Deanship of Graduate Studies

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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.

Acknowledgments

I have no words to express my deepest gratitude to Allah, the most compassionate and the most merciful, who enabled me to accomplish this work.

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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).

1.2 Flora in Palestine

Palestine is unique, not only in its geographical location between Asia, Africa, and Europe, but also due to its diverse topographical features which lead to weather and climate changes which in turns leads to biodiversity (Mendelsohn, 1999). The Palestinian Mountains are rich in plant species. More than 2953 plant species are growing in the hills and mountains of Palestine of which more than 700 are noted for their uses as medicinal herbs or as botanical pesticides (Dafni, Yaniv, & Palevitch, 1984; Said, Khalil, Fulder, & Azaizeh, 2002).

Herbal medicine is considered as an integral part of Palestinian culture and plays a pivotal and indispensable role in the current public healthcare. There are lots of medicinal plants which is used to treat several diseases but the efficacy, safety, toxicity, dosage and the usage instructions haven't ever been investigated scientifically and almost always are verbally inherited from one generation to another (Sawalha, Sweileh, Zyoud, & Jabi, 2008).

1.3 Essential oils (secondary metabolites)

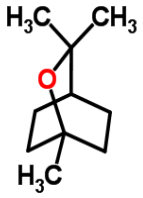
Plants are essential for the life of other creatures because they provide oxygen. In addition, they provide non-nutritional compounds or secondary metabolites (essential oils). These compounds are formed from precursors which are mediated by enzymatic reactions and are generally concentrated in one particular region of the plant such as leaves, roots, bark, fruits or glandular hairs (Harbone, 1993).

There were different believes regarding the role of these oils in plants. Some thought that they might help in both protecting the plant from attacks by other hostels while other thought that they may help in attracting some animals to help in pollination and seed dispersion (Packham, 1997).

Salvia species contain various secondary metabolites particularly essential oils and to a lesser extent, compounds such as sterols, flavonoids, phenols, monoterpens, sesquiterpenoids, sesterpenoids, diterpenoids and triterpenoids. Some of secondary metabolites concomitantly carry out physiological functions (Cioffi *et al.*, 2008, Wink, 2003).

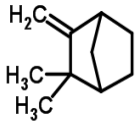
There are different factors that may determine the composition and yield of the essential oils. These variables include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized, post-harvest drying and storage conditions (Perry *et al*., 1999).

It was reported that the main components of *Salvia* essential oils are mostly the following four compounds; eucalyptol (1, 8 cineol), camphor, camphene and α-thujene which are usually responsible of the pharmacological activity of it. As was aforementioned, different factors affect the yield of the essential oils and their components (Abu-Darwish *et al*., 2013; Boszormenyi *et al*., 2009).

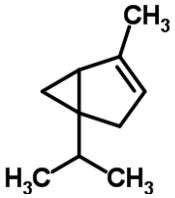


Eucalyptol

Camphor



Camphene



α-Thujene

Essential oils composition is important not only due to its biological activity, but also as a tool to resolve taxonomic problems and discrepancies (Wink, 2003). It was reported that trichomes are responsible for the essential oils accumulation and secretion. Trichomes are used in plant taxonomy, as they have been used to classify the species of the *Lamiaceae*. Therefore, studying the essential oil composition can be useful in distinguishing *Salvia* species and origin (Wester *et al*., 2009).

1.4 *Salvia* (Sage)

One of the most popular Palestinian herbal plants is *Salvia* (Sage in English and مريمية Miramya in Arabic). It is the largest genus of *Lamiaceae* or (*Labiatae*) family and comprises about 900 species (Hedege, 1992). The name is derived from Latin (*salvare)* which means 'to save or to cure' referring to the curable medicinal properties of it (Keller, 1978).

Several species of *Salvia* are used in folk medicine all around the world to treat microbial infections, cancer, malaria , inflammation, etc. (Kamatou, Makunga,

Ramogola, & Viljoen, 2008) due to its biological activities, such as antimicrobial, antimalarial, antioxidant, antitumor, antidiabetic, anxiolytic, sedative and anti-inflammatory activities (Esmaeili *et al*., 2008; Kelen & Tepe, 2008; Loizzo *et al.*, 2008; Loizzo, Tundis, Menichini, Saab, & Statti, 2007; Jaber, 2013).

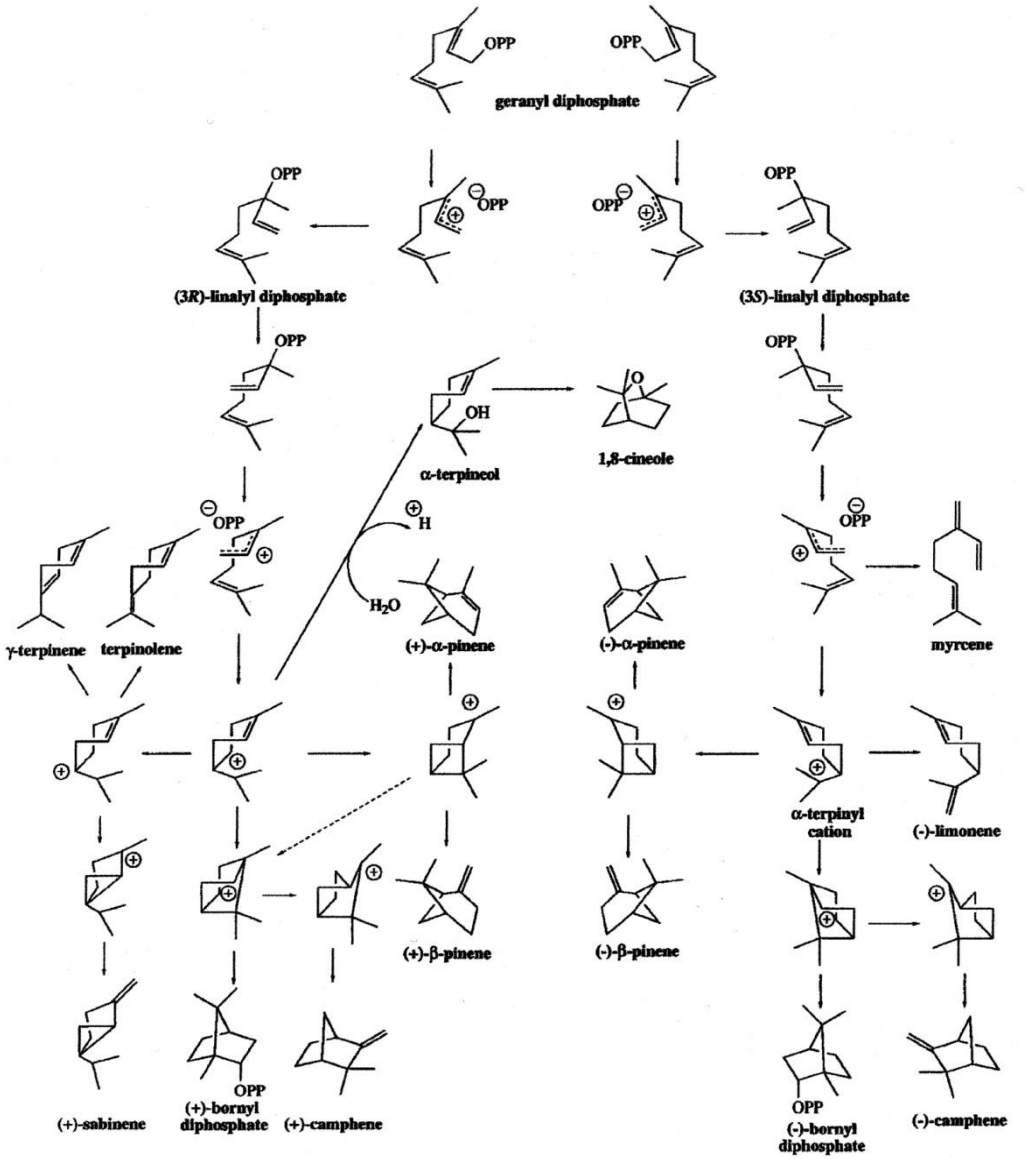
From its name, wild *S. palaestina* is native to Palestine. It was described and named by George Bentham in 1835. *Palaestina* is referring to its geographical distribution in the mountains between Gaza and Jerusalem. (http://en.wikipedia.org/wiki/Salvia_palaestina).

It grows 1-2 ft (0.30 to 0.60 m) in tall. The green leaves vary in shape and size, with light hairs on both sides. It has glands that release secondary metabolites of essential oils when rubbed, crushed or heated. The hairy leaves can be nearly 8–10 cm long. Its flowers are white to whitish lilac in color. Flowering season starts from April. In addition to Palestine, this plant is commonly found in Egypt, Syria, Lebanon, Southern Turkey, Iran, Northern Iraq and Jordan (Tukan, Takruri, & al-Eisawi, 1998).

1.5 Biosynthesis of *Salvia* components

Salvia is unique in synthesizing a broad range of components all of which utilize the same substrate by variations on a single reaction mechanism through multifunctional enzyme reactions. The biosynthesis of the monocyclic and bicyclic of *Salvia* components is illustrated in scheme 1 (Wise *et al*., 1998). Initially, the precursor geranyl diphosphate is generated from primary metabolites through the activation of mevalonate pathway. This precursor is ionized and isomerized to form either (3*R*) or (3*S*)-linalyl diphosphate which upon subsequent transformations gives the reactive intermediate α-terpinyl. Further transformations of this reactive intermediate may be effected by additional intramolecular electrophilic additions, hydride shifts, or other rearrangements before termination of the sequence by deprotonation of the final cation or capture by an external nucleophile, such as hydroxyl ion or diphosphate group.

At the end, different components are produced including, (±) α -pinene, (±) β-pinene, camphene, sabinene, limonene, myrcene, eucalyptol, α-terpineol, γ-terpinene, terpinolene and (±)-bornyl diphosphate (Scheme 1) (Wise *et al.*, 1998).



Scheme 1: The conversion of the precursor geranyl diphosphate to other components in *Salvia*.

1.6 Minerals in *Salvia*

Plants are essential sources of micronutrients and minerals for human beings and monitoring their levels are highly required for both dietary and medicinal purposes. Metallic and non-metallic elements are very important for the human body; they are required in certain amount to maintain healthy growth (Pier, 1975). Herbs contain certain amount of metals; therefore using them in treating illness might affect the general health and even more, it can be a factor of disease generation in certain cases (Annan *et al.*, 2010). In addition, herbs might contain metals as contaminants, which have negative impact on health. The determination of metals in plants can be considered as a marker of safety and purity (Li, Gao, & Zhao, 2002; Nasim & Dhir,

2010). To the best of our knowledge, there are no studies reporting about metal type and amount in *Salvia* species at large including Palestine.

1.7 Pharmacological activities of *Salvia*

Salvia is acknowledged for its curable activities. A proverb assures that a man who has Sage in his garden needs no doctor (Toussaint-Samat, 1996).

Numerous compounds have been reported to occur in *Salvia* species, some of which possess interesting biological activities. Phytochemical investigations have shown that *Salvia* species are mainly rich in volatile oils, polyphenols, diterpenoids and triterpenoids (Miguel *et al*., 2011).

As we aforementioned, there are many pharmacological activities of *Salvia*, however, we will concentrate on two of them:

I) Antimicrobial activity

Infectious diseases represent an important cause of morbidity and mortality among world population, especially in developing countries (Enwonwu & Salako, 2012). WHO summarized the top ten causes of death in low income countries in **Figure 1**, infectious disease ranked first.

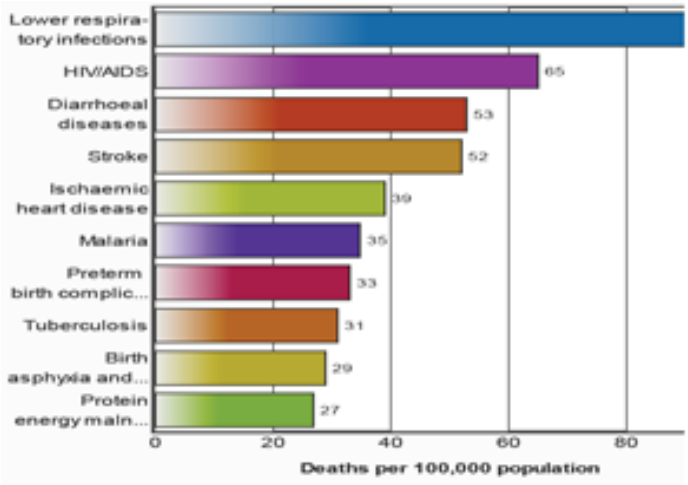


Fig. 1: The Ten leading causes of death in low-income countries, 2012. (http://www.who.int/mediacentre/factsheets/fs310/en/index1.html).

As a consequence, pharmaceutical companies were motivated to develop new antimicrobial drugs, especially because of the emergence of microorganisms resistant to available antimicrobials. It is obvious that bacterial species have genetic ability to develop and transmit resistance against currently available antimicrobials which can be noticed from the frequent reports on the isolation of bacteria that are known to be sensitive to routinely used drugs and became multiresistant to available antimicrobial at the usual therapeutic dose. Thus, natural antimicrobial products have gained special attention due to safety issues and to increasing resistance to antibiotics (Palombo & Semple, 2002).

Since centuries, man has used medicinal plants as antimicrobial while in fact he was unable to explain their effects. In the 19th century, a huge development in organic chemistry and pharmacology enables him to determine which active group is responsible of the antimicrobial therapeutic effect. The beneficial medicinal effects of plants resulted mostly from secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and localized in certain parts in the plant (Stary, 1996).

It was thought that these secondary metabolites may exert their action by resembling endogenous metabolites, hormones, ligands, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites (Briskin, 2000).

As other members of *Lamiaceae* family, *Salvia* species are reported to have antimicrobial activity. This reported activity was due to the presence of synergy between compounds such as linalool, eucalyptol, α-pinene, β-pinene, β-caryophyllene and limonene and other constituents of the essential oil. It was reported that linalool and eucalyptol had the highest antimicrobial activity (Sonboli *et al.*, 2006).

II) Antioxidant activity of essential oil

Developments in biomedical science emphasize the involvement of free radicals in many diseases, in particularly, degenerative diseases which can occur as a result of cellular damage caused by these free radicals. They are produced either from normal

cell metabolisms or from external sources including: pollution, cigarette smoking, radiation and medication, etc. (Devasagayam *et al.*, 2004).

Free radicals are generally reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both have toxic and beneficial effects and eventually balance is vital to achieve good health. Low or moderate levels of both, ROS and RNS, have positive impact on cellular responses and immune function. At high levels, oxidative stress is generated, which can damage cell structures and have great effect on developing chronic and degenerative conditions such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Emerit & Michelson, 1982; Gutteridge & Halliwell, 1992). The human body counteracts oxidative stress by producing antioxidants, which are either naturally produced, or externally supplied through foods and/or supplements. Both act as "free radical scavengers" in order to prevent and repair damages caused by ROS and RNS, and as consequence result enhancing the immune defense and lowering the risk of degenerative diseases and cancer (Halliwell, 2001; Valko *et al.*, 2006).

Thus, antioxidants may play an important role in disease prevention and they are defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Their antioxidant activity is based on their ability to donate hydrogen atoms to free radicals and mainly, they are phenolic compounds with potent scavenging activity (Aruoma, 1994).

Plants have played a significant role in the development of new drugs and there are strong attitudes to explore natural substances as substitutes for synthetic compounds. Moreover, the commonly synthetic chemicals used as antioxidants, butylated hydroxyanisol and butylated hydroxytolune, have possible toxicity which limit their use (Papas, 1999). Thus, natural antioxidants is highly demanded due to safety issues(Lobo, Patil, Phatak, & Chandra, 2010).

The essential oil of *Salvia* is used in some countries as antioxidant due to its scavenging activity of free radicals to prevent and repair related degenerative diseases (Miguel, *et al*., 2011).

1.8. Analytical methods for the analysis of essential oils

1.8.1 Gas Chromatography (GC)

Gas Chromatography is a highly recognized tool used for separation and quantification of volatile compounds in an oily mixture.

In all chromatography, separation occurs after injection of the sample mixture into a stationary phase with the aid of the mobile phase. In gas chromatography (GC), the mobile phase is an inert carrier gas such as helium or nitrogen.

The mobile phase carries the sample mixture through the stationary phase. The stationary phase is an open tubular long capillary column covered with certain chemical at the surface to interact selectively with the components of the sample mixture.

The sample components carried by the carrier gas interact with the stationary phase at a different rate. By controlling the properties of both mobile phase and the stationary phase, different mixtures of components can be separated. Further improvements on the quality of separation technique can be achieved by changing either the temperature of stationary phase or pressure of the mobile phase.

The capillary column is kept in an oven that can be programmed to increase the temperature gradually (ramped). While column's temperature increases, compounds with low boiling points elute faster than those with higher boiling points. Therefore, controlling the separating forces, temperature and stationary phase interactions will enhance separation.

Separated compounds elute from the column and enter a detector, which is capable of creating an electronic signal in response to the presence of a compound. The signal size is proportional to the concentration of the compound in the sample. At the end, signal is processed by a computer (Chattoraj & Tietz, 1969).

1.8.2 Mass Spectrometry (MS)

Mass spectrometer detector can be connected in tandem with GC chromatograph. Once the compounds elute from the GC capillary column, they enter the electron ionization chamber in which, they are bombarded with a stream of electrons causing them to break dawn into fragments. These fragments are positively charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass-to-charge ratio (m/z). The quadrupole mass analyzer focuses each of the fragments through a slit and into the electron multiplier detector (Costello, 1986).

1.8.3 Methods of isolation and identification of essential oils

Traditional steam distillation (SD) technique is used for extraction of thermoslabile volatile compounds from natural products. It allows these compounds to distil and subsequently recovered at a temperature below that of the boiling points of the individual constituents. Essential oils contain substances with boiling points up to 250^{o}C. In the presence of boiling water, these substances are volatilized at a temperature close to 100^{o}C at atmospheric pressure. The mixture of hot vapors will, if allowed to pass through a cooling system, condense to form a liquid in which the oil and water comprise two distinct layers. Most essential oils are lighter than water and form the top layer.

After separating the oil from water using n-hexane solvent, the oil composition and constituents are determined directly by GC-MS (Romanik *et al.*, 2007).

1.8.4 Inductively coupled plasma-optical emission spectrometry (ICP-OES)

ICP-OES is a precise, sensitive and accurate atomic emission tool to determine minerals in different matrices.

The sample solution is first nebulized into a radiofrequency (RF)-induced argon plasma. The small divided drops of the sample that reached the plasma are immediately dried, vaporized, and energized through collisional excitation at extremely high temperature. The atomic emission from the plasma is viewed in either a radial or axial configuration, focused by a lens or mirror, and imaged onto the entrance slit of a certain wavelengths.

The particular wavelength excited the monochromator which converted it to an electrical signal by a photodetector. The signal is amplified and processed by the detector then registered and stored by the computer.

The number of photons is proportional to the concentration of the element in the sample. Up to 70 elements can be detected at single run with the combination of a polychromator and an array detector (C.B. Boss, 1997; Fassel, 1986).

1.9 Problem statement and motivation of the study

A few studies about Palestinian herbs are available, the composition, efficacy and safety is still unexplored. In addition, due to the undesirable side effects of the orthodox synthetic medications such as toxicity and carcinogenicity and the emerging microbial resistance to available antimicrobial agents, attention has considerably increased to find out naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine.

S. palaestina was chosen as a model for this study because of its long medicinal reputation among Palestinians. The lack of phytochemical composition of its volatiles, semivolatiles and minerals and the scarcity in pharmacological studies on indigenous *Salvia* in Palestine motivated this research.

1.10 Aim of the study

The aim of this study is to screen Palestinian *S. palaestina* secondary metabolites and minerals by using SD-GC-MS and ICP-OES and to examine some of their claimed pharmacological activities. Test will include anti-oxidant, anti-bacterial and anti-fungal biological activities.

1.11 Objectives of the study

1. To extract the major crude essential oils present in *S. palaestina* by SD and to analyze the extract by GC-MS technology at the electron impact (EI) mode.
2. To compare the essential oil type and composition of the wild vs. cultivated *S. palaestina's* leaves from different locations.
3. To assess a relation between *S. palaestina's* growing location and availability of certain essential oils.

4. To estimate the anti-oxidant activity of *S. palaestina* essential oils using a spectrophotometric method.
5. To estimate the inhibitory effects of the essential oils on the growth of selected bacteria and fungus in comparison with positive controls.
6. To study the *S. palaestina* leaves minerals content by ICP-OES.

Chapter Two

Literature Review

2. Literature Review

The essential oils produced by *Salvia* are acknowledged worldwide because of their beneficial uses (Sylvestre *et al.*, 2006). There are number of reports in literature on the analyses of essential oils by using GC-MS technology from plants of other *Salvia* species with morphological and genetic variations according to their geographical origin (Farhat, Affara, & Gali-Muhtasib, 2001; Schnitzler, Nolkemper, Stintzing, & Reichling, 2008; Zhong *et al*., 2009).

Although, *Salvia* species have been scientifically studied in many parts of the world for their biological activities including anti-inflammatory (Abu-Darwish *et al*., 2013; Baricevic *et al*., 2001) , antimicrobial (Matu & van Staden, 2003), antimalarial (Jaber, 2013) anticancer (Sertel, Eichhorn, Plinkert, & Efferth, 2011) , anti-oxidant (Kolak *et al*., 2009), anticholinesterase (Chan *et al*., 2011) etc. Palestinian *Salvia* has not yet been fully investigated for its pharmacological effectiveness.

In Palestine, few studies are available mainly to evaluate the relative efficacy of medicinal plants for treatment of certain conditions such as skin, prostate and urinary disorders (Ali-Shtayeh, Yaniv, & Mahajna, 2000).

Among the few available investigations of volatile natural products in Palestine, the wild and cultivated thyme (*Majorana syriaca*) from different Palestinian locations were studied using SD and HS-GC-MS technologies (Abu-Lafi *et al*., 2007). It was found that HS technology offered faster, reliable, and simultaneous method for the determination of the more essential oils present in Palestinian thyme.

A survey among students in Palestinian universities was performed to find out the most commonly used medicinal herbs in Palestine, it found out that Sage ranked first (Sawalha *et al*., 2008).

In 2008, a study was performed to investigate the efficacy of aqueous and ethanol extracts of some Palestinian medicinal plants for potential antibacterial activity, but *S. palaestina* was not included (Shanab, 2008).

At the mid of 2013, different medicinal Palestinian plants were investigated to test their antimicrobial activity against acne inducing bacteria. However, *S. palaestina* was not included in the study (Ali-Shtayeh, 2013).

At the end of 2013, at Al-Quds University, the crude alcoholic extract of *S. palaestina* leaves was examined for the antimalarial activity. The revealed activity was about 75% of that from the positive control (chloroquine) (Jaber, 2013).

In the last two years, intensive researches were conducted in Jordan on this plant. However, other species was investigated rather than *S. palaestina*. For example, one research was aimed to study the composition of *S. officinalis* by using SD-GC-MS. It was reported that there was two main components (eucalyptol and camphor) in the oil. The activity of the oil was examined on fungus only, and dermatophyte strains were reported to be sensitive (Abu-Darwish *et al*., 2013).

Fresh and dried aerial parts of wild Jordanian, *S. palaestina* essential oil composition were studied after SD by GC-MS. The main component in both leaves oil was germacrene D and the percentages were 21.18% and 26.02%, respectively. Air-drying of the leaves resulted in an increase in sesquiterpene hydrocarbons and a decrease in the monoterpene hydrocarbons content. However, cultivated leaves was not included in this study (Al-Jaber, *et al.,* 2012).

Moreover, *S. palaestina* growing wild in Iran was examined by GC-MS. The main components were germacrene D (14.0%), *β*-bisabolene (11.9%), 1-*epi*-cubenol (9.8%), decanal (7.0%), *β*-caryophyllene (6.1%) and isobornyl butanoate (5.8%) (Salehi, 2005).

Other studies were conducted on *Salvia* from Mediterranean neighboring countries such as Turkey, Lebanon, Algeria, and Saudi Arabia (Al-Howiriny, 2007; Senatore, 2005; Gürsoy, 2011). All of these studies have examined native *Salvia* and none has examined *Salvia* grown in Palestine.

An interesting study that is utilized HS technology was performed in Poland. Twenty species of *Salvia* were investigated by HS-GC-MS. The main components were α-

pinene, camphene, β-pinene, thujol, camphor. The aim of this study was to find out compounds that can be used as chemotaxonomic markers, however, *S .palaestina* was not one of these twenty species (Rzepa *et al*., 2009).

Differences between the conducted studies revealed that there are remarkable variations in essential oils types, yields and even the pharmacological activities and were attributed mainly to different geographic locations (Al-Howiriny, 2007; F. Senatore, 2005; Gürsoy, 2011). For example, GC-MS analyses of the essential oils from Turkey resulted in the determination of 70 different compounds. The major compounds were caryophyllene oxide (16.1%) and (*E*)-caryophyllene (4.5%) (Gürsoy, 2011).

In Lebanon, the essential oils of *S. palaestina* was obtained by hydrodistillation of aerial parts and analyzed by GC-MS. Thirty-four compounds were identified and the major components were sclareol (20.2%), β-caryophyllene (16.6%) and linalool (8.6%). It is reported that the oil shows activity against Gram-positive bacteria (Senatore, 2005).

Another investigation from Saudi Arabia showed that out of thirty-eight GC peaks, thirty-four components were identified. The major components were sclareol (26.8 %), β-caryophyllene (16.9 %), linalool (7.8 %), guaiol (5.4 %) and eucalyptol (5.2 %) (Al-Howiriny, 2007).

In the last decade, several clinical trials have been developed in order to investigate the actual value of *Salvia* species. Results obtained are promising and confer scientific basis in the use of medicinal plants in traditional medicine. The antimicrobial activity of *Salvia's* essential oils was pronounced against Gram-positive more than Gram-negative bacteria (Fraternale, *et al.*, 2012; Kamatou, *et al*., 2008; Miguel, *et al*., 2011; Sonboli, *et al*., 2006). Unfortunately, no single research was conducted on Palestinian *S. palaestina*.

Due to the increasing health concerns and the toxicity of synthetic available antioxidants, natural antioxidants have been extensively examined in recent years. The antioxidant activity of *Salvia* species was reported in many researches. In Jordan,

for example, butanol fraction was examined for the antioxidant activity by using DPPH radical scavenging activity and was reported to be (91.9%) for the flavonoid luteolin 7-O-(2″-p-hydroxybenzoyl)-β-glucuronide) (Al-Qudah, Al-Jaber, Abu Zarga, & Abu Orabi, 2014). However, to the best of our knowledge, literature survey revealed that there is no single reference on the antioxidant activity of *S. palaestina* in Palestine.

Heavy metals in medicinal herbs have been studied in many countries as an indicator of pollution and mainly to evaluate the impact of traffic on metal concentration in herbs growing nearby roads. One of these researches was conducted in Montenegro on *S. officinalis* leaves and the outcome of this research was that the concentration of heavy metals decreases at 50-100 m from the edge of a major roads. Therefore, the impact of road traffic through the pollution of aromatic herbs was noted (Adamo *et al.*, 2002; Annan *et al*., 2010; Bakircioglu, Kurtulus, & Ibar, 2011; Blagojeviae, 2009). Moreover, researches showed that the ability of different medicinal plants to accumulate minerals depends on the plant species, the geographical location and environmental conditions and thus it was advised to examine the availability of certain minerals in plants before using them (Annan.k, 2013). Intensive literature survey indicated that there is no single study on the *S. palaestina* minerals in Palestine.

Chapter Three

Methodology

3. Methodology

3.1 Collection of plant materials

Wild and cultivated *S. palaestina* leaves were collected from seven different governorates (9 locations) in Palestine between April and May 2013 (**Table 1**).

Figure 2 shows the investigated sample's location from northern to southern of Palestine.

Fig. 2

Fig. 2: Map showing the sites of collected *S. palaestina* (google maps).

Some of *S. palaestina* leaves were air dried in the absence of light at room temperature for a about one week untill constant weight is achieved.

Dried samples were stored in sealed paper bags protected from light.

Table 1 Sample's location, type and harvesting time.

Location Village/District	**Native of *S. palaestina***	**Harvesting Date**
Anabta/Tulkarem	Wild	28/4/2013
	Cultivated	28/4/2013
Beita/Nablus	Wild	29/4/2013
	Cultivated	29/4/2013
Kafr Na'ma/Ramallah	Wild	5/5/2013
	Cultivated	5/5/2013
Halhul/Hebron	Wild	19/5/2013
	Cultivated	12/5/2013
Al-Khadr/Bethlehem	Wild	19/5/2013
	Cultivated	19/5/2013
Ya'bad/Jenin	Wild	22/5/2013
	Cultivated	22/5/2013
Jericho	Cultivated	19/5/2013
Deir Istiya/Salfit	Wild	30/4/2013
Abud/Ramallah	Wild	19/5/2013

5.3 Examination of *S. palaestina* leaves by scanning electron microscopy (SEM)

Scanning electron microscope (SEM) was employed to analyze the morphology of fresh S. *palaestina* wild and cultivated leaves. The experiments were performed on high resolution scanning electron microscope (HR SEM) Sirion (FEI Company) using Shottky-type field emission source and secondary electron (SE) detector. The images were scanned at voltage of 5kV.

3.3 Isolation and extraction of *S. palaestina's* essential oils, sample preparation and GC-MS analysis

3.3.1 Reagents

GC grade n-hexane solvent and anhydrous sodium sulfate salt were purchased from Sigma-Aldrich Inc. (USA). Kovats retention index (KI) reagent that consist of alkane standard mixture were between C_{10}-C_{40} (even numbered) were purchased from Fluka, Switzerland. All the reference standards and the drugs used in the thesis were kindly supplied by the Central Public Health Laboratory, Ministry of Health, Ramallah, Palestine.

3.3.2 Equipments

The following equipments were used during the entire investigation, simple distillation system (clevenger apparatus), analytical balance (Sartorius, accuracy ±0.0001g, Germany), rotary evaporator (Steroglass-strike202, Italy), Whatman filter papers #1, separatory funnels, glass funnels, graduated cylinders, micropipete, erlenmeyer flasks, brown glass bottels (300 and 25 ml) and brown 2ml-GC vials.

3.3.3 Preparation of SD samples and essential oils isolation

The essential oils of the *S. palaestina* leaves were isolated by distillation using a Clevenger type apparatus according to the following procedure:-

1- About 10 gm of the leaves from each governorate were grounded and mixed with 250 ml distilled water.
2- The sample was subjected to steam distillation for three hours at atmospheric pressure.
3- The water distillate was extracted twice with 100 ml hexane using separator funnel.
4- The hexane fractions were combined and dried over anhydrous sodium sulfate.
5- 300 µL of hexane extract was diluted to 1 mL with hexane and 1 µL of the resulted diluted sample was injected to GC-MS using optimized method.
6- The remaining hexane fractions was evaporated under vacuum by rotary evaporator and the **oil was collected, weighed and kept in closed amber vials at deep freezing conditions** for antimicrobial and antioxidant activity examination.

3.3.4 Instrumentation

Essential oils were analyzed using Perkin Elmer, Clarus Gas Chromatography connected to Clarus 600 C mass spectrometer (USA). The GC-MS was operated in the electron impact ionization mode (EI) at 70 eV. Perkin Elmer autosampler was used with 2ml vials. The GC is equipped with a fused silica capillary column; DB-5 MS consisted of (5% diphenyl polysiloxane, 95% dimethyl polysiloxane) 28 m x 0.25 mm, coating film thickness is 0.25 µm (Restck, USA).

GC-MS chromatographic condition for steam distillation samples

Perkin Elmer GC-MS at electron impact mode (EI) was used. The flow rate of the carrier gas was 1 ml He/min. Injector temperature was set at 235^{o}C, the source temperature was at 250^{o}C and the interface temperature was at 260^{o}C. Split ratio of 1:20 was adopted during the entire analysis.

The column gradient temperature was held at 50°C for 2 minutes, then raised from 50°C to 180°C at a ramp of 5°C/min and from 180° to 280°C at a ramp rate of 15°C/min and held there for extra 5min. Solvent cut time of 4.5 minutes was used to eliminate the solvent gigantic peak. The mass range was from 50 up to 480 Da, and of scan interval of 0.2 seconds.

Peaks identification

The identification of compounds was based mainly on matching their MS spectra with NIST mass spectral library. Moreover, Kovats Retention Index **I** calculation was used to support the identification of our results according to the following equation (Kovats, 1958):

$$I = 100[n + (N - n)\frac{\log t_r^{'}(unknown) - \log t_r^{'}(n)}{\log t_r^{'}(N) - \log t_r^{'}(n)}]$$

where
n is the number of carbonatoms in the smaller alkane
N is the number of carbonatoms in the larger alkane
$t_r^{'}$ is in all cases the adjusted retentiontime (measured time minus the time of the undelayed methane or small compound.

KI values were compared with literature NIST values. Excellent agreement was obtained even using different chromatographic conditions.

Quantitative analysis of the essential oils was performed once the identities of the compounds are known by the MS.

3.4 Evaluation of the anti-oxidant activity

Electrons donation ability of *S. palaestina* essential oils was measured from the bleaching of purple colored methanol solution of 2, 2'-diphenyl-1-picrylhydrazyl stable radical (DPPH) using spectrophotometric assay. After incubation period, the absorbance was measured at 517nm.

Different concentrations of *S. palaestina* essential oil in methanol were prepared, 50 microliters of each concentration were added to 2 ml of DPPH solution (DPPH concentration was 6×10^{-5} M), all samples were warped with aluminum foil and kept in dark place (drawer). Absorbance at wavelength 517 nm was measured at three different time points, namely after 30 min, 1 hour and 1.5 hour.

Percentage of the antioxidant scavenging activity (AI %) was calculated from the following equation and then plotted against concentration to calculate the AI_{50} or IC_{50} (Brand-Williams , 1995):

(AI%), %DPPH radical scavenging activity = [1-(As/Ac)]*100

Where,
AI: Antioxidant index
As: Sample absorbance Ac: Control absorbance
The control was DPPH. The blank was methanol.

