisonings in dogs and cats in southern Africa. Journal of the South African Veterinary Association, 80 (2), 63–74. DOI number: 10.4102/jsava.v80i2.173.
10. Campbell, A., and Chapman, M., 2000. Handbook of poisoning in dogs and cats. London: Blackwell Science.
11. Campbell, A., and Chapman, M., 2008. Handbook of poisoning in dogs and cats: John Wiley & Sons.
12. Chiu, K., Lee, Y., and Yung, K., 1992. Bioactive substances from the Chinese daffodil, Narcissus tazetta. Phytotherapy Research, 6 (5), 231-236.
13. Cooper, R.G., 2007. Poisoning in ostriches following ingestion of toxic plants-field observations. Tropical Animal Health and Production, 39 (6), 439-442. DOI number: 10.1007/s11250-007-9044-y.

14. Cortinovis, C., and Caloni, F., 2013. Epidemiology of intoxication of domestic animals by plants in Europe. Veterinary Journal, 197 (2), 163–168. DOI number: 10.1016/j.tvjl.2013.03.007.
15. Cortinovis, C., and Caloni, F., 2017. Plants Toxic to Farm and Companion Animals. Page: 107-134.
16. Doan, L.G., 2004. Ricin mechanism of toxicity, clinical manifestation, and vaccine development. Clinical Toxicology, 42 (2), 201-208. DOI number: 10.1081/clt-120030945.
17. Ferreiro, D., Orozco, J.P., Mirón, C., Real, T., Hernández-Moreno, D., Soler, F., Perez-Lopez, M., 2010. Chinaberry tree (Melia azedarach) poisoning in dog: a case report. Topics in Companion Animal Medicine, 25 (1), 64–67. DOI number: 10.1053/j.tcam.2009.07.001
18. Fitzgerald, K.T., 2010. Lily toxicity in the cat. Topics in Companion Animal Medicine, 25 (4), 213-217. DOI number: 10.1053/j.tcam.2010.09.006
19. Ge, J-J., Wang, L-T., Chen, P., Zhang, Y., Lei, X-X., Ye, X-X., 2016. Two new tetracyclic triterpenoids from the barks of Melia azedarach. Journal of Asian Natural Products Research, 18 (1), 20–25. DOI number: 10.1080/10286020.2015.1124866
20. Gwaltney-Brant, S.M., 2013. Christmastime plants. In: Petersen ME, Talcott PA, editors. Small Animal Toxicology. 3rd ed. St. Louis: Saunders.
21. Hare, W., Garland, T., and Barr, A., 1998. Chinaberry (Melia azedarach) poisoning in animals. Toxic Plants and Other Natural Toxicants. CAB International, Wallingford, 514-516.
22. Kızıl, Ö., and Çiftçi, Ü., 2018. Kedi ve köpeklerde bitkisel zehirlenmeler. Fırat University Journal of Health Sciences (Veterinary), 32 (1), 69-73.
23. Knight, A.P., 2007. A guide to poisonous house and garden plants. Jackson: Teton, NewMedia.
24. Kretzing, S., Abraham, G., Seiwert, B., Ungemach, F.R., Krugel, U., and Regenthal, R., 2011. Dose-dependent emetic effects of the Amaryllidaceous alkaloid lycorine in beagle dogs. Toxicon, 57 (1), 117-124. DOI number:10.1016/j.toxicon.2010.10.012
25. Lord, J.M., Roberts, L.M., and Robertus, J.D., 1994. Ricin: structure, mode of action, and some current applications. FASEB Journal, 8 (2), 201-208.
26. Loretti, A.P., da Silva Ilha, M.R., and Ribeiro, R.E., 2003. Accidental fatal poisoning of a dog by Dieffenbachia picta (dumb cane). Veterinary Human Toxicology, 45 (5), 233–239.
27. Mahdi, A., and van der Merwe, D., 2013. Dog and cat exposures to hazardous substances reported to the Kansas State Veterinary Diagnostic Laboratory: 2009-2012. Journal of Medical Toxicology, 9 (2), 207-211. DOI number:10.1007/s13181-013-0289-8
28. Méndez, C., Aragão, M., Fabiana, E., Riet-Correa, F., and Juan Gimeno, E., 2002. Experimental intoxication by the leaves of Melia azedarach (Meliaceae) in cattle. Pesquisa Veterinária Brasileira, 22 (1), 19-24.
29. Müller, N., Glaus, T., and Gardelle, O., 1998. Extensive stomach ulcers due to Dieffenbachia intoxication in a cat. Tierarztl Prax Ausg K Kleintiere Heimtiere, 26, 404-407.
30. Pan, X., Matsumoto, M., Nishimoto, Y., Ogihara, E., Zhang, J., Ukiya, M., Tokuda, H., Koike, K., Akihisa, M., and Akihisa, T., 2014. Cytotoxic and nitric oxide production-inhibitory activities of limonoids and other compounds from the leaves and bark of Melia azedarach. Chemistry and Biodiversity, 11 (8), 1121–1139. DOI number: 10.1002/cbdv.201400190
31. Panter, K.E., Welch, K.D., Gardner, D.R., and Green, B.T., 2013. Poisonous plants: effects on embryo and fetal development. Birth Defects Research Part C Embryo Today, 99, 223–234.
32. Peterson, K., Beymer, J., Rudloff, E., and O'Brien, M., 2009. Airway obstruction in a dog after Dieffenbachia ingestion. Journal of Veterinary Emergency Critical Care (San Antonio), 19 (6), 635-639. DOI number: 10.1111/j.1476-4431.2009.00486.x
33. Phua, D.H., Tsai, W.J., Ger, J., Deng, J.F., and Yang, C.C., 2008. Human Melia azedarach poisoning. Clinical Toxicology, 46 (10), 1067-1070. DOI number:10.1080/15563650802310929
34. Plumlee, K., 2003. Clinical Veterinary Toxicology-E-Book: Elsevier Health Sciences.
35. Poppenga, R.H., 2017. Chapter 19. Toxicological emergencies. Lesley G. King, Amanda Boag (eds). BSAVA Manual of Canine and Feline Emergency and Critical Care, Second edition, p. 278-294.
36. Oelrichs, P.B., Hill, M.W., Vallely, P.J., MacLeod, J.K., and Molinski, T.F., 1983. Toxic tetranortriterpenes of the fruit of Melia azedarach. Phytochemistry, 22 (2), 531-534.
37. Severino L., 2009. Toxic plants and companion animals. CAB Reviews, 4, 1-8.
38. Saxon-Buri, S., 2004. Daffodil toxicosis in an adult cat. Canadian Veterinary Journal, 45 (3), 248-250.
39. Soto-Blanco, B., Sinhorini, I.L., Gorniak, S.L., and Schumaher-Henrique, B., 2002. Ricinus communis cake poisoning in a dog. Veterinary Human Toxicology, 44 (3), 155-156.
40. Sueda, K.L.C., Hart, B.L., and Cliff, K.D., 2008. Characterisation of plant eating in dogs. Applied Animal Behaviour Science, 111 (1-2), 120-132.
41. Tiwari, R.M., and Sinha,M., 2010. Veterinary Toxicology. Oxford Book Company, Jaipur, India.
42. Worbs, S., Köhler, K., Pauly, D., Avondet, M.A., Schaer, M., Dorner, M.B., and Dorner, B.G., 2011. Ricinus communis intoxications in human and veterinary medicine-a summary of real cases. Toxins, 3 (10), 1332–1372. DOI number: 103390/toxins3101332
43. Yılmaz, H., Akpınar, E., and Yılmaz, H. 2006. Peyzaj mimarlığı çalışmalarında kullanılan bazı süs bitkilerinin toksikolojik özellikleri. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi, 7 (1), 82-95.

Research Article
Curr. Pers. MAPs, (2020); 3(2): 113-120

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Antioxidant and Anticancer Effects of *Malva verticillata* Methanolic Extract

Duygu GENÇALP[1], Namık Refik KERKÜKLÜ[2], Özde BUDA[3], Göktürk BİNER[3], Emre TURGAL[3], Dilara POLAT[3], Fatma İRDEM[3], Nazife KASAPOĞLU[3], Ergül MUTLU ALTUNDAĞ[1*]

[1]Eastern Mediterranean University, Dr. Fazıl Küçük Faculty of Medicine, Department of Medical Biochemistry, Via Mersin 10, 99628, Turkey
[2]Eastern Mediterranean University, Faculty of Arts and Sciences, Department of Chemistry, Via Mersin 10, 99628, Turkey
[3]Eastern Mediterranean University, Dr. Fazıl Küçük Faculty of Medicine, Via Mersin 10, 99628, Turkey
*Corresponding author: ergul.altundag@emu.edu.tr

https://doi.org/10.38093/cupmap.828637

Received : 20/11/2020
Accepted : 18/12/2020

Abstract

Objectives: *Malva verticillata* (*M. verticillata*), growing in North Cyprus, is an edible plant known as "mallow" in public. Current literature does not contain any data about the anticancer and antioxidant activities of the *M. verticillata* plant grows in Northern Cyprus.

Materials and Methods: In this study, *M. verticillata* methanolic extract was used to investigate the *in vitro* antioxidant and anticancer effects of the *M. verticillata* plant. The antioxidant potential of the Malva extract was determined using the α-diphenyl-p-picrylhydrazyl (DPPH) free radical scavenging method, the total phenolic content (TPC) test, and the total flavonoid content (TFC) test. Besides, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was used to investigate the anticancer potential of *M. verticillata* methanolic extract on MCF-7 cells.

Results: The highest antioxidant activity of *M. verticillata* methanolic extract was found to be 69.35 ± 3.3% at 70 mg/ml. TFC and TPC contents were calculated as 502 ± 1.8 µg/mg and 499 ± 7.5 µg/mg extract at 70 mg/ml, respectively. Evaluation of anticancer activity revealed that MCF-7 cell proliferation was significantly inhibited with increased extract concentrations of *M. verticillata*.

Conclusion: In this study, methanolic extract of *M. verticillata* plant has shown to possess significant antioxidant and anticancer activity.

Key Words: Antioxidant, Anticancer, Breast cancer (MCF-7), *Malva verticillata*

1. Introduction

Breast cancer is known to be the primary cause of cancer death in women worldwide. Although it is rare in men, 25.4 % of the total new cancer cases in women were diagnosed as breast cancer in 2018 (Bray et al., 2018). According to a study carried out by North Cyprus Cancer Registry, Ministry of Health, breast cancer was found to be the most common cancer type for female population in North Cyprus between 2007 and 2012 (Pervaiz et al., 2017). Breast cancer can be

treated in several ways including chemotherapy, radiation therapy, hormonal therapy and mastectomy (Maughan et al., 2010) (Radecka and Litwiniuk, 2016). However, all treatment strategies for breast cancer has some side effects and they have negative effects on patients quality of life (Taylor and Kirby, 2015) (Zurrida and Veronesi, 2015) (Klassen et al., 2017). Pain, lymphedema, hair loss, diarrhoea and peeling of skin are some of the side effects that decrease patients' quality of life. Therefore, the development of new treatment strategies with low side effects is needed to improve the survival rate of these patients. Nowadays, there is an increased interest in herbal medicine and natural products to treat diseases, and laboratory studies have gained momentum. Recent studies showed that different *Malva* species have anti-cancer, anti-inflammatory and antioxidant effects (Rayssan and Shawkat, 2019) (Khoury et al., 2020) (Mousavi et al., 2020) (DellaGreca et al., 2009).

M. verticillata, also known as "cluster mallow" or "Chinese mallow", is an edible plant which is a member of Malvaceae family. It grows in terrestrial habitats and it is mostly found in South East Asian countries and China (Ashok et al., 2020). Studies indicated that M. verticillata grows in China is a valuable source of natural antioxidants. Free radical scavenging activity of *M. verticillata* ethanol extract has been reported, and it is found to have a significant reducing power (Bao et al., 2018). In another study, water extract of *M. verticillata* seeds showed bone resorption suppression and osteoclastogenesis (Shim et al., 2016). Glycosyl glycerides isolated from the *M. verticillata* demonstrated cytotoxicity to A549, HCT-15, AGS, HepG2 cancer cells (Ko et al., 2018). However, there is no evidence about the antioxidant and anti-cancer potential of *M. verticillata* grows in North Cyprus. *M. verticillata* is a part of the natural flora of North Cyprus and is widely used in Cypriot cuisine. In the current study, we aimed to evaluate the potential antioxidant and in vitro anti-cancer properties on MCF-7 cells of *M. verticillata* methanolic extract.

2. Material and Methods

2.1. Chemicals: Methanol (CN: 24229), Gallic acid (CN: 398225), 1,1-diphenyl-2-picrylhydrazyl (DPPH, CN: D9132), Sodium carbonate (CN: 13418), Folin reagent (CN: F9252) and Quercetin (CN: Q4951)) were obtained from Sigma-Aldrich. Aluminum chloride (PC:10558030) was purchased from Thermo Fisher-Scientific.

2.2. Plant Material: Plant samples were collected from Taşkent, Kyrenia, Northern Cyprus (35.265204, 33.397465) in April 2019. Herbarium Botanists Prof. Dr. Neriman Özhatay from the Eastern Mediterranean University (EMU), Faculty of Pharmacy, identified the plant material as *M. Verticillata*. The herbarium specimen (Voucher No: DE 002) was pressed and deposited in the herbarium. Plant name was checked from http://www.theplantist.org.

2.3. Preparation of *M. verticillata* methanolic extract by Soxhlet extraction: Leaves of *M. verticillata* were separated carefully and washed with distilled water. Plant material was then dried at room temperature (25 °C) and crushed into powder. For Soxhlet extraction, 13 grams of *M. verticillata* powder and 300 ml of methanol was used. The process was completed in a total of 16 hours; two cycles at 70 °C each lasting for eight hours. Extracts were kept in the refrigerator at 4 °C until analysis.

