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GC-MS Analysis of the Secondary Metabolites from the Leaves of Wild/ Cultivated *Salvia palaestina* and their *in vitro* Antioxidant and Antimicrobial Activities

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Abstract

Herbal medicine is widely practiced in Palestine. In particular, *S. palaestina* (Sage in English and **مريمية** in Arabic) is intensively used but its usage merely relied on traditional heritage rather than scientific basis. *S. palaestina* contains secondary metabolites which have wide applications in food flavoring, preservation and folk medicine. The production and accumulation of these secondary metabolites are affected by different factors that may determine the composition and yield. Recently, these oils are gaining much recognition as potential source of natural and safer bioactive agents, especially due to microbial resistance arising against available antimicrobial agents.

Leaves of *S. palaestina* (cultivated and wild) were collected from seven different governorates in Palestine. Air dried leaves were subjected to steam distillation (SD) and the composition of essential oils was determined for the first time by GC-MS in the electron impact mode. The antioxidant activity was estimated by DPPH method while the antimicrobial activity was examined by disc diffusion method. ICP-OES was used to determine the content of minerals in dried leaves.

Twenty volatile and semivolatile components were identified using GC-MS. The major components in all the cultivated samples were eucalyptol and camphor except for Jericho's sample in which the main component was camphor (30.65%) which was not more than (9.1%) in other samples. Moreover, thujone derivatives in Jericho's sample were abundant in high concentrations (28.9%), while in other samples they were not more than (2%). In wild leaves, however, eucalyptol was the major component in all locations and its concentration was higher than that in cultivated, while the later has higher amount of camphor.

The antioxidant activity of *S. palaestina* oil was examined using the DPPH method. The IC_{50} was 2.333 mg/ml after 30 min, while after 90 min it was 1.585 mg/ml, which means that the antioxidant activity of *S. palaestina* oil increased with time and with increasing concentration.

Moreover, the antimicrobial activity of 5 µl of *S. palaestina* essential oil was found to be greater than the activity of gentamicin in case of *Satphylococcus aureus* while it was nearly the same as gentamicin against *E.coli*. Furthermore, this concentration was two times more active than nystatin against *Candida albicans*.

S. palaestina leaves are rich in minerals particularly, potassium, but it turned out that the sample examined contained remarkable amount of aluminum, which might affect the health due to its accumulation properties. Therefore, further work on *Salvia's* minerals is recommended to examine other locations rather than the selected location (Ramallah), hence the general believe that medicinal plants are safe and devoid of toxicity could be misconstrued.

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I would like to express my appreciation to Al-Quds University for the role it is playing in fulfilling the needs of the Palestinian society and the efforts to upgrade and promote the students to have a share in developing the Palestinian industry through this master program of pharmaceutical sciences.

Special appreciation to the Central Palestinian Health Laboratories in which this research was conducted. My deepest respect and thanks also to my colleagues for their valuable help and guidance. I am intensely grateful in particular to Ali Jahajha (Quality Assurance Coordinator), Hashem Jaes (Head of Pesticide Residue Unit), Suha Al-khras (Head of Drug and Cosmetics Microbiology Unit) and Nour Halayka (Head of Water Unit). My sincere thanks to you all.

I am grateful to my husband and children who inspired and encouraged me to explore the best in me. I thank them for dedication and patience.

Special thanks for my father and mother, your prayer for me was what sustained me thus far.

Finally, I take great privilege to express my heartfelt thanks to all dignitaries who have been involved directly or indirectly with the successful completion of this work.

Dedication

This thesis is dedicated to:

The sake of Allah, my Creator and my Master.
The teacher of teachers, Prophet of humanity Mohammad -peace be upon him.
My beloved parents, who never stop giving themselves in countless ways.
My dearest husband: for his endless support and encouragement.
My beloved kids: Amin, Basil and Mohammad, who lighten my life up and give me the power to keep on.
At last, I dedicate this research, to all the people in my life who touch my heart.

Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……..

Reem Nimer Mohammad Sabboubeh

Date:

Contents

List of Abbreviations

AI %	Antioxidant scavenging activity
ATCC	American Type Culture Collection
CFU	Colony forming unit
Cm	Centimeter
DPPH	2, 2'-diphenyl-1-picrylhydrazyl
DRI	Daily Required Intake
EI	Electron Impact
EPA	Environmental Protection Agency
Fig.	Figure
Ft	Feet
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HS	Head Space
IC_{50}	Inhibitory Concentration 50
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
KI	Kovats Index
LOD	Limit of Detection
LOQ	Limit of Quantitation
MS	Mass Spectrometry
NIST	National Institute of Standards and Technology
RF	Radiofrequency
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RSD	Relative Standard Deviation
RT	Retention Time
SD	Steam Distillation
SEM	Scanning Electron Microscopy
TDI	Tolerable Daily Intake
TIC	Total ion chromatogram
TWI	Tolerable Weekly Intake
WHO	World Health Organization
α	Alfa
β	Beta
µ	Micro

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Chapter One

Introduction

1. Introduction

1.1 Medicinal plants and traditional medicine

Throughout ages, humans have relied on plants for their availability as source of food, clothing, flavors, fragrances, medicine, etc (Gurib-Fakim, 2006). Using medicinal plants is as old as mankind and the sophisticated traditional medicine practices have been based on plants and used for thousands of years all over the world. The oldest written evidence of medicinal plants' usage (5000 years old), contains more than 250 plants was found on a Sumerian clay slab from Nagpur (Petrovska, 2012).

Basically, traditional medicine using natural products was the only way for formal health care in ancient countries (Oumeish, 1998) and was based on observations of their efficacy to discover their therapeutic properties and therefore were used and prescribed, even if their chemical active components were not completely known (Cowan, 1999).

Up to present, herbal medicines which formed the basis of healthcare throughout the world since the earliest days of mankind are still widely practiced in many developing countries especially in Middle East (Azaizeh, Saad, Cooper, & Said, 2010). WHO estimated that about 80% of the populations in these countries are still relying on medicinal plants for their primary health care need. This originates from economic reasons and from their deep believes that herbs aren't harmful (Matu & van Staden, 2003). However, it is nearly always goes side by side with medicine (Maha & Shaw, 2007).

Nowadays, although pharmaceutical industries are well developed in most of the world, it can't face the decreased efficacy of synthetic drugs and the increasing contraindications of their usage. Thus, in the last few decades, interest in natural therapies has increased greatly. In particularly, the essential oils and herbs-derived extracts are gaining much recognition as potential source of natural and safer bioactive agents, especially because of the growing microbial resistance against available chemically infective agents (Kelen & Tepe, 2008).