3.5 Antimicrobial activity

3.5.1 Reagents

Nutrient agar (Difco), sabouraud dextrose agar (Difco) for *Candida albicans*, purified water, 0.9 % sodium chloride AR solution, ciprofloxacin standard, gentamicin standard, nystatin standard, barium chloride AR and sulfuric acid AR were kindly supplied by the Central Public Health Laboratory, Ministry of Health, Ramallah, Palestine.

3.5.2 Instrumentation

The incubator used during this work was of B-series (bd15) with mechanical control, (Binder- Germany), while the autoclave was 3870e model (Tuttnauer- USA) and the UV-Vis spectrophotometer was lambda 25 (Perkin Elmer-USA).

3.5.3 Microbial strains and their American Type Culture Collection (ATCC#)

- *Staphylococcus aureus* (25923)
- *Staphylococcus epidermidis* (12228)
- *Candida albicans* (10231)
- *E.coli* (8739)
- *Salmonella Typhimurium* (14028)

All the above strains were from Becton Dickinson, France.

3.5.4 Microbial suspension preparation

1- Each strain was suspended in 0.9% NaCl solution until reaching to 0.5 Macfarland standards this was achieved by measuring transmittance by UV-vis spectrophotometer.

2- Macfarland standards were used to standardize the approximate number of microbes in the liquid suspension by visually comparing the turbidity of microbial suspension with the turbidity of 0.5 Macfarland standard (**Figure 3**) and was prepared as follows:

- 85 ml of 1% (v/v) sulfuric acid was added into a 100 volumetric flask.
- 0.5 ml of 1.175% barium chloride solution was added dropwisely to the above flask with continuous swirling and stirring.
- The volume was completed to 100 ml by 1% (v/v) sulfuric acid.
- The optical density (amount of light scattered by bacteria) was measured at 625nm and should be in the range (0.08-0.1).

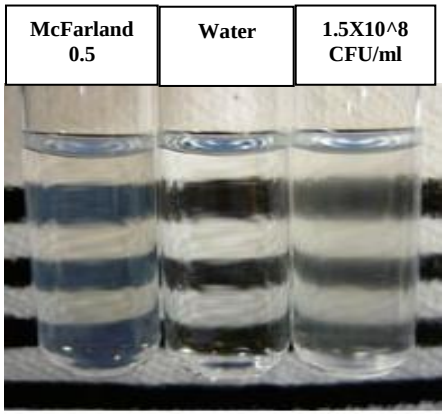


Fig. 3: Visual comparing of microbial suspension turbidity with 0.5 Macfarland standards by using Wickerham card as background.

3.5.5 Media preparation

1- 1 liter of nutrient agar (Difco) and sabouraud dextrose agar (Difco) was prepared in water, dissolved, boiled and sterilized at 121 °C for 20 min.
2- 20 ml of agar was added to each plate.
3- 0.1 ml of microbial NaCl suspensions was diluted with NaCl to 10 ml then 0.1 ml of the new suspension was spread with glass rod on the surface of the agar plate by streaking.
4- The final concentration of both bacteria and fungi on each plate was about 1.5×10^6 CFU/ ml.

3.5.6 Positive control preparation

Solutions of gentamicin, ciprofloxacin and nystatin (10µg/ml) were prepared and were used as positive control.

3.5.7 Procedure

1- The test was carried out using disk diffusion method.
2- Four disks were spread on the surface of the media in each plate.
3- 5 µl of sample (*S. palaestina* oil)/positive control was added to each disk.
4- Each plate was incubated at 37 °C for 24 hr for bacteria while for *Candida albicans* it was incubated at 25 °C for 72 hr.
5- The zone of inhibition was measured and the result was documented.

3.6 Minerals analysis

3.6.1 Reagents

Milli.Q ultra-pure water (Resistivity > 18 megaohm (MΩ·cm), high Purity Nitric Acid 3% optima grade was purchased from Fisher chemicals and Fluka multielement standard solution 5 for ICP (Trace cert.) Lot # BCBH5213V.

3.6.2 Instruments

Perkin Elmer ICP-OES (DV7300), muffle furnace 6000 (USA).

1. The optimized instrumental condition were as listed below:
    - Power 1450 watt
    - Plasma Gas flow : 15 L/Min
    - Auxiliary Gas flow: 0.2 L/Min

- Nebulizer Gas Flow : 0.8 L /min
- Peak Algorithm : Peak Area
- Number of Replicate: 3
- Read Time: 2 -10 sec
- Plasma View: Axial and Radial

3.6.3 Procedure

3.6.3.1 Sample preparation

Two samples of dried leaves of *S. palaestina* (1g and 0.5 g) was transferred into a silica crucible and kept into a muffle furnace for ashing at 450 °C for 7 hours and then 2 ml of 3% HNO_3 was added to crucible. Care was taken to ensure that all ash was in contact with the acid. Then, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 3% HNO_3 solution and made up to 25 ml. Working standard solutions were prepared by diluting the stock solution with 3% HNO_3.

3.6.3.2 Blank and standard solution preparation

In order to prepare the blank and the standard solutions, a 3 % HNO_3 solution was used as diluent (42 ml of High Purity HNO_3 Acid 70% diluted into 1000 ml volumetric flask (V.F.) with Milli.Q water used as blank and as diluent in standards preparation)

All standards were prepared from stock Multielements standard solution 5 for ICP (**Table 2** below summarized all elements concentration).

Standard # 1: Dilute 0.05 ml from stock Multielements standard solution 5 into 100 ml V.F. using diluent 3% HNO_3 and complete to mark.

Standard # 2: Dilute 0.5 ml from stock Multielements standard solution 5 into 100 ml V.F. using diluent 3% HNO_3 and complete to mark.

Standard # 3: Dilute 1.0 ml from stock Multielements standard solution 5 into 100 ml V.F. using diluent 3% HNO_3 and complete to mark.

Standard # 4: Dilute 2.5 ml from stock Multielements standard solution 5 into 100 ml V.F. using diluent 3% HNO_3 and complete to mark.

Standard # 5: Dilute 5.0 ml from stock Multielements standard solution 5 into 25 ml V.F. using diluent 3% HNO_3 and complete to mark.

Table 2 Summary of the concentrations of standards used in the ICP analysis of minerals

Element	Stock Std. ppb	Std # 1	Std # 2	Std # 3	Std # 4	Std # 5
Ag	10010	5.005	50.05	100.1	500.5	2002
Al	10010	5.005	50.05	100.1	500.5	2002
Ba	10010	5.005	50.05	100.1	500.5	2002
Ca	100100	50.05	500.5	1001	5005	20020
Cd	10010	5.005	50.05	100.1	500.5	2002
Co	10010	5.005	50.05	100.1	500.5	2002
Cr	10010	5.005	50.05	100.1	500.5	2002
Cu	10010	5.005	50.05	100.1	500.5	2002
Fe	100100	50.05	500.5	1001	5005	20020
K	100100	50.05	500.5	1001	5005	20020
Mg	10010	5.005	50.05	100.1	500.5	2002
Mn	100100	50.05	500.5	1001	5005	20020
Mo	10010	5.005	50.05	100.1	500.5	2002
Na	100100	50.05	500.5	1001	5005	20020
Ni	10010	5.005	50.05	100.1	500.5	2002
Pb	10010	5.005	50.05	100.1	500.5	2002
Sr	10010	5.005	50.05	100.1	500.5	2002
Zn	10010	5.005	50.05	100.1	500.5	2002

Chapter Four

Results and Discussion

4. Results and Discussion

4.1 Examination of *S. palaestina* leaves morphology by scanning electron microscopy (SEM)

Aromatic plants in general exhibit significant morphological and phytochemical variabilities. Even among the same genus, *Salvia*, morphology is expected to be very variable since it is reported that it has more than 900 species (Hedege, 1992).

It is well known that the secondary metabolites are preserved in the trichomes of *Salvia's* leave in order to avoid the direct contact of them with the vital leave tissues. Since our research aimed at investigating these secondary metabolites, scanning electron microscopy (SEM) of *S. palaestina* wild and cultivated fresh leaves were performed and comparison between both was conducted as follows:

Upper vs. lower surface of the leaves:

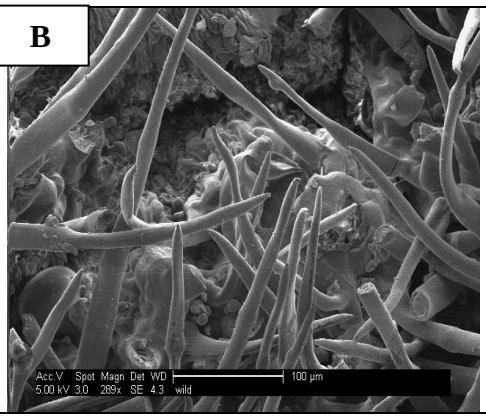
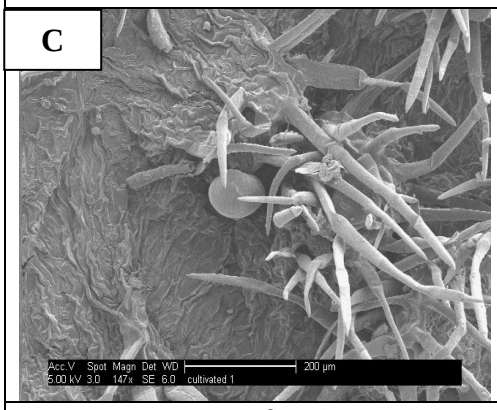
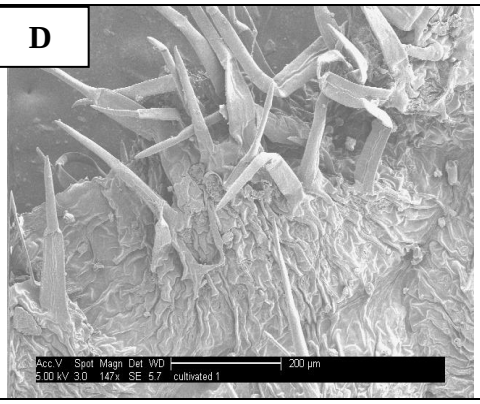
<table>
<tr><td>A
  </td><td>B
  </td></tr>
<tr><td>A: SEM image of wild lower leaf surface</td><td>B: SEM image of wild upper leaf surface</td></tr>
<tr><td>C
  </td><td>D
  </td></tr>
<tr><td>C: SEM image of cultivated lower leaf surface</td><td>D: SEM image of cultivated upper leaf surface</td></tr>
</table>

Fig. 4: SEM of wild vs. cultivated *S. palaestina*

Figure 4 highlights the comparison between A and B, which shows that the intensity of trichomes in the lower surface is higher than that in the upper surface. The same observation was also noticed for cultivated sample when C is compared to D.

Figure 4 also illustrates the comparison between A and C, which shows that the intensity of trichomes in the lower surface is higher than that in the cultivated sample. The same was also noticed for the upper surface when B is compared to D.

Wild vs cultivated leaves:

SEM images of both wild and cultivated leaves surface is illustrated in **Figure 5** below:

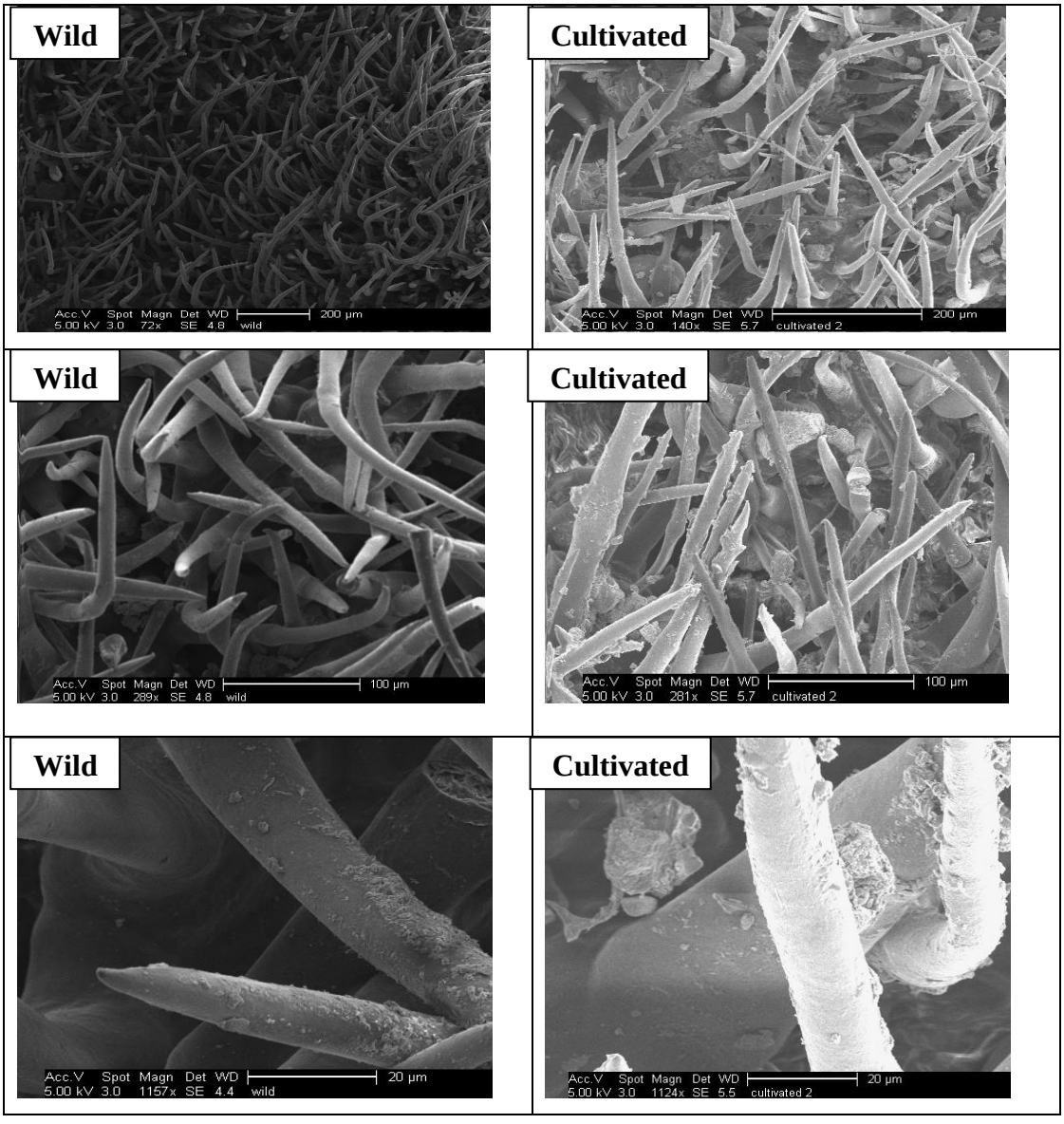
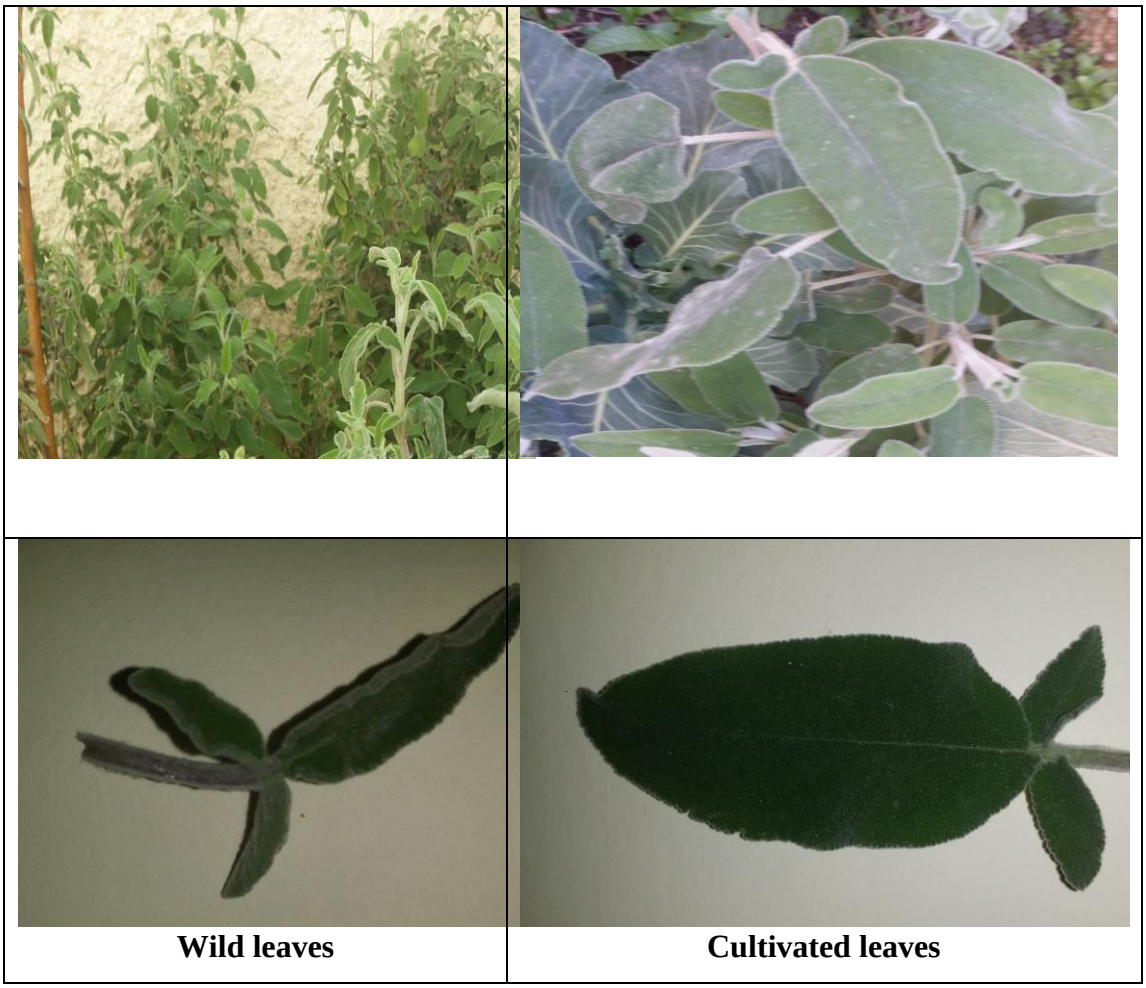


Fig. 5: SEM of wild and cultivated leaves.

Comparison between wild and cultivated leaves showed that not only the intensity of trichomes of leaves is different but also the surface structure is different. It is obvious that wild leaves contains more intensive, branched, thinner, smoother and darker trichomes than that in the cultivated leaves.

The above results are consistent with the fact that all creatures seek to adapt and cope with its environmental conditions. Thus, the morphology of the wild leaves of *S. palaestina* was found to be different from cultivated in order to avoid harsh environmental conditions and to avoid loss of water and essential oils. This also observed from the width of the leaf which is much smaller in wild and the edges of wild leaf are curved inside as a cover.

Although the cultivated leaves are larger but they are thicker than the wild which might help in preservation of the oil and delay the evaporation to surrounding environment **(Fig. 6)**.



Wild leaves | **Cultivated leaves**

Fig. 6: Wild vs. cultivated leaves

4.2 Yield of dry *S. palaestina* leaves oils

S. palaestina leaves were collected from seven different Palestinian governorates between April 2013 and May 2013.

Prior extracting the oil of *S. palaestina*, part of fresh leaves was dried and the water loss was calculated to acquire information about water content in the leaves. The essential oils of dried leaves were then isolated by steam distillation (SD). The harvesting time, location, water loss percent and the oil yield were summarized in **Table 3**.

Table 3: *S. palaestina* leaves location, type, harvesting date, water loss% and oil yield%

Location	***S. palaestina* type**	**Harvesting date**	**Dried/Fresh Weight Ratio**	**Average of water loss w/w%± SD (*n=3*)**	**Average of oil yield w/w%± SD (*n=3*)**
Anabta/ Tulkarem	Wild	28/04/2013	33.2/100	66.8±1.153	ND*
	Cultivated	28/04/2013	31.0/100	69±1.007	ND
Beita/ Nablus	Wild	29/04/2013	33.4/100	66.6±1.168	0.074±0.17
	Cultivated	29/04/2013	23.1/100	76.9±0.971	0.275±0.067
Kafr Ni'ma/ Ramallah	Wild	05/05/2013	49.6/100	50.4±0.985	0.304±0.096
	Cultivated	05/05/2013	45.7/100	54.3±0.814	0.64±0.054
Halhul/ Hebron	Wild	19/05/2013	34.6/100	65.4±0.954	0.698±0.085
	Cultivated	12/05/2013	25.4/100	74.6±1.026	0.7±0.104
Al-Khader/ Bethlehem	Wild	19/05/2013	30.7/100	69.3±1.079	0.72±0.131
	Cultivated	19/05/2013	29.5/100	70.5±1.179	1.58±0.199
Ya'bad/ Jenin	Wild	22/05/2013	46.6/100	53.4±0.874	0.552±0.095
	Cultivated	22/05/2013	26.9/100	73.1±1.124	0.735±0.136
Jericho	Cultivated	19/05/2013	31.2/100	68.8±0.954	0.183±0.066
Deir Istiya/ Salfit	Wild	30/04/2013	63.5/100	36.5±0.493	0.434±0.081
Abud/ Ramallah	Wild	19/05/2013	45.3/100	54.7±0.862	0.234±0.076

*ND: Not determined.

Figure 7 and **Table 3** revealed that the water loss was higher in the cultivated samples vs. the wild in all the selected locations as was expected. However, the water loss in the wild leaves apparently depends mostly on the topographical nature, rainfall and relative humidity of the location.

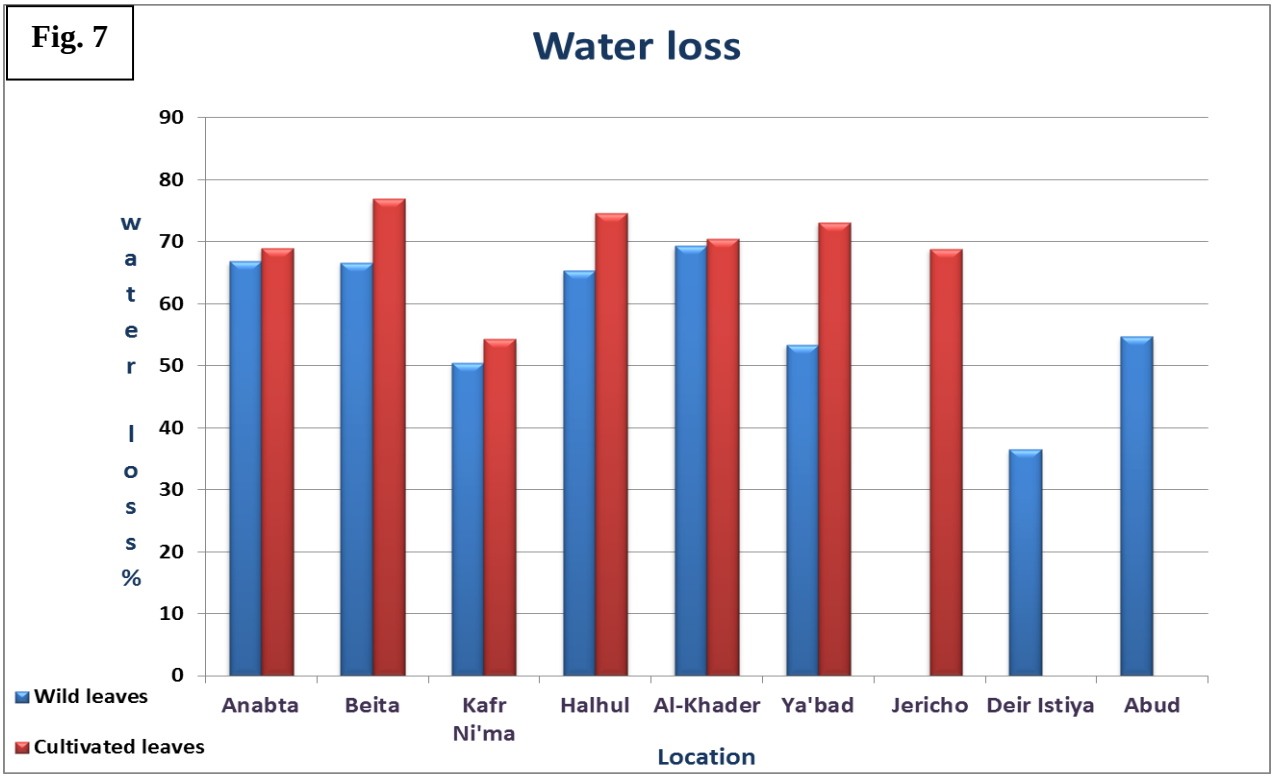


Fig. 7: Water loss of *S. palaestina* leaves.

The oil yield was calculated based on weight of oil to weight of each dried sample. The average oil yield (w/w) was approximately 0.46 % for the wild samples while it was 0.63% for the cultivated.

It was noticed from **Figure 8** that the oil yield was higher in cultivated vs wild samples, which was not quite expected, but it seems that at the vegetative stage at this time of the year, the cultivated leaves are more worthful.

Fig. 8

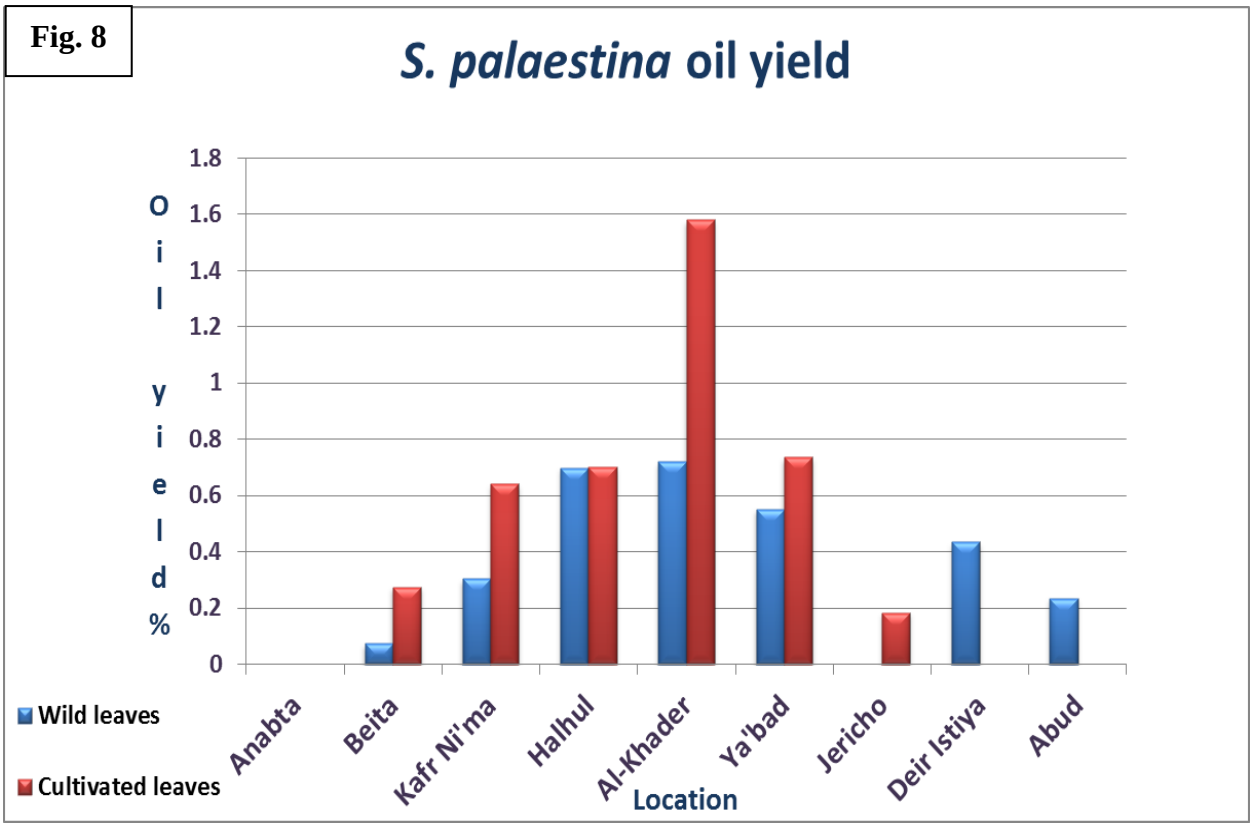


Fig. 8: Oil yield of *S. palaestina* leaves.

4.3 GC-MS analysis:

4.3.1 Identification of separated components

The essential oils were analyzed by GC-MS in the Electron Impact (EI) mode and identified by comparing with NIST library and by calculating Kovats Index (KI). Twenty major components were identified. The structure, molecular formula, retention time and KI values are summarized in **Table 4**.

The following are the total ion chromatograms (TIC) GC-MS that comprises some of the main components in cultivated and wild oil sample collected from different locations in Palestine. All the complementary detailed TICs are present in the appendix of this thesis.

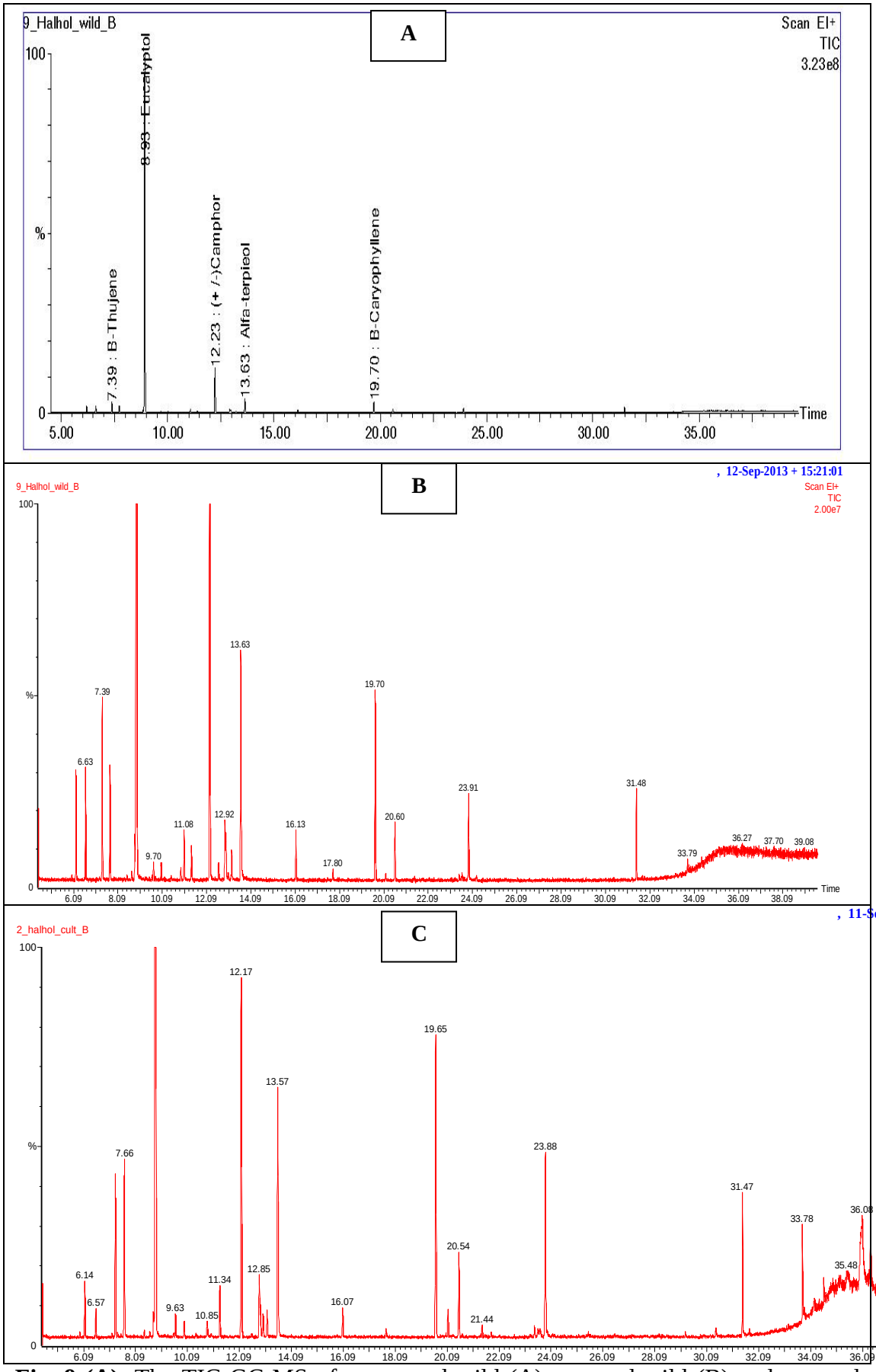


Fig. 9 (A): The TIC GC-MS of unzoomed wild (A), zoomed wild (B) and zoomed cultivated (C) *S. palaestina* samples collected from Halhul.