2.4. Antioxidant Tests by α,α-diphenyl-β-picrylhydrazyl (DPPH) Assay: The antioxidant activities of *M. verticillata* methanol extract were determined using a DPPH reduction method with minor modifications (Alara et al., 2018a). *M. verticillata* extract was dissolved in distilled

water, and 5 µl from different concentrations (10, 20, 30, 40, and 70 mg/mL) was mixed with 195 µl DPPH solution in 96-well-plate. Then, the 96-well-plate was incubated at room temperature for 30 minutes in darkness. Gallic acid solution with varying concentrations (50, 100, 200, 499,600, 800 and 1000 µg/mL) was used as a standard. Following the incubation, the absorbance of the samples was measured at 517 nm with Varioskan Flash Multimode Microplate Reader (Thermo Scientific, USA). Following formula was used to calculate the inhibition percentages of the radical scavenging activity:

DPPH scavenging activity (% Inhibition) = [(Acontrol-Asample) /Acontrol] x 100

Acontrol =The absorbance of methanol mixed with DPPH solution
Asample =The absorbance of M. verticillata extract mixed with DPPH solution

2.5. TPC (Total phenolic content) Determination: To determine the TPC of *M. verticillata* extracts, Folin-Ciocalteu reagent method was used with modifications (Alara et al., 2018a) (Alara et al., 2018b). First, 50 µl from extract concentrations (10, 20, 30, 40 and 70 mg/ml) was added into 96-well plates. Then, 100 µl from folin reagent and 100 µl from Na2CO3 (sodium carbonate) solution were added to reaction mixtures. Gallic acid solution with varying concentrations (50, 100, 150, 200, 250, 1000 µg/ml) was used for the generation of the standard calibration curve *(y=0,0074x+0,2664; r2=0.978)*. Samples were incubated for 30 minutes at 25 °C prior to the absorbance measurement. Absorbance measurements were done at 765 nm using a Varioskan Flash Multimode Microplate Reader (Thermo Scientific, USA). The results are represented as µg gallic acid equivalent (GAE) per mg of *M. verticillata* extract (µg GAE/mg extract).

2.6. TFC (Total Flavonoid Content) Determination: AlCl3 (Aluminum chloride) colorimetric method was used to determine the TFC of the *M. verticillata* extracts with minor modifications(Kim et al., 2003). 50 µl from each extract solution (10, 20, 30, 40 and 70 mg/mL) was mixed with 50 µl of 2 % AlCl3 in 96-well plate. Then, the reaction mixtures were incubated at room temperature for 60 minutes. Different concentrations of quercetin (50, 100, 150, 200 and 250 µg/ml) were used for the generation of the standard calibration curve (y=0,0074x–0,0158; r2=0,9709). At the end of the incubation period, absorbance was measured at 420 nm via Varioskan Flash Multimode Microplate Reader (Thermo Scientific, USA). The results were expressed as µg quercetin equivalent (QE) per mg *M. verticillata* extract (µg QE/mg extract).

2.7. MCF-7 Cell Culture and Cell Viability Assay: Cell culture experiment was performed on MCF-7 human breast cancer cell line obtained from the American Type Culture Collection (ATCC, HTB-22). Dulbecco's modified Eagle's medium (DMEM- Merck, Germany) supplemented with 1 % L-glutamine, 1 % penicillin-streptomycin, 10 % fetal bovine serum (FBS; Hyclone Laboratories, USA) was used as cell culture media and cells were grown in a 5 % CO2 incubator at 37 °C. In order to determine the cell viability, MTT (Sigma, M2003) assay was performed (van der Heijden et al., 2004), and 5- Fluorouracil (5-FU) treated group was used as a positive control. Prior to the cell viability assay, 1x104 cells/well were seeded in 96 well plates in 100 µl of fresh culture medium and incubated for 24 hours at 37 °C. At the end of the incubation, cells were treated with *M. verticillata* extract at various concentrations (5,10, 20, 50, 100, 200 µg/mL) and 5 µM Fluorouracil (5-FU) for 24 hours. Then, 10 µl of 5 mg/ml MTT solution in PBS was added to each well, and left to incubate at 37 °C for four hours. At the end of incubation period,

the supernatant was removed, and DMSO (100 µl) was added to each well. The 96-well plates were placed into a microplate shaker for 5 minutes prior to the absorbance measurement. Absorbance was measured at 570 nm with a Varioskan Flash Multimode Microplate Reader (Thermo Scientific, USA). The following formula was used to calculate the percentage of cell viability:

% Viable cells= (Asample- Ablank) / (Acontrol-Ablank) x 100
Ablank= The absorbance of blank
Acontrol =The absorbance of control
Asample =The absorbance of M. verticillata extract

The experiment was performed in triplicate, and CalcuSyn Software program (Biosoft, Ferguson, USA) was used to calculate the concentration of extract required to inhibit 50 % of MCF-7 cell viability (IC50)

2.8. Statistical Analysis: Experiments were performed in triplicate and $P<0.05$ was considered to be statistically significant. Test results were calculated in Microsoft Excel 2015 software (Microsoft, Redmond, USA), and results were expressed as mean ± standard deviation (SD). GraphPad Prism Version 8 software was used to perform the statistical analyses. ANOVA/Dunnet's Multiple Comparison Test was used to determine the differences among groups.

3. Results and Discussion

Antioxidant activity of *M. verticillata* methanolic extract was determined by DPPH free radical scavenging assay, and reducing power of extract was determined by measuring its ability to act as a free radical scavenger. Gallic acid was used as standard and antioxidant activities of standard gallic acid concentrations at 50 and 800 mg/ml were determined as 6.13 % and 91.91 %, respectively (data not shown). *M. verticillata* methanolic extracts demonstrated simultaneous increase in DPPH radical scavenging activities in a dose-dependent manner. DPPH radical scavenging activity was calculated as 16,3 % ± 4,2 at 10 mg/mL of *M. verticillata* extract concentration. The highest DPPH scavenging activity of methanolic extract of *M. verticillata* was 69,35 ± 3,3 % at 70 mg/mL. The mean percentage of DPPH free-radical scavenging activity at different concentrations of extracts is shown in Table 1. As a basis, the total phenolic content of *M. verticillata* methanolic extract was determined using Folin-Ciocalteu reagent. The total phenolic content of the extract concentrations at 10, 20, 30, 40 and 70 mg/ml was determined as 455±14, 490±6.4, 492±2.4, 497±2.9 and 499±7.5 µg GAE /mg extract, respectively (Table 2). Total flavonoid content of *M. verticillata* methanolic extract was determined using aluminium chloride in a colorimetric method. The results were derived from the calibration curve (Data not shown) ($y = 0.0074x - 0.0158$, $R2 = 0.9709$) of quercetin (10–250 µg/mL) and expressed as µg Quercetin per mg *M. verticillata* extract (µg Quercetin / mg *M. verticillata* extract). Our results showed that flavonoid content of *M. verticillata* was 448±1.8 µg/mg quercetin equivalent at 10 mg/mL extract concentration. TFC of 20, 30, 40 and 70 mg/mL extracts was determined as 475±0.3, 481±0.2, 486±0.2 and 502±1.8 µg/mL, respectively (Table 3). To evaluate the cytotoxic effects, the methanolic extract of *M. verticillata* was subjected to MTT assay using breast cancer cell line MCF-7. MTT assay results showed concentration-dependent growth inhibition in MCF-7 breast cancer cell lines following *M. verticillata* methanolic extract (5-200 µg/ml) application. Results of the cytotoxicity evaluation against MCF-7 cells of the *M. verticillata* extract are shown in Figure 1. The lowest concentration (5 µg/mL) of the extract exhibited 22.92 % ($P=0.0008$) cell viability inhibition compared to control. In addition, increasing concentrations of the extract from 10, 20, 50, 100 to 200 µg/mL

resulted in an increase in cell viability inhibition on MCF-7 cells from 30.22, 38.39, 46.6, 49.74 % to 62.98 % compared to control (P<0.0001), respectively.

Table 1. DPPH radical scavenging activity of the different concentrations of *M. verticillata* methanolic extract. Results are expressed as % radical scavenging activity relative to 100 % radical scavenging activity of gallic acid as a reference. Values are expressed as mean ± standard deviation (n=3).

***M. verticillata* extract concentration (mg/mL)**	**DPPH radical scavenging activity (%)**
10 mg/mL	16,3 ± 4,2
20 mg/mL	29,67 ± 6,5
30 mg/mL	29,08 ± 2,2
40 mg/mL	37,84 ± 2,3
70 mg/mL	69,35 ± 3,3

Table 2. Table shows the total phenolic content of *Malva verticillata* extract expressed as µg gallic acid/mg of extract. Values are expressed as means ± standard deviation (n=3).

	***M. verticillata* extract concentration (mg/mL)**				
	10 mg/mL	**20 mg/mL**	**30 mg/mL**	**40 mg/mL**	**70 mg/mL**
GAE/mg of extract	455±14	490±6,4	492±2,4	497±2,9	499±7,5

Table 3: Table shows the total flavonoid content of *Malva verticillata* extract expressed as quercetin/mg of extract. Values are expressed as means ± standard deviation (n=3).

	***M. verticillata* extract concentration (mg/mL)**				
	10 mg/mL	**20 mg/mL**	**30 mg/mL**	**40 mg/mL**	**70 mg/mL**
µg quercetin/mg extract	448±1.8	475±0.3	481±0.2	486±0.2	502±1.8

Positive control 5µM 5-FU was found to decrease cell viability by 50.48 % (P<0.0001). Furthermore, methanolic extract of *M. verticillata* exhibited significant cell viability inhibition against the MCF-7 cells with an IC50 value of 71.39 µg/mL at 24 hours. Some medicinal plants have attracted attention as alternative cancer therapies because of their low toxicity and ease of affordability (Cassileth and Chapman, 1996). The discovery of novel potential products from bioactive plant

extracts for cancer treatment is the subject of various researches. The current study is focused on gathering information about the antioxidant activity of the *M. verticillata* and demonstrating the potential anticancer effect on MCF-7 breast cancer cell line.

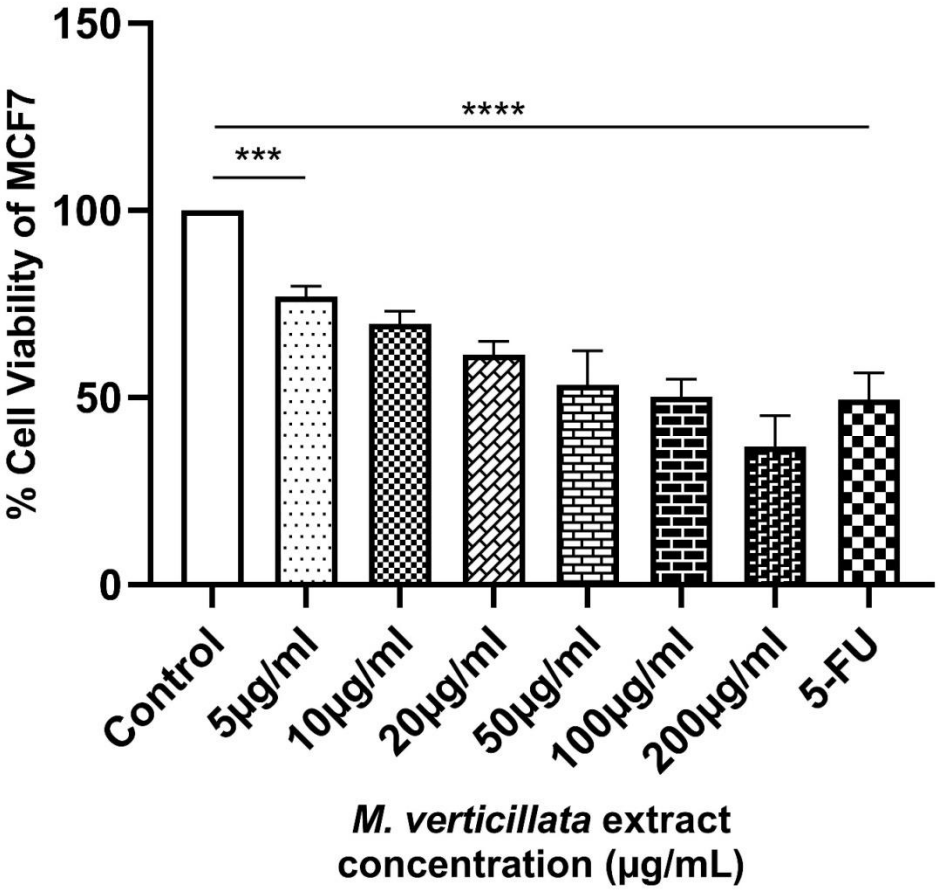


Figure 1: Effects of *M. verticillata* on MCF-7 cell viability. MCF-7 breast cancer cells were treated with different concentrations of *M. verticillata* methanol extract for 24 hours (p=0.0008***, p<0,0001****).

In this study, DPPH, TPC, and TFC tests were used to determine the antioxidant potential of *M. verticillata* methanolic extract. Previous studies have shown that other subtypes of the Malvaceae family have significant DPPH radical scavenging activity, high amount of total phenolic and total flavonoid contents (DellaGreca et al., 2009) (Güder and Korkmaz, 2012) (Choukri Beghdad et al., 2014). In one study, DPPH radical scavenging activity of *M. verticillata* leaves, seed and stem was reported (Bao et al., 2018). Based on our experimental results, *M. verticillata* methanolic leaf extract was found to have 29.67 % DPPH scavenging activity at 20 mg/mL. Total phenolic content evaluation also revealed phenolic content of the extract to be between 455±14 to 499±7.5 µg GAE /mg extract. The results suggest that *M. verticillata* methanolic extract has the potential antioxidant capability. Previous studies also reported that phenolic components have anticancer properties and capability to inhibit different types of cancer formation (Carocho and Ferreira, 2013) (Benetou et al., 2008) (Chahar et al., 2011). To understand the cytotoxicity effect of *M. verticillata* methanolic extract on breast cancer cells, MCF-7 cell line was selected to be

investigated throughout this study. Earlier findings support that M. sylvestris methanolic leaves extract inhibits cell viability of lymphoma cells by 68.65 % and melanoma cells by 76.53 % at 200 µg/mL concentration (Rayssan and Shawkat, 2019). Similarly, in this study, *M. verticillata* methanolic extract exhibited comparable effect with M. sylvestris and inhibited the MCF-7 cell viability by 62.98 % at 200 µg/mL extract concentration. As a result, we investigated the potential antioxidant and anticancer properties of *M. verticillata* which grows in North Cyprus, for the first time. The present findings provide preliminary data exposing *M. verticillata* grows in North Cyprus have potent cytotoxic activity against MCF-7 cells.

4. Conclusion

In summary, this study provides evidence for the significant antioxidant activity and anticancer effect of *M. verticillata* methanolic extract. *M. verticillata* has the potential antioxidant properties that could be beneficial to health either as potential therapeutic agent or as a dietary component. Further studies are required in order to reveal its mechanism, investigate the anticancer effects of *M. verticillata* on different cancer cell lines. al., 2010).

Acknowledgments

-

Conflict of Interest

No conflict of interest was reported by the authors.

References

1. Alara, O.R., Abdurahman, N.H., Olalere, O.A., 2018a. Ethanolic extraction of bioactive compounds from Vernonia amygdalina leaf using response surface methodology as an optimization tool. J. Food Meas. Charact. 12, 1107–1122. https://doi.org/10.1007/ s11694- 018-9726-3
2. Alara, O.R., Abdurahman, N.H., Olalere, O.A., 2018b. Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. Food Bioprod. Process. 107, 36–48. https://doi.org/10.1016/j.fbp.2017.10.007
3. Ashok, A., Ravivarman, J., Kayalvizhi, K., 2020. Underutilized leafy vegetables of India and their pharmaceutical value to provoke human immune system. ~ 1319 ~ J. Pharmacogn. Phytochem. 9. https://doi.org/10.22271/phyto.2020.v9.i3v.11493
4. Bao, L., Bao, X., Li, P., Wang, X., Ao, W., 2018. Chemical profiling of Malva verticillata L. by UPLC-Q-TOF-MSE and their antioxidant activity in vitro. J. Pharm. Biomed. Anal. 150, 420–426. https://doi.org/10.1016/j.jpba.2017.12.044
5. Benetou, V., Orfanos, P., Lagiou, P., Trichopoulos, D., Boffetta, P., Trichopoulou, A., 2008. Vegetables and fruits in relation to cancer risk: Evidence from the Greek EPIC cohort study. Cancer Epidemiol. Biomarkers Prev. 17, 387–392. https://doi.org/10.1158/1055-9965.EPI-07-2665
6. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. Cancer J. Clin. 68, 394–424. https://doi.org/10.3322/caac.21492
7. Carocho, M., Ferreira, I., 2013. The Role of Phenolic Compounds in the Fight against Cancer – A Review. Anticancer. Agents Med. Chem. 13, 1236–1258. https://doi.org/10.2174/18715206113139990301
8. Cassileth, B.R., Chapman, C.C., 1996. Alternative and complementary cancer therapies. Cancer 77, 1026–1034. https://doi.org/10.1002/(SICI)1097-0142(19960315)77:6<1026::AID-CNCR4>3.0.CO;2-F
9. Chahar, M.K., Sharma, N., Dobhal, M.P., Joshi, Y.C., 2011. Flavonoids: A versatile source of anticancer drugs. Pharmacogn. Rev. https://doi.org/10.4103/0973-7847.79093
10. Choukri Beghdad, M., Benammar, C., Bensalah, F., Sabri, F.-Z., Belarbi, M., Chemat, F., 2014. Antioxidant activity, phenolic and flavonoid content in leaves, flowers, stems and seeds of mallow (Malva sylvestris L.) from North Western of Algeria. African J. Biotechnol. 13, 486–491. https://doi.org/10.5897/AJB2013.12833
11. DellaGreca, M., Cutillo, F., D'Abrosca, B., Fiorentino, A., Pacifico, S., Zarrelli, A., 2009. Antioxidant and radical scavenging properties of Malva sylvestris. Nat. Prod. Commun. 4, 893–896. https://doi.org/10.1177/1934578x0900400702
12. Güder, A., Korkmaz, H., 2012. Evaluation of in-vitro antioxidant properties of hydroalcoholic solution extracts Urtica dioica L., Malva neglecta Wallr. and their mixture. Iran. J. Pharm. Res. 11, 913–923. https://doi.org/10.22037/ijpr.2012.1159

13. Khoury, M. El, Haykal, T., Hodroj, M.H., Najem, S.A., Sarkis, R., Taleb, R.I., Rizk, S., 2020. Malva pseudolavatera leaf extract promotes ROS induction leading to apoptosis in acute myeloid leukemia cells in vitro. Cancers (Basel). 12. https://doi.org/10.3390/cancers12020435
14. Kim, D.-O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem.81,321–326. https://doi.org/10.1016/S0308-8146(02)00423-5
15. Klassen, O., Schmidt, M.E., Ulrich, C.M., Schneeweiss, A., Potthoff, K., Steindorf, K., Wiskemann, J., 2017. Muscle strength in breast cancer patients receiving different treatment regimes. J. Cachexia. Sarcopenia Muscle 8, 305–316. https://doi.org/10.1002/jcsm.12165
16. Ko, J.H., Cho, S.M., Joo, S.W., Kim, H.G., Lee, Y.G., Kang, S.C., Baek, N.I., 2018. Glycosyl glycerides from the aerial parts of Malva verticillata and their chemopreventive effects. Bioorg. Chem. 78, 381–392. https://doi.org/10.1016/j.bioorg.2018.03.013
17. Maughan, K.L., Lutterbie, M.A., Ham, P.S., 2010. Treatment of breast cancer. Am. Fam. Physician 81, 1339–1346. https://doi.org/10.1056/nejm199810013391407
18. Mousavi, S.M., Hashemi, S.A., Zarei, M., Bahrani, S., Savardashtaki, A., Esmaeili, H., Lai, C.W., Mazraedoost, S., Abassi, M., Ramavandi, B., 2020. Data on cytotoxic and antibacterial activity of synthesized Fe3O4 nanoparticles using Malva sylvestris. Data Br. 28. https://doi.org/10.1016/j.dib.2019.104929
19. Pervaiz, R., Tulay, P., Faisal, F., Serakinci, N., 2017. Incidence of cancer in the turkish republic of Northern Cyprus. Turkish J. Med. Sci. 47, 523–530. https://doi.org/10.3906/sag-1510-145
20. Radecka, B., Litwiniuk, M., 2016. Breast cancer in young women. Ginekol. Pol. https://doi.org/10.5603/GP.2016.0062
21. Rayssan, R., Shawkat, M.S., 2019. Cytotoxicity Assessment of Malva Sylvestris Crude Extract on Melanoma and Lymphoma Cell Lines. J. Pharm. Sci. Res. 11, 70–74.
22. Shim, K.-S., Lee, C.-J., Yim, N.-H., Ha, H., Ma, J.Y., 2016. A water extract of Malva verticillata seeds suppresses osteoclastogenesis and bone resorption stimulated by RANK ligand. https://doi.org/10.1186/s12906-016-1295-6
23. Taylor, C.W., Kirby, A.M., 2015. Cardiac Side-effects From Breast Cancer Radiotherapy. Clin. Oncol. 27, 621–629. https://doi.org/10.1016/j.clon.2015.06.007
24. van der Heijden, A.G., Jansen, C.F.J., Verhaegh, G., O'Donnell, M.A., Schalken, J.A., Witjes, J.A., 2004. The Effect of Hyperthermia on Mitomycin-C Induced Cytotoxicity in Four Human Bladder Cancer Cell Lines. Eur. Urol. 46, 670–674. https://doi.org/10.1016/J.EURURO.2004.06.009
25. Zurrida, S., Veronesi, U., 2015. Milestones in breast cancer treatment. Breast J. 21, 3–12. https://doi.org/10.1111/tbj.12361

Research Article
Curr. Pers. MAPs, (2020); 3(2): 121-127

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

A Study on Antimicrobial and Antioxidant Activities of *Cyclamen coum Colchicum turcicum* and *Colchicum bornmuelleri* Species

Fatih ÖZCAN, Alican Bahadır SEMERCİ, Kenan TUNÇ*

Department of Biology, Faculty of Arts and Sciences, Sakarya University, Sakarya, TURKEY
*Corresponding author : ktunc@sakarya.edu.tr

https://doi.org/10.38093/cupmap.834545

Received : 03/12/2020
Accepted : 19/12/2020

Abstract

In this study, antimicrobial effects of *Cyclamen coum* Mill., *Colchicum bornmuelleri* Freyn and *Colchicum turcicum* Janka extracts prepared with ethanol against *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *S.typhimurium* ATCC 14028, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 1029 and *S. aureus* ATCC 29213 microorganism were investigated by using Disk Diffusion method. In additon, DPPH scavening activities, antioxidant activities and total phenolic content were determined with Folin-Ciocalteu method. It was determined that extracts obtained from *C. coum* plant showed moderate antifungal activity on *C. albicans*. It has been determined that aerial extracts of *C. bornmuelleri* and *C. turcicum* plants have antibacterial effects on *E. faecalis*. These extracts did not showed antibacterial activty on other test microorganisms.The highest and lowest antioxidant activity results (IC_{50} values) obtained from the extracts were determined as *Colchicum turcicum* aerial parts 14.2 µg/mL, *Colchicum bornmuelleri* aerial parts 768,65 µg/mL. When the extracts used in this study were compared with the standart antioxidant ascorbic acid, it was found that the aboveground extracts of *C. bornmuelleri* and *C. turcicum* showed high antioxidant activity. The total amounts of phenolic substances was determined as 191,85 mg GA/100 g in the extracts obtained from the aerial part of the *C. bornmuelleri* plant.

Key Words: Antimicrobial, Antioxidant, Geophyt, Total phenolic content

1. Introduction

Geophytes are bulbous, tuberous and rhizome plants whose stems can store nutrients by differentiating under the ground. These plants are spread around the world in regions where the Mediterranean climate prevails, with warm and humid winters and hot and dry summers (Tanrıverdi, 2019). In addition to being used as an ornamental plant with its beautiful leaves and flowers, it has also been widely used medicinally and aromatically. Medicinal aromatic plants are known to have antimicrobial and antioxidant properties due to the alkaloids, tannins, flavonoids, phenolic compounds and fatty acids they contain (İnceçayır et. al., 2019). These compounds have mechanisms of action, such as scavenging free radicals, chelating metal, reducing or inhibiting reactive oxygen generation. In addition, they prevent the formation of free radicals with the aromatic rings they contain (Çoban and Batır, 2010).

Cylamen coum, which is a geophyte plant species, has flattened spherical tubers that

are usually smaller than 3.5 cm (Davis, 1984). This plant is known with local names such as ground nut and pork apple in Anatolia (Güner, 2012). It is frequently preferred as an ornamental plant with its heart-shaped leaves and pink flowers. Apart from its use as an ornamental plant, it is also used in the pharmaceutical and chemical industry due to its secondary metabolites such as coumoside, cyclominorin, deglucocyclamine, cyclacumin, mirabilin lacton, cyclamiretin and cyclamigenin (Çalış et. al., 1997; Yaylı et. al., 1998; Bokov et. Al., 2020). Although the *Colchicum* genus is more common in the Northern Hemisphere, especially in the Mediterranean Region, it spreads to the North of Europe, North Africa and the Himalayas (Düşen, 2005). Some of the *Colchicum* species bloom in the spring, while others bloom in the fall. Fall-blooming species usually have larger bulbs and seeds than spring-blooming varieties. *Colchicum*, one of the natural geophytes of Anatolia, is frequently used both as an ornamental plant and as a medicinal plant with various alkaloids it contains. *Colchicum* species are given local names such as Bitterberry, Belladonna, Orchid Flower, Lycophor and Tarhan Flower (Düşen, 2005).

Colchicine, which is the best known of the alkaloids obtained from the Colchicum plant, is used as a drug of choice in the treatment of Gout Disease, Familial Mediterranean Fever, Behçet's Disease because it has anti-inflammatory properties and is known to have antitumor properties (Akbulut, 2009; Brossi, 1990). Anticholinesterase, isoquinoline alkaloids and phenolic acids such as coumaric acid, ferulic acid, kaffeic acid, vanillic acid, 2-hydroxybenzoic acid have been isolated from different *Colchicum* species (Azadbakht et. al., 2020).

In this study, the antimicrobial activities of ethanolic extracts prepared using bulb, leaves and flowers of *Cyclamen coum* species and corm and aerial parts of *Colchicum turcicum* and *Colchicum bornmuelleri* were investigated against test microorganisms by disk diffusion method. In addition, it was aimed to determine the antioxidant activities and total phenolic contents of the extracts obtained by DPPH scavenging method.

2. Material and Methods

2.1. Material: *Cyclamen coum* Mill. and *Colchicum turcicum* Janka species used in this study were collected from the under forest vegetation of Çilekli village (Sakarya). *Colchicum bornmuelleri* Freyn species was collected from the Tuzla Village (Sakarya)(see Fig. 1). The plants were washed and left to dry in the shade at room temperature for seven days.

Figure 1. A: *Cyclamen coum*, B: *Colchicum turcicum*, C: *Colchicum bornmuelleri*

All the chemicals and reagents (Ethanol, Methanol, Gallic Acid, Ascorbic Acid, Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Triptic Soy Broth, Mueller Hinton Agar (MH Agar) and Sodium Carbonate) used in the study were of the analytical grades and obtained from SIGMA company Germany.

2.2. Preparation of extracts: All the milled plant parts were placed in screw-capped glass bottles to be 100 g and 100 ml of

ethanol was added on them. These blends were mixed in a magnetic stirrer for 72 hours. A rotary evaporator was used at 40-45 °C to remove plant extracts from the solutions. For antimicrobial activity experiments 6400 µg/disk and for antioxidant activity and total phenolic content 1000 µg/mL ethanol extract stocks were prepared.

2.3. Disc diffusion method: All the strains of bacteria used in this study were purchased from Microbiology Research Laboratory of Sakarya University. To determine the antimicrobial activities of the extracts Disk Diffusion Method was used. 15 µL of the plant extracts prepared were taken and absorbed into sterile empty discs. The discs were allowed to dry for 2 hours at room temperature in the dark. 0,5 Mcfarland density suspension was prepared with previously activated microorganisms by using a densitometer. Inoculation was carried out in a sterile environment with a swab from prepared microorganism suspensions to MHA media. The discs impregnated with plant extract were left on the planted MHA medium with forceps and incubated at 37 °C for 24 hours. Ethanol impregnated discs were used as negative control, and Gentamicin loaded discs were used as positive control. After 24 hours, the antibacterial effect of plant extracts against microorganisms was evaluated by measuring the inhibition zone diameters around the disc using a digital caliper.