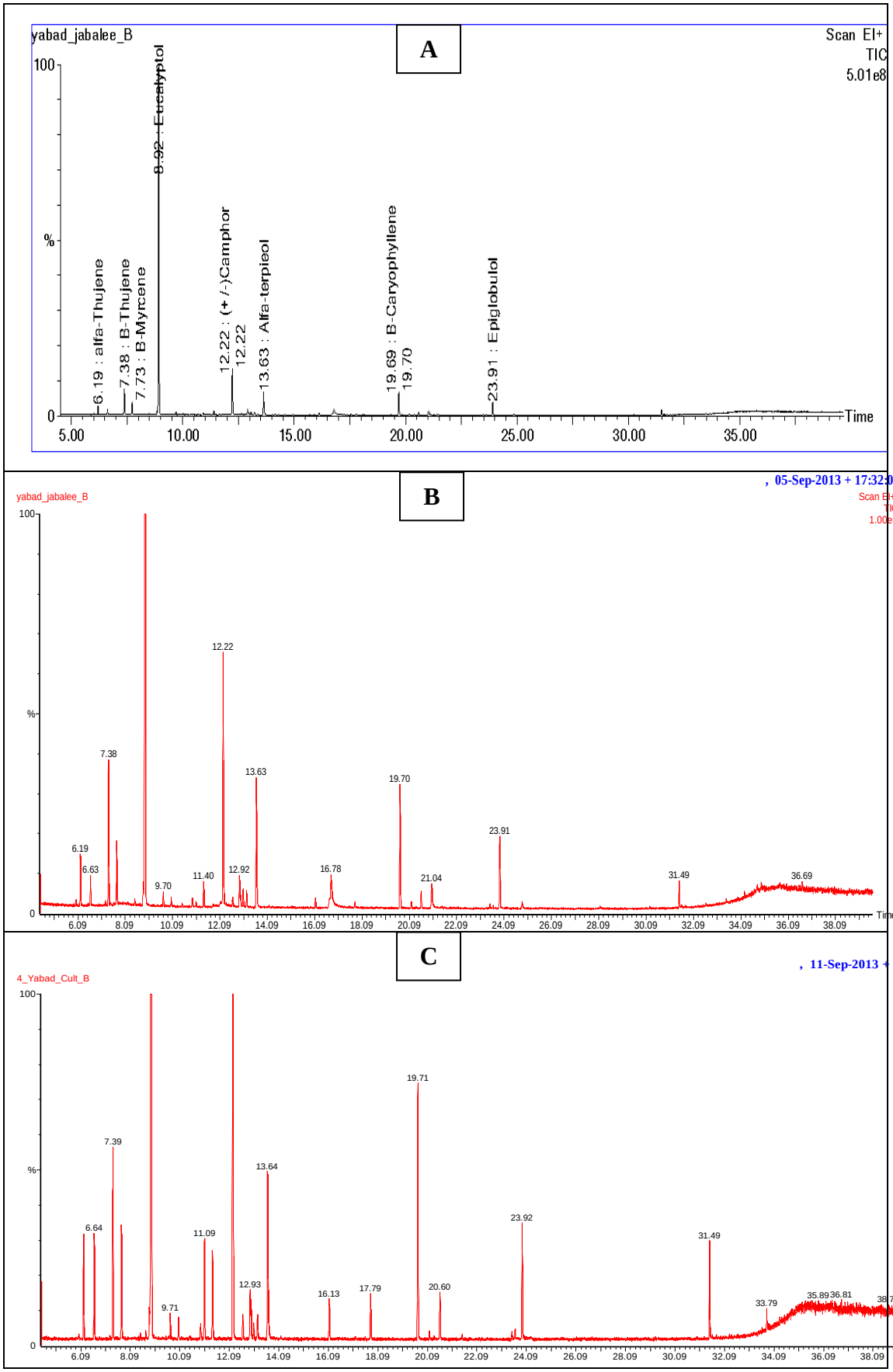
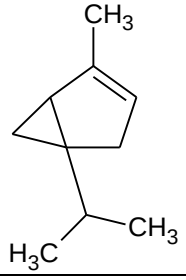
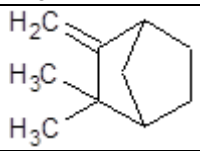
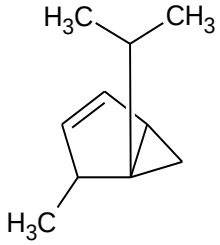
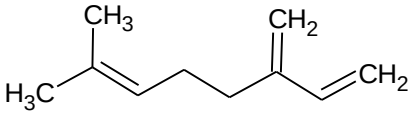
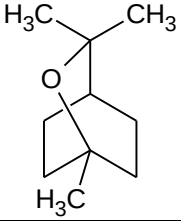
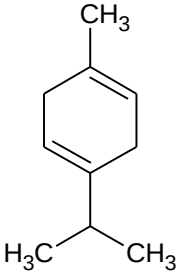
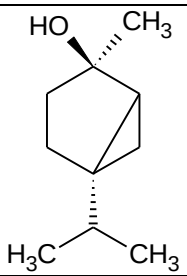
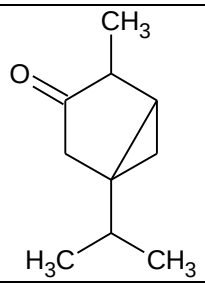
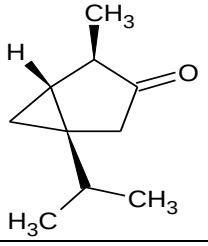
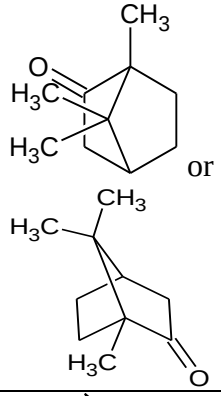
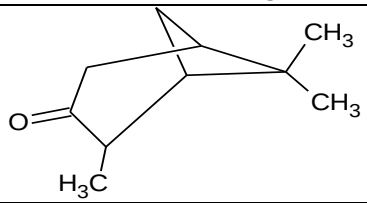
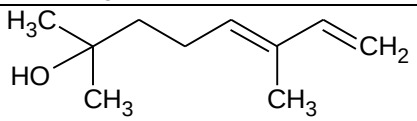
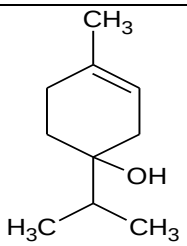
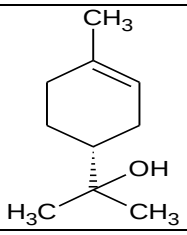
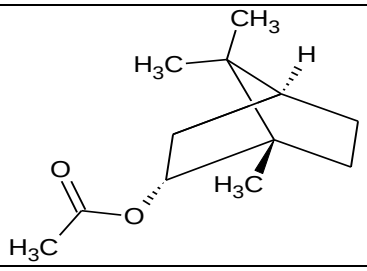
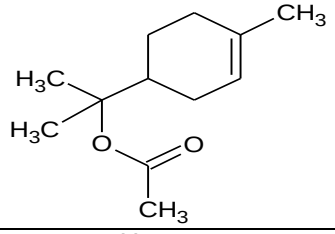
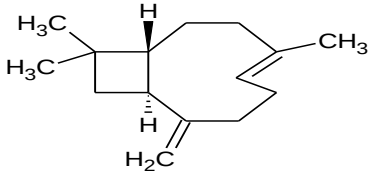
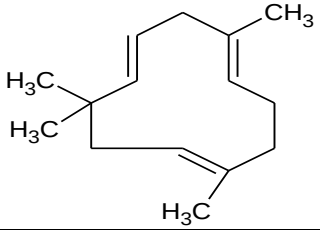
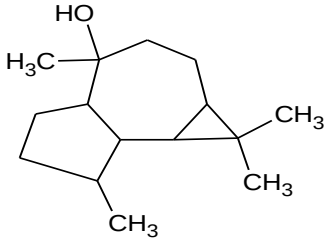
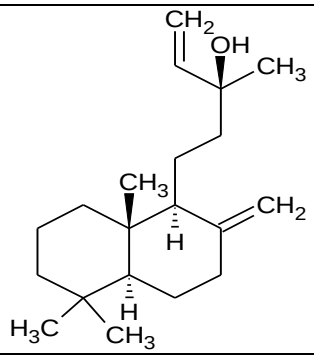


Fig. 9 (B): The TIC GC-MS of unzoomed wild (A), zoomed wild (B) and zoomed cultivated (C) *S. palaestina* samples collected from Ya'bad.

Table 4 Identified component, structure, molecular formula, retention times and KI values					
#	**Component**	**Chemical Structure**	**Molecular Formula**	**t_R (mins)**	**KI value**
1	α-Thujene		$C_{10}H_{16}$	6.209	
2	Camphene		$C_{10}H_{16}$	6.635	
3	β-Thujene		$C_{10}H_{16}$	7.392	
4	β-Myrcene		$C_{10}H_{16}$	7.736	
5	Eucalyptol		$C_{10}H_{18}O$	8.95	1042
6	γ-Terpinene		$C_{10}H_{16}$	9.707	1072
7	*trans*-4-Thujanol		$C_{10}H_{18}O$	10.04	1084

8	3-Thujanone		$C_{10}H_{16}O$	11.08	1120
9	α-Thujone		$C_{10}H_{16}O$	11.41	1131
10	(±)-Camphor		$C_{10}H_{16}O$	12.25	1156
11	3-Pinanone		$C_{10}H_{16}O$	12.64	1168
12	Ocimenol		$C_{10}H_{18}O$	12.94	1176
13	L-Terpinen-4-ol		$C_{10}H_{18}O$	13.24	1184
14	α-Terpineol		$C_{10}H_{18}O$	13.67	1196

15	L-Bornyl acetate		$C_{12}H_{20}O_2$	16.13	1293
16	Terpinyl acetate		$C_{12}H_{20}O_2$	17.84	1353
17	β-Caryophyllene		$C_{15}H_{24}$	19.71	1419
18	α-Caryophyllene		$C_{15}H_{24}$	20.6	1458
19	Epiglobulol		$C_{15}H_{26}O$	23.92	1591
20	13-Epi-manool		$C_{20}H_{34}O$	31.49	2066

As seen in **Table 4** and in the figures [**Fig. 9 (A)** and **Fig. 9 (B)**], the essential oils contain mainly monoterpenoids, oxygenated monoterpenoids and to less extent sesquiterpens and diterpens. Among all, eucalyptol (oxygenated monoterpenoid) was the abundant component in average concentration exceeding 50%.

All the essential oils were analyzed by GC-MS in triplicate. The relative standard deviation percentage (RSD %) were calculated for both the retention time and the peaks areas (**Table 5, 6**). The RSD % values were within acceptable limits for retention time and for peak areas in particular, since in case of low concentrations (in ppms), the accepted limit usually is <15%.

Table 5 The RSD % of retention time (RT) for each peak area *(n=3)*

Component	RT 1	RT 2	RT 3	Average	SD	RSD%
α-Thujene	6.209	6.209	6.209	6.209	0.000	0.000
Camphene	6.636	6.636	6.633	6.635	0.002	0.026
β-Thujene	7.391	7.391	7.395	7.392	0.002	0.031
β –Myrcene	7.733	7.736	7.74	7.736	0.004	0.045
Eucalyptol	8.93	8.929	8.934	8.931	0.003	0.030
γ-Terpinen	9.707	9.707	9.706	9.707	0.001	0.006
trans-4-Thujanol	10.041	10.04	10.041	10.041	0.001	0.006
3-Thujanone	11.081	11.088	11.085	11.085	0.004	0.032
α-Thujone	11.413	11.415	11.412	11.413	0.002	0.013
Camphor(+-)	12.235	12.234	12.235	12.235	0.001	0.005
3-Pinanone	12.641	12.637	12.641	12.640	0.002	0.018
Ocimenol	12.926	12.929	12.926	12.927	0.002	0.013
L-Terpinen-4-ol	13.233	13.232	13.229	13.231	0.002	0.016
α-Terpineol	13.639	13.637	13.642	13.639	0.003	0.018
L-Bornyl acetate	16.132	16.127	16.128	16.129	0.003	0.016
Terpinyl acetate	17.795	17.793	17.801	17.796	0.004	0.023
β-Caryophyllene	19.708	19.706	19.703	19.706	0.003	0.013
α -Caryophyllene	20.595	20.596	20.6	20.597	0.003	0.013
Epiglobulol	23.918	23.915	23.916	23.916	0.002	0.006
13-Epimanool	31.486	31.491	31.491	31.489	0.003	0.009

The very low RSD % values of retention time advocate that the GC-MS system is reproducible.

Table 6 The RSD % of the peaks areas (*n=3*)

Component	Area 1	Area 2	Area 3	Average	SD	RSD%
α-Thujene	160224.800	169973.400	167367.600	165855.267	5047.194	3.043
Camphene	187267.600	187555.600	188309.100	187710.767	537.809	0.287
β-Thujene	321089.900	336303.600	330685.400	329359.633	7693.010	2.336
β –Myrcene	187078.500	193205.500	190275.900	190186.633	3064.475	1.611
Eucalyptol	8645684.000	8854943.000	8714939.000	8738522.000	106604.178	1.220
γ-Terpinen	32761.100	37752.850	42744.600	37752.850	4991.750	13.222
***trans*-4-Thujanol**	41015.600	45605.600	46686.800	44436.000	3011.080	6.776
3-Thujanone	193122.800	198262.300	201001.500	197462.200	3999.825	2.026
α-Thujone	159311.000	162141.300	159073.600	160175.300	1706.739	1.066
Camphor(+-)	1585102.900	1672623.800	1603166.800	1620297.833	46206.939	2.852
3-Pinanone	45752.600	50176.100	48769.300	48232.667	2260.049	4.686
Ocimenol	91275.500	95996.200	94554.400	93942.033	2419.193	2.575
L-Terpinen-4-ol	51632.400	57496.500	56299.600	55142.833	3098.467	5.619
α-Terpineol	386500.700	411812.900	403870.300	400727.967	12945.368	3.230
L-Bornyl acetate	75375.400	78315.300	73051.000	75580.567	2638.140	3.491
Terpinyl acetate	87171.500	95924.700	87308.300	90134.833	5014.638	5.563
B-Caryophyllene	527274.200	537789.500	536841.800	533968.500	5816.767	1.089
α-Caryophyllene	98672.000	106413.100	100311.800	101798.967	4079.204	4.007
Epiglobulol	218009.900	243778.100	235330.300	232372.767	13136.220	5.653
13-Epimanool	112737.000	132372.400	130777.000	125295.467	10905.166	8.704

All the RSD % of peaks areas are within accepted limit <15% which indicate that even in very low concentrations, the amount of oil components determined is accurate.

4.3.2.1 Interpretation of the GC-MS results

The GC-MS results were interpreted mainly based on their EI-MS and in comparison to typical NIST stored MS's. The following **Table 7** shows the MS of the major isolated volatiles present in *S. palaestina*. It is worthwhile mentioning that each compound has some other synonyms.

Table 7 MS of the isolated volatiles from *S. palaestina* and their major fragments.

#	**Compound name**	**Nominal Molecular ion ($M^{\cdot+}$)**	**Other m/z major fragments**
1	**α-Thujene**	136	121, 119, 105, 94, 93, 92, 91, 77, 65, 53, 51, 43, 41 and 39
2	**Camphene**	136	121, 107, 93, 91, 79, 77, 67, 41, 39 and 27
3	**β-Thujene**	136	93, 91, 79 and 77
4	**β–Myrcene**	136	93, 69, 41,39 and 27
5	**Eucalyptol**	154	139, 111, 108, 96, 93, 84, 83, 81, 71, 69, 68, 67, 55, 43, 41 and 39
6	**γ-Terpinen**	136	121, 93, 92, 91 and 77
7	***trans*-4-Thujanol**	154	136, 121, 111, 93, 91, 81, 79, 77, 71, 69, 55, 43, 41, 39 and 27
8	**3-Thujanone**	152	110, 109, 95, 82, 81, 79, 70, 69, 68, 67, 55 and 41
9	**α-Thujone**	152	110, 109, 95, 82, 81, 79, 70, 69, 68, 67, 55 and 41
10	**Camphor(+-)**	152	109, 108, 95, 83, 81, 69, 67, 55, 41, 39 and 27
11	**3-Pinanone**	152	97, 95, 83, 81, 69, 67, 55, 41 and 39
12	**Ocimenol**	154	121, 93, 71, 69, 67, 55, 43 and 41
13	**L-Terpinen-4-ol**	154	111, 93, 86, 71, 69, 43 and 41
14	**α-Terpineol**	154	121, 93, 92, 91, 81, 79, 77, 68, 67, 59, 43, 41 and 39
15	**L-Bornyl acetate**	196	136, 121, 108, 95, 93, 43 and 41
16	**Terpinyl acetate**	196	136, 121, 93, 68, 59, 43 and 41
17	**B-Caryophyllene**	204	161, 148, 147, 134, 133, 121, 120, 119, 109, 107, 106, 105, 95, 94, 93, 92, 91, 81, 79, 77, 69, 67, 55, 53, 41 and 39
18	**α-Caryophyllene**	204	121, 93, 80 and 41
19	**Epiglobulol**	222	161, 121, 119, 109, 108, 107, 105, 95, 93, 91, 82, 81, 79, 77, 71, 69, 67, 55, 43, 41, 39, 29 and 27
20	**13-Epimanool**	290	257, 137, 136, 123, 121, 109, 107, 95, 94, 93, 91, 81, 80, 71, 69, 67, 55, 43 and 41

The molecular ions and the fragmentation patterns were found to have full match with the NIST library. For example, the three major peaks that share the same molecular weight of 152 Da, namely, camphor, 3- thujonone and α-thujone when matched with NIST authentic MS gave excellent conformity as shown in **Figure 10** below.

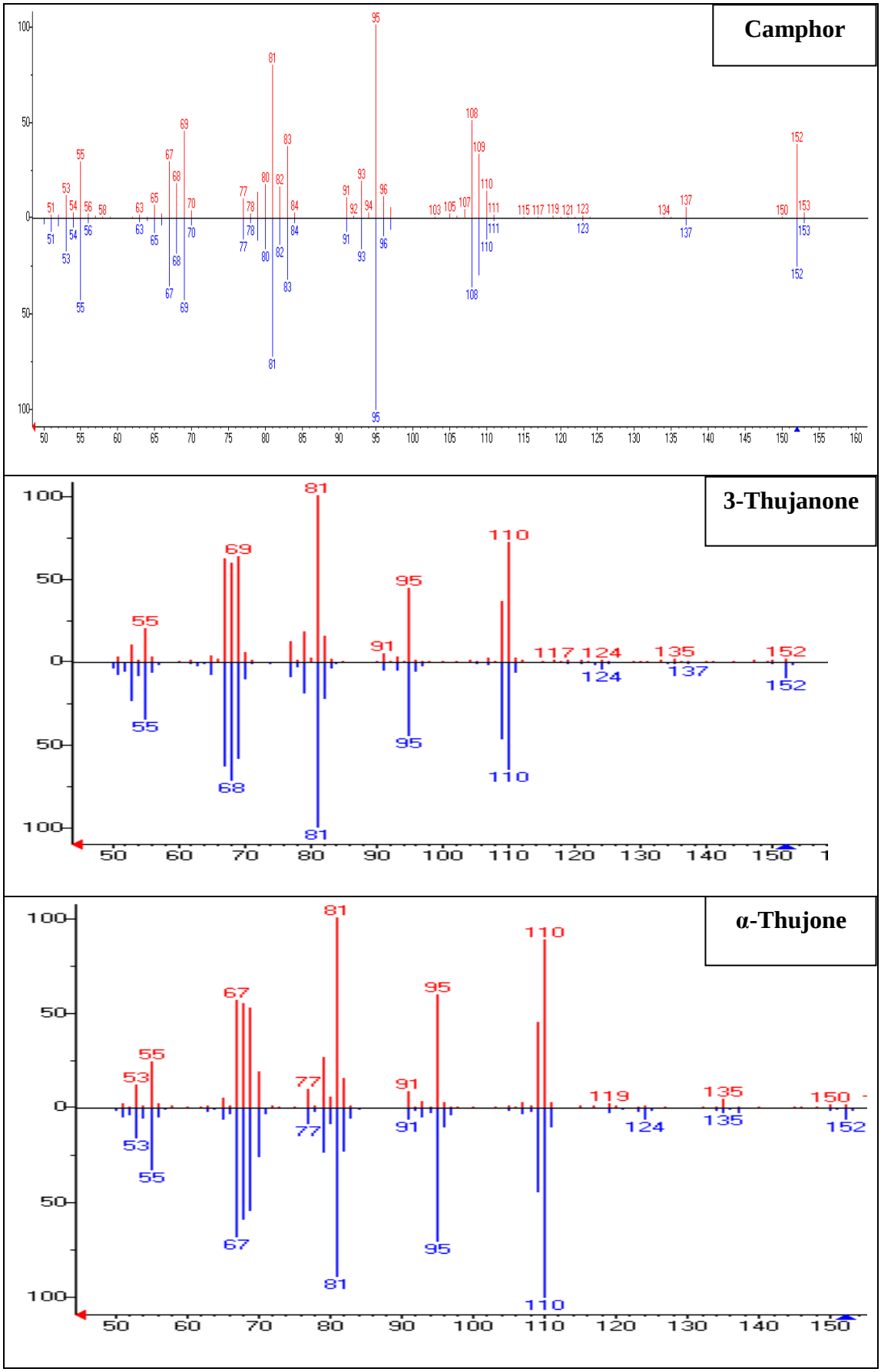


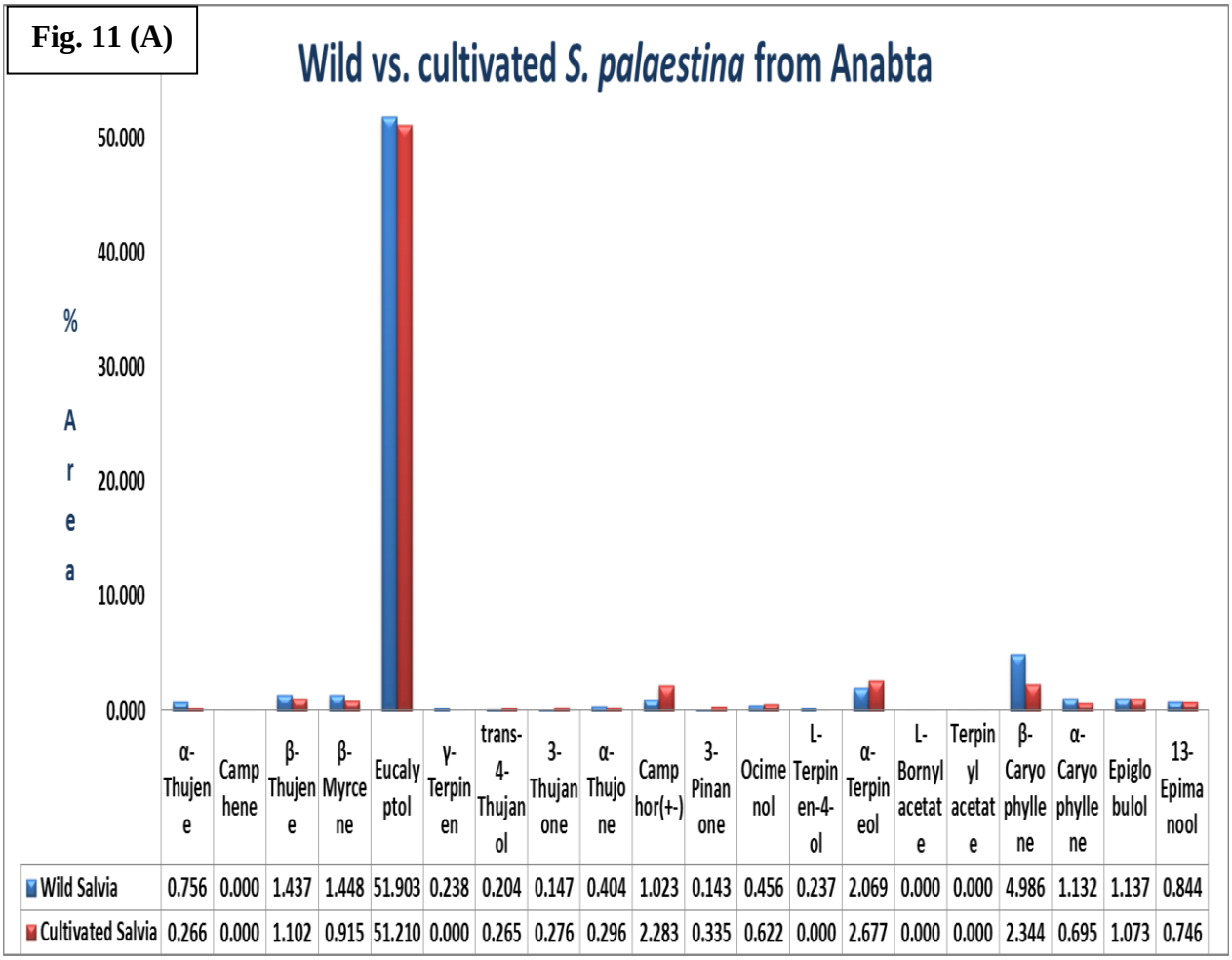
Fig. 10: Head to tail MS of camphor, 3- thujonone and α -thujone from *S. palaestina* sample (red) and NIST MS (blue).

4.3.2.1.1 Wild vs. cultivated for each location

Both wild and cultivated essential oil samples at each location were determined, compared and represented with histograms as the following:

1. **Anabta/Tulkarem**

Fig. 11 (A)



	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	0.756	0.000	1.437	1.448	51.903	0.238	0.204	0.147	0.404	1.023	0.143	0.456	0.237	2.069	0.000	0.000	4.986	1.132	1.137	0.844
Cultivated Salvia	0.266	0.000	1.102	0.915	51.210	0.000	0.265	0.276	0.296	2.283	0.335	0.622	0.000	2.677	0.000	0.000	2.344	0.695	1.073	0.746

Fig. 11 (A): Histogram of the percentage of essential oils of *S. palaestina* from **Anabta/Tulkarem** as determined by GC-MS.

From the above histogram, it seems that there is only a subtle difference between wild and cultivated leaves in Anabta's sample. Both leaves contain nearly the same amount of eucalyptol as a major component but the wild leaves contain doubled amount of caryophyllene as in the cultivated. Conversely, cultivated leaves contain more α-Terpieol and camphor, which is illustrated in **Figure 11 (B).**

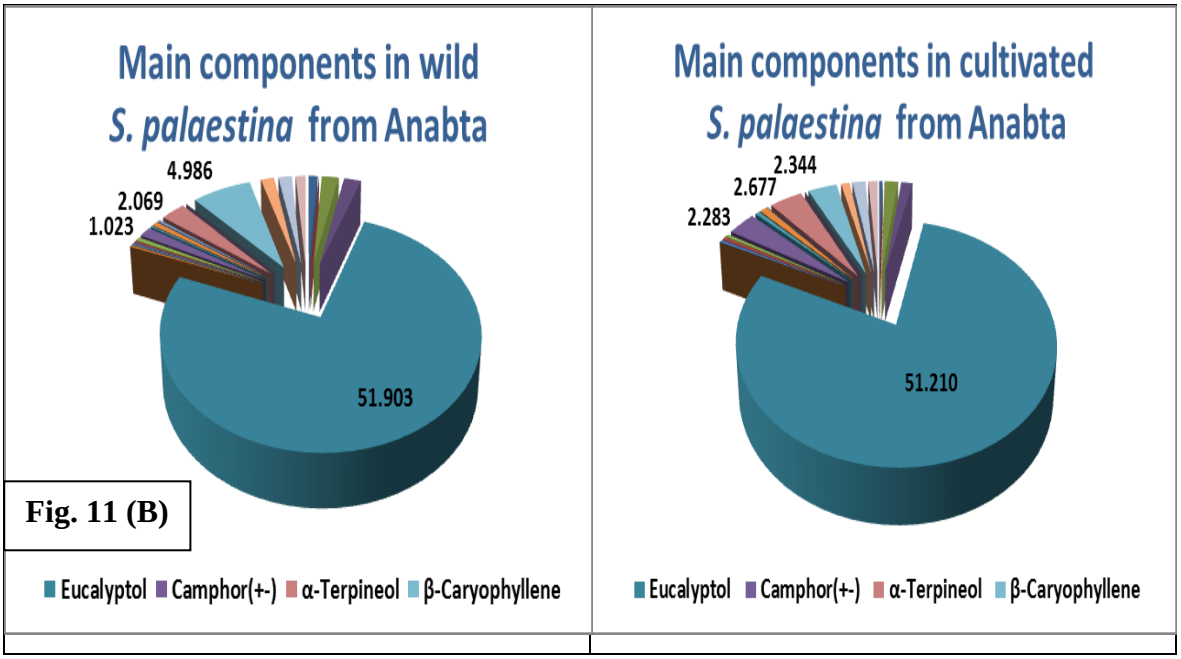
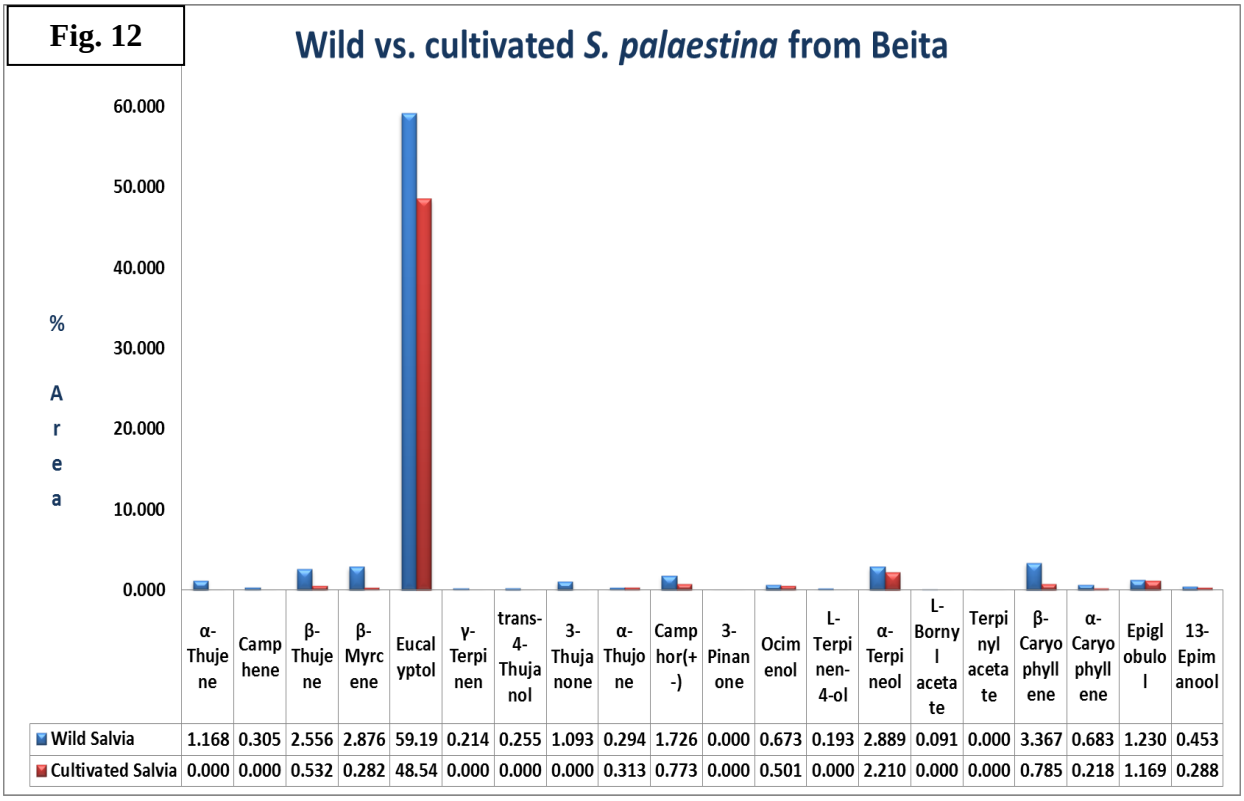


Fig. 11 (B)

Fig. 11 (B): Pie chart of Anabta oil sample by GC-MS.

2. Beita/Nablus

Fig. 12



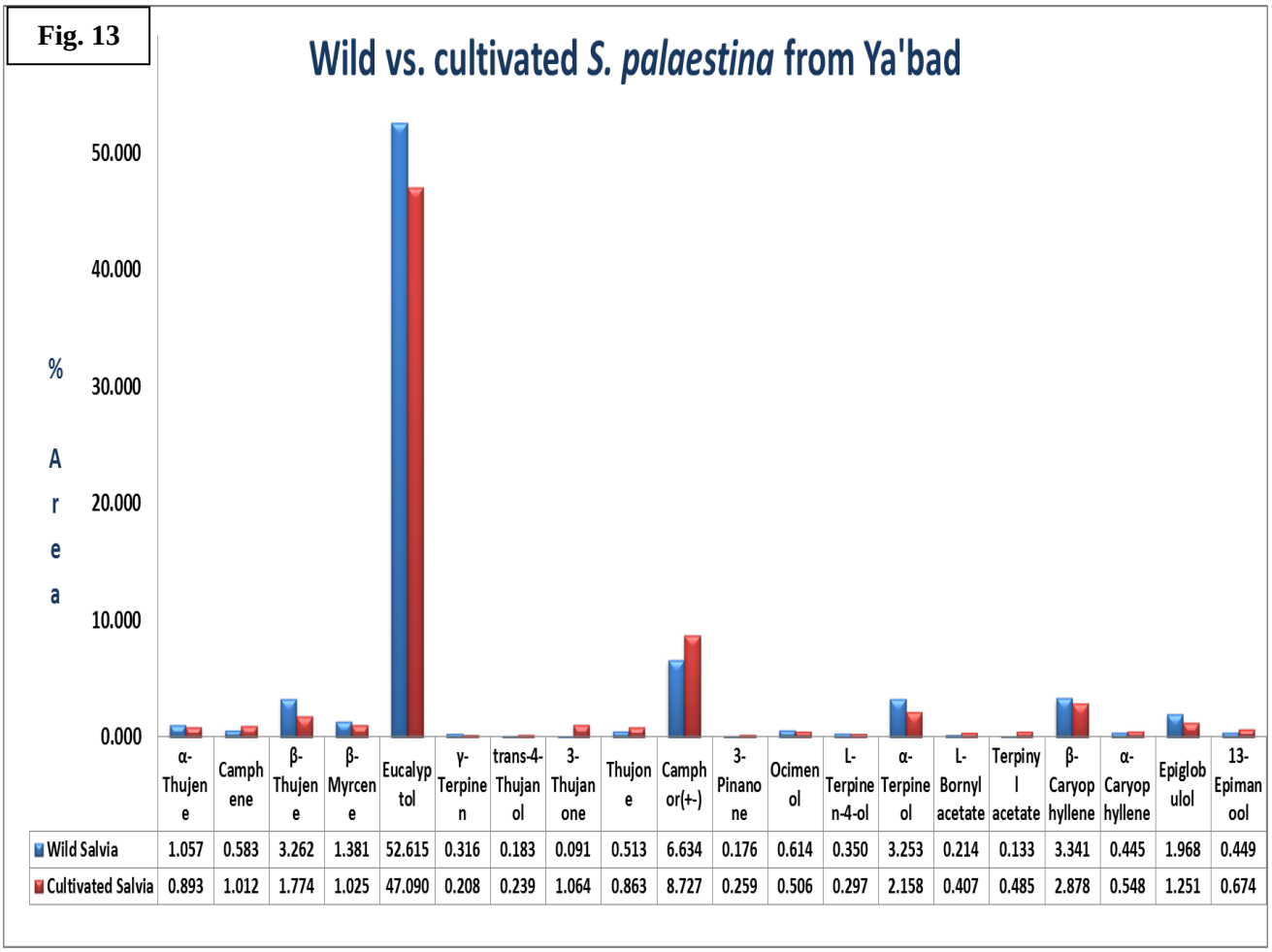
	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	1.168	0.305	2.556	2.876	59.19	0.214	0.255	1.093	0.294	1.726	0.000	0.673	0.193	2.889	0.091	0.000	3.367	0.683	1.230	0.453
Cultivated Salvia	0.000	0.000	0.532	0.282	48.54	0.000	0.000	0.000	0.313	0.773	0.000	0.501	0.000	2.210	0.000	0.000	0.785	0.218	1.169	0.288

Fig. 12: Histogram of the percentage of essential oils of *S. palaestina* from **Beita/Nablus** as determined by GC-MS.