2.4. Antioxidant activity: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated according to the Blois (1958) method with minor modifications. Briefly, 1 mL of ethanolic extracts prepared in different concentrations was taken and 1 mL of 0.04 % solution of DPPH radical in ethanol was added on it. After mixing with vortex, it was kept at room temperature in the dark for 30 minutes and absorbances were recorded at 517 nm in the spectrophotometer. For the control, 1 mL of DPHH was added to 1 mL of ethanol solution. The percentage of DPPH scavenging activity of the extract was calculated using the following equation:

%DPPH radical scavenging = [(control absorbance- extract absorbance)/control absorbance] x 100

2.5. Total phenolic content: Total amount of phenolic content was determined by making minor changes in the Folin-Ciocalteu method of Singleton and Rossi (1965). 100 µL of the prepared extract was taken, 200 µL of 50% Folin-Ciocalteu reagent was added and left for 2 minutes. 1 mL of 2% sodium carbonate solution was added and the absorbance was read at 76 nm after one hour of incubation at room temperature in the dark. The total phenolic content was determined in mg / 100 g using Gallic Acid Standard.

2. Results and Discussion

The emergence of multidrug resistance in human and animal to pathogenic bacteria, as well as the undesirable side effects of some antibiotics, has increased interest in researching new antimicrobial drugs of plant origin. Traditionally used herbs produce a variety of compounds with known therapeutic properties. Plant-derived antimicrobial compounds can be formed in the branches, roots, leaves, bark, flowers or fruits of the plants (Ahmad and Beg, 2001; Albayrak and Kaya, 2019; De zoysa et. al., 2019). Considering the great potential of plants that are sources of antimicrobial drugs, this study will make significant contributions to the literature. In our study, the antimicrobial activities of extracts obtained from Cyclamen coum bulb, leaf and flower parts, Colchicum bornmuelleri and Colchicum turcicum from corm and aerials parts were evaluated, and the results are given in Table 1.

Table 1. Antimicrobial activity of extracts used in the study on test microorganisms

Samples (6400 µg/disk)		Test microorganism (Inhibision zone diameters, mm±SD)						
		Bs	**Ec**	**Ef**	**Se**	**Sa**	**St**	**Ca**
C. turcicum	Aerial parts	0	0	16.5±0.3	0	0	0	-
	Corm	0	7±0.3	0	0	0	0	-
C. bornmüelleri	Aerial parts	0	0	10.5±0.8	0	0	0	-
	Corm	0	7±0.7	0	0	0	0	-
C. coum	Bulb	0	0	8	0	0	0	8±1.0
	Leaf	0	0	0	0	0	0	0
	Flower	0	0	0	0	0	0	11±0.3
P. control	Gc	17	19	20	21	20	21	-
	Amp	-	-	-	-	-	-	16
N. control	Etanol	0	0	0	0	0	0	0

Ec*: Escherichia coli*, Se: *Staphylococcus epidermidis*, Ef: *Enterococcus faecalis*, Ca: *Candida albicans*, Bs: *Bacillus subtilis*, Sa: *Staphylococcus aureus*, St: *Salmonella typhimurium*, Gc: Gentamicin, Amp: Amphotericin B, - no tested.

The results revealed that aerial part extracts of both *Colchicum* species had moderate antibacterial effect against *E. faecalis* bacteria. Aerial extracts of *C. turcicum* and *C. bornmuelleri* species did not show antibacterial activity against other test microorganisms. The aerial extracts of the same plant species had showed a low level of antimicrobial effect against E. coli bacteria only.

The study revealed that the ethanolic extract prepared using all parts of *Colchicum balaense* plant created an inhibition zone diameter of 10 mm on *S. aureus*, 8 mm on *E. faecalis* and 9 mm on *E. coli*. (Mammadov et. al., 2009). In our study, it was observed that extracts obtained from the corm parts of *Colchicum* species formed 7 mm on *E. coli* and the extracts obtained from the aerial parts formed 10.5 mm and 16.5 mm inhibition zone diameters on *E. faecalis*. The difference between the studies may be due to the different plant species, as well as the place where the plants are collected, extract preparation methods, and the use of different plant parts while preparing the extract. As similar as our study, Hanif et al. (2010) reported that extracts obtained from *Colchicum autumnale* L. species did not show antimicrobial activity on *B. subtilis* bacteria. Türker and Usta (2008) reported that aqueous extracts of *Cyclamen coum* leaves did not show antibacterial effects on *E. coli, P. aeruginosa*, *S. epidermidis* and *S. aureus*. In our study, it was determined that *Cyclamen coum* leaf extracts did not show antimicrobial activity on the test microorganisms used.

Semerci et al. (2019) reported that methanolic extracts of *Cyclamen coum* flowers created an inhibition zone diameter of 9.5 mm on *C. albicans*. Similarly, it was observed that ethanol flower extracts created an inhibition zone diameter of 11 mm on *C. albicans*. Low antimicrobial activity does not mean that the plant does not contain bioactive compounds or that the plant has no activity against microorganisms. The fact that the extracts contain insufficient amounts of active ingredient to show antimicrobial activity can also cause negative results. Medicinal plants are generally rich in flavonoids, tannins, coumarins, lignans and phenolic compounds. The antioxidant properties of polyphenols are due to their redox properties such as being a reducing agent and hydrogen donor, acting as a metal chelator, and binding reactive oxygen. Polyphenolics exhibit a wide variety of biological effects such as antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic (Piluzza and Bullitta, 2011; Ben Yakoub et. al., 2018). In this study, it was determined that aerial part extracts of *Colchicum* species have higher antioxidant activity and total phenolic

content (Table 2). The highest antioxidant activity was found in the extract prepared from the aerial part of the *Colchicum turcicum* plant. The lowest antioxidant activity was observed in the extracts obtained from the corm parts of *Colchicum bornmuelleri* plant.

Table 2. Antioxidant activities and total phenolic contents of the extracts

Samples	TPC(mgGA/100g)±SD	IC_{50} µg/mL(±SD)
C. bornmuelleri aerial parts	191.85±1.2	23.78±2.6
C. bornmuelleri corm	17.76±0.6	768.65±1.0
C. turcicum aerial parts	162.2±2.5	14.2±3.0
C. turcicum corm	13.3±1.3	530.92±5.9
C. coum bulb	43.41±0.2	145.15±1.1
C. *coum* leaf	46.4±0.8	151.68±1.9
Ascorbic acid	-	3.6±0.1

Kılıç et al. (2014) calculated the IC_{50} values of water and acetone extracts obtained from the root and stem parts of the *Colchicum turcicum* plant as 29.98 µg/mL and 45.74 µg/mL, respectively. In the same study, the total phenolic content of the extract prepared with water was 0.454 mg/g, and the total phenolic content of the extract prepared with acetone was 2.172 mg/g. In our study, the IC_{50} value of the aerial extract prepared with ethanol was calculated as 530.92 µg/mL and the total phenolic value was calculated as 13.3 mg GA/100 g. The differences between these studies may vary according to the place where the plant is collected, the solutions used in the extract preparation and the plant parts used. Mammadov et al. (2009) examined the antioxidant activities of extracts obtained from the corm and aerial parts of the *Colchicum balaense* plant by using percentage of DPPH scavenging ratio. At the end of the study, it was reported that the extracts obtained from the aerial parts of the *C. balaense* plant showed higher antioxidant activity. In another study, the scavenging rate of the ethanolic corm extracts prepared from *Colchicum autumnale* plant was 34% and the scavenging rate of flower extracts was 52% (Suica-Bungez et al., 2017). Similar to the literature data our study revealed that the aerial parts of *Colchicum* species show higher antioxidant activity compared to the corm parts.

Jaradat et al. (2017) calculated the IC50 value as 31 µg/mL and the total phenolic content amount as 32.7 mg GA/100 g in the methanol extracts they obtained using the aerial parts of the *Cyclamen coum* plant. In this study, IC_{50} value of *Cyclamen coum* plant leaf extracts prepared with ethanol was calculated as 151.68 µg/mL and total phenolic content amount as 46.4 mg GA/100 g. While the total amount of phenolic matter was similar in those two studies, it was observed that there were differences in DPPH scavenging activity which could be linked with differences in the used plant parts and type of the extracting agent.

4. Conclusions

The study revealed that the aerial parts of *Colchicum turcicum* and *Colchicum bornmuelleri* species showed higher antioxidant activity compared to the ascorbic acid as the standard antioxidant. It

is thought that these herbs can be used in a wide variety of fields such as the pharmaceutical industry, food supplements and food preservatives as potential sources of antioxidants. Although it is seen that the plants used in the study do not have high antimicrobial activity, we think that it is important to clarify the chemical structures by purifying the contents of bioactive substances with different solutions.

Acknowledgements
Thanks Dr. Mehmet Sağıroğlu for identification of plants

Conflict of Interest
The authors declare that they have no conflict of interest.

References

1. Ahmad, I. Beg, A.Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74: 113–123.
2. Akbulut, S. 2009. Hamsiköy Yöresinde Odun Dışı Bitkisel Ürün Olarak Alchemilla Spp. Ve Colchicum Speciosum' un Envanteri Üzerine Bir Araştırma. Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Orman Mühendisliği Bölümü, Doktora Tezi.
3. Albayrak, S. Kaya, O. 2019. Antioxidant, Antimicrobial and Cytotoxic Activities of Endemic Astragalus argaeus Boiss. from Turkey. Hacettepe J. Biol. & Chem., 47 (1): 87–97
4. Azadbakht , M. Khoshvishkaie, E., Davoodi, A., Hosseinimehr, S.J., Azadbakht, M., Emami, S., Jouybari, H.B., Mirzae, F., Ghadiri, K. 2020. Optimization of Ultrasound-Assisted Acidic-Solvent Extraction of Colchicine from Colchicum kurdicum (Bornm.) Stef. Using Response Surface Methodology, Research Journal of Pharmacognosy ,7(3): 5-13
5. Ben Yakoub,A.R. Abdehedic, O., Jridic, M.,Elfallehd, W., Nasric, M., Ferchichi,A. 2018. Flavonoids, phenols, antioxidant, and antimicrobial activities in variousextracts from Tossa jute leave (Corchorus olitorus L.). Industrial Crops & Products 118: 206–213
6. Blois MS, (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181 (1958) 1199. 13
7. Bokov, D. O. Krasikova, M. K., Sergunova, E. V., Bobkova, N. V., TYu, K., Bondar, A, Marakhova A, Morokhina S, Krasnyuk I, Moiseev D. 2020. Pharmacognostic, Phytochemical and Ethnopharmacological Potential of Cyclamen coum Mill. Pharmacognosy Journal, 12(1): 204-212.
8. Brossi, A. 1990. Bioactive Alkaloids. 4. Results of Recent Investigations with Colchicine andPhysostigmine., Journal Of Medicinal Chemistry, 33(9).
9. Çaliş, İ. Yürüker, A., Tanke,r N., Wright, A.D., Sticher, O. 1997. Triterpene Saponins from Cyclamen coum var. coum. Planta Med., 63(2): 166-70.
10. Çoban, Ö.E. Batır, B. 2010. Antioksidan Etkili Bazı Bitki ve Baharatların Gıdalarda Kullanımı, Electronic Journal of Food Technologies 5(2) 7-19.
11. Davis, P.H. 1978 Flora of Turkey and The East Aegean Islands, 6. Cilt, Edinburgh Univ. Press, London, 128–135.
12. De Zoysa, M.H.N. Rathnayake, H., Hewawasam, R.P., Wijayaratne, W.M.D.G.B. 2019. Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. Hindawi International Journal of Microbiology
13. Düşen, O.D., 2005. Akdeniz Bölgesi'nde Yayılış Gösteren Colchicum L. (Liliaceae) Cinsine Ait Türlerin Taksonomik Yönden Araştırılması. Akdeniz Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi.
14. Güner, A., Aslan, S., Ekim, T., Vural, M. ve Babaç, M.T. (edlr.). 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul
15. Hanif, M.A. Bhatti, H.N., Jamil, M.S., Anjum, R.S., Jamil, A., Khan, M.M. 2010. Antibacterial and Antifungal Activities of Essential Oils Extracted from Medicinal Plants Using CO2 Supercritical Fluid Extraction Technology Asian Journal of Chemistry, 22(10): 7787-7798.
16. İnceçayır, D. Semerci, A.B., Mustafa, N., Tunç, K. 2019. Catalpa bignonioides Metanolik Çiçek Ekstraktının Biyolojik ve Kimyasal Aktivitesi. Türk Tarım ve Doğa Bilimleri Dergisi, 6(2): 230–234.
17. Jaradat, N.A. Al-Masri, M., Hussen, F., Zaid, A.N., Ali, I., Tammam, A., Odeh, D.M., Shakarneh, O.H., Rajabi, A. 2017. Preliminary Phytochemical and Biological Screening of Cyclamen coum a Member of Palestinian Flora. Pharmaceutical Sciences, 23, 231-237.
18. Kılıç,I. Yesiloglu, Y., Bayrak, Y. 2014. Investigation on the Antioxidant Activity of Roots and Stem of Colchicum turcicum L. Asian Journal of Chemistry; 26(1), 5-9.

19. Mammadov, R. Düşen, O., Demir, D.U, Köse, E. 2009. Antioxidant and antimicrobial activities of extracts from tubers and leaves of Colchicum balansae Planchon. Journal of Medicinal Plants Research ,3(10): 767-770.
20. Piluzza, G. Bullitta, S. 2011. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharmaceutical Biology, 49(3): 240–247.
21. Semerci, A.B. Inceçayir, D., Tunç, K., Sağıroğlu, M. 2019. Antimicrobial Activity of Different Flower Extracts. Curr. Pers. MAPs, 2: 53-58.
22. Singleton VL, Rossi JA, (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Viticult, 16 (2017) 144.
23. Suica-Bunghez, I.R. Ion, R.M., Teodorescu, S., Sorescu, A.A., Stirbescu, R.M., Stirbescu, N.M. 2017. Fitochemical And Antioxidant Characterization Of autumn Crocus (Colchicum Autumnale) Flowers and Roots Plant Extracts. Journal of Science and Arts, 3 (40), 539-546.
24. Tanrıverdi O, D. 2019. Yalova İli Geofitleri ve Peyzajda Kullanım Olanakları. Bursa Uludağ Üniversitesi, Fen Bilimleri Enstitüsü, Peyzaj Mimarlığı Bölümü, Yüksek Lisans Tezi.
25. Türker, A. U. Usta, C. 2008. Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. Natural Product Research, 22 (2):136–146.
26. Yaylı, N. Baltaci, C., Zengin, A., Küçükislamoglu, M., Genç, H. 1998. Triterpenoid saponin from Cyclamen coum. Phytochemistry, 48(5): 88, 1-4

Research Article
Curr. Pers. MAPs, (2020); 3(2): 128-134

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

In vitro Antimitotic Activity of Gall Extract of *Pistacia terebinthus*

Bellifa NAZIM[1*], Benhaddou ISMAIL[2], Toumi HOUARI[3], Norredine ZAKARIA[4], Ferkous HOUSSEM[5]

[1]Department of Pharmacy, Pharmacognosy Laboratory, Faculty of Medecin, University of Djilali Liabes, 22000, Sidi Bel Abbes, ALGERIA
*Corresponding author: nazim.bellifa@univ-sba.dz
[2]Department of Pharmacy, Pharmacognosy Laboratory, Faculty of Medecin, University of Djilali Liabes, 22000, Sidi Bel Abbes, ALGERIA
[3]LRDP Pharmacology unit EHU Oran, University of ORAN, 31000, Oran, ALGERIA
[4]Department of Pharmacy, Analytical Chemistry Laboratory, Faculty of Medecin, University of Tlemcen, 13000, Tlemcen, ALGERIA
[5]Department of Pharmacy, Laboratory of Botany, Faculty of Medecin, University of Djilali Liabes, 22000, Sidi Bel Abbes, ALGERIA

10.38093/cupmap.830383

Received : 23/11/2020
Accepted : 22/12/2020

Abstract

Pistacia terebinthus L. - turpentine tree - is a perennial flowering plant in the Mediterranean region, some aphids species induce the formation of galls in *Pistacia terebinthus* formerly called Carobs of Judea whose tannin content equals 60%, used in traditional medicine as a stimulant, diuretic, astringent for the treatment of asthma and other respiratory and urological affections. In the present study, the antimitotic activity of *Pistacia terebinthus* galls was evaluated using meristematic cells of *Allium cepa* roots assay our results reveal that the methanolic extract decreased the root length and dividing cell number significantly after 96 h and compared to control ($p < 0.05$) the mitotic index of extract at the concentration 4 mg/ml was 31% and has significant activity near to the standard methotrexate. Overall, the methanol extract of *P. terebinthus* galls revealed the presence of the phytochemical's compounds such as gallic acid, caffeoylquinic acid, which affect plant mitosis and can be used as an antimitotic drug.

Key Words: *Allium cepa*, Antimitotic activity, Galls, Caffeoylquinic acid, *Pistacia terebinthus*

1. Introduction

Pistacia terebinthus L. (the Terebinth) is a deciduous tree or shrub from North Africa, referring to the Anacardiaceae, a fairly large family that includes 600 species and 70 genera (Bozorgi, 2013). The genus *Pistacia* has ten species, characterized by having alternate, pinnate leaves (Lin., 1984) and secretory cavities that contain resinous compounds. The plant owes its name to the oleoresin known as CHIO turpentine, it is the first turpentine known by Discords and this name was later extended to the oleoresin of conifers (Denoël., 1958). This tree is one of the components of the Mediterranean shrub, particularly in Algeria, where there are large stands, especially in the Tessala park, known

as Betoum el Kiffan in Arabic and Hejji Kabyle (Lapie,1914).

Pistacia terebinthus produces a rich mixture of substances, including resin, essential oils, proteins, organic acids, sugars, flavonoids, and tannins. The leaves are often attacked by aphids, which stimulate the plant to form galls in the leaves. The gall contains a mixture of 60% resin, 36% tannins and 4% essential oil (Pulaj. 2019). Several studies have been engaged in different locations where the species grows in the wild to evaluate and compare its composition and biological proprieties, including as a bioherbicide and antifungal. anti-inflammatory (Giner, 2002; Kordali, 2003; Kıvçak, 2004; Remila, 2015). While the antimitotic properties of this species have never been evaluated.

Some plant-derived compounds such as combretastatin, paclitaxel, colchicine, and vincristine are important antimitotic agents of cytotoxic drugs (Mukhtar, 2014). A large number of new drugs isolated from plants have been used for treating cancer. Most of these drugs are the secondary plant metabolites including phenolics and alkaloid (Calzada, 2020) These phytoconstituents are usually active against various types of cancers (Conforti, 2008).

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals which modify the normal course of mitosis, In *Allium cepa* L. root tip model root system of plant cells, it is commonly used as a test for investigating environmental pollution factors, the toxicity of chemical compounds and evaluating potential anticancer properties. It is easy to make preparations of onion roots; they contain rather homogenous meristematic (Kuras, 2016). This study aimed to evaluate the composition of the methanol extracts of galls from *Pistacia trebinthus* and to test them for potential antimitotic activity on meristematic cells of Allium cepa roots.

2. Material and Methods

Pistacia terebinthus galls induced by aphids were collected by Dr. Bellifa Nazim in September and November in Tessala (35°16'22.9"N 0°47'08.8"W) state of Sidi Bel Abbes Western Algeria, the plant material was authenticated by Dr. Ferkous Houssem botanist of the pharmacy department Sidi bel abbes and Pr Alvarez R university Leon Spain, A specimen has kept in the herbarium of the department (Fig. 1).

Galls were dried and reduced into powder (100 g) separated into 3; Soxhlet extraction technique was adopted to get crude extracts using methanol as solvent. The extract obtained was concentrated in a rotary evaporator at low pressure and temperature to give crude methanol extracts, tannins were precipitated by ammoniac to give 48 g than the residue was extracted by chloroform according to the Stas Otto Method (Cortes, 2019). TLC was carried (DCM- MeOH-H2O, 77:13:10) to give gallic acid Rutin and caffeoylquinic acid, these compounds were determined using UV visible and Infrared spectroscopy (Jahangirian, 2011) using the spectral database (Bio-Rad) as well as a direct comparison of TLC with authentic samples (Sigma) available in our laboratory.

Ultraviolet-Visible spectroscopy: The chloroform fraction (7 g) was resolved by column chromatography (silica gel, 180 g) using a step gradient of a DCM-MeOH solvent system to give carboxylic phenol acid. The evaluation of the anti-mitotic activity of the MeOH fraction was made as described by Sehgal et al. (2006) with modifications (Grant, 1982; Fiskesjo, 1988; Melappa, 2017). Using *Allium cepa* root meristematic cells which have been used extensively in the screening of drugs with a natural antitumoral origin (Fig 2).

Fig 1 : Galls of *Pistacia terebinthus*

Fig 2 Antimitotic Activity after treatment of *A. cepa* root with different MeoH extracts of Galls

A. cepa bulbs (70 ± 10 g) were purchased from the local market and grown in beakers at room temperature for 48 h the bulbs with roots measuring 2–3 cm were transferred to beakers containing extracts at different dilutions (0.5, 1, 2 and 4 mg/mL) in tap water.

A blank with water was used as control. Methotrexate was used as a standard control. All the groups were incubated at 25±2 °C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h. After, the root tips were fixed with fixing the solution of acetic acid and alcohol. Squash preparations were made by staining the treated roots with acetocarmine stain and Giemsa and May Grünwald stain. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 3-5 fields of view using high resolution (100x) light microscopy (Leica). The mitotic index was calculated as

Mitotic Index = Number of dividing cells/Total number of cells x 100

3. Results and Discussion

In the present work, we displayed the strong anti-mitotic effect of methanol extract of the galls from Pistacia terebinthus towards *Allium cepa* root development; this model has been used for evaluating anti-mitotic activity (Raheel, 2017).

As already mentioned, aphids induce the host plant to secrete a range of phytochemical compounds such as polyphenols and terpenes, which under normal conditions, the plant would not generate this explains the difference in content between the leaf of *P. terebeinthus* and the extract of the galls, this can be explained by the interference of the metabolism of the plant and the aphid for example the pathways of development of auxin and cytokinin in plants. The high phenolic content helps explain the use of this plant in traditional medicine. Indeed, these compounds are widely known for their antiviral, antispasmodic, anti-tumor, hypocholesterolemic, anti-inflammatory, anti-hypertensive and antimicrobial activities. The extract was purified by TLC or CC and pure compounds were obtained: Hydroxamic acid and evaluated for antimitotic activity showed significant activity near to the standard. The MeOH extract of galls from *Pistacia terebinthus* presented strong and dose-dependent anti-mitotic activity in terms of decrease in mitotic index, mean root length and the number of dividing cells, the results were correlated with water used as a control in which 78% mitotic index was observed with actively dividing cells at various stages of mitosis (Fig. 3).

The results were analyzed based on mitotic index and are presented in Table 1 reflect the effect of various extracts on mitotic index. The methanolic extract of *Pistacia terebinthus* galls decrease the root length and dividing cell number significantly after 96h and compared to control ($p< 0.05$) as shown in the Table 1.

The mitotic index of *P. terebinthus* extract at the concentration 4 mg/ml was 31% and has significant activity proximate to the standard methotrexate 30% (0.1 mg/ml) Table1 shows the antimitotic activity of different extracts and methotrexate. Our results on these meristematic cells model corroborate previous works in which the mitotic index of extract decrease dividing cells number as root length was described Raheel, R 2017. And had an excellent anti-mitotic activity that was comparable to the activity of methotrexate. A maximum number of non-dividing cells were observed.

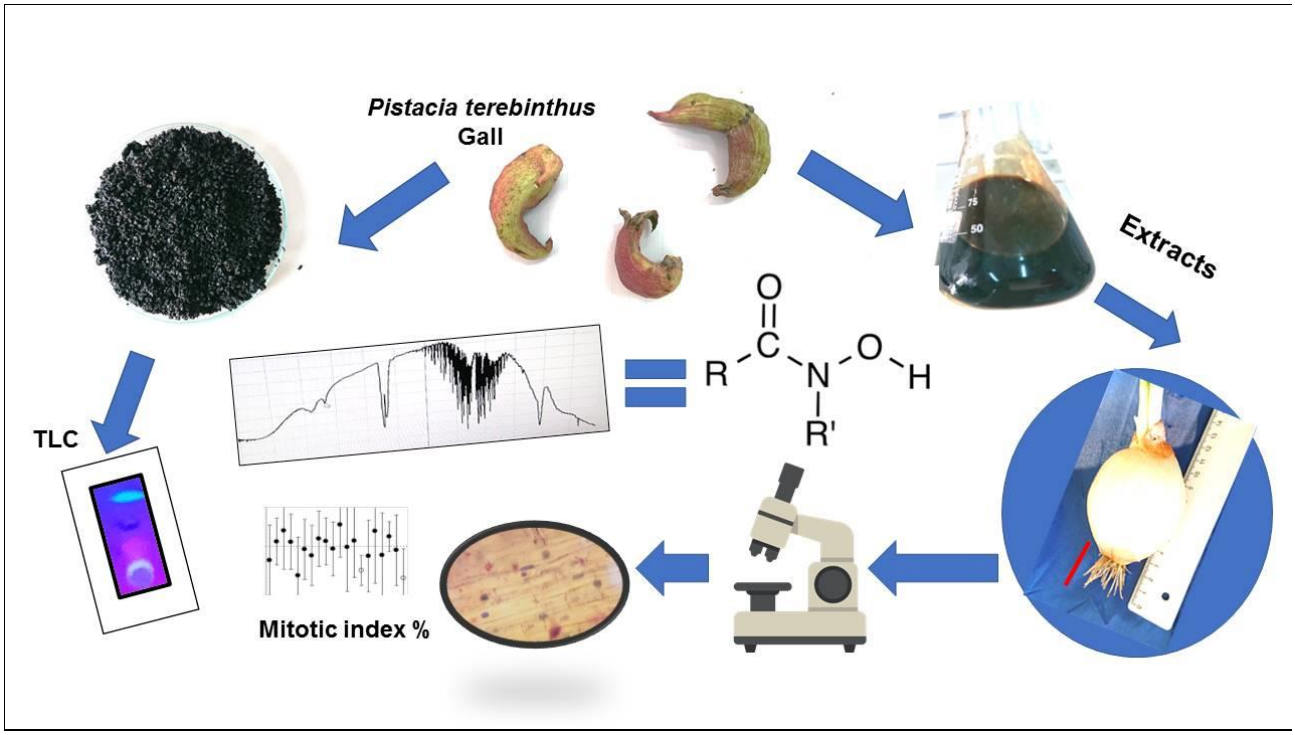


Figure 3. Dividing cells at various stages of mitosis

Table 1: The effect of different extracts on mitotic index

Extract concentration (mg/mL)	Mean root Length (mm)	Number of dividing cells	Average number of total cells	Mitotic Index (%)
Water	45 ±1.5	47	60	78
0.5	40 ±2	41	60	68
1	40 ±1.2	37	60	61
2	30 ±2.3	23	60	38
4	20 ±1.6	19	60	31
Methotrexate (0.1 mg/mL)	15 ± 1.5	18	60	30

±Values are expressed as Mean± SD for triplicates

Cytotoxicity tests using in vivo plant systems, such as Allium cepa, are validated by several researchers, who have jointly conducted in vitro animal tests and the results obtained are similar. The *Allium cepa* test is one of the few direct methods of measuring cellular damage and analysis of cytotoxicity and genotoxicity because the roots are in direct contact with the extract often associated with microtubular disturbances.