Sample from Beita revealed that the cultivated comprises fewer components in comparison to the wild one. Moreover, the quantities of these components are higher in the wild leaves.

3. Ya'bad/Jenin

Fig. 13



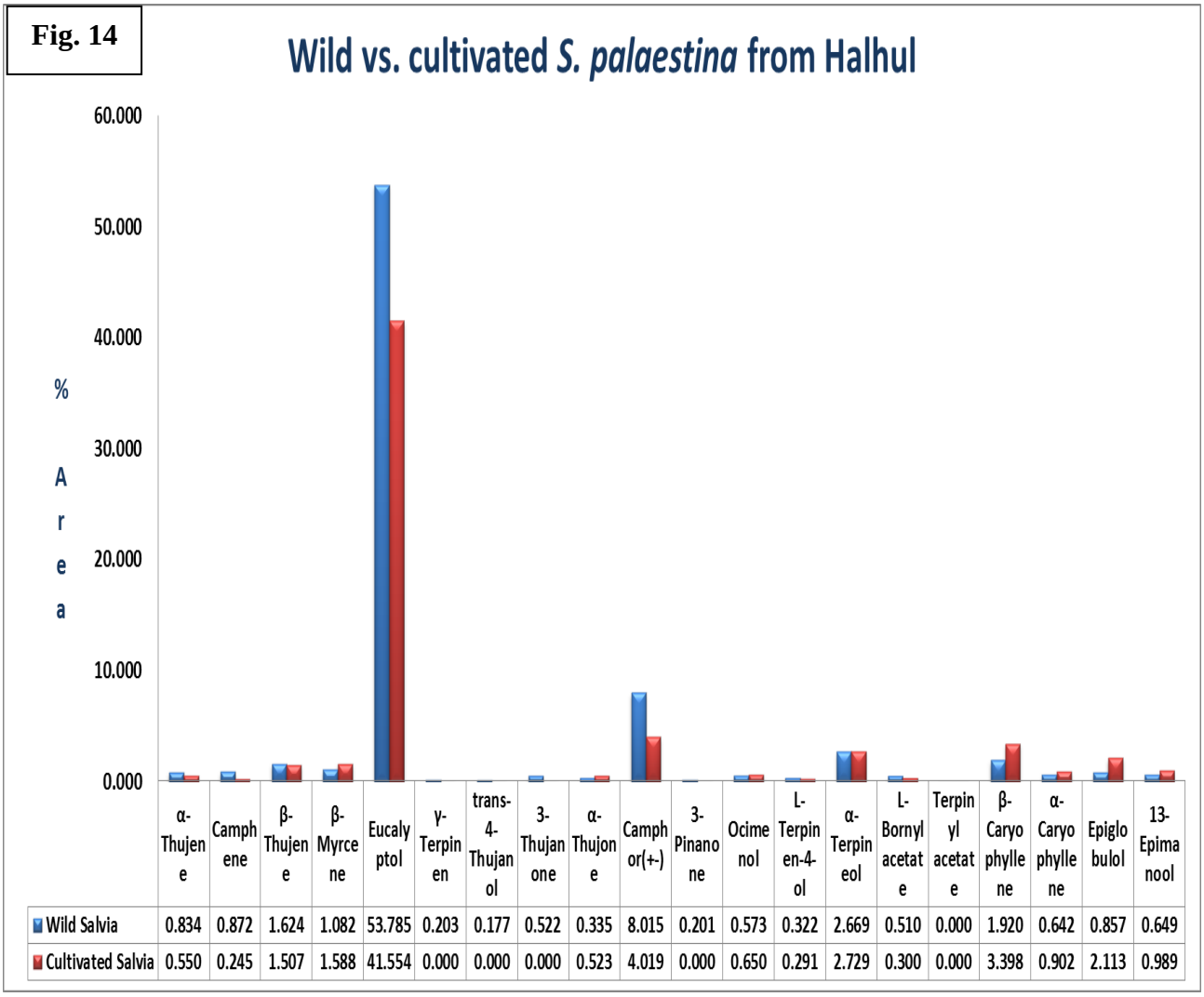
	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	1.057	0.583	3.262	1.381	52.615	0.316	0.183	0.091	0.513	6.634	0.176	0.614	0.350	3.253	0.214	0.133	3.341	0.445	1.968	0.449
Cultivated Salvia	0.893	1.012	1.774	1.025	47.090	0.208	0.239	1.064	0.863	8.727	0.259	0.506	0.297	2.158	0.407	0.485	2.878	0.548	1.251	0.674

Fig. 13: Histogram of the percentage of essential oils of *S. palaestina* from **Ya'bad/Jenin** as determined by GC-MS.

Components variety was nearly the same in both samples but the amount of components were higher in wild sample excluding camphor which was higher in cultivated *Salvia.*

4. Halhul/Hebron

Fig. 14



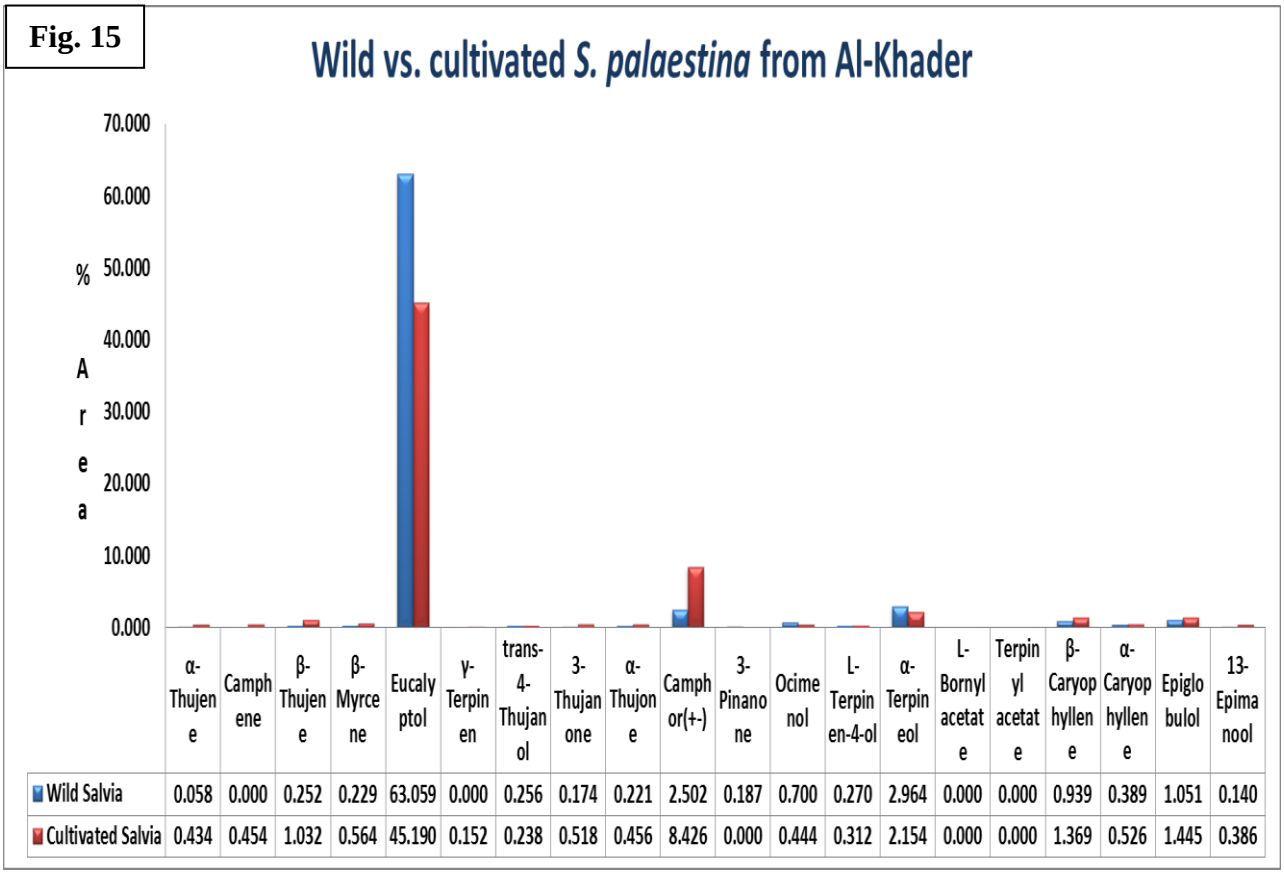
	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	0.834	0.872	1.624	1.082	53.785	0.203	0.177	0.522	0.335	8.015	0.201	0.573	0.322	2.669	0.510	0.000	1.920	0.642	0.857	0.649
Cultivated Salvia	0.550	0.245	1.507	1.588	41.554	0.000	0.000	0.000	0.523	4.019	0.000	0.650	0.291	2.729	0.300	0.000	3.398	0.902	2.113	0.989

Fig. 14: Histogram of the percentage of essential oils of *S. palaestina* from **Halhul/Hebron** as determined by GC-MS.

Figure 14 revealed that the number of identified volatiles was less than other investigated locations. The concentration of identified volatiles was higher in wild leaves except for β-caryophyllene, β -myrcene and epiglobulol.

5. Al-Khader/Beithlaham

Fig. 15



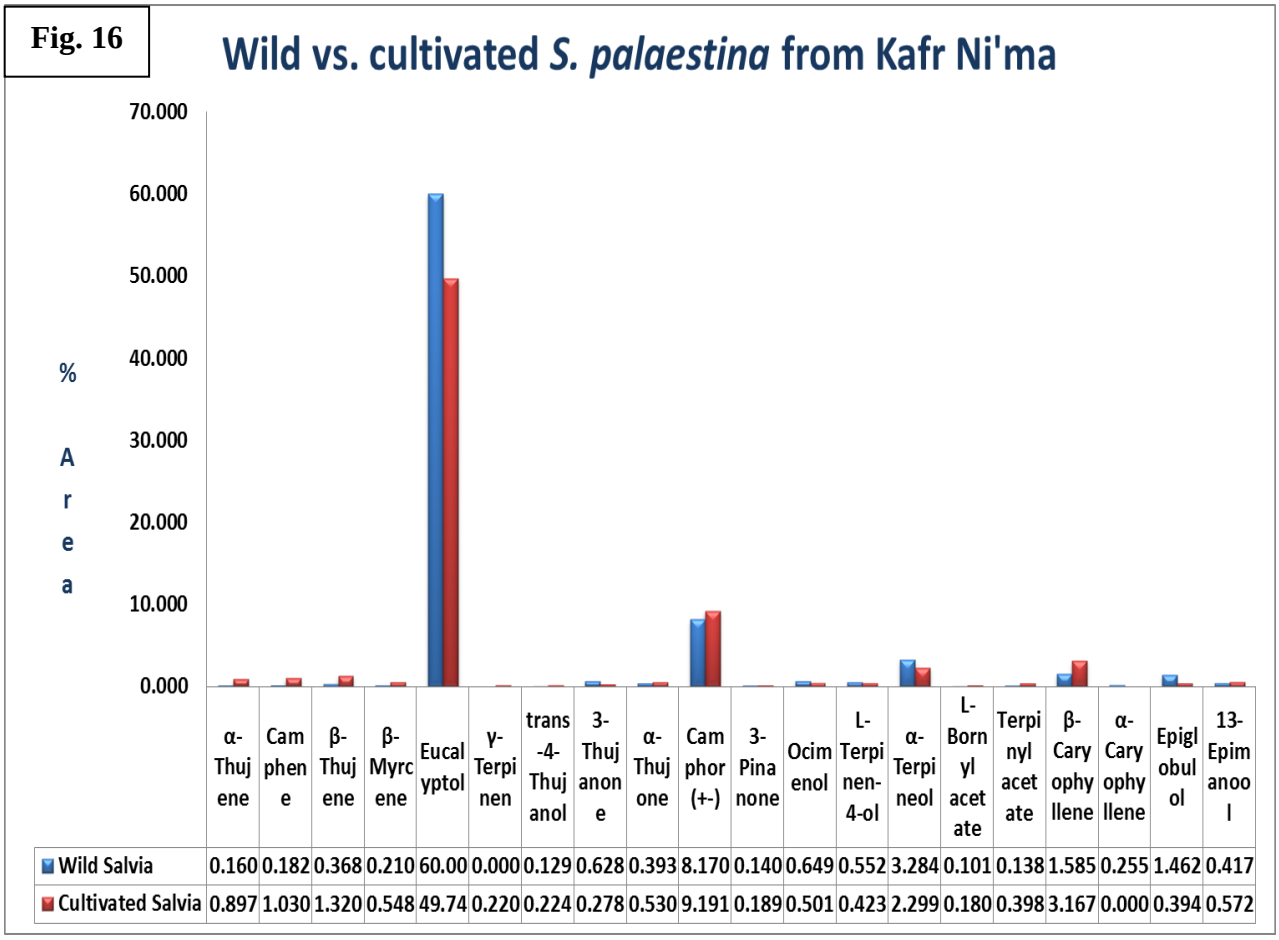
	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	0.058	0.000	0.252	0.229	63.059	0.000	0.256	0.174	0.221	2.502	0.187	0.700	0.270	2.964	0.000	0.000	0.939	0.389	1.051	0.140
Cultivated Salvia	0.434	0.454	1.032	0.564	45.190	0.152	0.238	0.518	0.456	8.426	0.000	0.444	0.312	2.154	0.000	0.000	1.369	0.526	1.445	0.386

Fig. 15: Histogram of the percentage of essential oils of *S. palaestina* from **Al-Khader/Beithlaham** as determined by GC-MS.

Sample from Al-Khader was especially distinguished with its higher levels of eucalyptol (63.059%) in wild leaves and high levels of camphor (8.426%) in cultivated ones.

6. Kafr Ni'ma/Ramallah

Fig. 16



	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor (+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Wild Salvia	0.160	0.182	0.368	0.210	60.00	0.000	0.129	0.628	0.393	8.170	0.140	0.649	0.552	3.284	0.101	0.138	1.585	0.255	1.462	0.417
Cultivated Salvia	0.897	1.030	1.320	0.548	49.74	0.220	0.224	0.278	0.530	9.191	0.189	0.501	0.423	2.299	0.180	0.398	3.167	0.000	0.394	0.572

Fig. 16: Histogram of the percentage of essential oils of *S. palaestina* from **Kafr Ni'ma/Ramallah** as determined by GC-MS.

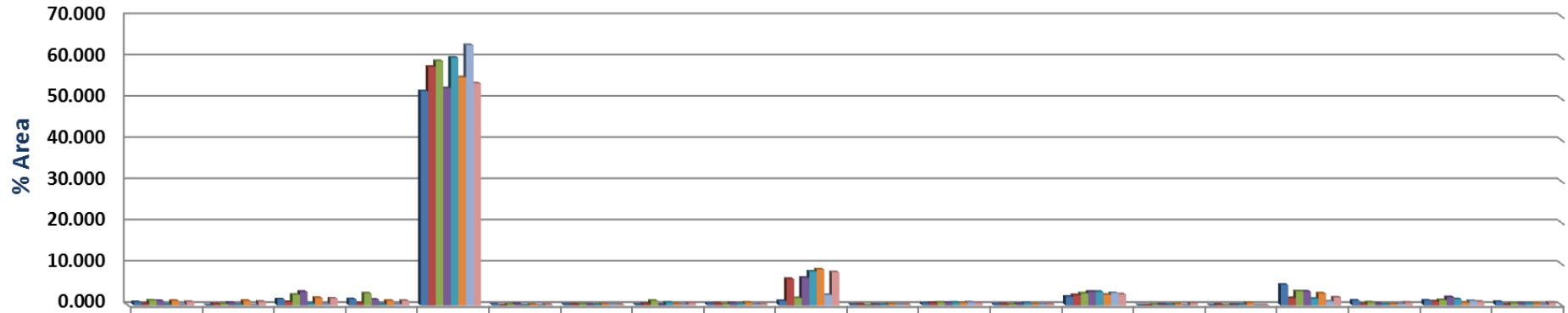
As in previous samples, the main component in *S. palaestina* from Kafr Ni'ma was eucalyptol and its level is higher in wild *S. palaestina* than in cultivated, conversely, the later has higher levels of camphor and caryophyllene.

In general, comparison between wild/cultivated *S. palaestina* leaves components at certain location revealed that the concentration of eucalyptol was always higher in wild leaves, while in the majority the concentrations of camphor, caryophellene and β -myrcene were higher in cultivated samples.

4.3.2.1.2 Wild *S. palaestina* from all locations

In histogram below (**Fig. 17**), we compared wild *S. palaestina* samples from all locations collected between April and May 2013. It was obvious that there were certain indigenous volatiles which distinguished each location. For example, Al-Khader wild sample was characterized by high levels of eucalyptol, while Beita was differentiated with β –myrcene. *S. palaestina* from Abud was characterized with high levels of camphene and camphor and Anabta however, was distinguished with the sesquiterpene caryophyllene. In future work, further studies on more samples and at different stages of plant life cycle could be conducted to use these components as markers to distinguish the origin of *S. palaestina*.

Fig. 17



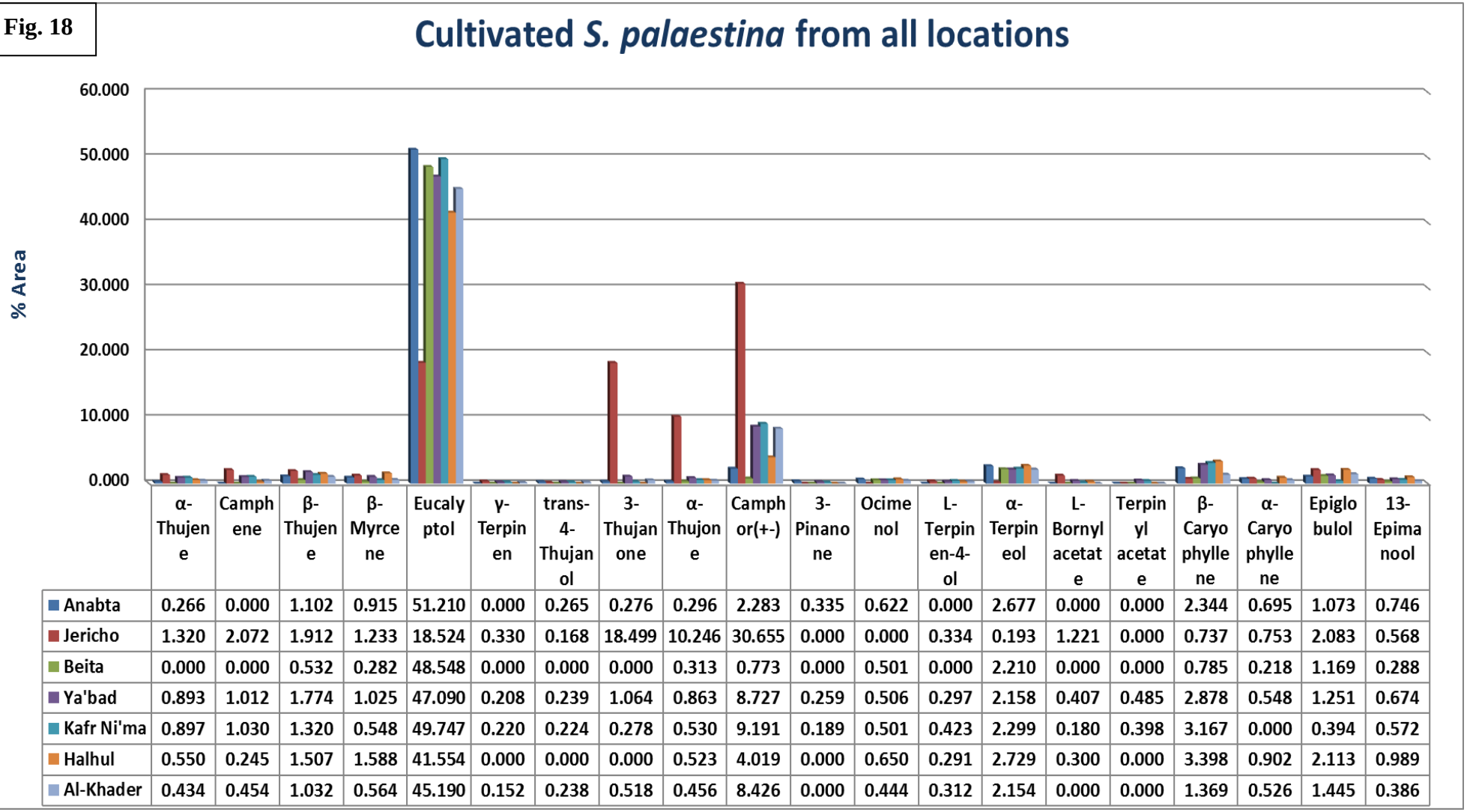
	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Anabta	0.756	0.000	1.437	1.448	51.903	0.238	0.204	0.147	0.404	1.023	0.143	0.456	0.237	2.069	0.000	0.000	4.986	1.132	1.137	0.844
Deir Istiya	0.354	0.283	0.784	0.498	57.785	0.000	0.205	0.315	0.421	6.387	0.190	0.539	0.272	2.451	0.000	0.142	1.722	0.283	0.907	0.159
Beita	1.168	0.305	2.556	2.876	59.197	0.214	0.255	1.093	0.294	1.726	0.000	0.673	0.193	2.889	0.091	0.000	3.367	0.683	1.230	0.453
Ya'bad	1.057	0.583	3.262	1.381	52.615	0.316	0.183	0.091	0.513	6.634	0.176	0.614	0.350	3.253	0.214	0.133	3.341	0.445	1.968	0.449
Kafr Ni'ma	0.160	0.182	0.368	0.210	60.000	0.000	0.129	0.628	0.393	8.170	0.140	0.649	0.552	3.284	0.101	0.138	1.585	0.255	1.462	0.417
Abud	1.066	1.078	1.793	1.064	55.260	0.140	0.290	0.358	0.628	8.703	0.318	0.569	0.324	2.564	0.288	0.562	2.895	0.304	0.646	0.429
Al-Khader	0.058	0.000	0.252	0.229	63.059	0.000	0.256	0.174	0.221	2.502	0.187	0.700	0.270	2.964	0.000	0.000	0.939	0.389	1.051	0.140
Halhul	0.834	0.872	1.624	1.082	53.785	0.203	0.177	0.522	0.335	8.015	0.201	0.573	0.322	2.669	0.510	0.000	1.920	0.642	0.857	0.649

4.3.2.1.3 Cultivated *S. palaestina* from all locations

Comparison was performed between all cultivated samples from all locations collected between April and May 2013. The comparison revealed that the sample from Jericho was unique due to its high concentration of camphor, thujone, 3-thujanone, L-bronyl acetate and camphene. Jericho is the deepest point in the world and its soil is different from other places in Palestine in term of texture, moisture content and acidity which probably affects the composition of *S. palaestina* leaves essential oils.

In histogram below (**Fig. 18**), few differences were observed in volatiles from other locations rather than that of Jericho.

Fig. 18

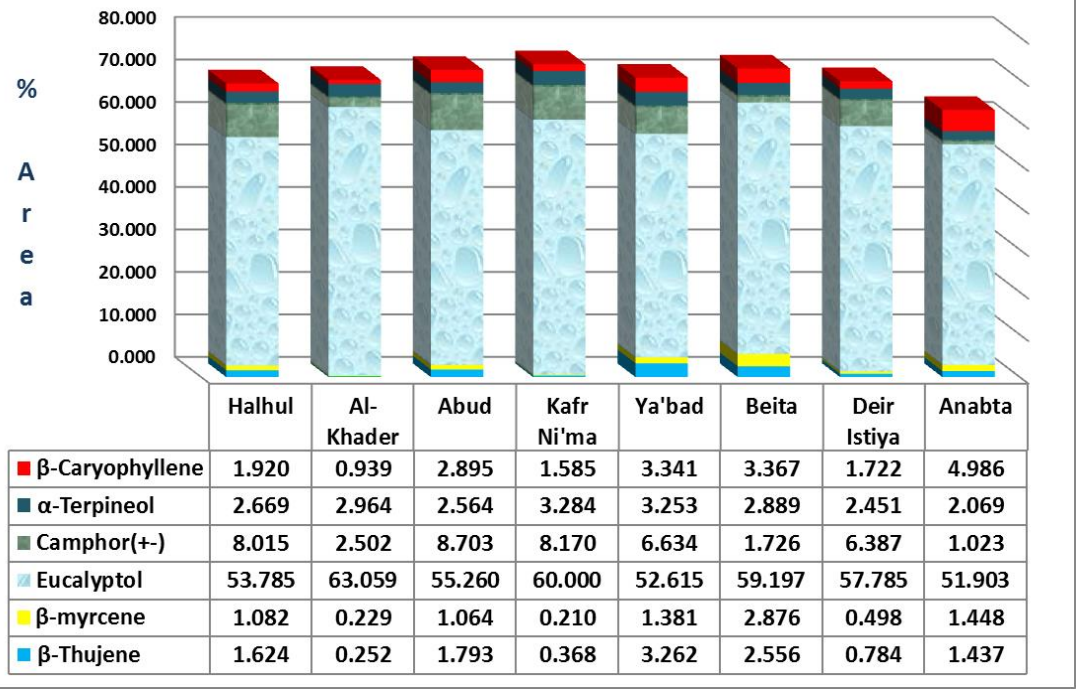


	α-Thujene	Camphene	β-Thujene	β-Myrcene	Eucalyptol	γ-Terpinen	trans-4-Thujanol	3-Thujanone	α-Thujone	Camphor(+-)	3-Pinanone	Ocimenol	L-Terpinen-4-ol	α-Terpineol	L-Bornyl acetate	Terpinyl acetate	β-Caryophyllene	α-Caryophyllene	Epiglobulol	13-Epimanool
Anabta	0.266	0.000	1.102	0.915	51.210	0.000	0.265	0.276	0.296	2.283	0.335	0.622	0.000	2.677	0.000	0.000	2.344	0.695	1.073	0.746
Jericho	1.320	2.072	1.912	1.233	18.524	0.330	0.168	18.499	10.246	30.655	0.000	0.000	0.334	0.193	1.221	0.000	0.737	0.753	2.083	0.568
Beita	0.000	0.000	0.532	0.282	48.548	0.000	0.000	0.000	0.313	0.773	0.000	0.501	0.000	2.210	0.000	0.000	0.785	0.218	1.169	0.288
Ya'bad	0.893	1.012	1.774	1.025	47.090	0.208	0.239	1.064	0.863	8.727	0.259	0.506	0.297	2.158	0.407	0.485	2.878	0.548	1.251	0.674
Kafr Ni'ma	0.897	1.030	1.320	0.548	49.747	0.220	0.224	0.278	0.530	9.191	0.189	0.501	0.423	2.299	0.180	0.398	3.167	0.000	0.394	0.572
Halhul	0.550	0.245	1.507	1.588	41.554	0.000	0.000	0.000	0.523	4.019	0.000	0.650	0.291	2.729	0.300	0.000	3.398	0.902	2.113	0.989
Al-Khader	0.434	0.454	1.032	0.564	45.190	0.152	0.238	0.518	0.456	8.426	0.000	0.444	0.312	2.154	0.000	0.000	1.369	0.526	1.445	0.386

4.3.2.1.4 Wild/cultivated *S. palaestina* main components from all locations

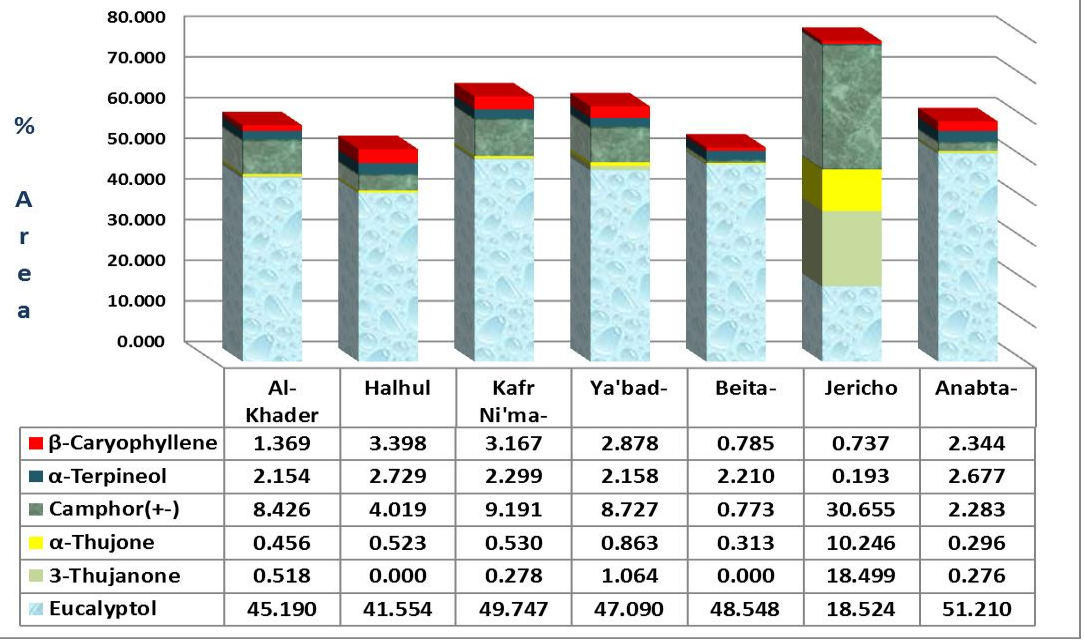
Focused comparison between main components percentages was summarized as in the following histograms (**Fig. 19 & 20**):

Fig. 19



	Halhul	Al-Khader	Abud	Kafr Ni'ma	Ya'bad	Beita	Deir Istiya	Anabta
β-Caryophyllene	1.920	0.939	2.895	1.585	3.341	3.367	1.722	4.986
α-Terpineol	2.669	2.964	2.564	3.284	3.253	2.889	2.451	2.069
Camphor(+-)	8.015	2.502	8.703	8.170	6.634	1.726	6.387	1.023
Eucalyptol	53.785	63.059	55.260	60.000	52.615	59.197	57.785	51.903
β-myrcene	1.082	0.229	1.064	0.210	1.381	2.876	0.498	1.448
β-Thujene	1.624	0.252	1.793	0.368	3.262	2.556	0.784	1.437

Fig. 20



	Al-Khader	Halhul	Kafr Ni'ma-	Ya'bad-	Beita-	Jericho	Anabta-
β-Caryophyllene	1.369	3.398	3.167	2.878	0.785	0.737	2.344
α-Terpineol	2.154	2.729	2.299	2.158	2.210	0.193	2.677
Camphor(+-)	8.426	4.019	9.191	8.727	0.773	30.655	2.283
α-Thujone	0.456	0.523	0.530	0.863	0.313	10.246	0.296
3-Thujanone	0.518	0.000	0.278	1.064	0.000	18.499	0.276
Eucalyptol	45.190	41.554	49.747	47.090	48.548	18.524	51.210

Fig. 19 & Fig. 20: Main components in wild and cultivated *S. palaestina*.

The main components in wild *S. palaestina* were eucalyptol, camphor, caryophyllene, terpineol, β -thujene and β -myrcene, while the main components in cultivated leaves were: eucalyptol, camphor, caryophyllene, α-terpineol, thyjone and 3-thujanone. The above major six components represented about 80% of cultivated *S. palaestina* components as in Jericho, while in wild *S. palaestina* they represented about 70% of all components which probably indicated that there is variety in components in wild leaves more than that in the cultivated. Moreover, the concentration of similar components varies between cultivated and wild samples; in general the wild *S. palaestina* has higher concentration of eucalyptol, while the cultivated has higher concentration of camphor. This means that it is advisable to choose wild *S. palaestina* when the eucalyptol effects are desired, while cultivated *S. palaestina* is the one to be chosen when camphor effect is required.

Eucalyptol, which is the main component in the wild leaves, is mainly used as an active ingredient in mouthwash, lozenges, ointments inhalants, body powder and cough suppressant preparations. It controls airway mucus hypersecretion and asthma via inhibition of cytokine production in human monocytes (Juergens *et al.,* 2003; Juergens, Stober, & Vetter, 1998). In addition, it stimulates immune system response by enhancing the phagocytic ability of human monocytes (Juergens *et al*., 2004). Eucalyptol has noticeable antimicrobial activity with minimal side effects either when applied topically or systemically. Therefore, it is used in many preparations as an active antiseptic and to reduce inflammation and pain. Due to its pleasant smell, it is used as a fragrance to impart a fresh and clean aroma in soaps, lotions, detergents and cosmetics. Recently, several studies revealed that it might have anti-tumour activity since it kills leukaemia cells *in vitro* (Moteki *et al.*, 2002). Thus, it is advisable to use wild *S. palaestina* leaves in the preparation of pharmaceutical dosage forms that containing eucalyptol as active or inactive ingredient.

Camphor, which is available in relatively high concentrations in cultivated leaves, has a long history of use as analgesic, antiseptic, antipruritic, counterirritant and rubefacient. Its success in medical topical use is mainly related to its mild local anesthesizing effect and to the production of heat sensation then a feeling of cooling (Dodds, 1936; Moqrich *et al.,* 2005). Nowadays, camphor is mostly used in the form of inhalant preparations for home treatment of colds, nasal decongestant(Inoue & Takeuchi, 1969) and as an active ingredient in topical pharmaceutical preparations used as analgesic and anti-inflammatory to treat sprains and

swellings (Vicks VapoRub) (Dodds, 1936; Lehtinen, 1975; Martin, Valdez, Boren, & Mayersohn, 2004).

Systemic use is associated with tachycardia, vasodilation in skin (flushing), slower breathing, reduced appetite, increased secretions and excretions such as perspiration and diuretic effects (Schenone *et al.*, 2000). Moreover, it can modulate the activities of hepatic enzymes involved in phase I and phase II drug metabolism and inhibit mitochondrial respiration (Banerjee, Welsch, & Rao, 1995; Kadkhodayan, Coulter, Maryniak, Bryson, & Dawson, 1995). Camphor can also be a potential radiosensitizing agent in radiotherapy (Goel & Roa, 1988). Nevertheless, medicinal use of camphor is discouraged by the FDA, except for skin-related uses in relatively small amounts ("Camphor revisited: focus on toxicity. Committee on Drugs. American Academy of Pediatrics," 1994; Emery & Corban, 1999; Horikawa & Okada, 1975; Yim, Kim, & Kim, 2014). Thus, it is advisable to use cultivated leaves in skin pharmaceutical preparations when there is need to use camphor.

In order to qualitatively understand the accumulated results one must keep in mind that secondary metabolites biosynthesis and accumulation in plants is strongly influenced by various biotic and abiotic factors (Smetanska, 2008). Plants are exposed to various degrees of stress, which might be either natural or human-induced factors. Drought, salinization, water, light, radiation, humidity, atmosphere, pressure, sound waves, soil type and the presence of heavy metals in the soil which all might cause substantial effect on yield, type and quality of bioactive components in the oil (Mohammadkhani & Heidari, 2008).

Due to the aforementioned factors, differences between *S. palaestina* components from one governorate to another are justified. In case of cultivated sample from Jericho, significant differences were observed probably due to the fact that Jericho is the deepest place in the world which affects the atmospheric temperature and pressure. An important recent study compared Jericho's soil to Tulkarem's soil. The study revealed that the Jericho's soil texture comprises sand 60.5%, gravel 23.5%, slit 11.2% and clay 4.8%, while Tulkarem's soil comprises sand 9.56%, gravel 0%, slit 66.92% and clay 23.52%. Moreover, the total dissolved substances (TDS) in Jericho's soil was 65 mg/L while in Tulkarem it was 185 mg/L and the moisture content was about 3.73% in Jericho soil, while in Tulkarem it was 19.57% (Shehdeh Jodeh, 2013). Even the acidity value was different between both it was more acidic in Tulkarem sample. Thus, it's not surprising that *S. palaestina* from Jericho is different.

S. palaestina from Jericho is rich in thujone and its isomers, which all have some structural similarities to tetrahydrocannabinol, the main psychoactive substance found in marijuana. It was hypothesized, few decades ago, that it might act on the same way on the brain receptors (Del Castillo *et al.*, 1975). Later on, thujone was considered as a gamma-amino butyric acid (GABA) receptor inhibitor (Olsen, 2000). By inhibiting GABA receptor activation, neurons may fire more easily also it works on 5-HT_3 receptors (Hold *et al.*, 2000).

Moreover, thujone and its derivatives are considered toxic substances due to its adverse reactions on brain, liver and kidney cells. It might cause convulsions (muscle spasms), rapid heart rate, restlessness, anxiety, sleeplessness, vomiting, kidney damage, vertigo, epileptic seizures, and psychedelic effects if ingested in high doses. It's contraindicated during pregnancy because it might cause abortion.(Naser *et al.*, 2005).

Since *S. palaestina* from Jericho is completely distinguished with its high levels of thujone which if ingested might cause abortion in addition to many undesirable effects as aforementioned, its usage should be controlled and restricted as in other countries in European Union, Canada and United states (Services, 2014).

4.4 Antioxidant activity of *S. palaestina*

Antioxidants are important in many aspects especially in medical field as well as in food industry. Recently, researches on natural antioxidants from plants and food materials have been received great attention. The oxidation induced by reactive oxygen species (ROS) may cause cell damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as liver injury, cancer and cardiovascular diseases(Bohm *et al.*, 1998; Liao & Yin, 2000; Sanches-Silva *et al.*, 2014).

The antioxidant activity of *S. palaestina* essential oil was examined by the DPPH method. Series of concentrations of the oil in methanol were prepared, 50 µL of each concentration were taken and 2 ml of DPPH solution (DPPH concentration $6*10^{-5}$) was added to each concentration, final concentrations ranging from 0.122 to 1.35 mg/ml, all samples were warped with aluminum foil and kept in dark place (drawer). Absorbance at wavelength 517 nm was measured at three different points (after 30 min, 1 hour and 1.5 hour). At the same time tert-butyl-4-hydroxy toluene (BHT) was used as positive control, series of concentrations were

prepared, 5 µL of each concentration was taken and to 2 ml of DPPH was added as in *S. palaestina* essential oils, final concentrations for BHT ranged from 0.015 to 0.125 mg/ml.

The antioxidant index (AI) was calculated from the following equation:

(AI%), %DPPH radical scavenging activity = [1-(As/Ac)]*100

As: Sample absorbance
Ac: Control absorbance
Note that the control was DPPH and the blank was methanol (Brand-Williams , 1995).

All AI% values were plotted against its corresponding concentration for both oil sample and the positive control as following:

- **Antioxidant activity after 30 min.**

Figure 21 represents the antioxidant activity of Palestinian *S. palaestina* oil, while **Figure 22** represents the antioxidant activity of the BHT after 30 min.

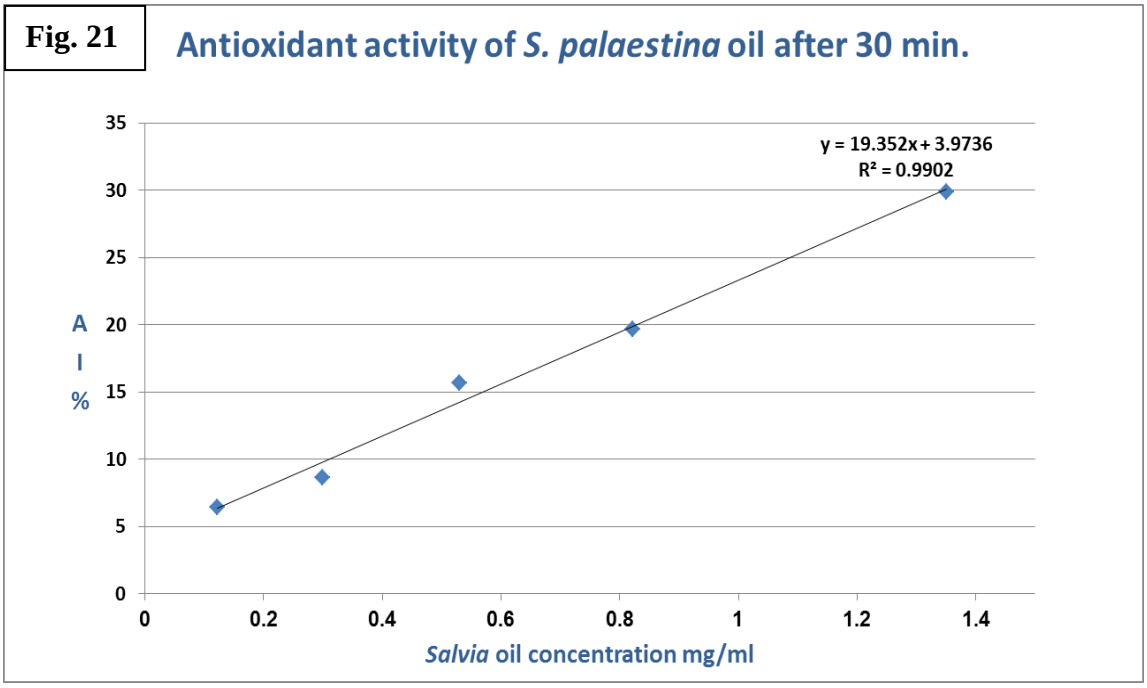


Fig. 21: Antioxidant activity of *S. palaestina* oil after 30 min.

Fig. 22

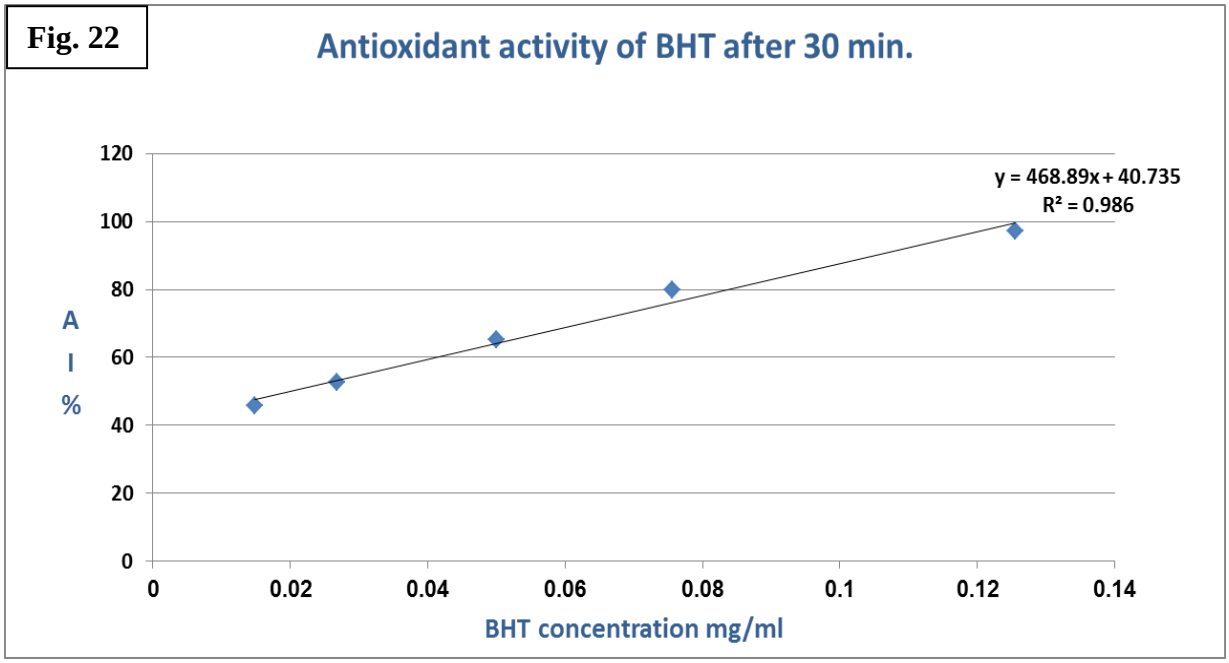


Fig. 22: Antioxidant activity of the positive control (BHT) after 30 min.

From the figures above, linear relation between concentration and antioxidant activity was observed. The inhibition concentration (IC_{50}) was calculated and it was 2.333 mg/ml for the oil and 0.02 mg/ml for BHT which means that the antioxidant activity of the oil is less than that of the positive control.

- **Antioxidant activity after 60 min.**

Figure 23 illustrated the antioxidant activity of the *S. palaestina* oil, while **Figure 24** represents the antioxidant activity of the BHT after 60 min.

Fig. 23

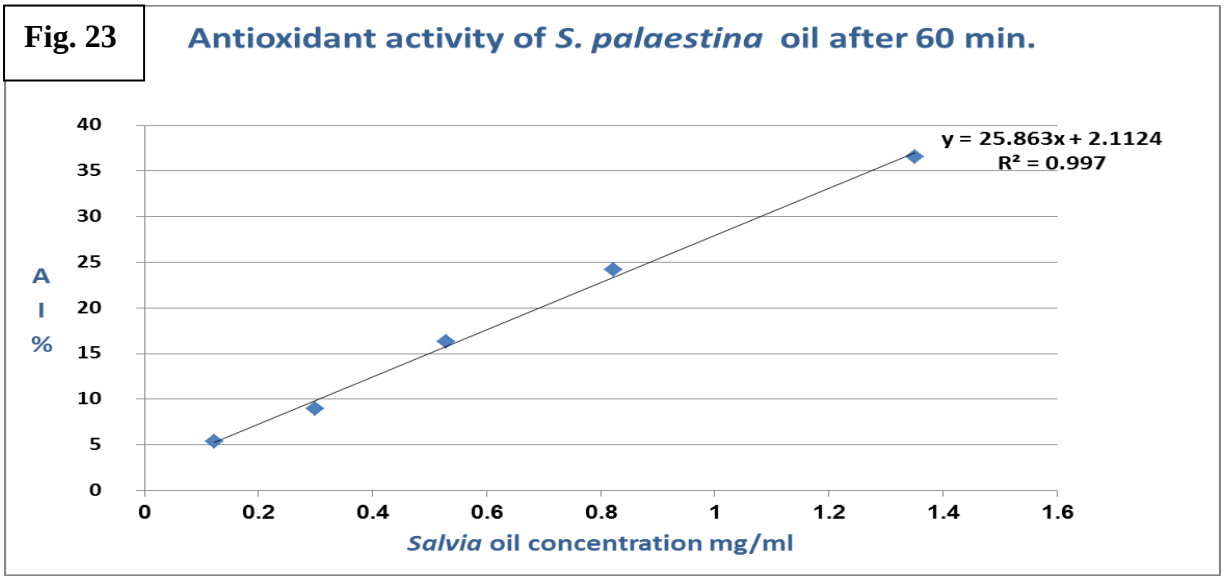


Fig. 23: Antioxidant activity of *S. palaestina* oil after 60 min.

Fig. 24

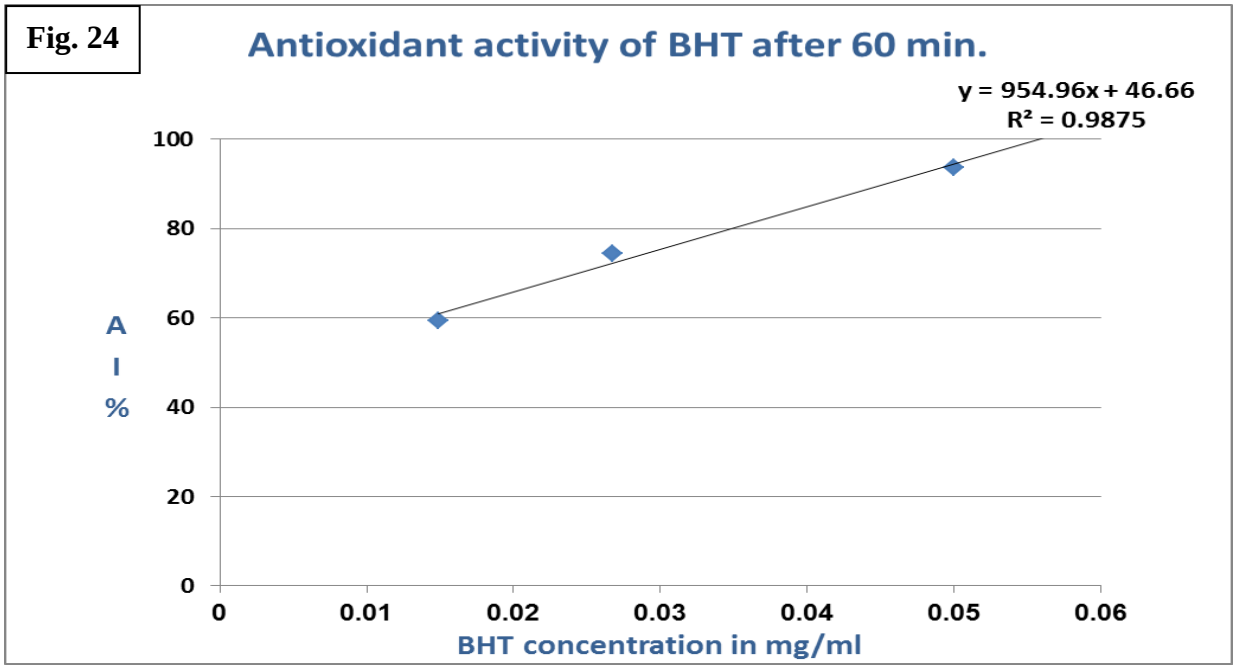


Fig. 24: Antioxidant activity of the positive control (BHT) after 60 min.

IC_{50} after 60 min. was calculated and it was 1.852 mg/ml for *S. palaestina* oil, while it was 0.004 mg/ml for BHT which means that the activity of the oil is still less than that of the positive control.

- **Antioxidant activity after 90 min.**

The antioxidant activity of the oil after 90 min. is illustrated in **Figure 25** below.

Fig. 25

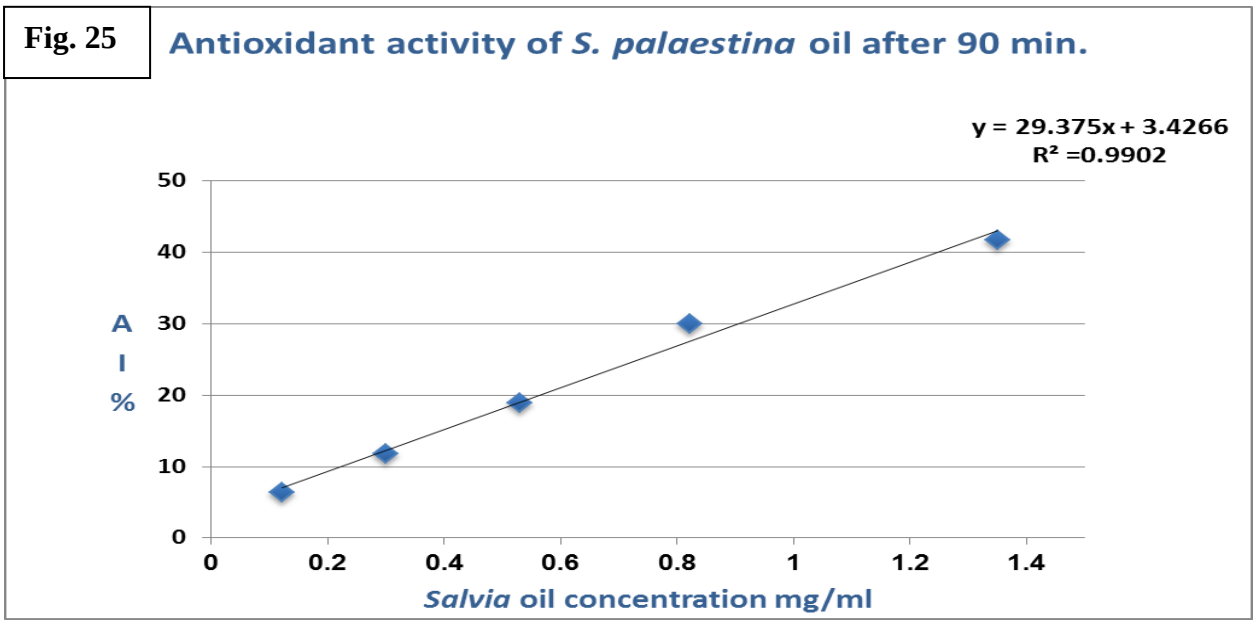


Fig. 25: Antioxidant activity of *S. palaestina* oil after 90 min.

IC_{50} for *S. palaestina* oil was 1.585 mg/ml, while it was excluded for BHT because of un-harmonized values.

IC_{50} values for both sample and positive control were represented in the following histogram **(Fig. 26)**.

Fig. 26

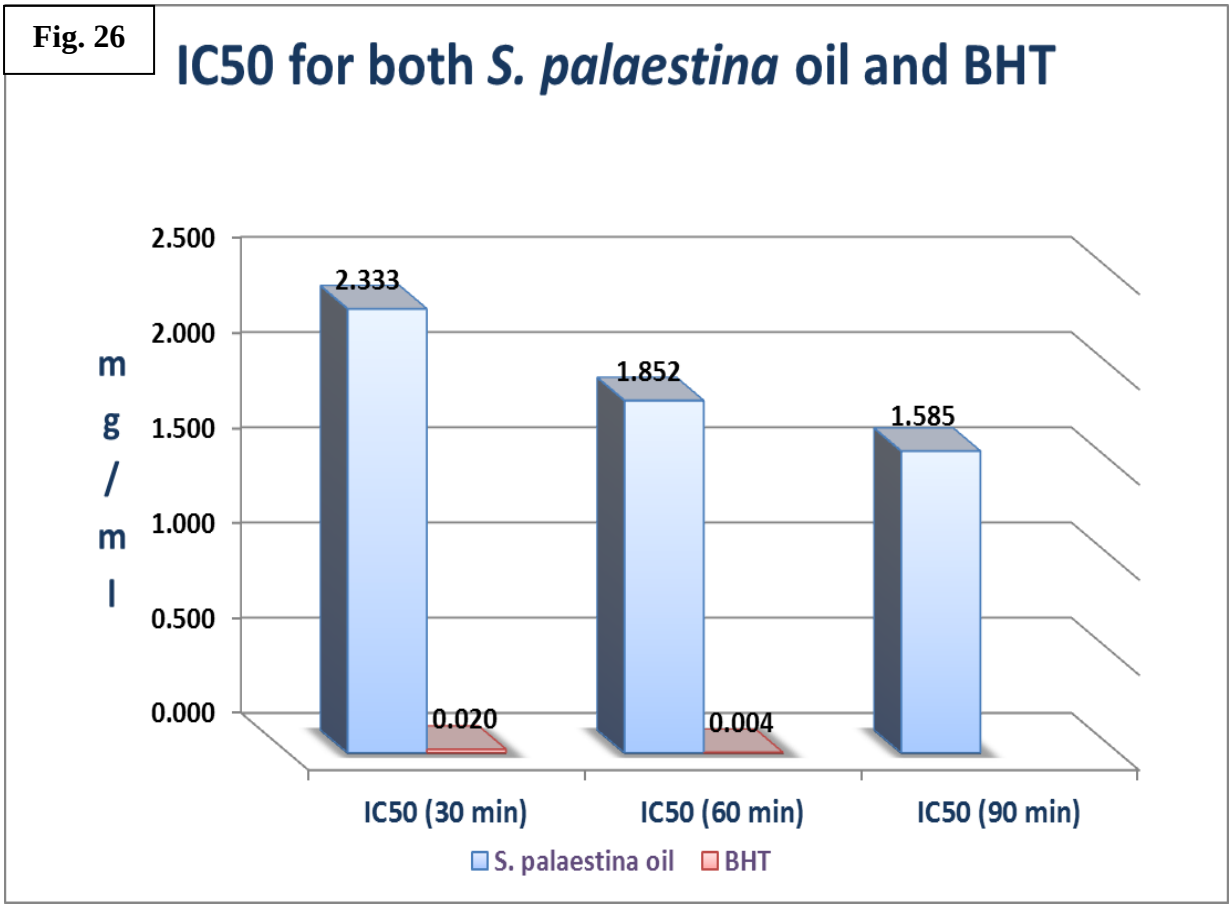


Fig. 26: IC_{50} for both *S. palaestina* oil and the positive control

It is obvious from the above histogram that *S. palaestina* oil has antioxidant activity which is less than that of the positive control; hence the activity of 2.333 mg/ml of the oil is equal to 20 µg/ml of BHT after 30 min. Furthermore, after 60 min. 1.852 mg/ml of the oil will give the same antioxidant activity as that of 4 µg/ml of BHT.

Within the tested range of concentrations, free radical scavenging capacity of the tested *S. palaestina* oils increased in a concentration dependent manner.

DPPH scavenging activities (%) were increased significantly with increasing the concentration from 0.122 to1.35 mg/ml of *S. palaestina* essential oil. In addition, the activity of the oil is increased with time and to achieve a good antioxidant activity sufficient time is required.

This activity was mainly attributed to the major effective components such as oxygenated compounds and terpenes. Preliminary GC-MS screening indicated the presence of these compounds in *S. palaestina's* oil which are considered as free radical scavengers (Al-Qudah, *et al*., 2014; Gürsoy, 2011; Tenore *et al*., 2011).

Although native *S. palaestina* (at vegetative stage) doesn't have antioxidant activity as that of BHT but currently there is considerable interest in new natural antioxidants to replace the synthetic especially in food and cosmetic products. Several studies were conducted on the available synthetic antioxidants (BHT and butylated hydroxyanisole (BHA)) to evaluate the safety of both substances. They revealed that long exposure might cause thyroid, liver, kidney dysfunctions and might affect the lung function and blood coagulation. Moreover, it was suggested that high doses of BHT might mimic estrogen, the main female six hormones, causing reproductive system dysfunctions (Clapp *et al*., 1979; Kahl & Kappus, 1993).

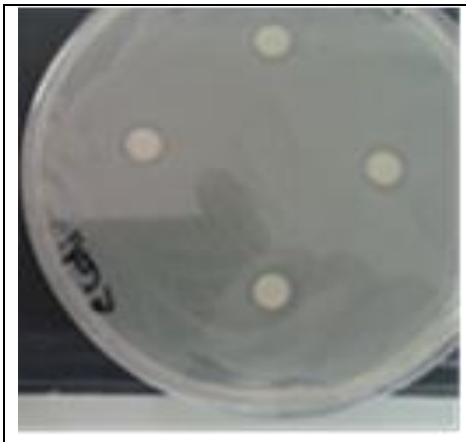
However, further research is needed to investigate the bioactivity and toxicity of *S. palaestina* essential oils and to test the activity of native *S. palaestina* at different stages of the plant life cycle and from different geographical locations.

4.5 Antimicrobial activity

Plants have been used throughout ages to cure infections through herbal supplier and self-medications. In particular, the usage of plants in Palestine is a tradition and relies mainly on observations rather than scientific experimental data. Nevertheless, humanity always seeks for new emerging antimicrobial agents due to the fact that the effective life span of any antibiotic is limited by the microbial resistance and toxicity. Therefore, in the following work, initial screening of the *S. palaestina* essential oil antimicrobial activity against different types of organisms was performed.

The antimicrobial activity of **5 µl** *of S. palaestina* essential oil was examined on gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermis*), gram negative bacteria (*Salmonella typhimurium, E.coli*) and fungus (*Candida albicans*) in the presence of

positive control (gentamicin, ciprofloxacin and nystatin) by using disc diffusion method. The zones of inhibition (**Fig. 27**) were measured and the average results of zones of inhibition were summarized in **Table 8**.



S. palaestina oil antimicrobial activity against *E.coli*

S. palaestina oil antimicrobial activity against *Candida*

Fig. 27: Zone of inhibition of *S. palaestina* oils

Table 8 The antimicrobial activity results

Average zone of inhibition (mm)					
	Staphylococcus aureus	***Staphylococcus epidermis***	***Salmonella typhimurium***	***E. coli***	***Candida albicans***
***S.palaestina* oil 5 µl**	10.39	ND*	ND	8.57	14.04
Gentamicin 10 µg/ml	8.72	12.36	9.98	9.47	NT
Ciprofloxacin 10 µg/ml	NT*	NT	21.73	23.79	NT
Nystatin 115 IU/ml	NT	NT	NT	NT	6.79
Blank	ND	ND	ND	ND	ND

*NT: Not tested *ND: Not detected.

Comparison between antimicrobial activities is illustrated in **Fig. 28**.

Fig. 28

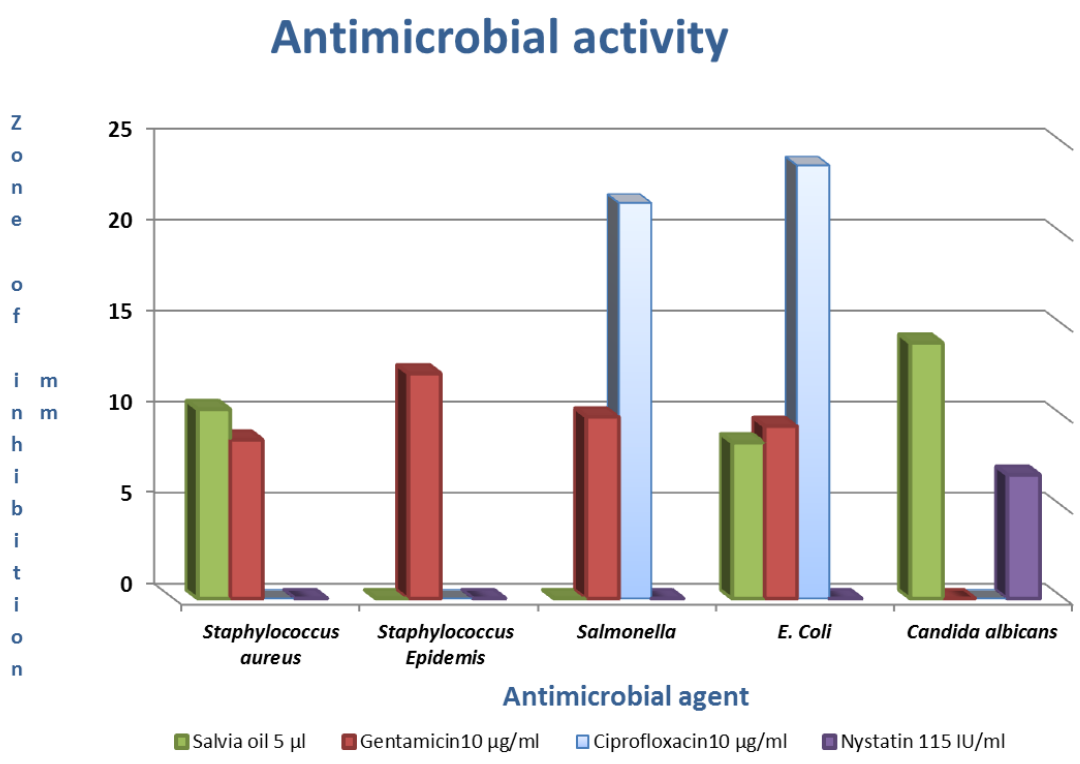


Fig. 28: Antimicrobial activity of Palestinian *S. palaestina* oil.

It is clear that **5 µl** of *S. palaestina* essential oil exhibits notable antimicrobial activity against some gram positive bacteria (*Staphylococcus aureus*) and some gram negative bacteria (*E.coli*). This observed activity is superior to gentamicin in case of *Staphylococcus aureus* and almost nearly the same as that of gentamicin in case of *E.coli*. Moreover, this volume of *S. palaestina* oil was **two times more effective** than nystatin in case of ***Candida albicans***. Conversely, this volume of tested oil doesn't exhibit any activity neither on *Staphylococcus epidermis* nor on *Salmonella typhimurium*.

Regardless effectiveness, the usage of gentamicin is restricted by its toxicity which includes ototoxicity and nephrotoxicity. This reported toxicity remains a major problem in clinical use (Andreu *et al*., 1985; Dulon *et al*., 1988).

It's well known that nystatin resistance was reported after gradual exposure, seven isolates of *Candida* species became resistant (Athar & Winner, 1971). In addition, it has no appreciable activity against bacteria, protozoa, or viruses. It is contraindicated in patients with history of hypersensitivity which might include the following side effects; tachycardia, bronchospasm,

facial swelling, sever skin reactions which all required immediate emergency admission. Minor adverse reactions might include diarrhea (in some cases bloody diarrhea), vomiting, nausea, rashes, urticaria, Stevens-Johnson syndrome, and nonspecific myalgia ("http://www.drugs.com,").

Although ciprofloxacin's antimicrobial activity is greater than that of **5 µl** of the oil against tested organisms, but it's contraindicated in ages less than 18 years and its adverse reactions might be very sever such as tendon rupture which was reported recently in a number of cases. Achilles tendon rupture has been reported to the FDA in 25 cases. Some ruptures have also occurred in hands and shoulder ("http://www.drugs.com,").

In general, the essential oils are hydrophobic in nature, and it was proposed that the cell membrane is the primary target of their antimicrobial action. *S. palaestina* essential oil seems to accumulate in the cell membrane causing leakage of enzymes, ions and metabolites (Inoue *et al.*, 2004). In case of gram negative bacteria there might be resistance to essential oil due to the additional outer membrane of their cell wall, which acts as a barrier to many substances including antibiotics (Palombo & Semple, 2001). However, the antimicrobial activity of *S. palaestina* essential oil on *Candida* is mainly due to the antimicrobial constituents especially eucalyptol, which are capable of changing the structure and moisture of mucous membranes of fungal cells, interfering with the respiratory processes, and thus eliminate the pathogen (Chen *et al.*, 2013).

It was suggested that the antimicrobial activity of *S. palaestina* oil was probably due to its constituents (thujone, eucalyptol, camphor, camphene and caryophylline). But when comparing the antimicrobial activity of each constituent with the antimicrobial activity of the whole oil, the oil activity was superior. This indicates that their antimicrobial effect probably involves some type of synergism between many constituents (Dragana Mitic-Ćulafic, 2005; Fraternale *et al.*, 2012).

Thus, the overall therapeutic effect of *S. palaestina* oil could be attributed to the synergistic interactions of individual components and to the anti-inflammatory approved effect of this oil which can lead to easier passage of the essential oils through mucous membrane.

Initial screening of oil's activity revealed that this oil might be active against both bacteria and fungus which might be superior to the available antibiotics which usually act on one type only.

Further studies are needed to estimate the minimum inhibitory concentration (MIC) and the safety of this oil.

4.6 Minerals analysis

Due to the increasing use of herbal medicines worldwide, the safety, efficacy and quality of medicinal plants has become a major concern. There are several works have been reported in many developed countries on minerals content of medicinal plants, unfortunately there is no single report regarding minerals content in these herbs in Palestine.

Our present work was carried out to screen the availability of minerals in *S. palaestina* leaves by using inductively coupled plasma optical emission spectrometry (ICP-OES) which is a trustable tool for the determination of various elements in liquid and solid samples. Eighteen minerals were screened namely; silver (Ag), aluminum (Al), calcium (Ca), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), potassium(K), sodium (Na), barium (Ba), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), strontium (Sr) and zinc (Zn).

Five different concentrations of standard solution for each mineral were prepared as in methodology (**Table 2**) then standard calibration curves were established and correlation coefficients were calculated. All the calibration curves are present in the appendix of this thesis.

There was clear Linear relation between concentrations and ems for the studied element, the limit of detection (LOD) and the limit of quantitation (LOQ) were determined for each element as in **Table 9**.

Table 9 The detected mineral, related wavelength, LOD, LOQ and the concentration in *S. palaestina* leaves.