Phytochemical characterization of the MeOH extract of Gall from Pistacia revealed the presence of the phytochemicals- Alkaloid, polyphenols, flavonoids, terpen, saponin glycosides and reducing sugars. In our study, it may be suggested that the extract may be acting through the pathway inhibiting tubulin that is required for DNA synthesis that arrest cell division. Methotrexate is known as an anticancer drug which competes with folic acid for the enzyme reductase Also Adamaskis et al had reviewed the antimitotic activity of Bisphenol and Taxol by immunofluorescence microscopy in meristematic root cells their study reported an elevation of tubulin

acetylation on *A. cepa* root. caffeoylquinic acid is a potent moiety not only in the field of cancer therapy but also as a mutagenic agent. Among the various derivatives of carboxylic phenol acid, is considered as a potent anticancer agent. Scientists from different corners synthesized different phenolic acid moieties groups and have been evaluated as antimitotic agents. (Zhu, 2011).

Caffeoylquinic acids have attracted considerable interest recently because of their capacity to inhibit a variety of enzymes such as metalloproteases, some carboxylic phenol acid, such as caffeic acid have been used clinically for the treatment of cancer or iron-overload diseases. Much of the activities of these carboxylic phenol acids were thought to be due to their metal chelating properties (Witte, 2000; Jahangirian, 2011).

4. Conclusion

In conclusion, our results indicate the following: caffeoylquinic acid affects plant mitosis it maybe explains by disrupting microtubule organization. this result can explain the resistance of plants against aphids and open the possibility of exploiting *P. terebinthus* galls as a source of therapeutic agents.

Acknowledgments

The authors thank Dr. Benouala for his assistance and all the members of pharmacognosy Laboratory. Thanks, are also due to Pr Alvarez R for his contribution and assistance. UV-Vis spectra were recorded on a SHIMADZU 600 spectrophotometer in the range of 200 nm to 700 nm with 1 nm data interval. Scan speed was set to 400 nm min−1. All samples were prepared in methanol. Infrared spectroscopy. FT-IR spectra were recorded using a SHIMADZU IR Affinity-1S in the range of 500 to 3750 cm−1.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Adamakis, Ioannis-Dimosthenis S.; Panteris, Emmanuel; Eleftheriou, Eleftherios P. 2019. "Tubulin Acetylation Mediates Bisphenol A Effects on the Microtubule Arrays of Allium cepa and Triticum turgidum." Biomolecules 9, no. 5: 185.
2. Bozorgi, M., Memariani, Z., Mobli, M., Salehi Surmaghi, M. H., Shams-Ardekani, M. R., & Rahimi, R. 2013. Five Pistacia species (P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus): a review of their traditional uses, phytochemistry, and pharmacology. TheScientificWorldJournal, 219815. https://doi.org/10.1155/2013/219815
3. Calzada, F., Merlin-Lucas, VI, Valdes, M. 2020 Métabolites secondaires et propriétés biologiques d'Annona muricata .Rev., Bras. Farmacogn. https://doi.org/10.1007/s43450-020-00012-w
4. Conforti, F., Ioele, G., Statti, G. A., Marrelli, M., Ragno, G., & Menichini, F. 2008. Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. Food and chemical toxicology, 46(10), 3325-3332.
5. Cortes, Diego, Moreno, Laura, Párraga, Javier, Galán, Abraham et Cabedo, Nuria. 2019. Nouveaux médicaments inspirés des annonacées. Revista Brasileira de Fruticultura , 36 (spe1), 22-31. https://doi.org/10.1590/S0100-29452014000500003
6. Denoël A 1958 Matière médicale végétale (pharmacognosie). Presses Universitaires de Liège, 2e éd.
7. Fiskesjö, G. 1988. The Allium test—an alternative in environmental studies: the relative toxicity of metal ions. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 197(2), 243-260.
8. Giner-Larza, E. M., Máñez, S., Giner, R. M., Recio, M. C., Prieto, J. M., Cerdá-Nicolás, M., & Ríos, J. 2002. Anti-inflammatory triterpenes from Pistacia terebinthus galls. Planta Medica, 68(04), 311-315.
9. Grant, W. F. 1982. Chromosome aberration assays in Allium: A report of the US Environmental Protection Agency gene-tox program. Mutation Research/Reviews in Genetic Toxicology, 99(3), 273-291.
10. Jahangirian, H., Haron, M. J., Yusof, N. A., Silong, S., Kassim, A., Rafiee-Moghaddam, R., Peyda, M., & Gharayebi, Y. 2011. Enzymatic synthesis of fatty hydroxamic acid derivatives based on palm kernel oil. Molecules (Basel, Switzerland), 16(8), 6634–6644. https://doi.org/10.3390/molecules16086634
11. Kıvçak, B., Akay, S., Demirci, B., & Başer, K. 2004 Chemical composition of essential oils from leaves and twigs of Pistacia lentiscus, Pistacia lentiscus var. chia, and Pistacia terebinthus from Turkey. Pharmaceutical biology, 42(4-5), 360-366.

12. Kordali, S., Cakir, A., Zengin, H., & Duru, M. E. 2003. Antifungal activities of the leaves of three Pistacia species grown in Turkey. Fitoterapia, 74(1-2), 164-167.
13. Kuras M, Nowakowska J, Sliwinska E, Pilarski R 2016 Changes in chromosome structure, mitotic activity and nuclear DNA content from cell of Allium Test induced by bark water extract of Uncaria tementosa (Willd.) DC. Journal of Ethanopharmacology 107: 211-221.
14. Lapie G., et Maige A., 1914 La flore forestière illustrée de l'Algérie. Paris.p581
15. Lin TS, Crane JC, Ryugo K, Polito VS, Djong TM 1984 Comparative study of leaf morphology, photosynthesis and leaf conductance in selected Pistacia species. J Amer Soc Hortic Sci 109: 325–
16. Melappa, G., Cb, S., & Channabasava, B. P. 2017. In vitro antimitotic, antiproliferative and GC-MS studies on the methanolic extract of endophytic fungi, Penicillium species of Tabebuia Argentea Bur & K. SCH. Sch. Farmacia, 65, 301-309.
17. Mukhtar, E., Adhami, V. M., & Mukhtar, H. 2014. Targeting microtubules by natural agents for cancer therapy. Molecular cancer therapeutics, 13(2), 275–284. https://doi.org/10.1158/1535-7163.MCT-13-0791
18. NAZIM, B , HOUARI, T , ISMAIL, B . 2020. Ethnobotanical Survey of Some Plants Used in Tessala Region, Algeria. Current Perspectives on Medicinal and Aromatic Plants (CUPMAP) , 3 (1) , 18-24 . DOI: 10.38093/cupmap.652708
19. Pulaj B, Mustafa B, Nelson K, Quave CL, Hajdari A. 2019 Chemical composition and in vitro antibacterial activity of Pistacia terebinthus essential oils derived from wild populations in Kosovo. BMC Complementary and Alternative Medicine. DOI: 10.1186/s12906-016-1135-8.
20. Raheel, R., Saddiqe, Z., Iram, M., & Afzal, S. 2017. In vitro antimitotic, antiproliferative and antioxidant activity of stem bark extracts of Ficus benghalensis L. South African Journal of Botany, 111, 248-257.
21. Remila, S., Atmani-Kilani, D., Delemasure, S., Connat, J. L., Azib, L., Richard, T., & Atmani, D. 2015. Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of Pistacia lentiscus (Anacardiaceae) leaf and fruit extracts. European Journal of Integrative Medicine, 7(3), 274-286.
22. Sehgal, R., Roy, S., & Kumar, V. L. 2006. Evaluation of cytotoxic potential of latex of Calotropis procera and Podophyllotoxin in Allum cepa root model. Biocell, 30(1), 9-13.
23. Witte, I., Zhu, B. Z., Lueken, A., Magnani, D., Stossberg, H., & Chevion, M. 2000. Protection by desferrioxamine and other hydroxamic acids against tetrachlorohydroquinone-induced cyto-and genotoxicity in human fibroblasts. Free Radical Biology and Medicine, 28(5), 693-700.
24. Zhu, B. Z., Zhu, J. G., Fan, R. M., & Mao, L. 2011 Metal-independent pathways of chlorinated phenol/quinone toxicity. In Advances in Molecular Toxicology (Vol. 5, pp. 1-43). Elsevier.

Research Article
Curr. Pers. MAPs, (2020); 3(2): 135-142

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

Effects of *Tribulus terrestris*, *Avena sativa* and White Ginseng on Adiponectin, Leptin, Resistin, Fatty Acid Binding Protein 4, Homocysteine and Paraoxonase-1 Levels in Hypercholesterolemic Rats

Ahmet NALBANT[1], Ali BİLGİLİ[2*], Başak HANEDAN[3], Mehmet AKDOĞAN[4]

[1]Department of Internal Medicine, Faculty of Medicine, University of Sakarya, 54050, Sakarya, TURKEY
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ankara, 06110, Ankara, TURKEY
*Corresponding author: abilgili@gmail.com
[3]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ataturk, 25240, Erzurum, TURKEY
[4]Department of Medical Biochemistry, Faculty of Medicine, University of Sakarya, TR-54050, Sakarya, TURKEY

https://doi.org/10.38093/cupmap.829184

Received : 21/11/2020
Accepted : 22/12/2020

Abstract

This study was aimed to evaluate the effects of *Tribulus terrestris* (TT), *Avena sativa* (AS), white ginseng (WG), and a triple combination (TC) powder on adipokines, homocysteine and paraoxonase-1 levels in hypercholesterolemic rats. Hypercholesterolemia in rats was induced by diet added 2% cholesterol. Herbal treatment groups consisted of Group III (TT), Group IV (AS), Group V (WG) and Group VI (triple combination of TT, AS and WG). Significant increase in total cholesterol, LDL-C, homocysteine, leptin and resistin levels ($P<0.05$) and insignificant decrease in adiponectin, and paraoxonase-1 levels ($P>0.05$) were found in hypercholesterolemic rats. The treatment combination with TT, AS and WG significantly reduced total cholesterol, LDL-C, homocysteine, leptin and resistin levels in hypercholesterolemic rats ($P<0.05$). In conclusion, it was found that TT, AS and WG had positive effects on reversing the effects of hypercholesterolemia in rats. The combination treatment with TT, AS and WG may have therapeutic effects in the treatment of hypercholesterolemia.

Key Words: Hypercholesterolemia, Adipokines, Homocysteine, Paraoxonase-1, Herbal treatment

1. Introduction

Obesity incidence is appeared to increase during recent decades. It may be accompanied with adipose tissue dysfunction, fatty liver disease, dyslipidemia, and atherosclerosis (Bluher, 2009). Hypercholesterolemia causes adipocyte hypertrophy, and the inflammation and endocrine dysfunction of adipose tissue (Aguilar and Fernandez, 2014). Adipokines are secreted by adipose tissue (Cobbold, 2019). Some adipokines are adiponectin, leptin, resistin and fatty acid binding protein 4 (FABP4) (Tu et al., 2017; Li and Shen, 2019).

Adiponectin enhances that skeletal muscles utilize glucose and fatty acids and is linked with lipoprotein metabolism (Yanai and Yoshida, 2019). Leptin has a role on

suppression of appetite, enhancing of energy expended, and regulation of body weights (Mechanick et al., 2018). Resistin levels are increased in obesity induced by diet (Zieba et al., 2020). It has been also determined that resistin levels are increased in obese women (Alissa et al., 2019). FABP4 is highly expressed in adipocytes and macrophages (Lamounier-Zepter et al., 2013), and involved in the coordination of lipid transportation in the cellular level (Tu et al., 2017).

Hyperhomocysteinemia causes increase of hydrogen peroxide production, endothelial dysfunction and increase of oxidation of low-density lipoproteins (Jamwal and Sharma, 2018). Serum paraoxonase-1 (PON1) has the protective effects against atherogenesis by contributing to the antioxidant properties of high density lipoprotein (Shekhanawar et al., 2013). In addition, PON-1 acts on suppressing the inflammatory response of macrophages, and attenuating plaque progression (Aharoni et al., 2013).

Chronic diseases caused by hypercholesterolemia lead to an important rate of deaths in the developed countries (Gouveia et al., 2004). Pharmacological agents for the treatment of hypercholesterolemia include cholesterol synthesis inhibition, reduction of fat absorption in the gastrointestinal system, and degradation of fatty acids. These agents may present side effects, therapeutic insufficiency, and reduced tolerability (Klein-Szanto and Bassi, 2019). For example, statins are used for lowering serum lipid levels in hypercholesterolemic patients. However, statins have adverse effects such as decrease of insulin sensitivity and adiponectin levels (Koh et al., 2008; Koh et al., 2010; Koh et al., 2016), and liver toxicity (Abd El Aal et al., 2017). Thus, new plant-derived treatment choices are explored for lowering blood lipid levels.

Tribulus terrestris (TT) has been used as a traditional medicine for a long time by virtue of chemical constituents that it contains flavonoids, flavonol glycosides and alkaloids. It has beneficial activities including hypolipidemic, antidiabetic, cardiotonic, hepatoprotective and anti-inflammatory (Chhatre et al., 2014).

Avena sativa (AS) is used for food and traditional medicine. It has various pharmacological activities such as cholesterol lowering effect, antioxidant activity, anti-atherogenic activity, and lowering the obesity risk (Singh et al., 2013). Ginseng is used for centuries for treating diseases (Davis and Behm, 2019). Ginseng has therapeutic effects on cardiovascular diseases (Xu et al., 2018), immune function (Kim et al., 2018), obesity (Lee et al., 2013a), hyperlipidemia, and hyperglycemia (Chung et al., 2016) via bioactive compounds (Liu et al., 2018).

The treatment of hypercholesterolemia with herbal medicines is extensively evaluated. However, there is need to know the effects of herbal medicines on adipokines in the treatment of hypercholesterolemia. Thus, in this study, the effects of TT, AS, white ginseng (WG), and a triple combination (TC) powder were evaluated on adipokines, homocysteine and PON-1 levels in hypercholesterolemic rats.

2. Material and Methods

2.1. Animals and Materials: The present study material consisted of 42 Wistar albino rats, 2.5 months old. The rats were kept in a standard condition (12 h light/dark; temperature: 25°C). All rats were acclimated before experiment. The approval of Kobay's Local Ethics Committee was obtained for this experimental study. The study consisted of six groups. The rats of group I were fed with pellet feed. Other groups were fed with 2% added cholesterol (Sigma Aldrich C-

75209). 15% TT (milled seed); 7.5% AS (seed); 5% WG (root); and 7.5 TT%, 3.75% AS, 2.5% WG were added to the diet of Group III, IV, V, VI, respectively. Upon completion of the study period, the rats were administered with xylazine (10 mg/kg) and ketamine (90 mg/kg). Centrifugation of blood samples and freezing serum samples were made.