#	Analyte Name	Wavelength (nm)	LOD (ppb)	LOQ (ppb)	Analyte concentration (ppm)
1	**Ag**	328.065	1.3893	4.6311	ND
2	**Al**	394.395	5.2188	17.3960	310.9
3	**Ba**	455.386	0.1401	0.4670	14.665
4	**Ca**	315.885	25.9416	86.4719	12365
5	**Cd**	228.797	2.2294	7.4313	0.0413
6	**Co**	238.885	2.2912	7.6373	ND
7	**Cr**	267.704	2.6253	8.7510	0.7078
8	**Cu**	324.752	1.5875	5.2918	8.49
9	**Fe**	259.933	1.8094	6.0313	217.0375
10	**K**	766.46	20.3046	67.6820	22207.5
11	**Mg**	285.211	5.2790	17.5968	1908.5
12	**Mn**	257.608	0.4265	1.4218	13.3088
13	**Mo**	202.03	4.4794	14.9314	0.8969
14	**Na**	589.571	3.1390	10.4634	490.125
15	**Ni**	231.599	4.3459	14.4862	0.5543
16	**Pb**	216.999	28.9402	96.4674	0.2267
17	**Sr**	421.525	0.0333	0.1110	22.7188
18	**Zn**	213.856	1.61035	5.3678	27.41

Sixteen elements were detected and quantified in dried *S. palaestina* leaves. Detected elements were arranged in decreasing order as follows:

K, Ca, Mg, Na, Al, Fe, Zn, Sr, Ba, Mn, Cu, Mo, Cr, Ni, Pb, Cd.

The concentration of both silver and cobalt was below detection limit. **Figure 29** represents the availability of minerals in *S. palaestina* leaves.

Fig. 29

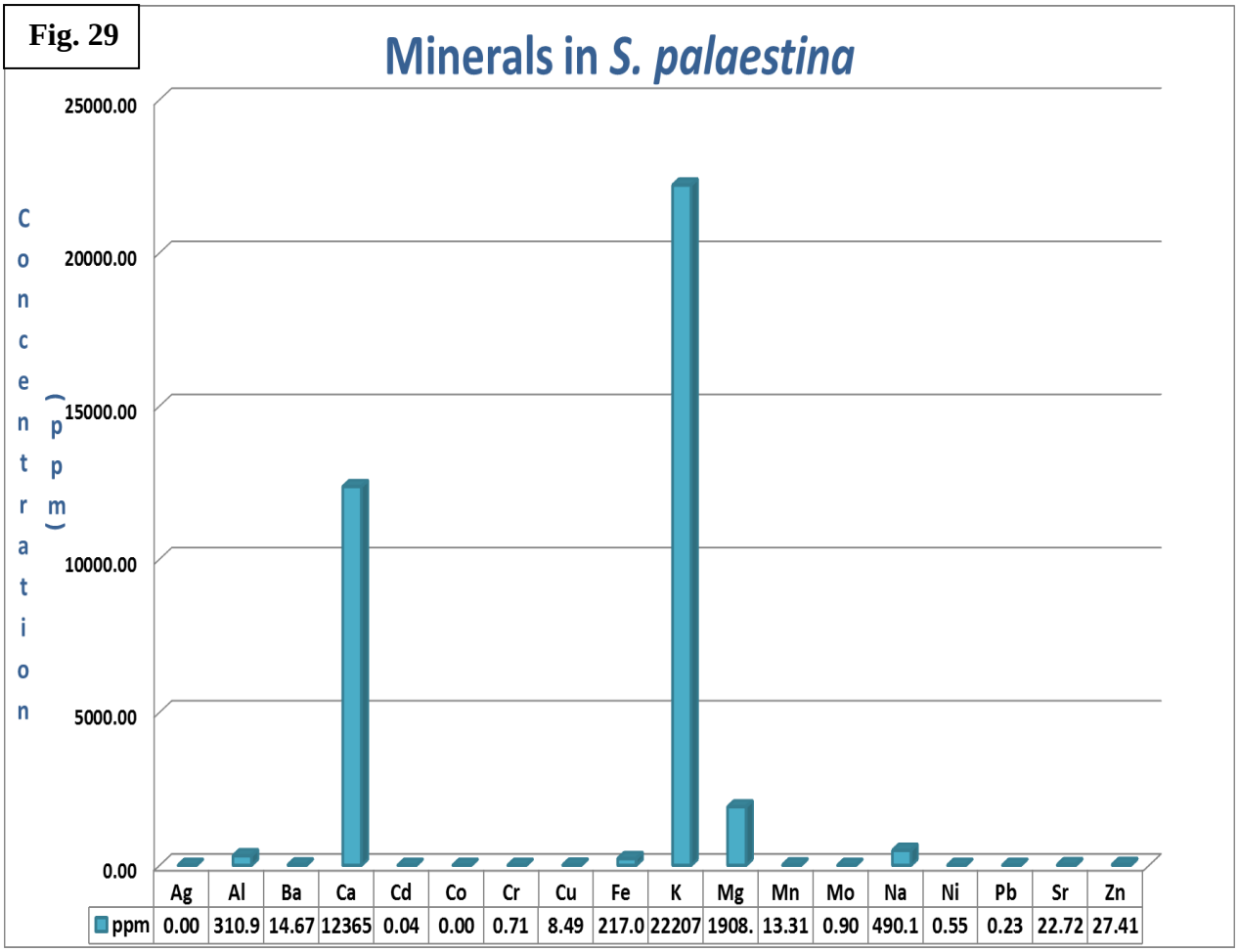


Fig. 29: Minerals in *S. palaestina* leaves.

The abundant mineral was potassium in the tested leaves and high levels of it is consistent with its important role in biosynthesis of primary and secondary metabolic products in plants (Ibrahim, Jaafar, Karimi, & Ghasemzadeh, 2012).

The main fifth minerals were lightened by pie chart in **Figure 30.**

Fig. 30

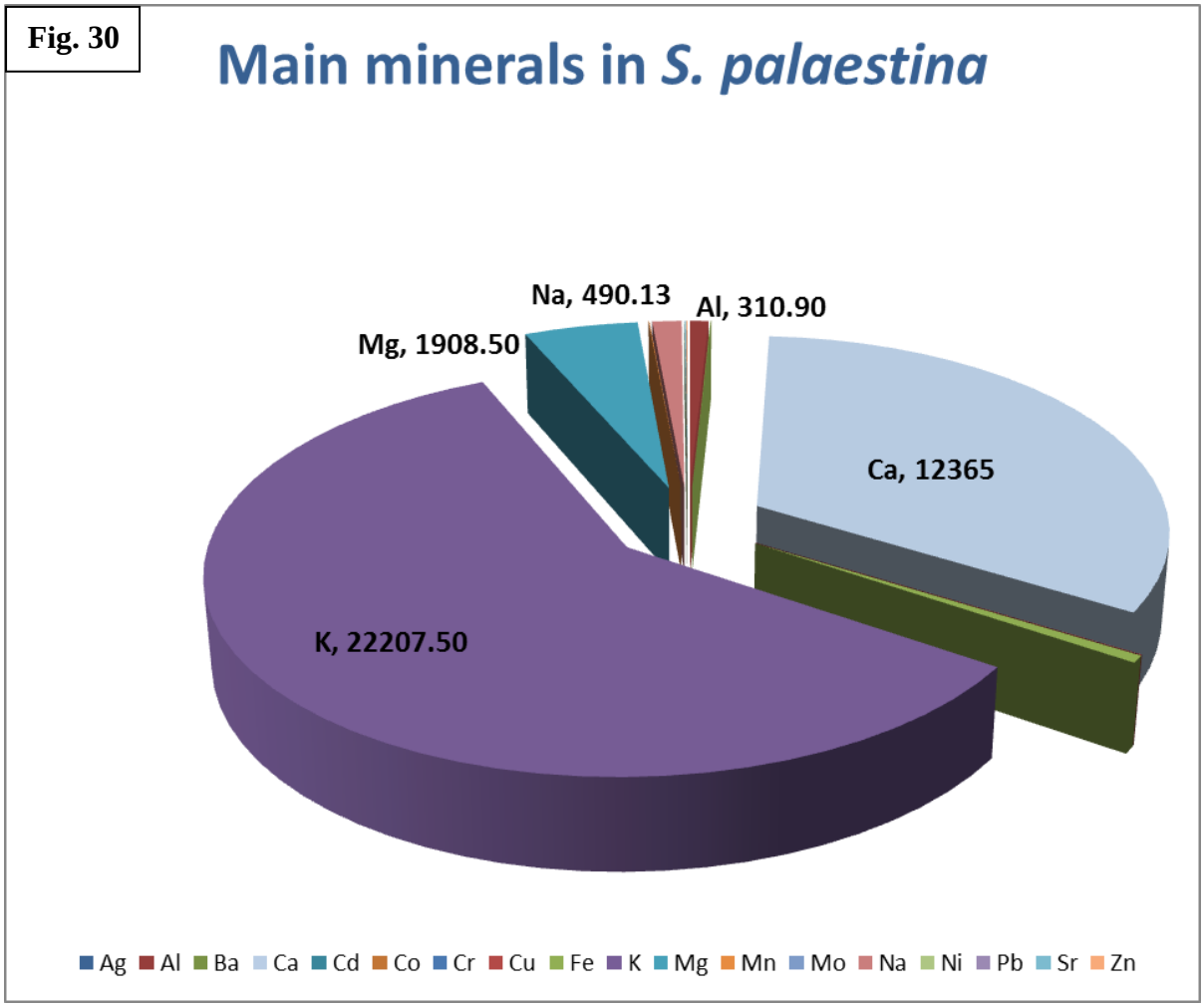


Fig. 30: Main minerals in *S. palaestina* leaves.

Minerals are elements that originate in the soil and can't be created by living creatures. But all creatures need minerals in certain amounts to maintain proper health. Plants absorb minerals from the soil then animals get their minerals from the plants or other animals they eat. Human beings obtain minerals either directly from plants or indirectly from animal sources.

In *S. palaestina* leaves the abundant mineral was potassium (22207.5 mg/kg) which is the major ion inside every living plant and animal cell. It is involved in nerve impulses, muscle contractions, including the heart muscle and in influencing osmotic balance (Bessman & Pal, 1980; Clay & Shlesinger, 1977; Dhalla, Singh, Fedelesova, Balasubramanian, & McNamara, 1974; Stolkowski & Reinberg, 1959). The ion pump (Na+/K+-ATPase) uses ATP to pump sodium ions out of the cell and potassium ions into the cell (Wambach *et al.*, 1980). The optimal daily required intake (DRI) of potassium was estimated by the Institute of Medicine in 2004 as 4,700 mg of potassium. Several researches related stroke with low potassium

intake (Burgess *et al.*, 1999; Khan, Spiers, & Khan, 2013; Levine & Coull, 2002; Singhal, 2002).

In practice, banana is well known as a rich source of potassium, 100 gram of banana contains about 358 mg of potassium this means that each gram of banana contains 3.58 mg while *S. palaestina* leaves contains about 22.2075 mg/gram. In other word, one weight of *S. palaestina* leaves contains potassium approximately seven times more than that of the same weight of banana (Harvey & Theuer, 1991).

If we assumed that about 2 grams of leaves is needed to make one cup of *S. palaestina* tea, then quantities of minerals in one cup is illustrated as in **Table 10**.

Table 10 Approximate minerals concentration in one cup of *S. palaestina* tea.

#	Analyte Name	Analyte concentration (ppm)	Concentration mg/2g (cup)
1	**Ag**	ND	ND
2	**Al**	310.9	0.6218
3	**Ba**	14.665	0.0293
4	**Ca**	12365	24.73
5	**Cd**	0.0413	0.0001
6	**Co**	ND	ND
7	**Cr**	0.7078	0.0014
8	**Cu**	8.49	0.0170
9	**Fe**	217.0375	0.4341
10	**K**	22207.5	44.415
11	**Mg**	1908.5	3.817
12	**Mn**	13.3088	0.0266
13	**Mo**	0.8969	0.0018
14	**Na**	490.125	0.9803
15	**Ni**	0.5543	0.0011
16	**Pb**	0.2267	0.0005
17	**Sr**	22.7188	0.0454
18	**Zn**	27.41	0.0548

When talking about potassium always comes to mind sodium which is the main cation in the outer cells in all creatures. It regulates blood volume, pressure and pH. Also it is important in neuron function and in osmoregulation between cells and the extracellular fluids (Bobik, Neylon, Little, Cragoe, & Weissberg, 1990; Kesteloot & Joossens, 1988). The minimum requirement of sodium is about 500 milligrams per day. *S. palaestina* leaves contain relatively high concentration of potassium and low concentration of sodium which is optimal to maintain good health (Drewnowski, Maillot, & Rehm, 2012; Smith *et al.*, 1950).

The second abundant mineral in the leaves was calcium which is very important for life. It plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life. Also, it has other significant roles such as in neurotransmitter release and muscle contraction. Long-term calcium deficiency can lead to rickets and osteoporosis (Ortega *et al*., 1998). Some individuals are allergic to dairy products; others (vegetarians) avoid dairy products for ethical and health reasons. In these categories plants could be the main source of calcium which is required daily intake for adults is nearly about 1000 mg. *S. palaestina* leaves contain about 12365 ppm, while it contains about 1908.5 ppm of magnesium for which the daily recommended values is about 300 mg for men and 270 mg for women and ratio between both minerals is required to maintain good health. Magnesium is vital since it works as coenzyme and as catalyst (Levitan *et al*., 2013; Martinez-Ferrer, Peris, Reyes, & Guanabens, 2008; Smith *et al.*, 1950; Tibbetts & Aub, 1937).

Other minerals are needed in relatively very small amounts, while some of them such as Cd, Co, Pb and Cr might deteriorate human health and their availability in herbal medicines are controlled in several countries as recommended by the WHO guidelines for herbal assessing quality (Pier, 1975; WHO, 2007).

Table 11 below was adopted from the WHO guidelines and illustrated the proposed national limits for toxic metals in various types of herbal products.

Table 11 Examples of national limits for toxic metals in herbal medicines and products

		Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Mercury (Hg)	Copper (Cu)	Total toxic metals as lead
For herbal medicines								
Canada	raw herbal materials	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
	finished herbal products	0.01 mg/ day	0.02 mg/ day	0.006 mg/ day	0.02mg/ day	0.02 mg/ day		
China	herbal materials	2 ppm	10 ppm	1 ppm		0.5 ppm		20 ppm
Malaysia	finished herbal products	5 mg/kg	10 mg/ kg			0.5 mg/kg		
Republic of Korea	herbal materials							30 ppm
Singapore	finished herbal products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand	herbal material, finished herbal products	4 ppm	10 ppm	0.3 ppm				
WHO recommendations (2)			10 mg/kg	0.3 mg/kg				
For other herbal products								
National Sanitation Foundation draft proposal (Raw Dietary supplement)[a]		5 ppm	10 ppm	0.3 ppm	2 ppm			
National Sanitation Foundation draft proposal (Finished Dietary Supplement)[a]		0.01 mg/ day	0.02 mg/ day	0.006 mg/ day	0.02 mg/ day	0.02 mg/ day		

The most common heavy metals implicated in human toxicity include lead, mercury, arsenic, and cadmium, although aluminum and cobalt may also cause toxicity (Keen, 1974). The accumulation of these metals in herbs is highly dependent on their availability in the soil. Moreover, cross-contamination might occur also during drying and processing of the herb (Hoagland, 1931; Lindsay, 1972; Rengel, 2004).

In Palestinian tested *S. palaestina*, all results for these metals were within allowable limits except for aluminum, which is not used in any biochemical process in living systems. It accumulates in the brain and, to a lesser extent, in the bones (Rengel, 2004; Tripathi & Pandey, 2007). This accumulation can persist for very long time in various organs and tissues. High levels of aluminum may produce DNA damage, which might cause

carcinogenicity (Darbre *et al.*, 2013). Aluminum has shown neurotoxicity and it was suggested that aluminum is implicated in the etiology of Alzheimer's disease and associated with other neurodegenerative diseases in humans ("Aluminium and Alzheimer," 1976; Exley, 2006; Swegert, Dave, & Katyare, 1999; van der Voet, Marani, Tio, & de Wolff, 1991). Even more, in vivo studies indicated that there was relation between aluminum and dysfunction of male reproductive system(Singh, Singh, & Singh, 1998). In addition, clinical studies conducted on maternal exposure had shown that aluminum might cause embryo toxicity and affect the developing nervous system in fetus (Paternain, Domingo, Llobet, & Corbella, 1988).

The environmental protection agency (EPA) has set the maximum acceptable level of aluminum in fresh water at 750 µg / liter (Kg). In *S. palaestina* leaves, the result of aluminum was 310.9 mg/Kg, which means that one cup prepared by 2 grams of the leaves will contain about 621.8 µg of aluminum and two cups of this tea will exceed the maximum acceptable limit (1243.6 µg /2 cups).

Nevertheless, due to the cumulative nature of aluminum, it was suggested that a tolerable weekly intake (TWI) for aluminum rather than a tolerable daily intake (TDI) is strongly recommended.

Aluminum concentration in medicinal plants depends upon geographical location, soil, air and water contamination. In general, the uptake of metals by plants is influenced by a number of factors including metal concentrations in soils, cation-exchange capacity, soil pH, organic matter content, types and varieties of plants and plant age. However, the most important factor is the concentration of the metal in the soil and the existing environmental conditions (Annan.k, 2013; Karak *et al*., 2015; Losfeld *et al.*, 2014; Rengel, 2004; Wang, Takematsu, & Ambe, 2000).

Finally, the general believe that medicinal plants are safe and devoid of toxicity could be misapprehended and therefore, WHO recommended that medicinal plants should be checked for the presence of certain metals which might deteriorate the general health (WHO, 2007). *S. palaestina* sample was obtained from a very famous herbal shop in Ramallah and further work is needed to screen the minerals availability in other Palestinian governorates.

Chapter Five

Conclusions & Future Work

5. Conclusions and Future Work

5.1 Conclusions

We examined for the first time the chemical constituents of *S. palaestina* from different locations in Palestine and some of their pharmacological activities.

SEM of wild and cultivated leaves showed that not only the intensity of the trichomes of leaves is different but also their surface structure. It was found that wild leaves contain more intensive, branched, thinner, smoother and darker trichomes when compared to the cultivated ones.

The above results are consistent with the fact that all creatures seek to adapt and cope with its environmental conditions. Thus, the morphology of the wild leaves of *S. palaestina* was found to be different from cultivated in order to avoid harsh environmental conditions and to avoid loss of water and essential oils. This also observed from the width of the leaf which is much smaller in wild and the edges of wild leaf are curved inside as a cover.

The oil obtained by SD was analyzed by GC-MS technology and the yield for each sample was calculated based on dry basis. Particularly, in spring, the cultivated leaves contains higher amount of essential oils than wild leaves and this can be observed from oil yield.

The GC-MS technique was utilized and found to be precise, accurate and reliable in the separation and identification of the components of *S. palaestina* complex volatile mixtures.

Twenty volatile and semivolatile components were detected and identified in *S. palaestina* leaves. Comparison between wild and cultivated leaves at the same location revealed that there are few differences between both. The wild samples were characterized by eucalyptol unequivocal dominance while the cultivated was distinguished with higher levels of camphor. Therefore, when camphor pharmacological activity is required (topical applications), the usage of cultivated leaves is recommended, while the wild leaves will be appropriated when eucalyptol pharmacological activity is required. In other word, it is advisable to use wild *S. palaestina* leaves in the preparation of pharmaceutical dosage forms that containing eucalyptol as active or inactive ingredient and to use cultivated leaves in skin preparations to benefit from camphor.

Jericho's cultivated sample is unique, in which camphor volatile compound was the main constituent. Its amount was three times more than other samples from different locations. Therefore, it might be advantageous to benefit from its high content in topical applications. On the other hand, Jericho's sample contains high amount of thujone derivatives, which is harmful if ingested. Thus, special restrictions for the usage of Jericho's *S. palaestina* are recommended.

The antioxidant activity results revealed that the activity of *S. palaestina* oil is less than that of the positive control (BHT). Nevertheless, it increased with time and special sustained release formulations might be helpful.

The *S. palaestina* oil antimicrobial activity revealed activity against both bacteria and fungus. Moreover, it was superior to the available antibiotics in certain cases. It is obvious that 5 µl of tested essential oil exhibits notable antimicrobial activity against some gram positive bacteria (*Staphylococcus aureus*) and some gram negative bacteria (*E.coli*). The observed activity is superior to gentamicin in case of *Staphylococcus aureus* and almost the same as gentamicin in case of *E.coli*. Furthermore, this volume of *S. palaestina* oil was two times more effective than nystatin in case of *Candida albicans*. This promising result is important due to the increasing resistance against available antimicrobial agents in addition to its already known toxicity.

Although there are several works that have been reported on minerals content of medicinal plants in many developed countries, however, this work was the first to be conducted in Palestine. The efficacy, safety and quality of herbs are of important concerns for both consumers and health authorities.

Screening of native *S. palaestina* leaves minerals revealed that it is rich in potassium. One gram of leaves contains seven times more potassium than that of the same weight of banana. In addition, these leaves were rich in calcium and magnesium. Therefore, special recommendations for consumers might be necessary in order to prevent *S. palaestina* minerals from binding with drugs such as tetracycline or ciprofloxacin and affecting their absorption (take the drug 1 hour before or 2 hours after this plant).

The general believe that medicinal plants are safe and devoid of toxicity could be misconstrued. Hence, the amount of aluminum in tested sample was exceeding the permissible limits and due to the accumulation properties of this element, long-term usage may deteriorate health.

5.2 Future Work

The following are some specific suggestions concerning future investigations:

1. The current research investigated *S. palaestina* essential oils merely at their vegetative stage The components of *S. palaestina* at different stages of plant life cycle will be explored in order to study the seasonal variations. The same methodology should be utilized and compared to the accumulated results at hand.
2. The antioxidant activity, bioactivity and toxicity of the essential oils at different stages of the plant life cycle from different extracts will be thoroughly investigated.
3. Further studies are recommended to estimate the minimum inhibitory concentration (MIC) and the safety of the essential oils.
4. The effect of combined *S. palaestina* oil with available antimicrobial agents will be tested.
5. The screening of minerals of *S. palaestina* in other locations all over Palestine is highly recommended to draw general decisive conclusions.
6. Since the safety and efficacy of herbal medicine is closely related to the quality of the source, we recommend to conduct a deepen discussion with the Palestinian regulatory bodies in order to warrant safer consumption of these herbs.

References

1. Abu-Darwish, M. S., Cabral, C., Ferreira, I. V., Goncalves, M. J., Cavaleiro, C., Cruz, M. T., .Salgueiro, L. (2013). Essential oil of common Sage (*Salvia officinalis L.*) from Jordan: assessment of safety in mammalian cells and its antifungal and anti-inflammatory potential. *Biomed Res Int, 2013,* 538940.

2. Abu-Lafi, S., Odeh, I., Dewik, H., Qabajah, M., Imam, A., Dembitsky, V. M., & Hanus, L. O. (2007). Natural compounds of Palestine flora. Comparison analysis by static headspace and steam distillation GC-MS of semivolatile secondary metabolites from leaves of cultivated Palestinian *Majorana syriaca*. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 151*(1), 21-29.

3. Adamo, P., Arienzo, M., Bianco, M. R., Terribile, F., & Violante, P. (2002). Heavy metal contamination of the soils used for stocking raw materials in the former ILVA iron-steel industrial plant of Bagnoli (southern Italy). *Sci Total Environ, 295*(1-3), 17-34.

4. Al-Howiriny. (2007). Composition and antimicrobial activity of the essential oil of S*alvia palaestina* bentham growing in Saudi Arabia.*Saudi Pharmaceutical Journal, 15*,p.3-4.

5. Al-Jaber, H. I., Al-Qudah, M. A., Barhoumi, L. M., Abaza, I. F., & Afifi, F. U. (2012). Essential oil composition of the aerial parts of fresh and air-dried *Salvia palaestina* Benth. (Lamiaceae) growing wild in Jordan. *Nat Prod Res, 26*(13), 1179-1187.

6. Al-Qudah, M. A., Al-Jaber, H. I., Abu Zarga, M. H., & Abu Orabi, S. T. (2014). Flavonoid and phenolic compounds from *Salvia palaestina L.* growing wild in Jordan and their antioxidant activities. *Phytochemistry, 99,* 115-120.

7. Ali-Shtayeh Mohammed S, Jamous Rana Majed. (2013). Antimicrobial Activity of Palestinian Medicinal Plantsagainst Acne-Inducing Bacteria. African journal of microbiology research, 7(21), 2560-2573.

8. Ali-Shtayeh, M. S., Yaghmour, R. M., Faidi, Y. R., Salem, K., & Al-Nuri, M. A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *J Ethnopharmacol, 60*(3), 265-271.

9. Ali-Shtayeh, M. S., Yaniv, Z., & Mahajna, J. (2000). Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. *J Ethnopharmacol, 73*(1-2), 221-232.

10. Aluminium and Alzheimer. (1976). *Lancet, 1*(7972), 1281-1282.

11. Andreu, J., Bodet, B., Garand, G., Dabo, B., & Leroy, G. (1985). [Ototoxicity and nephrotoxicity of gentamicin and tobramycin]. *Cah Anesthesiol, 33*(6), 515-521.

12. Annan, K., Kojo, A. I., Cindy, A., Samuel, A. N., & Tunkumgnen, B. M. (2010). Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. *Pharmacognosy Res, 2*(1), 41-44.

13. Annan.k, R. A. D., Isaac K. Amponsah, Isaac K. Nooni. (2013). The heavy metal contents of some selected medicinal plants sampled from different geographical locations. *Pharmacognosy Res, 5*(2), 103-108.

14. Aruoma, O. I. (1994). Nutrition and health aspects of free radicals and antioxidants. *Food Chem Toxicol, 32*(7), 671-683.

15. Athar, M. A., & Winner, H. I. (1971). The development of resistance by *Candida* species to polyene antibiotics in vitro. *J Med Microbiol, 4*(4), 505-517.

16. Azaizeh, H., Saad, B., Cooper, E., & Said, O. (2010). Traditional Arabic and Islamic Medicine, a Re-emerging Health Aid. *Evid Based Complement Alternat Med, 7*(4), 419-424.

17. Bakircioglu, D., Kurtulus, Y. B., & Ibar, H. (2011). Investigation of trace elements in agricultural soils by BCR sequential extraction method and its transfer to wheat plants. *Environ Monit Assess, 175*(1-4), 303-314. doi: 10.1007/s10661-010-1513-5

18. Banerjee, S., Welsch, C. W., & Rao, A. R. (1995). Modulatory influence of camphor on the activities of hepatic carcinogen metabolizing enzymes and the levels of hepatic and extrahepatic reduced glutathione in mice. *Cancer Lett, 88*(2), 163-169.

19. Baricevic, D., Sosa, S., Della Loggia, R., Tubaro, A., Simonovska, B., Krasna, A., & Zupancic, A. (2001). Topical anti-inflammatory activity of *Salvia officinalis L.* leaves: the relevance of ursolic acid. *J Ethnopharmacol, 75*(2-3), 125-132.

20. Bessman, S. P., & Pal, N. (1980). Phosphate metabolic control of potassium movement - its effect on osmotic pressure of the cell. *Adv Exp Med Biol, 128*, 175-186.

21. Blagojeviae, N. (2009). Heavy Metals Content in Leaves and Extracts of Wild-Growing *Salvia Officinalis* from Montenegro. *Polish J. of Environ. Stud., 18*(2), 167-173.

22. Bobik, A., Neylon, C. B., Little, P. J., Cragoe, E. J., Jr., & Weissberg, P. L. (1990). Sodium-dependent, ethylisopropylamiloride-sensitive mechanisms regulate intracellular pH in human vascular smooth muscle. *Clin Exp Pharmacol Physiol, 17*(4), 297-301.

23. Bohm, H., Boeing, H., Hempel, J., Raab, B., & Kroke, A. (1998). [Flavonols, flavone and anthocyanins as natural antioxidants of food and their possible role in the prevention of chronic diseases]. *Z Ernahrungswiss, 37*(2), 147-163.

24. Boszormenyi, A., Hethelyi, E., Farkas, A., Horvath, G., Papp, N., Lemberkovics, E., & Szoke, E. (2009). Chemical and genetic relationships among Sage (*Salvia officinalis L.*) cultivars and JudeanSage (*Salvia judaica Boiss.*). *J Agric Food Chem, 57*(11), 4663-4667.

25. Brand-Williams W. , C. M. E., Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensm–Wiss A-Technol.

26. Briskin, D. P. (2000). Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol, 124*(2), 507-514.

27. Burgess, E., Lewanczuk, R., Bolli, P., Chockalingam, A., Cutler, H., Taylor, G., & Hamet, P. (1999). Lifestyle modifications to prevent and control hypertension. 6. Recommendations on potassium, magnesium and calcium. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Centre for Disease Control at Health Canada, Heart and Stroke Foundation of Canada. *CMAJ, 160*(9 Suppl), S35-45.

28. C.B. Boss, K. J. F. (1997). Concept, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry. *Perkin-Elmer*(2nd edition).

29. Camphor revisited: focus on toxicity. Committee on Drugs. American Academy of Pediatrics. (1994). *Pediatrics, 94*(1), 127-128.

30. Chan, H. H., Hwang, T. L., Su, C. R., Reddy, M. V., & Wu, T. S. (2011). Anti-inflammatory, anticholinesterase and antioxidative constituents from the roots and the leaves of *Salvia nipponica Miq*. var. formosana. *Phytomedicine, 18*(2-3), 148-150.

31. Chattoraj, S. C., & Tietz, N. W. (1969). Gas chromatography: fundamentals and application. Hosp Top, 47(10), 81-100.

32. Chen, Y., Zeng, H., Tian, J., Ban, X., Ma, B., & Wang, Y. (2013). Antifungal mechanism of essential oil from Anethum graveolens seeds against *Candida albicans*. *J Med Microbiol, 62*(Pt 8), 1175-1183.

33. Cioffi, G., Bader, A., Malafronte, A., Dal Piaz, F., & De Tommasi, N. (2008). Secondary metabolites from the aerial parts of *Salvia palaestina* Bentham. *Phytochemistry, 69*(4), 1005-1012.

34. Clapp, N. K., Satterfield, L. C., & Bowles, N. D. (1979). Effects of the antioxidant butylated hydroxytoluene (BHT) on mortality in BALB/c mice. *J Gerontol, 34*(4), 497-501.

35. Clay, J. R., & Shlesinger, M. F. (1977). Random walk analysis of potassium fluxes associated with nerve impulses. *Proc Natl Acad Sci U S A, 74*(12), 5543-5546.

36. Costello, C. E. (1986). Fundamentals of present-day mass spectrometry. J Clin Pharmacol, 26(6), 390-395.

37. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin Microbiol Rev, 12*(4), 564-582.

38. Dafni, A., Yaniv, Z., & Palevitch, D. (1984). Ethnobotanical survey of medicinal plants in northern Israel. *J Ethnopharmacol, 10*(3), 295-310.

39. Darbre, P. D., Bakir, A., & Iskakova, E. (2013). Effect of aluminium on migratory and invasive properties of MCF-7 human breast cancer cells in culture. *J Inorg Biochem, 128*, 245-249.

40. del Castillo, J., Anderson, M., & Rubottom, G. M. (1975). Marijuana, absinthe and the central nervous system. *Nature, 253*(5490), 365-366.

41. Devasagayam, T. P., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S., & Lele, R. D. (2004). Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Physicians India, 52*, 794-804.

42. Dhalla, N. S., Singh, J. N., Fedelesova, M., Balasubramanian, V., & McNamara, D. B. (1974). Biochemical basis of heart function. XII. Sodium-potassium stimulated adenosine triphosphatase activity in the perfused rat heart made to fail by substrate-lack. *Cardiovasc Res, 8*(2), 227-236.

43. Dodds, E. C. (1936). The Pharmacological Action and Clinical Use of Drugs with a Camphor- and Coramine-like Action: (Section of Therapeutics and Pharmacology). *Proc R Soc Med, 29*(6), 655-657.

44. Dragana Mitic-Ćulafic, B. V.-G., Jelena Knezevic-Vukcevic, S. Stankovic and Draga Simic. (2005). Comparative study on the antibacterial activity of volatiles from Sage (*Salvia officinalis L.*). *Arch. Biol. Science, 57*(3), 173-178.

45. Drewnowski, A., Maillot, M., & Rehm, C. (2012). Reducing the sodium-potassium ratio in the US diet: a challenge for public health. *Am J Clin Nutr, 96*(2), 439-444.

46. Dulon, D., Aurousseau, C., Erre, J. P., & Aran, J. M. (1988). Relationship between the nephrotoxicity and ototoxicity induced by gentamicin in the guinea pig. *Acta Otolaryngol, 106*(3-4), 219-225.

47. Emerit, J., & Michelson, A. M. (1982). [Free radicals in medicine and biology]. *Sem Hop, 58*(45), 2670-2675.

48. Emery, D. P., & Corban, J. G. (1999). Camphor toxicity. *J Paediatr Child Health, 35*(1), 105-106.

49. Enwonwu, C. O., & Salako, N. (2012). The periodontal disease-systemic health-infectious disease axis in developing countries. *Periodontol 2000, 60*(1), 64-77.

50. Esmaeili, A., Rustaiyan, A., Nadimi, M., Larijani, K., Nadjafi, F., Tabrizi, L., . . . Amiri, H. (2008). Chemical composition and antibacterial activity of essential oils from leaves, stems and flowers of *Salvia reuterana* Boiss. grown in Iran. *Nat Prod Res, 22*(6), 516-520.

51. Exley, C. (2006). A vexing Commentary on the important issue of aluminium and Alzheimer' disease. *J Alzheimers Dis, 10*(4), 451-452.

52. Farhat, G. N., Affara, N. I., & Gali-Muhtasib, H. U. (2001). Seasonal changes in the composition of the essential oil extract of East Mediterranean Sage (*Salvia libanotica*) and its toxicity in mice. *Toxicon, 39*(10), 1601-1605.

53. Fassel, V. A. (1986). Analytical Inductively Coupled Plasma Spectroscopies – Past, Present, and Future *Fresenius Z.Anal. Chem., 324(6)*, 511-518.

54. Fraternale, D., Flamini, G., Bisio, A., Albertini, M. C., & Ricci, D. (2012). Chemical composition and antimicrobial activity of *Salvia x jamensis* essential oil. *Nat Prod Commun, 7*(9), 1237-1240.

55. Goel, H. C., & Roa, A. R. (1988). Radiosensitizing effect of camphor on transplantable mammary adenocarcinoma in mice. *Cancer Lett, 43*(1-2), 21-27.

56. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med, 27*(1), 1-93.

57. Gürsoy. (2011). Chemical Composition and Antioxidant Activity of the Essential Oils of *Salvia palaestina* (Bentham) and S. ceratophylla (L.). *A C G publication*, p. 278-287.

58. Gutteridge, J. M., & Halliwell, B. (1992). Comments on review of Free Radicals in Biology and Medicine, second edition, by Barry Halliwell and John M. C. Gutteridge. *Free Radic Biol Med, 12*(1), 93-95.

59. Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging, 18*(9), 685-716.

60. Harbone, J. B. (1993). Introduction to Ecological Biochemistry. *Academic Press, London.*(4th edition).

61. Harvey, R. A., & Theuer, R. C. (1991). Potassium as an index of fruit content in baby food products. Part I. Banana-containing and apricot-containing products. *J Assoc Off Anal Chem, 74*(6), 929-932.

62. Hedege, I. C. (1992). A gLobal survey of the biogeography of the *lamiaceae*. Advances in Labiate science, 7-18.

63. Hoagland, D. R. (1931). Absorption of Mineral Elements by Plants in Relation to Soil Problems. *Plant Physiol, 6*(3), 373-388.

64. Horikawa, E., & Okada, T. (1975). [Experimental study on acute toxicity of phenol camphor (author's transl)]. *Shikwa Gakuho, 75*(6), 934-939.

65. http://en.wikipedia.org/wiki/*Salvia palaestina*.

66. https://maps.google.com.

67. http://www.drugs.com.

68. http://www.who.int/mediacentre/factsheets/fs310/en/index1.html.

69. Ibrahim, M. H., Jaafar, H. Z., Karimi, E., & Ghasemzadeh, A. (2012). Primary, secondary metabolites, photosynthetic capacity and antioxidant activity of the Malaysian Herb Kacip Fatimah (*Labisia Pumila Benth*) exposed to potassium fertilization under greenhouse conditions. *Int J Mol Sci, 13*(11), 15321-15342.

70. Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H., & Shimada, J. (2004). The antibacterial effects of terpene alcohols on Staphylococcus aureus and their mode of action. *FEMS Microbiol Lett, 237*(2), 325-331.

71. Inoue, Y., & Takeuchi, S. (1969). [Expectorant-like action of camphor derivatives]. *Nihon Ika Daigaku Zasshi, 36*(4), 351-354.

72. Jaber.S, S. A.-L., Asharif A. , Qutob M., Aburemeleh Q. and Akkawi. M. (2013). Potential Antimalarial Activity from Alcoholic Extracts of Wild *Salvia palaestina* Leaves. British Journal of Pharmacology and Toxicology, 4(5), 201-206

73. Juergens, U. R., Dethlefsen, U., Steinkamp, G., Gillissen, A., Repges, R., & Vetter, H. (2003). Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. *Respir Med, 97*(3), 250-256.

74. Juergens, U. R., Engelen, T., Racke, K., Stober, M., Gillissen, A., & Vetter, H. (2004). Inhibitory activity of 1,8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes and monocytes. *Pulm Pharmacol Ther, 17*(5), 281-287.

75. Juergens, U. R., Stober, M., & Vetter, H. (1998). Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes in vitro. *Eur J Med Res, 3*(11), 508-510.

76. Kadkhodayan, S., Coulter, E. D., Maryniak, D. M., Bryson, T. A., & Dawson, J. H. (1995). Uncoupling oxygen transfer and electron transfer in the oxygenation of camphor analogues by cytochrome P450-CAM. Direct observation of an intermolecular isotope effect for substrate C-H activation. *J Biol Chem, 270*(47), 28042-28048.

77. Kahl, R., & Kappus, H. (1993). [Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E]. *Z Lebensm Unters Forsch, 196*(4), 329-338.

78. Kamatou, G. P., Makunga, N. P., Ramogola, W. P., & Viljoen, A. M. (2008). South African *Salvia* species: a review of biological activities and phytochemistry. *J Ethnopharmacol, 119*(3), 664-672.

79. Karak, T., Sonar, I., Paul, R. K., Frankowski, M., Boruah, R. K., Dutta, A. K., & Das, D. K. (2015). Aluminium dynamics from soil to tea plant (Camellia sinensis L.): is it enhanced by municipal solid waste compost application? *Chemosphere, 119*, 917-926

80. Keen, P. (1974). Trace elements in plants and soil in relation to cancer. *S Afr Med J, 48*(57), 2363-2364.

81. Kelen, M., & Tepe, B. (2008). Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. *Bioresour Technol, 99*(10), 4096-4104.

82. Keller, M. S. (1978). *Mysterious herbs & roots : ancient secrets for beautie, health, magick, prevention and youth, 1600 B.C.-1900 A.D*. Culver City, Calif.: Peace Press.

83. Kesteloot, H., & Joossens, J. V. (1988). Relationship of dietary sodium, potassium, calcium, and magnesium with blood pressure. Belgian Interuniversity Research on Nutrition and Health. *Hypertension, 12*(6), 594-599.

84. Khan, E., Spiers, C., & Khan, M. (2013). The heart and potassium: a banana republic. *Acute Card Care, 15*(1), 17-24.

85. Kolak, U., Kabouche, A., Ozturk, M., Kabouche, Z., Topcu, G., & Ulubelen, A. (2009). Antioxidant diterpenoids from the roots of *Salvia barrelieri*. *Phytochem Anal, 20*(4), 320-327.

86. Kovats, E. (1958). Gas-chromatographische charakterisierung organischer verbindungen. Teil 1: Retentions indices aliphatischer halogenide, alkohole, aldehyde und ketone. . Helv. Chim. Acta., 1915-1932.

87. Lehtinen, R. (1975). Analgesic effect of apernyl and phenol-camphor solutions on alveolitis. *Int J Oral Surg, 4*(4), 157-159.

88. Levine, S. R., & Coull, B. M. (2002). Potassium depletion as a risk factor for stroke: will a banana a day keep your stroke away? *Neurology, 59*(3), 302-303.

89. Levitan, E. B., Shikany, J. M., Ahmed, A., Snetselaar, L. G., Martin, L. W., Curb, J. D., & Lewis, C. E. (2013). Calcium, magnesium and potassium intake and mortality in women with heart failure: the Women's Health Initiative. *Br J Nutr, 110*(1), 179-185.

90. Li, X., Gao, J., & Zhao, J. (2002). [Determination of heavy metal in Chinese herbs]. *Wei Sheng Yan Jiu, 31*(4), 295-297.

91. Liao, K., & Yin, M. (2000). Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: importance of the partition coefficient. *J Agric Food Chem, 48*(6), 2266-2270.

92. Lindsay, W. L. (1972). Influence of the soil matrix on the availability of trace elements to plants. *Ann N Y Acad Sci, 199*, 37-45.

93. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev, 4*(8), 118-126.

94. Loizzo, M. R., Saab, A. M., Tundis, R., Menichini, F., Bonesi, M., Piccolo, V., . . . Houghton, P. J. (2008). In vitro inhibitory activities of plants used in Lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. *J Ethnopharmacol, 119*(1), 109-116.

95. Loizzo, M. R., Tundis, R., Menichini, F., Saab, A. M., & Statti, G. A. (2007). Cytotoxic activity of essential oils from labiatae and lauraceae families against in vitro human tumor models. *Anticancer Res, 27*(5A), 3293-3299.

96. Losfeld, G., L'Huillier, L., Fogliani, B., Coy, S. M., Grison, C., & Jaffre, T. (2014). Leaf-age and soil-plant relationships: key factors for reporting trace-elements hyperaccumulation by plants and design applications. *Environ Sci Pollut Res Int*.

97. Maha, N., & Shaw, A. (2007). Academic doctors' views of complementary and alternative medicine (CAM) and its role within the NHS: an exploratory qualitative study. *BMC Complement Altern Med, 7*, 17.

98. Martin, D., Valdez, J., Boren, J., & Mayersohn, M. (2004). Dermal absorption of camphor, menthol, and methyl salicylate in humans. *J Clin Pharmacol, 44*(10), 1151-1157.

99. Martinez-Ferrer, A., Peris, P., Reyes, R., & Guanabens, N. (2008). [Intake of calcium, magnesium and sodium through water: health implications]. *Med Clin (Barc), 131*(17), 641-646.

100. Matu, E. N., & van Staden, J. (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J Ethnopharmacol, 87*(1), 35-41.

101. Mendelsohn, H., & Yom-Tov, Y. (1999). Fauna Palaestina: Mammalia of Israel. Jerusalem. *The Israel Academy of Sciences and Humanities.*

102. Miguel, G., Cruz, C., Faleiro, M. L., Simoes, M. T., Figueiredo, A. C., Barroso, J. G., & Pedro, L. G. (2011). *Salvia officinalis L*. essential oils: effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial activities. *Nat Prod Res, 25*(5), 526-541.

103. Mohammadkhani, N., & Heidari, R. (2008). Water stress induced by polyethylene glycol 6000 and sodium chloride in two maize cultivars. Pak J Biol Sci, 11(1), 92-97.

104. Moqrich, A., Hwang, S. W., Earley, T. J., Petrus, M. J., Murray, A. N., Spencer, K. S., Patapoutian, A. (2005). Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. *Science, 307*(5714), 1468-1472.

105. Moteki, H., Hibasami, H., Yamada, Y., Katsuzaki, H., Imai, K., & Komiya, T. (2002). Specific induction of apoptosis by 1,8-cineole in two human leukemia cell lines, but not a in human stomach cancer cell line. *Oncol Rep, 9*(4), 757-760.

106. Naser, B., Bodinet, C., Tegtmeier, M., & Lindequist, U. (2005). Thuja occidentalis (Arbor vitae): A Review of its Pharmaceutical, Pharmacological and Clinical Properties. *Evid Based Complement Alternat Med, 2*(1), 69-78.

107. Nasim, S. A., & Dhir, B. (2010). Heavy metals alter the potency of medicinal plants. *Rev Environ Contam Toxicol, 203*, 139-149.

108. Ortega, R. M., Requejo, A. M., Encinas Sotillos, A., Andres, P., Lopez-Sobaler, A. M., & Quintas, E. (1998). [Implication of calcium deficiency in the progress of periodontal diseases and osteoporosis]. *Nutr Hosp, 13*(6), 316-319.

109. Oumeish, O. Y. (1998). The philosophical, cultural, and historical aspects of complementary, alternative, unconventional, and integrative medicine in the Old World. *Arch Dermatol, 134*(11), 1373-1386.

110. P. Salehi, F. S., L. Bazzaz Tolami and A. Sonboli. (2005). Essential oil composition of *Salvia palaestina Benth*. from Iran. *Flavour and Fragrance Journal, 20*(5), 525-527.

111. Packham, C. L. (1997). Re: Essential oils and 'aromatherapy': their role in healing. *J R Soc Health, 117*(6), 400.

112. Palombo, E. A., & Semple, S. J. (2001). Antibacterial activity of traditional Australian medicinal plants. *J Ethnopharmacol, 77*(2-3), 151-157.

113. Palombo, E. A., & Semple, S. J. (2002). Antibacterial activity of Australian plant extracts against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE). *J Basic Microbiol, 42*(6), 444-448.

114. Papas, A. M. (1999). Diet and antioxidant status. *Food Chem Toxicol, 37*(9-10), 999-1007.

115. Paternain, J. L., Domingo, J. L., Llobet, J. M., & Corbella, J. (1988). Embryotoxic and teratogenic effects of aluminum nitrate in rats upon oral administration. *Teratology, 38*(3), 253-257.

116. Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A. J., McGimpsey, J. A., & Smallfield, B. M. (1999). Essential oils from dalmatian Sage (*Salvia officinalis l.*): variations among individuals, plant parts, seasons, and sites. *J Agric Food Chem, 47*(5), 2048-2054.

117. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacogn Rev, 6*(11), 1-5.

118. Pier, S. M. (1975). The role of heavy metals in human health. *Tex Rep Biol Med, 33*(1), 85-106.

119. Rengel, Z. (2004). Aluminium cycling in the soil-plant-animal-human continuum. *Biometals*, *17*(6), 669-689.

120. Romanik, G., Gilgenast, E., Przyjazny, A., & Kaminski, M. (2007). Techniques of preparing plant material for chromatographic separation and analysis. J Biochem Biophys Methods, 70(2), 253-261.

121. Rzepa, J., Wojtal, L., Staszek, D., Grygierczyk, G., Labe, K., Hajnos, M., . . . Waksmundzka-Hajnos, M. (2009). Fingerprint of selected *Salvia* species by HS-GC-MS analysis of their volatile fraction. *J Chromatogr Sci*, *47*(7), 575-580.

122. Said, O., Khalil, K., Fulder, S., & Azaizeh, H. (2002). Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. *J Ethnopharmacol, 83*(3), 251-265.

123. Sanches-Silva, A., Costa, D., Albuquerque, T. G., Buonocore, G. G., Ramos, F., Castilho, M. C., Costa, H. S. (2014). Trends in the use of natural antioxidants in active food packaging: a review. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, *31*(3), 374-395.

124. Sawalha, A. F., Sweileh, W. M., Zyoud, S. H., & Jabi, S. W. (2008). Self-therapy practices among university students in Palestine: focus on herbal remedies. *Complement Ther Med*, *16*(6), 343-349.

125. Schenone, S., Bruno, O., Ranise, A., Bondavalli, F., Filippelli, W., Falcone, G., & Rinaldi, B. (2000). O-[2-hydroxy-3-(dialkylamino)propyl]ethers of (+)-1,7,7-trimethyl

bicyclo[2.2.1]heptan-2-one oxime (camphor oxime) with analgesic and antiarrhythmic activities. *Farmaco, 55*(6-7), 495-498.

126. Schnitzler, P., Nolkemper, S., Stintzing, F. C., & Reichling, J. (2008). Comparative in vitro study on the anti-herpetic effect of phytochemically characterized aqueous and ethanolic extracts of *Salvia officinalis* grown at two different locations. *Phytomedicine, 15*(1-2), 62-70.

127. Senatore F., C. F., N. Apostolides Arnoldb & F.Piozzic. (2005). Essential Oils from Salvia sp. (*Lamiaceae*). III. Composition and Antimicrobial Activity of the Essential Oil of *Salvia palaestina Benth.* Growing Wild in Lebanon. Journal of Essential Oil Research, 17(4), 419-421.

128. Sertel, S., Eichhorn, T., Plinkert, P. K., & Efferth, T. (2011). [Anticancer activity of *Salvia officinalis* essential oil against HNSCC cell line (UMSCC1)]. *HNO, 59*(12), 1203-1208.

129. Services, D. o. H. a. H. (2014). CFR - Code of Federal Regulations Title 21, Food for Human Consumption. [21CFR172.510]. *FDA Regulations, 3*(April 1, 2014).

130. Shanab, B. A. A. (2008). Efficacy of Aqueous and Ethanol Extracts of Some Palestinian Medicinal Plants for Potential Antibacterial Activity

131. *The Islamic University Journal, 16*(1726-6807), 77-86.

132. Shehdeh Jodeh, M. S., Marwan Haddad. (2013). Impacts of biodegradable organics on soils and groundwater in West Bank. *Journal of the Association of Arab Universities for Basic and Applied Sciences, 14*(2013), 75-82.

133. Singh, H. P., Singh, C. K., & Singh, R. R. (1998). Effect of potash alum (aluminium potassium sulphate) on human semen and sperm. *Indian J Physiol Pharmacol, 42*(2), 311-314.

134. Singhal, M. K. (2002). Banana potassium and stroke. *Indian J Exp Biol, 40*(11), 1322.

135. Smetanska, I. (2008). Production of secondary metabolites using plant cell cultures. *Adv Biochem Eng Biotechnol, 111*, 187-228.

136. Smith, R. G., Craig, P., Bird, E. J., Boyle, A. J., Iseri, L. T., Jacobson, S. D., & Myers, G. B. (1950). Spectrochemical values for sodium, potassium, iron, magnesium and calcium in normal human plasma. *Am J Clin Pathol, 20*(3), 263-272.

137. Sonboli, A., Babakhani, B., & Mehrabian, A. R. (2006). Antimicrobial activity of six constituents of essential oil from *Salvia*. *Z Naturforsch C, 61*(3-4), 160-164.

138. Stary, F. (1996). The natural guide to medicinal herbs and plants,. *Barnes&Noble Inc.*

139. Stolkowski, J., & Reinberg, A. (1959). [Osmotic pressure of the medium, potassium movement & polymerization of ribonucleic acids in the animal cell]. *C R Hebd Seances Acad Sci, 248*(16), 2400-2402.

140. Swegert, C. V., Dave, K. R., & Katyare, S. S. (1999). Effect of aluminium-induced Alzheimer like condition on oxidative energy metabolism in rat liver, brain and heart mitochondria. *Mech Ageing Dev, 112*(1), 27-42.

141. Sylvestre, M., Pichette, A., Longtin, A., Nagau, F., & Legault, J. (2006). Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. *J Ethnopharmacol, 103*(1), 99-102.

142. Tenore, G. C., Ciampaglia, R., Arnold, N. A., Piozzi, F., Napolitano, F., Rigano, D., & Senatore, F. (2011). Antimicrobial and antioxidant properties of the essential oil of *Salvia lanigera* from Cyprus. *Food Chem Toxicol, 49*(1), 238-243.

143. Tibbetts, D. M., & Aub, J. C. (1937). Magnesium Metabolism in Health and Disease. I. The Magnesium and Calcium Excretion of Normal Individuals, Also the Effects of Magnesium, Chloride, and Phosphate Ions. *J Clin Invest, 16*(4), 491-501.

144. Toussaint-Samat. (1996). A history of food *Oxford*.

145. Tripathi, S. K., & Pandey, S. K. (2007). The effect of aluminium phosphide on the human brain: a histological study. *Med Sci Law, 47*(2), 141-146.

146. Tukan, S. K., Takruri, H. R., & al-Eisawi, D. M. (1998). The use of wild edible plants in the Jordanian diet. *Int J Food Sci Nutr, 49*(3), 225-235.

147. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact, 160*(1), 1-40.

148. Voet van der, G. B., Marani, E., Tio, S., & de Wolff, F. A. (1991). Aluminium neurotoxicity. *Prog Histochem Cytochem, 23*(1-4), 235-242.

149. Wambach, G., Helber, A., Bonner, G., Hummerich, W., Konrads, A., & Kaufmann, W. (1980). Sodium-potassium-dependent ATPase activity in erythrocyte ghosts of patients with primary and secondary hypertension. *Clin Sci (Lond), 59 Suppl 6*, 183s-185s.

150. Wang, H. F., Takematsu, N., & Ambe, S. (2000). Effects of soil acidity on the uptake of trace elements in soybean and tomato plants. *Appl Radiat Isot, 52*(4), 803-811.

151. Wester, K., Digiuni, S., Geier, F., Timmer, J., Fleck, C., & Hulskamp, M. (2009). Functional diversity of R3 single-repeat genes in trichome development. *Development, 136*(9), 1487-1496.

152. WHO. (2007). WHO guidelines for assessing quality of herbal medicines.

153. Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry, 64*(1), 3-19.

154. Wise, M. L., Savage, T. J., Katahira, E., & Croteau, R. (1998). Monoterpene synthases from common Sage (*Salvia officinalis*). cDNA isolation, characterization, and functional expression of (+)-sabinene synthase, 1,8-cineole synthase, and (+)-bornyl diphosphate synthase. *J Biol Chem, 273*(24), 14891-14899.

155. Yim, E. C., Kim, H. J., & Kim, S. J. (2014). Acute toxicity assessment of camphor in biopesticides by using Daphnia magna and Danio rerio. *Environ Health Toxicol, 29*, e2014008.

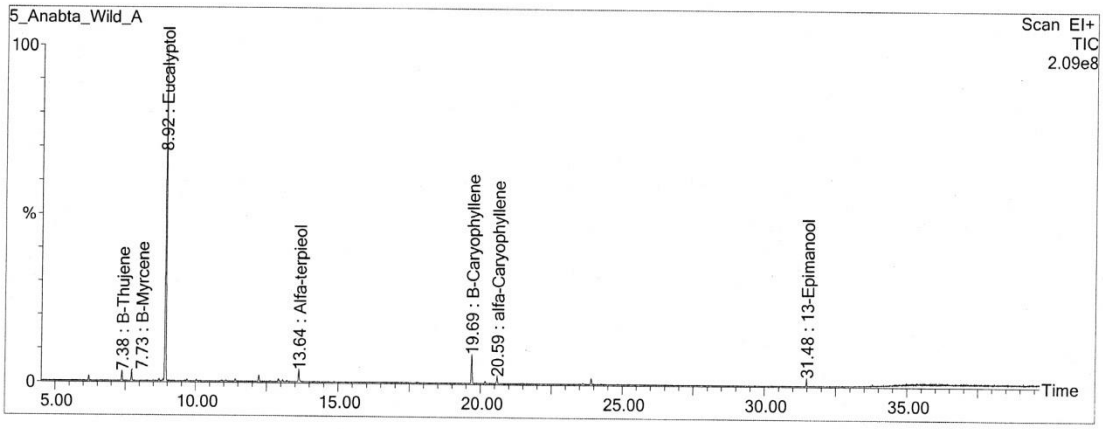
156. Zhong, G. X., Li, P., Zeng, L. J., Guan, J., Li, D. Q., & Li, S. P. (2009). Chemical characteristics of *Salvia miltiorrhiza* (Danshen) collected from different locations in China. *J Agric Food Chem, 57*(15), 6879-6887. doi: 10.1021/jf901462s

Appendix

Samples of TIC for GC-MS Analysis

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\5_Anabta_Wild_A.raw
Acquired: 11-Sep-13 11:37:58 PM Printed: 01-Jun-15 02:39 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 5



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.201	475	3,258,326	100,779.4	0.747	1.42	alfa-Thujene	
2	7.384	807	6,247,776	198,332.6	1.471	2.80	B-Thujene	
3	7.729	904	6,304,376	196,282.0	1.456	2.77	B-Myrcene	
4	8.709	1179	1,379,343	42,383.2	0.314	0.60		
5	8.852	1219	1,102,291	31,922.8	0.237	0.45		
6	8.923	1239	209,149,792	7,082,307.5	52.527	####	Eucalyptol	
7	9.632	1438	402,426	14,566.0	0.108	0.21		
8	9.699	1457	1,030,344	30,541.5	0.227	0.43	Gama-Terpinen	
9	10.038	1552	879,212	26,491.4	0.196	0.37	trans-4-Thujanol	
10	10.943	1806	496,774	22,685.9	0.168	0.32		
11	11.071	1842	484,114	17,845.4	0.132	0.25	3-Thujanone	
12	11.409	1937	1,440,714	51,303.7	0.381	0.72	alfa-Thujone	
13	12.232	2168	3,858,062	139,362.6	1.034	1.97	(+ /-)Camphor	
14	12.634	2281	514,967	18,354.9	0.136	0.26	3-Pinanone	
15	12.923	2362	1,653,517	57,743.1	0.428	0.82	Ocimenol	
16	13.073	2404	972,404	33,662.2	0.250	0.48		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

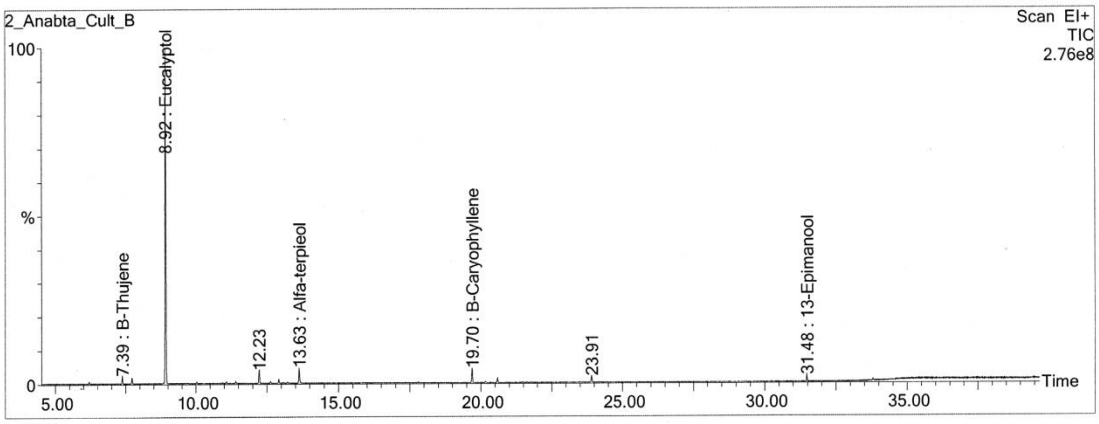
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\5_Anabta_Wild_A.raw
Acquired: 11-Sep-13 11:37:58 PM Printed: 01-Jun-15 02:39 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 5

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	13.226	2447	892,195	31,041.9	0.230	0.44	L-4-terpineneol	
18	13.635	2562	7,308,314	278,800.7	2.068	3.94	Alfa-terpieol	
19	19.694	4263	18,036,328	673,908.8	4.998	9.52	B-Caryophyllene	
20	20.172	4397	1,376,419	51,451.5	0.382	0.73		
21	20.588	4514	4,471,212	146,616.1	1.087	2.07	alfa-Caryophyllene	
22	23.498	5331	533,192	16,229.4	0.120	0.23		
23	23.619	5365	719,766	30,549.0	0.227	0.43		
24	23.907	5446	3,827,522	142,899.4	1.060	2.02	Epiglobulol	
25	31.481	7572	4,956,894	106,512.0	0.790	1.50	13-Epimanool	
26	33.785	8219	1,093,781	35,971.2	0.267	0.51		
27	35.299	8644	666,901	19,622.4	0.146	0.28		
28	35.356	8660	752,842	20,330.3	0.151	0.29		
29	35.428	8680	785,642	15,623.4	0.116	0.22		
30	35.495	8699	814,714	17,178.0	0.127	0.24		
31	35.574	8721	775,089	15,454.5	0.115	0.22		
32	35.617	8733	832,684	19,816.0	0.147	0.28		
33	36.404	8954	664,344	20,672.7	0.153	0.29		
34	36.867	9084	544,420	18,596.2	0.138	0.26		
35	37.451	9248	587,742	16,421.3	0.122	0.23		
36	37.568	9281	501,472	16,681.5	0.124	0.24		
37	37.750	9332	681,097	15,630.7	0.116	0.22		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\2_Anabta_Cult_B.raw
Acquired: 11-Sep-13 05:43:20 PM Printed: 05-Mar-15 04:54 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 2



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.198	474	1,687,784	49,670.4	0.292	0.55	alfa-Thujene	
2	7.388	808	6,312,884	189,824.8	1.115	2.11	B-Thujene	
3	7.734	905	4,665,290	159,372.5	0.936	1.77	B-Myrcene	
4	8.855	1220	891,100	28,406.7	0.167	0.32		
5	8.923	1239	275,440,960	8,981,907.0	52.779	####	Eucalyptol	
6	10.038	1552	1,353,268	45,304.7	0.266	0.50	trans-4-Thujanol	
7	11.085	1846	1,390,934	48,966.3	0.288	0.55	3-Thujanone	
8	11.409	1937	1,497,806	50,760.5	0.298	0.57	alfa-Thujone	
9	12.235	2169	10,710,850	398,271.2	2.340	4.43	(+ /-)Camphor	
10	12.630	2280	1,605,536	60,627.8	0.356	0.67	3-Pinanone	
11	12.919	2361	3,082,154	106,644.1	0.627	1.19	Ocimenol	
12	13.225	2447	993,141	33,423.0	0.196	0.37	L-4-terpineneol	
13	13.631	2561	12,675,564	462,392.1	2.717	5.15	Alfa-terpieol	
14	19.697	4264	11,797,609	410,426.8	2.412	4.57	B-Caryophyllene	
15	20.164	4395	1,164,327	46,637.2	0.274	0.52		
16	20.587	4514	3,575,483	119,434.6	0.702	1.33	alfa-Caryophyllene	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\2_Anabta_Cult_B.raw
Acquired: 11-Sep-13 05:43:20 PM Printed: 05-Mar-15 04:54 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 2

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	23.490	5329	680,800	30,421.9	0.179	0.34		
18	23.907	5446	5,413,349	194,147.2	1.141	2.16	Epiglobulol	
19	31.480	7572	5,729,163	128,631.7	0.756	1.43	13-Epimanool	
20	33.787	8220	1,681,897	45,506.7	0.267	0.51		
21	35.119	8594	1,158,438	35,032.1	0.206	0.39		
22	35.226	8624	1,066,083	35,586.9	0.209	0.40		
23	35.276	8638	1,052,880	28,682.5	0.169	0.32		
24	35.386	8669	1,079,173	29,718.6	0.175	0.33		
25	35.436	8683	1,219,324	39,581.5	0.233	0.44		
26	35.514	8705	1,122,771	41,798.9	0.246	0.47		
27	35.571	8721	1,171,255	43,171.6	0.254	0.48		
28	36.084	8865	928,437	30,819.9	0.181	0.34		
29	36.245	8910	1,044,052	30,894.6	0.182	0.34		
30	36.419	8959	1,226,741	39,315.3	0.231	0.44		
31	36.611	9013	865,424	30,421.5	0.179	0.34		
32	36.683	9033	894,394	34,646.4	0.204	0.39		
33	36.711	9041	965,825	30,877.1	0.181	0.34		
34	37.174	9171	826,910	36,181.6	0.213	0.40		
35	37.377	9228	923,167	30,151.5	0.177	0.34		
36	37.502	9263	1,058,818	41,223.0	0.242	0.46		
37	37.851	9361	915,298	31,628.3	0.186	0.35		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\13_Beita_wild_A.raw
Acquired: 12-Sep-13 11:28:38 PM Printed: 05-Mar-15 05:04 PM
Description:
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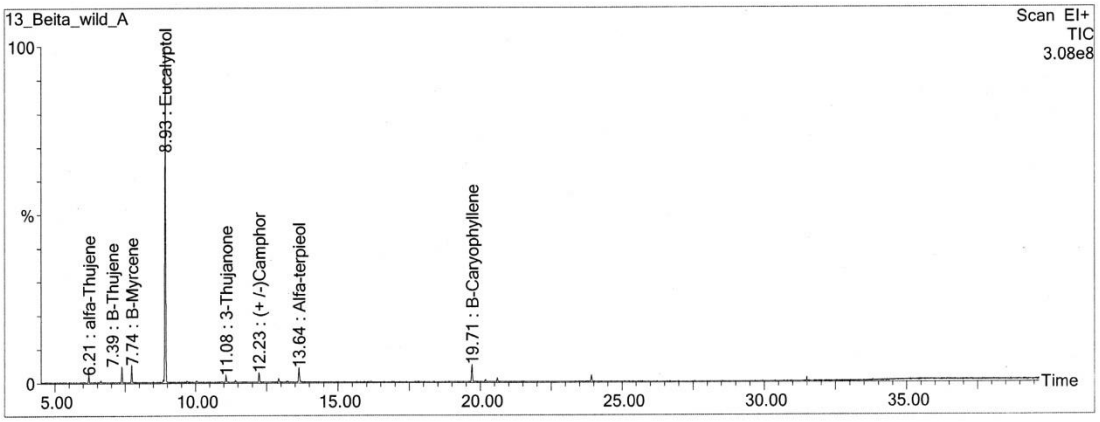
Sample ID: Vial Number: 13



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.020	424	575,979	13,443.7	0.076	0.13		
2	6.205	476	6,924,464	205,927.8	1.171	1.99	alfa-Thujene	
3	6.636	597	2,093,117	53,633.7	0.305	0.52	Camphene	
4	7.392	809	13,305,766	457,106.2	2.600	4.42	B-Thujene	
5	7.737	906	15,810,382	506,881.2	2.883	4.90	B-Myrcene	
6	8.499	1120	408,497	14,565.7	0.083	0.14		
7	8.859	1221	2,564,835	76,187.8	0.433	0.74		
8	8.927	1240	307,701,120	10,349,385.0	58.872	####	Eucalyptol	
9	9.707	1459	1,257,258	37,205.5	0.212	0.36	Gama-Terpinen	
10	10.034	1551	1,334,591	45,686.0	0.260	0.44	trans-4-Thujanol	
11	10.935	1804	670,318	25,764.7	0.147	0.25		
12	11.081	1845	6,123,456	190,537.1	1.084	1.84	3-Thujanone	
13	11.416	1939	1,605,678	51,244.4	0.291	0.50	alfa-Thujone	
14	12.235	2169	8,623,717	309,822.0	1.762	2.99	(+ /-)Camphor	
15	12.929	2364	3,237,932	118,839.9	0.676	1.15	Ocimenol	
16	13.236	2450	1,005,041	36,643.5	0.208	0.35	L-4-terpineneol	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\13_Beita_wild_A.raw
Acquired: 12-Sep-13 11:28:38 PM Printed: 05-Mar-15 05:04 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 13