2.2. Assessment of Sera Biochemical Parameters: The levels of sera total cholesterol, LDL-C, HDL-C, and triglyceride were measured using a COBAS-C501 chemistry analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Rat homocysteine levels by the quantitative sandwich enzyme immunoassay technique (Rat homocysteine Cusabio ELISA Kit), PON-1 activities by a novel automated photometric method developed by Erel (Aslan et al., 2007), adiponectin levels by the quantitative sandwich enzyme immunoassay technique (Rat adiponectin, ADP ELISA Kit), plasma leptin levels by the sandwich enzyme-linked immunosorbent method (Rat Leptin BioVendor), resistin levels by ELISA (Rat Resistin BioVendor) and FABP4 levels by ELISA technique (Rat fatty acid binding protein, BioVendor ELISA kit) were measured.

2.3. Statistical Analysis: Whether parameters were normally distributed was analyzed by Shapiro-Wilk test. Kruskal-Wallis test and one-way ANOVA was used for non-normally distributed parameters and normally distributed parameters, respectively. Pearson correlation for the correlation analyses of all parameters was used. $P < 0.05$ was significant.

3. Results and Discussion

The total cholesterol, HDL-C, LDL-C, triglyceride, homocysteine, PON-1, adiponectin, FABP4, leptin and resistin results are given in Table 1. There was significant increase of total cholesterol and LDL-C levels, and significant decrease of HDL-C levels in group II than group I ($P<0.05$). The rats of group VI had significant decreased total cholesterol and LDL-C levels than group I ($P<0.05$).

There was a significant increased homocysteine levels in group II than group I ($P<0.05$). The treatment of combination of TT, AS, and WG in Group VI significantly decreased homocysteine levels than group II of hypercholesterolemic rats ($P<0.05$). It was determined that PON-1 activities were insignificantly decreased in hypercholesterolemic rats and the treatment with TT, AS, and WG and TC insignificantly increased PON-1 activities in hypercholesterolemic rats ($P>0.05$). In hypercholesterolemic rats, adiponectin levels were non-significantly decreased. Herbal treatment insignificantly increased adiponectin levels in hypercholesterolemic rats ($P>0.05$). FABP4 levels were not significantly changed in the treatment groups ($P>0.05$). There was significant increase of leptin and resistin levels in group II than group I, but significant decrease of leptin and resistin levels in group VI than group II ($P<0.05$).

Significant correlation analyses for all measured parameters performed using Pearson correlation were given in Table 2. There was a correlation of total cholesterol with triglyceride, HDL-C, LDL-C, homocysteine, adiponectin, and leptin; of homocysteine with total cholesterol, triglyceride, HDL-C, LDL-C, PON-1, adiponectin, leptin and resistin; of PON-1 with homocysteine, and leptin; of adiponectin with total cholesterol, HDL-C, and homocysteine; of leptin with total cholesterol, triglyceride, HDL-C, LDL-C, homocysteine, PON-1, and resistin; and of resistin with homocysteine and leptin.

Table 1: The effects of TT, AS, WG and TC on adipokines, homocysteine and paraoxonase levels in hypercholesterolemic rats

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control	2% Chol	2% Chol + TT	2% Chol + AS	2% Chol + WG	2% Chol + TC
Total cholesterol (mg/dl)	69 (55-84)	91 (85-108)[a]	73 (67-110)	72 (68-80)	67 (56-88)[b]	70 (56-73)[c]
Triglyceride (mg/dl)	55.86±4.62	63.57±7.55	52±4.20	51.71±3.13	49.29±6.19	48.57±2.19
HDL-C (mg/dl)	41.00±2.25	30.86±1.37[d]	34.14±3.01	36.57±1.41	37.14±1.18	37.86±0.50
LDL-C (mg/dl)	33.00±1.27	39.43±0.68[e]	36.29±1.24	35.14±1.16	35.00±1.27	32.14±0.96[f]
Homocysteine	9.7 (8.7-11.9)	13.7 (12.4-15.8)[g]	10.9 (9.7-15.3)	11.6 (11.2-13.7)[h]	9.8 (9.2-14.8)	9.0 (8.5-10.7)[j]
Paraoxonase	242.86±19.10	190.86±4.94	208.43±11.13	215.57±11.25	217.43±5.76	239.57±14.59
Adiponectin	4.38 (2.59-5.22)	3.16 (2.58-3.85)	3.06 (2.39-4.13)	3.28 (2.94-3.51)	3.25 (3.05-4.37)	3.65 (2.87-3.88)
FAB4	2.70 (1.60-7.60)	3.10 (0.89-5.20)	3.70 (0.39-6.40)	3.50 (2.80-5.70)	2.90 (1.39-5.90)	2.40 (1.39-5.70)
Leptin	2.18 (1.87-2.51)	4.54 (4.13-5.42)[k]	2.64 (2.15-5.07)	3.10 (2.63-3.90)[l]	2.93 (1.96-3.14)	2.59 (2.31-2.93)[m]
Resistin	20.57±2.03	28.70±2.0[n]	25.48±0.73	24.77±1.79	22.30±1.57	18.95±2.07[o]

Notes: Group I: Control group; Group II: Cholesterol treatment group; Group III: 2% Cholesterol + TT treatment group; Group IV: 2% Cholesterol + AS treatment group; Group V: 2% Cholesterol + WG treatment group; Group VI: 2% Cholesterol + TC treatment group.
Each group consisted of 7 rats. All values were presented as mean ± SEM or median (minimum – maximum).
[a]P<0.05 versus Group I; [b]P<0.05 versus Group II; [c]P<0.05 versus Group II; [d]P<0.05 versus Group I; [e]P<0.05 versus Group I; [f]P<0.05 versus Group II; [g]P<0.05 versus Group I; [h]P<0.05 versus Group VI; [j]P<0.05 versus Group II; [k]P<0.05 versus Group I; [l]P<0.05 versus Group I; [m]P<0.05 versus Group II; [n]P<0.05 versus Group I; [o]P<0.05 versus Group II.

Table 2: Correlations among cholesterol, adipokines, homocysteine and paraoxonase levels in hypercholesterolemic rats

Pearson correlation levels and P values	Total cholesterol	Triglyceride	HDL-C	LDL-C	Homocysteine	Paraoxonase	Adiponectin	Leptin	Resistin
Total cholesterol		r=0.542 P=0.000	r=-0.494 P=0.001	r=0.625 P=0.000	r=0.735 P=0.000		r=-0.346 P=0.025	r=0.393 P=0.01	
Triglyceride	r=0.542 P=0.000		r=-0.361 P=0.019	r=0.442 P=0.003	r=0.468 P=0.002			r=0.309 P=0.047	
HDL-C	r=-0.494 P=0.001	r=-0.361 P=0.19		r=-0.606 P=0.000	r=-0.531 P=0.000		r=0.358 P=0.02	r=-0.419 P=0.006	
LDL-C	r=0.625 P=0.000	r=0.442 P=0.003	r=-0.606 P=0.000		r=0.493 P=0.001			r=0.536 P=0.000	
Homocysteine	r=0.735 P=0.000	r=0.468 P=0.002	r=-0.531 P=0.000	r=0.493 P=0.001		r=-0.430 P=0.004	r=-0.352 P=0.02	r=0.531 P=0.000	r=0.491 P=0.001
Paraoxonase					r=-0.430 P=0.004			r=-0.406 P=0.008	
Adiponectin	r=-0.346 P=0.02		r=0.358 P=0.02		r=-0.352 P=0.02				
Leptin	r=0.393 P=0.01	r=0.309 P=0.047	r=-0.419 P=0.006	r=0.536 P=0.000	r=0.531 P=0.000	r=-0.406 P=0.008			r=0.491 P=0.001
Resistin					r=0.491 P=0.001			r=0.491 P=0.001	

Notes: r: Pearson correlation; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol.

In this study, there were significant decreases of HDL-C, significant increase of total cholesterol and LDL-C levels and insignificant decrease of adiponectin levels in hypercholesterolemic rats. It was found that there was an inverse relation between high total cholesterol levels and low adiponectin levels and a positive correlation between HDL-C and adiponectin levels in hypercholesterolemic rats. Moreover, in this

study, hypercholesterolemia caused significant increase in homocysteine, leptin, and resistin levels, and insignificant decrease in adiponectin and PON-1 levels in rats. Increased resistin levels in hypercholesterolemic rats might be explained with promoting inflammation and disease conditions such as metabolic syndrome and causing the antagonistic activity on the protective adiponectin (Acquarone et al., 2019). High leptin levels in hypercholesterolemic rats might be associated with stimulating the production of proinflammatory cytokine (Berger and Polotsky, 2018). High homocysteine and low adiponectin levels might be attributed to tend possible inflammation and atherosclerosis (Lee et al., 2013b). In this study, homocysteine was found to be correlated with total cholesterol, triglyceride, HDL-C, LDL-C, PON-1, adiponectin, leptin, and resistin.

The treatment of TC in group VI provided beneficial effects by significantly decreasing homocysteine, leptin and resistin levels, and insignificantly increasing adiponectin levels in hypercholesterolemic rats. Beneficial effects by herbal treatments have been showed in hypercholesterolemic rats. For example, Abd El Aal et al. (2017) have demonstrated that carvacrol significantly decreases total cholesterol, triglycerides, LDL, leptin, and increases HDL and adiponectin. Metwally et al. (2019) have reported that reduction of visceral adiposity and serum leptin and resistin levels, increase in serum adiponectin levels, and reduction in serum total cholesterol, triglyceride, insulin and glucose levels are determined in M. alba leaf extract treated hypercholesterolemic rats for 8 weeks. Resveratrol has an increasing activity of adiponectin and decreasing activity of leptin and insulin in hypercholesterolemic rabbits and has a therapeutic potential for lipid-lowering effect (Jimoh et al., 2018).

Tribulus terrestris (1000 mg/d) decreased total cholesterol and LDL levels in women with type 2 diabetes (Samani et al., 2016). TT is reported to improve adiponectin levels but not significant in diabetic rats (Gandhi et al., 2013), and to decrease serum leptin and homocysteine levels in hypertensive rats (Jiang et al., 2017). In this study, TT in the diet of hypercholesterolemic rats insignificantly decreased LDL-C, homocysteine, leptin and resistin levels and insignificantly increased HDL-C, and PON-1 levels. This can be attributed to beneficial effects on its hypolipidemic activity.

Ginsenoside Rg1 of ginseng decreased lipid accumulation in obese mice (Liu et al., 2018). Fermented ginseng had improvement effect on hypercholesterolemia, and reduction effects in FABP4 expression, IL-1β and IL-6 expressions of adipose tissue (Li et al., 2018). Furthermore, it has been showed that black ginseng (200 mg/kg) decreases total serum cholesterol and LDL-C levels, and attenuates the lipogenesis genes in hypercholesterolemic rats (Saba et al., 2016). Chung et al. (2016) have reported that plasma adiponectin levels are increased and leptin and resistin levels are decreased with treatment of red ginseng in mice exposed to high fat diet. In addition, ginsenoside R1 treatment has been reported to improve central leptin sensitivity in obese mice (Wu et al., 2018). In this study, WG in the diet of hypercholesterolemic rats insignificantly decreased LDL-C, homocysteine, leptin and resistin levels and insignificantly increased HDL-C, and PON-1 levels. It is determined that white ginseng has positive effects on improvement of adipokines secreted by adipose tissue and improvement of homocysteine and PON-1 levels in rats. Oat protein and oat glucan can decrease plasma LDL-C levels by increasing of fecal total lipids in hypercholesterolemic hamsters (Tong et al., 2016). Furthermore, boiled oatmeal than brewed oatmeal has been reported to be more efficient on

lowering plasma and liver lipid levels in hypercholesterolemic rats (Ban et al., 2015). Oat β-glucan is reported to significantly decrease LDL-C and apoB in humans and to be a possible strategy on cardiovascular disease reduction (Ho et al., 2016). Similarly, oat beta glucan of ≥ 3 g added diet provided LDL cholesterol reduction (Whitehead et al., 2014). Oat-β glucan is reported to attenuate the intestinal FABP mRNA in rats (Drozdowski et al., 2010). However, in this study, no significant changes for FABP4 among groups were found.

Oat fiber decrease weight of body adipose tissues, increase serum adiponectin levels, increase protein expressions related to the lipolysis in mice fed a high-fat diet (Han et al., 2017), decrease serum lipids and leptin levels in mice fed a high-fat/cholesterol diet (Zhang et al., 2016). Oat beta glucan had no effects on changes of homocysteine levels and had a reduction effect on total cholesterol and LDL-C levels in hypercholesterolemic adults (Queenan et al., 2007). In this study, AS in the diet of hypercholesterolemic rats insignificantly decreased LDL-C, homocysteine, leptin, and resistin levels and insignificantly increased HDL-C, and PON-1 levels.

4. Conclusion

It was determined that TT, AS and WG had positive effects on reversing the effects of hypercholesterolemia in rats. In addition, the treatment of combination of TT, AS and WG was determined to have therapeutic potential in the treatment of hypercholesterolemia by significantly decreasing homocysteine, leptin and resistin levels, and insignificantly increasing adiponectin and PON-1 levels in hypercholesterolemic rats.

Acknowledgements

-

Conflict of interest

There is no any conflicts of interest in the course of conducting the research among authors.

References

1. Abd El Aal, H.A., Ahmed, L.A., Hassan, W.A., Fawzy, H.M., and Moawad H., 2017. Combination of carvacrol with simvastatin improves the lipid-lowering efficacy and alleviates simvastatin side effects. Journal of Biochemical and Molecular Toxicology, 31, e21981. DOI number: 10.1002/jbt.21981.
2. Acquarone, E., Monacelli, F., Borghi, R., Nencioni, A., and Odetti, P., 2019. Resistin: A reappraisal. Mechanisms of Ageing and Development, 178, 46-63. DOI number: 10.1016/j.mad.2019.01.004.
3. Aguilar, D., and Fernandez, M.L., 2014. Hypercholesterolemia induces adipose dysfunction in conditions of obesity and nonobesity. Advances in Nutrition, 5 (5), 497-502.
4. Aharoni, S., Aviram, M., and Fuhrman, B., 2013. Paraoxonase 1 (PON1) reduces macrophage inflammatory responses. Atherosclerosis, 228 (2), 353-361. DOI number: 10.1016/j.atherosclerosis.2013.03.005.
5. Alissa, E.M., Alzughaibi, L.S., and Marzouki, Z.M., 2019. Association between serum resistin, adiposity measures and inflammatory makers in women without cardiovascular diseases. Chemistry and Physics of Lipids, 218, 136-140. DOI number: 10.1016/j.chemphyslip.2018.12.005.
6. Aslan, M., Kosecik, M., Horoz, M., Selek, S., Celik, H., and Erel, O., 2007. Assessment of paraoxonase and arylesterase activities in patients with iron deficiency anemia. Atherosclerosis, 191 (2), 397-402. DOI number: 10.1016/j.atherosclerosis.2006.04.007.
7. Ban, Y., Qiu, J., Ren, C., and Li, Z., 2015. Effects of different cooking methods of oatmeal on preventing the diet-induced increase of cholesterol level in hypercholesterolemic rats. Lipids in Health and Disease, 14, 135. DOI number: 10.1186/s12944-015-0138-7.
8. Berger, S., and Polotsky, V.Y., 2018. Leptin and leptin resistance in the pathogenesis of obstructive sleep apnea: A possible link to oxidative stress and cardiovascular complications. Oxidative Medicine and Cellular Longevity, 2018, 5137947. DOI number: 10.1155/2018/5137947.
9. Bluher, M., 2009. Adipose tissue dysfunction in obesity. Experimental and Clinical Endocrinology and Diabetes, 117 (6), 241-250. DOI number: 10.1055/s-0029-1192044.
10. Chhatre, S., Nesari, T., Somani, G., Kanchan, D., and Sathaye, S., 2014. Phytopharmacological overview of

Tribulus terrestris. Pharmacognosy Reviews, 8 (15), 45-51. DOI number: 10.4103/0973-7847.125530.
11. Chung, S.I., Nam, S.J., Xu, M., Kang, M.Y., and Lee, S.C., 2016. Aged ginseng (Panax ginseng Meyer) reduces blood glucose levels and improves lipid metabolism in high fat diet-fed mice. Food Science and Biotechnology, 25 (1), 267-273. DOI number: 10.1007/s10068-016-0039-1.
12. Cobbold, C., 2019. Type 2 diabetes mellitus risk and exercise: is resistin involved? The Journal of Sports Medicine and Physical Fitness, 59 (2), 290-297. DOI number: 10.23736/s0022-4707.18.08258-0.
13. Davis, M.P., and Behm, B., 2019. Ginseng: A Qualitative review of benefits for palliative clinicians. The American Journal of Hospice and Palliative Care, 36 (7), 630-659. DOI number: 10.1177/1049909118822704.
14. Drozdowski, L.A., Reimer, R.A., Temelli, F., Bell, R.C., Vasanthan, T., and Thomson, A.B., 2010. Beta-glucan extracts inhibit the in vitro intestinal uptake of long-chain fatty acids and cholesterol and down-regulate genes involved in lipogenesis and lipid transport in rats. The Journal of Nutritional Biochemistry, 21 (8), 695-701. DOI number: 10.1016/j.jnutbio.2009.04.003.
15. Gandhi, S., Srinivasan, B.P., and Akarte, A.S., 2013. Potential nephrotoxic effects produced by steroidal saponins from hydro alcoholic extract of Tribulus terrestris in STZ-induced diabetic rats. Toxicology Mechanisms and Methods, 23 (7), 548-557. DOI number: 10.3109/15376516.2013.797533.
16. Gouveia, M., Borges, M., Costa, J., and Carneiro, A.V., 2004. Burden of disease from hypercholesterolemia in Portugal. Revista Portuguesa de Cardiologia, 23 (2), 255-270.
17. Han, S.F., Jiao, J., Zhang, W., Xu, J.Y., Zhang, W., Fu, C.L., and Qin, L.Q., 2017. Lipolysis and thermogenesis in adipose tissues as new potential mechanisms for metabolic benefits of dietary fiber. Nutrition, 33, 118-124. DOI number: 10.1016/j.nut.2016.05.006.
18. Ho, H.V., Sievenpiper, J.L., Zurbau, A., Blanco Mejia, S., Jovanovski, E., Au-Yeung, F., Jenkins, A.L., and Vuksan, V., 2016. The effect of oat beta-glucan on LDL-cholesterol, non-HDL-cholesterol and apoB for CVD risk reduction: a systematic review and meta-analysis of randomised-controlled trials. The British Journal of Nutrition, 116 (8), 1369-1382. DOI number: 10.1017/s000711451600341x.
19. Jamwal, S., and Sharma, S., 2018. Vascular endothelium dysfunction: a conservative target in metabolic disorders. Inflammation Research, 67, 391-405. DOI number: 10.1007/s00011-018-1129-8.
20. Jiang, Y.H., Guo, J.H., Wu, S., and Yang, C.H., 2017. Vascular protective effects of aqueous extracts of Tribulus terrestris on hypertensive endothelial injury. Chinese Journal of Natural Medicines, 15 (8), 606-614. DOI number: 10.1016/s1875-5364(17)30088-2.
21. Jimoh, A., Tanko, Y., Ayo, J.O., Ahmed, A., and Mohammed, A., 2018. Resveratrol increases serum adiponectin level and decreases leptin and insulin level in an experimental model of hypercholesterolemia. Pathophysiology, 25 (4), 411-417. DOI number: 10.1016/j.pathophys.2018.08.005.
22. Kim, J.K., Kim, J.Y., Jang, S.E., Choi, M.S., Jang, H.M., Yoo, H.H., and Kim, D.H., 2018. Fermented red ginseng alleviates cyclophosphamide-induced immunosuppression and 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice by regulating macrophage activation and T cell differentiation. The American Journal of Chinese Medicine, 46 (8), 1879-1897. DOI number: 10.1142/s0192415x18500945.
23. Klein-Szanto, A.J.P., and Bassi, D., 2019. Keep recycling going: new approaches to reduce LDL-C. Biochemical Pharmacology, 164, 336-341. DOI number: 10.1016/j.bcp.2019.04.003.
24. Koh, K.K., Oh, P.C., Sakuma, I., Lee, Y., Han, S.H., and Shin, E.K., 2016. Rosuvastatin dose-dependently improves flow-mediated dilation, but reduces adiponectin levels and insulin sensitivity in hypercholesterolemic patients. International Journal of Cardiology, 223, 488-493. DOI number: 10.1016/j.ijcard.2016.08.051.
25. Koh, K.K., Quon, M.J., Han, S.H., Lee, Y., Ahn, J.Y., Kim, S.J., Koh, Y., and Shin, E.K., 2008. Simvastatin improves flow-mediated dilation but reduces adiponectin levels and insulin sensitivity in hypercholesterolemic patients. Diabetes Care, 31 (4), 776-782. DOI number: 10.2337/dc07-2199.
26. Koh, K.K., Quon, M.J., Han, S.H., Lee, Y., Kim, S.J., and Shin, E.K., 2010. Atorvastatin causes insulin resistance and increases ambient glycemia in hypercholesterolemic patients. Jornal of the American College of Cardiology, 55 (12), 1209-1216. DOI number: 10.1016/j.jacc.2009.10.053.
27. Lamounier-Zepter, V., Look, C., Ehrhart-Bornstein, M., Bornstein, S.R., Fischer, S., and Julius, U., 2013. Lipoprotein apheresis reduces adipocyte fatty acid-binding protein serum levels. Atherosclerosis. Supplements, 14 (1): 129-134. DOI number: 10.1016/j.atherosclerosissup.2012.10.010.
28. Lee, M.R., Kim, B.C., Kim, R., Oh, H.I., Kim, H.K., Choi, K.J., and Sung, C.K., 2013a. Anti-obesity effects of black ginseng extract in high fat diet-fed mice. Jornal of Ginseng Research, 37 (3), 308-349. DOI number: 10.5142/jgr.2013.37.308.
29. Lee C.Y., Jan, M.S., Yu, M.C., Lin, C.C., Wei, J.C., and Shih, H.C., 2013b. Relationship between adiponectin and leptin, and blood lipids in hyperlipidemia patients treated with red yeast rice. Forschende Komplementarmedizin (2006), 20 (3), 197-203. DOI number: 10.1159/000351455.
30. Li, Z., Kim, H.J., Park, M.S., and Ji, G.E., 2018. Effects of fermented ginseng root and ginseng berry on obesity and lipid metabolism in mice fed a high-

fat diet. Journal of Ginseng Research, 42 (3), 312-319. DOI number: 10.1016/j.jgr.2017.04.001.
31. Li, J., and Shen, X., 2019. Oxidative stress and adipokine levels were significantly correlated in diabetic patients with hyperglycemic crises. Diabetology and Metabolic Syndrome, 11, 13. DOI number: 10.1186/s13098-019-0410-5.
32. Liu, H., Wang, J., Liu, M., Zhao, H., Yaqoob, S., Zheng, M., Cai, D., and Liu, J., 2018. Antiobesity effects of ginsenoside Rg1 on 3T3-L1 preadipocytes and high fat diet-induced obese mice mediated by AMPK. Nutrients, 10 (7), 830. DOI number: 10.3390/nu10070830.
33. Mechanick, J.I., Zhao, S., and Garvey, W.T., 2018. Leptin, an adipokine with central importance in the global obesity problem. Global Heart, 13 (2), 113-127. DOI number: 10.1016/j.gheart.2017.10.003.
34. Metwally, F.M., Rashad, H., and Mahmoud, A.A., 2019. Morus alba L. diminishes visceral adiposity, insulin resistance, behavioral alterations via regulation of gene expression of leptin, resistin and adiponectin in rats fed a high-cholesterol diet. Physiology and Behavior, 201, 1-11. DOI number: 10.1016/j.physbeh.2018.12.010.
35. Queenan, K.M., Stewart, M.L., Smith, K.N., Thomas, W., Fulcher, R.G., and Slavin, J.L., 2007. Concentrated oat beta-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. Nutrition Journal, 6, 6. DOI number: 10.1186/1475-2891-6-6.
36. Saba, E., Jeon, B.R., Jeong, D.H., Lee, K., Goo, Y.K., Kim, S.H., Sung, C.K., Roh, S.S., Kim, S.D., Kim, H.K., and Rhee, M.H., 2016. Black ginseng extract ameliorates hypercholesterolemia in rats. Journal of Ginseng Research, 40 (2), 160-168. DOI number: 10.1016/j.jgr.2015.07.003.
37. Samani, N.B., Jokar, A., Soveid, M., Heydari, M., and Mosavat, S.H., 2016. Efficacy of the hydroalcoholic extract of Tribulus terrestris on the serum glucose and lipid profile of women with diabetes mellitus: A double-blind randomized placebo-controlled clinical trial. Journal of Evidence-Based Complementary and Alternative Medicine, 21 (4), Np91-97. DOI number: 10.1177/2156587216650775.
38. Shekhanawar, M., Shekhanawar, S.M., Krisnaswamy, D., Indumati, V., Satishkumar, D., Vijay, V., Rajeshwari, T., and Amareshwar, M., 2013. The role of 'paraoxonase-1 activity' as an antioxidant in coronary artery diseases. Journal of Clinical and Diagnostic Research, 7, 1284-1287. DOI number: 10.7860/JCDR/2013/5144.3118.
39. Singh, R., De, S., and Belkheir, A., 2013. Avena sativa (Oat), a potential neutraceutical and therapeutic agent: an overview. Critical Reviews in Food Science and Nutrition, 53 (2), 126-144, 2013. DOI number: 10.1080/10408398.2010.526725.
40. Tong, L.T., Guo, L., Zhou, X., Qiu, J., Liu, L., Zhong, K., and Zhou, S., 2016. Effects of dietary oat proteins on cholesterol metabolism of hypercholesterolaemic hamsters. Journal of the Science of Food and Agriculture, 96 (4), 1396-1401. DOI number: 10.1002/jsfa.7236.
41. Tu, W.J., Zeng, X.W., Deng, A., Zhao, S.J., Luo, D.Z., Ma, G.Z., Wang, H., and Liu, Q., 2017. Circulating FABP4 (Fatty Acid-Binding Protein 4) Is a novel prognostic biomarker in patients with acute ischemic stroke. Stroke, 48 (6), 1531-1538. DOI number: 10.1161/strokeaha.117.017128.
42. Whitehead, A., Beck, E.J., Tosh, S., and Wolever, T.M., 2014. Cholesterol-lowering effects of oat beta-glucan: a meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition, 100 (6), 1413-1421. DOI number: 10.3945/ajcn.114.086108.
43. Wu, Y., Huang, X.F., Bell, C., and Yu, Y., 2018. Ginsenoside Rb1 improves leptin sensitivity in the prefrontal cortex in obese mice. CNS Neuroscience and Therapeutics, 24 (2), 98-107. DOI number: 10.1111/cns.12776.
44. Xu, Z.M., Li, C.B., Liu, Q.L., Li, P., and Yang, H., 2018. Ginsenoside Rg1 prevents doxorubicin-induced cardiotoxicity through the inhibition of autophagy and endoplasmic reticulum stress in mice. International Journal of Molecular Sciences, 19 (11), 3658. DOI number: 10.3390/ijms19113658.
45. Yanai, H., and Yoshida, H., 2019. Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression: Mechanisms and perspectives. International Journal of Molecular Sciences, 20 (5), 1190. DOI number: 10.3390/ijms20051190.
46. Zhang, R., Jiao, J., Zhang, W., Zhang, Z., Zhang, W., Qin, L.Q., and Han, S.F., 2016. Effects of cereal fiber on leptin resistance and sensitivity in C57BL/6J mice fed a high-fat/cholesterol diet. Food and Nutrition Research, 60, 31690. DOI number: 10.3402/fnr.v60.31690.
47. Zieba, D.A., Biernat, W., and Barc, J., 2020. Roles of leptin and resistin in metabolism, reproduction, and leptin resistance. Domestic Animal Endocrinology, 73, 106472. DOI number: 10.1016/j.domaniend.2020.106472.