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	13.638	2563	12,995,104	514,639.2	2.927	4.97	Alfa-terpieol	
18	16.131	3263	444,395	16,822.3	0.096	0.16	L-Bornyl acetate	
19	17.805	3733	471,363	14,556.9	0.083	0.14	Terpinyl acetate	
20	19.711	4268	15,774,648	585,266.5	3.329	5.66	B-Caryophyllene	
21	20.181	4400	1,812,220	60,687.8	0.345	0.59		
22	20.598	4517	3,433,211	120,339.2	0.685	1.16	alfa-Caryophyllene	
23	21.488	4767	526,790	19,745.5	0.112	0.19		
24	23.918	5449	5,787,380	220,056.4	1.252	2.13	Epiglobulol	
25	31.487	7574	3,605,978	83,359.5	0.474	0.81	13-Epimanool	
26	33.794	8222	674,967	13,254.6	0.075	0.13		
27	34.956	8548	532,395	12,673.4	0.072	0.12		
28	35.102	8589	466,347	11,384.0	0.065	0.11		
29	35.208	8619	434,544	11,776.1	0.067	0.11		
30	35.543	8713	694,001	12,639.9	0.072	0.12		
31	35.604	8730	435,897	11,810.9	0.067	0.11		
32	35.679	8751	465,981	11,771.7	0.067	0.11		
33	36.876	9087	401,486	12,507.1	0.071	0.12		
34	36.997	9121	612,210	16,192.4	0.092	0.16		
35	37.093	9148	504,734	11,624.2	0.066	0.11		
36	37.862	9364	387,732	13,531.1	0.077	0.13		
37	38.756	9615	442,220	12,556.4	0.071	0.12		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\3_Beita_Cult_A.raw
Acquired: 11-Sep-13 07:11:59 PM Printed: 05-Mar-15 04:55 PM
Description:
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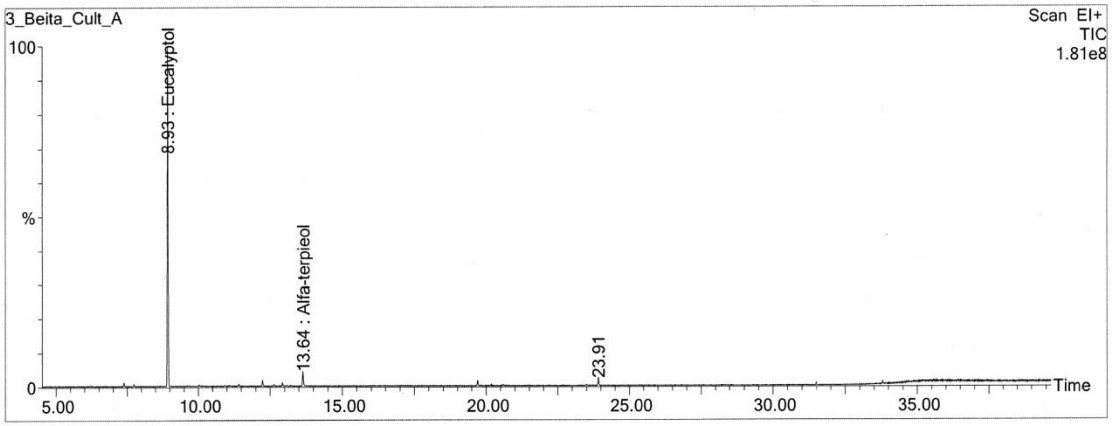
Sample ID: Vial Number: 3



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	7.389	808	2,092,758	68,895.4	0.535	1.11	B-Thujene	
2	7.741	907	1,270,750	38,602.8	0.300	0.62	B-Myrcene	
3	8.928	1240	180,920,368	6,227,731.0	48.323	####	Eucalyptol	
4	10.039	1552	687,049	26,366.0	0.205	0.42	trans-4-Thujanol	
5	11.414	1938	1,322,688	41,698.5	0.324	0.67	alfa-Thujone	
6	12.240	2170	2,994,307	101,329.4	0.786	1.63	(+ /-)Camphor	
7	12.927	2363	1,739,792	64,718.9	0.502	1.04	Ocimenol	
8	13.643	2564	7,729,605	284,679.6	2.209	4.57	Alfa-terpieol	
9	19.705	4266	2,739,574	97,647.9	0.758	1.57	B-Caryophyllene	
10	20.592	4515	694,209	28,056.3	0.218	0.45	alfa-Caryophyllene	
11	23.918	5449	4,150,654	153,806.8	1.193	2.47	Epiglobulol	
12	31.492	7575	1,669,893	39,364.0	0.305	0.63	13-Epimanool	
13	33.789	8220	1,480,151	44,487.8	0.345	0.71		
14	35.192	8614	1,253,841	34,628.5	0.269	0.56		
15	35.242	8628	1,111,641	39,510.2	0.307	0.63		
16	35.360	8661	1,139,884	55,335.6	0.429	0.89		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

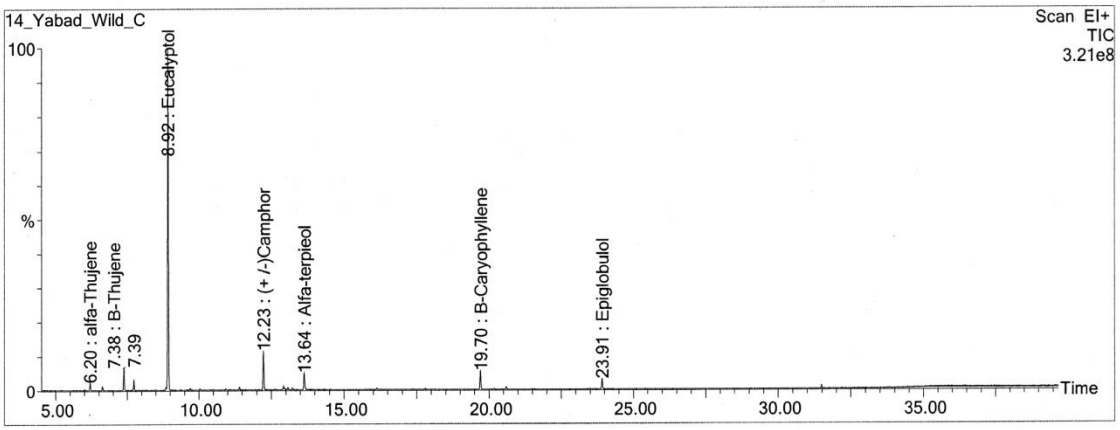
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\3_Beita_Cult_A.raw
Acquired: 11-Sep-13 07:11:59 PM Printed: 05-Mar-15 04:55 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 3

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	35.385	8668	1,158,598	28,384.0	0.220	0.46		
18	35.470	8692	1,186,902	54,155.8	0.420	0.87		
19	35.549	8714	1,093,251	41,692.8	0.324	0.67		
20	35.599	8728	1,192,702	43,732.3	0.339	0.70		
21	35.691	8754	1,189,867	31,594.7	0.245	0.51		
22	35.730	8765	1,110,389	31,163.2	0.242	0.50		
23	35.812	8788	1,105,375	53,407.7	0.414	0.86		
24	35.898	8812	992,352	27,138.8	0.211	0.44		
25	35.983	8836	1,165,714	50,314.7	0.390	0.81		
26	36.015	8845	1,329,065	30,165.4	0.234	0.48		
27	36.204	8898	1,067,901	35,334.2	0.274	0.57		
28	36.240	8908	1,016,117	53,246.2	0.413	0.85		
29	36.325	8932	1,051,855	31,829.7	0.247	0.51		
30	36.414	8957	1,279,580	58,168.1	0.451	0.93		
31	36.457	8969	1,134,991	31,500.7	0.244	0.51		
32	36.542	8993	1,059,790	50,186.3	0.389	0.81		
33	36.617	9014	1,036,332	27,754.9	0.215	0.45		
34	36.749	9051	877,530	35,473.8	0.275	0.57		
35	36.895	9092	882,858	32,634.2	0.253	0.52		
36	37.436	9244	904,375	35,929.9	0.279	0.58		
37	38.960	9672	693,954	27,532.8	0.214	0.44		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\14_Yabad_Wild_C.raw
Acquired: 13-Sep-13 03:10:20 AM Printed: 05-Mar-15 05:05 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 14



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.016	423	766,538	24,463.4	0.125	0.23		
2	6.202	475	7,015,936	213,273.5	1.093	1.98	alfa-Thujene	
3	6.633	596	3,899,123	115,441.6	0.592	1.07	Camphene	
4	7.256	771	410,064	14,447.7	0.074	0.13		
5	7.392	809	20,780,352	659,190.6	3.379	6.12	B-Thujene	
6	7.733	905	9,362,539	275,812.6	1.414	2.56	B-Myrcene	
7	8.499	1120	630,274	20,393.4	0.105	0.19		
8	8.855	1220	3,037,964	90,423.9	0.464	0.84		
9	8.923	1239	320,718,560	10,778,667.0	55.250	####	Eucalyptol	
10	9.703	1458	2,017,796	61,082.4	0.313	0.57	Gama-Terpinen	
11	10.034	1551	997,361	30,909.9	0.158	0.29	trans-4-Thujanol	
12	10.497	1681	447,049	11,176.7	0.057	0.10		
13	10.925	1801	906,515	37,161.1	0.190	0.34		
14	11.085	1846	523,238	18,068.5	0.093	0.17	3-Thujanone	
15	11.405	1936	2,751,458	94,516.2	0.484	0.88	alfa-Thujone	
16	12.232	2168	36,578,920	1,343,050.4	6.884	####	(+ /-)Camphor	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

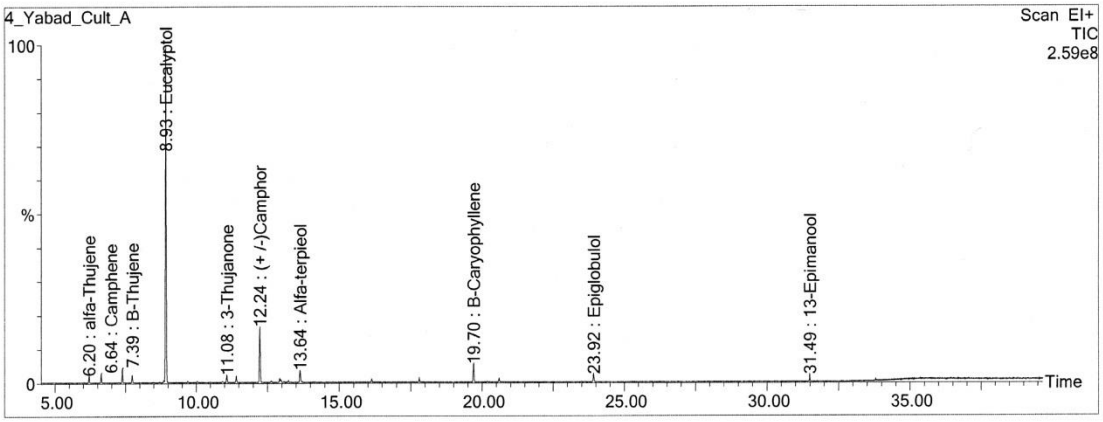
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\14_Yabad_Wild_C.raw
Acquired: 13-Sep-13 03:10:20 AM Printed: 05-Mar-15 05:05 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 14

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	12.630	2280	1,008,924	33,471.0	0.172	0.31	3-Pinanone	
18	12.926	2363	3,302,967	121,261.6	0.622	1.13	Ocimenol	
19	12.965	2374	1,346,189	41,769.0	0.214	0.39		
20	13.072	2404	2,096,917	73,194.2	0.375	0.68		
21	13.225	2447	1,794,814	65,915.6	0.338	0.61	L-4-terpineneol	
22	13.638	2563	15,875,531	644,155.1	3.302	5.98	Alfa-terpieol	
23	16.125	3261	1,209,643	40,516.0	0.208	0.38	L-Bornyl acetate	
24	17.799	3731	660,095	23,136.8	0.119	0.21		
25	19.704	4266	17,428,110	655,918.4	3.362	6.09	B-Caryophyllene	
26	20.171	4397	712,097	25,984.1	0.133	0.24		
27	20.591	4515	2,370,936	83,232.2	0.427	0.77	alfa-Caryophyllene	
28	21.482	4765	353,813	15,504.5	0.079	0.14		
29	23.501	5332	435,933	17,596.0	0.090	0.16		
30	23.915	5448	10,103,687	372,467.3	1.909	3.46	Epiglobulol	
31	31.487	7574	3,265,537	78,829.2	0.404	0.73	13-Epimanool	
32	35.384	8668	502,217	11,415.2	0.059	0.11		
33	35.587	8725	467,935	12,496.8	0.064	0.12		
34	36.834	9075	618,601	17,542.5	0.090	0.16		
35	37.343	9218	570,961	17,838.4	0.091	0.17		
36	37.703	9319	466,973	15,539.9	0.080	0.14		
37	38.283	9482	450,436	12,561.4	0.064	0.12		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\4_Yabad_Cult_A.raw
Acquired: 11-Sep-13 09:24:59 PM Printed: 05-Mar-15 04:56 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 4



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.209	477	5,541,668	160,224.8	0.905	1.85	alfa-Thujene	
2	6.636	597	6,570,077	187,267.6	1.057	2.17	Camphene	
3	7.391	809	10,712,278	321,089.9	1.813	3.71	B-Thujene	
4	7.733	905	5,669,316	187,078.5	1.056	2.16	B-Myrcene	
5	8.859	1221	1,968,394	58,896.9	0.333	0.68		
6	8.930	1241	259,022,768	8,645,684.0	48.817	####	Eucalyptol	
7	9.707	1459	1,041,282	32,761.1	0.185	0.38	Gama-Terpinen	
8	10.041	1553	1,198,603	41,015.6	0.232	0.47	trans-4-Thujanol	
9	10.932	1803	887,400	36,011.7	0.203	0.42		
10	11.081	1845	5,521,430	193,122.8	1.090	2.23	3-Thujanone	
11	11.413	1938	4,835,505	159,311.0	0.900	1.84	alfa-Thujone	
12	12.235	2169	42,701,764	1,585,102.9	8.950	####	(+ /-)Camphor	
13	12.641	2283	1,195,108	45,752.6	0.258	0.53	3-Pinanone	
14	12.926	2363	2,853,360	91,275.5	0.515	1.06	Ocimenol	
15	12.969	2375	1,938,314	63,857.3	0.361	0.74		
16	13.083	2407	819,926	29,541.2	0.167	0.34		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

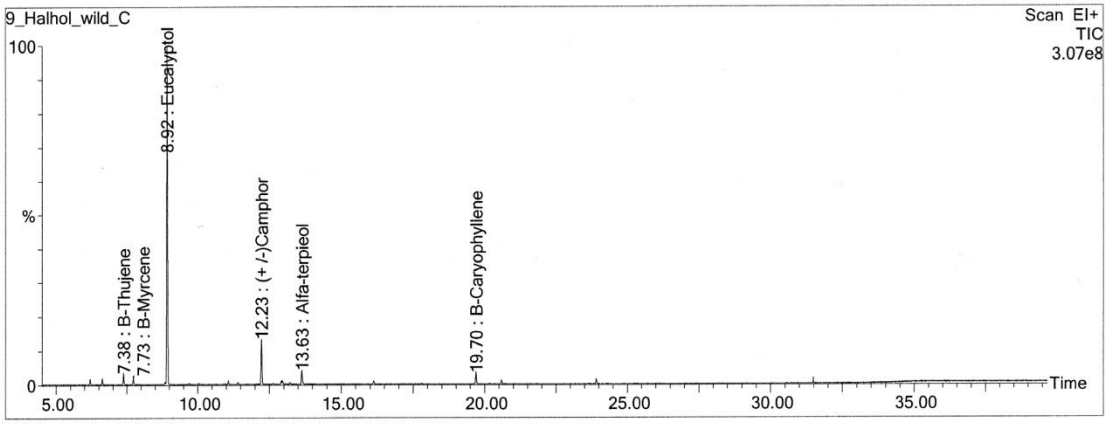
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\4_Yabad_Cult_A.raw
Acquired: 11-Sep-13 09:24:59 PM Printed: 05-Mar-15 04:56 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 4

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	13.233	2449	1,474,958	51,632.4	0.292	0.60	L-4-terpineneol	
18	13.639	2563	9,239,312	386,500.7	2.182	4.47	Alfa-terpieol	
19	16.132	3263	2,253,375	75,375.4	0.426	0.87	L-Bornyl acetate	
20	17.795	3730	2,813,466	87,171.5	0.492	1.01	Terpinyl acetate	
21	19.708	4267	14,075,664	527,274.2	2.977	6.10	B-Caryophyllene	
22	20.171	4397	609,875	27,123.7	0.153	0.31		
23	20.595	4516	2,751,025	98,672.0	0.557	1.14	alfa-Caryophyllene	
24	23.918	5449	6,172,754	218,009.9	1.231	2.52	Epiglobulol	
25	31.486	7574	5,416,039	112,737.0	0.637	1.30	13-Epimanool	
26	33.790	8221	1,388,642	27,873.3	0.157	0.32		
27	35.072	8581	678,696	20,690.0	0.117	0.24		
28	35.215	8621	899,612	33,792.5	0.191	0.39		
29	35.347	8658	1,007,580	50,266.8	0.284	0.58		
30	35.436	8683	891,514	22,770.9	0.129	0.26		
31	35.471	8693	720,074	20,064.7	0.113	0.23		
32	35.553	8716	946,447	21,374.9	0.121	0.25		
33	35.632	8738	965,349	21,102.0	0.119	0.24		
34	35.699	8757	940,129	28,513.7	0.161	0.33		
35	36.155	8885	658,248	24,549.7	0.139	0.28		
36	36.433	8963	881,208	25,090.6	0.142	0.29		
37	36.671	9030	691,260	19,701.5	0.111	0.23		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\9_Halhol_wild_C.raw
Acquired: 12-Sep-13 04:05:20 PM Printed: 05-Mar-15 05:00 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 1 of 2
Sample ID: Vial Number: 9



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.202	475	5,225,876	154,668.9	0.855	1.51	alfa-Thujene	
2	6.634	596	5,300,665	159,782.4	0.884	1.56	Camphene	
3	7.381	806	10,078,259	307,821.6	1.702	3.00	B-Thujene	
4	7.731	904	6,843,552	207,767.9	1.149	2.03	B-Myrcene	
5	8.503	1121	376,107	13,248.9	0.073	0.13		
6	8.710	1179	571,775	17,155.7	0.095	0.17		
7	8.860	1221	2,361,281	70,922.0	0.392	0.69		
8	8.924	1239	306,516,672	10,254,230.0	56.710	####	Eucalyptol	
9	9.700	1457	1,271,071	38,950.9	0.215	0.38	Gama-Terpinen	
10	10.042	1553	963,662	32,673.8	0.181	0.32	trans-4-Thujanol	
11	10.936	1804	690,773	32,494.5	0.180	0.32		
12	11.075	1843	3,015,898	93,222.3	0.516	0.91	3-Thujanone	
13	11.410	1937	1,795,200	61,894.7	0.342	0.60	alfa-Thujone	
14	12.226	2166	40,568,868	1,533,945.9	8.483	####	(+ /-)Camphor	
15	12.636	2281	1,020,088	36,469.9	0.202	0.36	3-Pinanone	
16	12.917	2360	2,999,739	100,917.1	0.558	0.98	Ocimenol	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

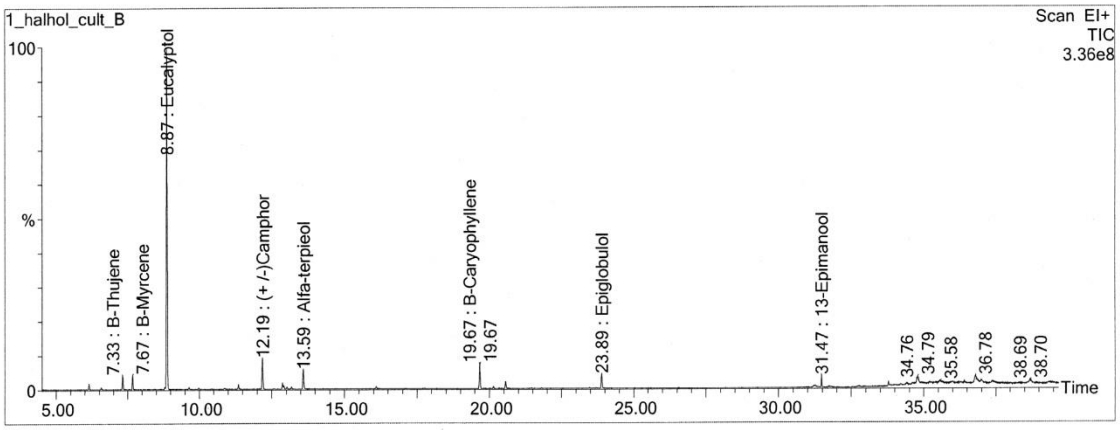
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\9_Halhol_wild_C.raw
Acquired: 12-Sep-13 04:05:20 PM Printed: 05-Mar-15 05:00 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 9

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	12.960	2372	2,801,570	92,011.4	0.509	0.90		
18	13.223	2446	1,562,486	57,270.3	0.317	0.56	L-4-terpineneol	
19	13.633	2561	12,179,330	506,496.9	2.801	4.94	Alfa-terpieol	
20	16.126	3261	2,693,084	94,444.7	0.522	0.92	L-Bornyl acetate	
21	17.789	3728	686,385	23,736.2	0.131	0.23	Terpinyl acetate	
22	19.697	4264	10,099,201	357,836.8	1.979	3.49	B-Caryophyllene	
23	20.168	4396	546,753	20,061.3	0.111	0.20		
24	20.584	4513	3,250,313	122,049.1	0.675	1.19	alfa-Caryophyllene	
25	23.490	5329	375,680	15,829.8	0.088	0.15		
26	23.619	5365	451,797	17,207.1	0.095	0.17		
27	23.907	5446	4,248,113	150,037.4	0.830	1.46	Epiglobulol	
28	31.487	7574	5,055,170	112,854.9	0.624	1.10	13-Epimanool	
29	33.795	8222	684,816	16,525.9	0.091	0.16		
30	33.873	8244	418,115	13,496.3	0.075	0.13		
31	35.526	8708	564,983	14,623.6	0.081	0.14		
32	36.267	8916	547,220	11,136.0	0.062	0.11		
33	37.428	9242	617,586	10,609.3	0.059	0.10		
34	37.606	9292	470,204	13,077.9	0.072	0.13		
35	37.906	9376	499,027	11,105.7	0.061	0.11		
36	39.045	9696	452,802	10,418.1	0.058	0.10		
37	39.572	9844	520,510	12,595.7	0.070	0.12		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\1_halhol_cult_B.raw
Acquired: 11-Sep-13 12:09:16 PM Printed: 05-Mar-15 04:46 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 1



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.148	460	6,113,269	154,331.1	0.579	1.33	alfa-Thujene	
2	6.576	580	2,204,147	65,512.9	0.246	0.57	Camphene	
3	7.331	792	13,309,930	425,225.0	1.595	3.67	B-Thujene	
4	7.673	888	14,100,892	438,373.9	1.645	3.79	B-Myrcene	
5	8.802	1205	2,294,083	68,783.1	0.258	0.59		
6	8.873	1225	335,997,568	11,580,124.0	43.449	####	Eucalyptol	
7	11.359	1923	4,248,019	142,824.1	0.536	1.23	alfa-Thujone	
8	12.189	2156	30,851,162	1,118,195.6	4.196	9.66	(+ /-)Camphor	
9	12.880	2350	5,398,308	186,711.1	0.701	1.61		
10	12.926	2363	2,571,836	68,907.1	0.259	0.60	Ocimenol	
11	13.033	2393	1,878,609	62,893.6	0.236	0.54		
12	13.186	2436	2,102,209	77,103.1	0.289	0.67	L-4-terpineneol	
13	13.592	2550	19,334,286	747,641.2	2.805	6.46	Alfa-terpieol	
14	16.092	3252	2,281,031	70,550.0	0.265	0.61	L-Bornyl acetate	
15	19.675	4258	24,528,370	926,038.0	3.475	8.00	B-Caryophyllene	
16	20.145	4390	2,166,316	71,091.5	0.267	0.61		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

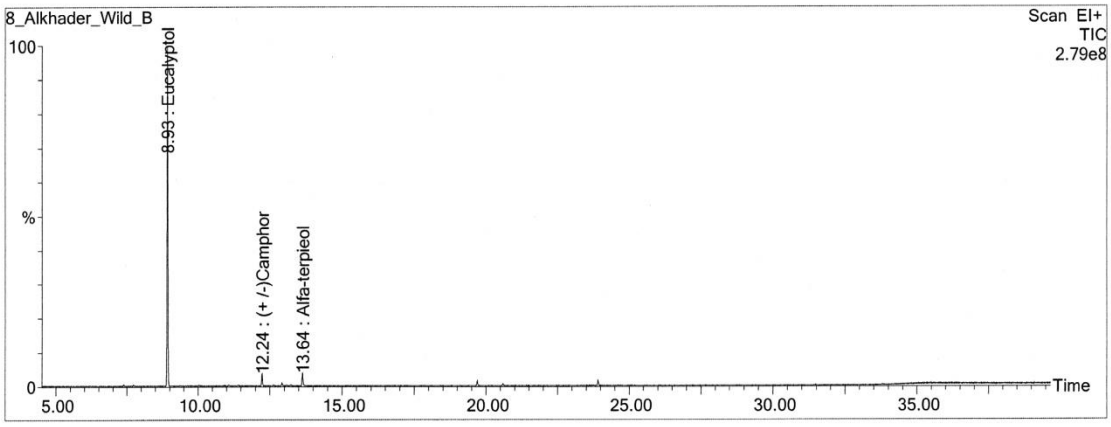
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\1_halhol_cult_B.raw
Acquired: 11-Sep-13 12:09:16 PM Printed: 05-Mar-15 04:46 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 1

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	20.558	4506	6,284,144	232,938.0	0.874	2.01	alfa-Caryophyllene	
18	23.888	5441	14,909,532	554,945.9	2.082	4.79	Epiglobulol	
19	31.233	7503	1,879,878	174,143.6	0.653	1.50		
20	31.472	7570	12,524,897	290,395.8	1.090	2.51	13-Epimanool	
21	33.779	8218	4,513,734	143,852.8	0.540	1.24		
22	33.851	8238	1,456,968	71,003.7	0.266	0.61		
23	34.207	8338	1,637,480	74,190.3	0.278	0.64		
24	34.410	8395	2,045,983	113,901.3	0.427	0.98		
25	34.613	8452	1,679,427	79,396.1	0.298	0.69		
26	34.791	8502	7,927,310	626,823.1	2.352	5.41		
27	35.204	8618	1,722,846	59,745.2	0.224	0.52		
28	35.577	8723	2,949,081	224,106.0	0.841	1.94		
29	35.624	8736	1,793,966	88,762.3	0.333	0.77		
30	36.411	8957	2,682,474	85,378.2	0.320	0.74		
31	36.785	9062	8,059,198	737,229.8	2.766	6.37		
32	36.870	9086	4,125,152	136,524.1	0.512	1.18		
33	36.984	9118	3,760,309	293,215.4	1.100	2.53		
34	37.354	9222	2,320,405	83,524.4	0.313	0.72		
35	37.387	9231	2,513,504	120,290.2	0.451	1.04		
36	38.701	9600	4,256,792	393,664.8	1.477	3.40		
37	39.331	9777	1,494,155	82,043.1	0.308	0.71		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\8_Alkhader_Wild_B.raw
Acquired: 12-Sep-13 07:01:15 AM Printed: 05-Mar-15 04:59 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 8



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.202	475	366,730	13,151.7	0.092	0.14	alfa-Thujene	
2	7.388	808	1,170,724	36,797.3	0.256	0.40	B-Thujene	
3	7.737	906	972,701	31,583.6	0.220	0.34	B-Myrcene	
4	8.926	1240	278,529,760	9,170,438.0	63.874	####	Eucalyptol	
5	10.034	1551	970,345	35,009.4	0.244	0.38	trans-4-Thujanol	
6	10.935	1804	631,902	25,483.3	0.177	0.28		
7	11.081	1845	732,957	26,575.9	0.185	0.29	3-Thujanone	
8	11.412	1938	876,931	32,473.8	0.226	0.35	alfa-Thujone	
9	12.235	2169	9,829,640	354,558.1	2.470	3.87	(+ /-)Camphor	
10	12.638	2282	761,754	28,413.8	0.198	0.31	3-Pinanone	
11	12.922	2362	2,507,512	99,390.2	0.692	1.08	Ocimenol	
12	13.076	2405	686,069	20,672.7	0.144	0.23		
13	13.225	2447	1,073,485	39,175.8	0.273	0.43	L-4-terpineneol	
14	13.638	2563	10,601,249	430,849.6	3.001	4.70	Alfa-terpieol	
15	19.700	4265	3,927,886	136,096.4	0.948	1.48	B-Caryophyllene	
16	20.597	4517	1,726,349	58,123.7	0.405	0.63	alfa-Caryophyllene	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

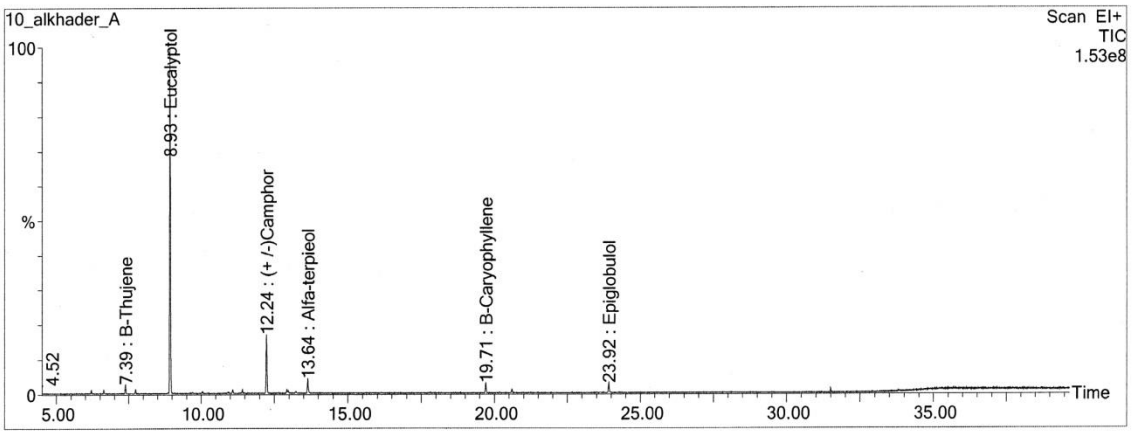
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\8_Alkhader_Wild_B.raw
Acquired: 12-Sep-13 07:01:15 AM Printed: 05-Mar-15 04:59 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 8

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	23.909	5447	4,097,827	152,740.8	1.064	1.67	Epiglobulol	
18	31.477	7572	871,482	21,783.9	0.152	0.24	13-Epimanool	
19	33.788	8221	659,358	15,806.2	0.110	0.17		
20	35.194	8616	562,163	18,769.9	0.131	0.20		
21	35.248	8631	558,206	15,818.2	0.110	0.17		
22	35.401	8674	647,182	21,166.0	0.147	0.23		
23	35.451	8688	617,637	15,163.9	0.106	0.17		
24	35.508	8704	617,553	12,663.8	0.088	0.14		
25	35.675	8751	639,729	18,726.6	0.130	0.20		
26	35.746	8771	606,383	14,613.5	0.102	0.16		
27	35.782	8781	605,382	13,814.1	0.096	0.15		
28	35.850	8800	544,702	13,144.1	0.092	0.14		
29	36.409	8957	603,103	13,593.8	0.095	0.15		
30	37.035	9133	461,995	13,177.4	0.092	0.14		
31	37.096	9150	567,428	13,026.2	0.091	0.14		
32	37.192	9177	646,162	13,301.6	0.093	0.15		
33	37.623	9298	579,572	12,835.3	0.089	0.14		
34	38.068	9423	532,921	13,889.7	0.097	0.15		
35	38.531	9553	435,585	16,861.0	0.117	0.18		
36	38.574	9565	471,567	16,211.8	0.113	0.18		
37	38.831	9637	608,355	12,432.2	0.087	0.14		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\10_alkhader_A.raw
Acquired: 12-Sep-13 04:49:39 PM Printed: 05-Mar-15 05:01 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 1 of 2
Sample ID: Vial Number: 10



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.209	477	1,636,470	48,052.5	0.401	0.94	alfa-Thujene	
2	6.636	597	1,868,704	51,616.1	0.430	1.01	Camphene	
3	7.395	810	4,006,534	121,771.2	1.015	2.38	B-Thujene	
4	7.744	908	2,028,884	65,923.1	0.550	1.29	B-Myrcene	
5	8.933	1242	153,096,672	5,124,432.5	42.717	####	Eucalyptol	
6	10.048	1555	780,619	24,710.3	0.206	0.48	trans-4-Thujanol	
7	11.088	1847	1,564,157	57,524.3	0.480	1.12	3-Thujanone	
8	11.420	1940	1,632,496	53,528.8	0.446	1.04	alfa-Thujone	
9	12.239	2170	25,989,504	942,291.1	7.855	####	(+ /-)Camphor	
10	12.934	2365	1,495,218	49,674.6	0.414	0.97	Ocimenol	
11	12.980	2378	1,035,075	33,017.9	0.275	0.64		
12	13.240	2451	902,810	36,286.9	0.302	0.71	L-4-terpineneol	
13	13.642	2564	6,462,966	241,745.5	2.015	4.72	Alfa-terpieol	
14	19.708	4267	4,402,646	157,473.1	1.313	3.07	B-Caryophyllene	
15	20.595	4516	1,703,291	58,330.5	0.486	1.14	alfa-Caryophyllene	
16	23.922	5450	4,387,604	158,738.0	1.323	3.10	Epiglobulol	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

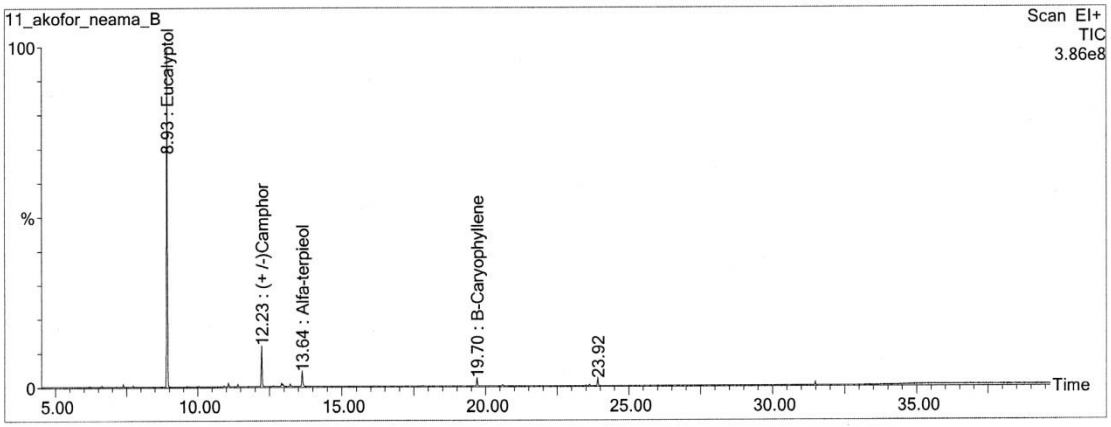
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\10_alkhader_A.raw
Acquired: 12-Sep-13 04:49:39 PM Printed: 05-Mar-15 05:01 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 10

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	31.489	7575	1,909,582	37,747.8	0.315	0.74	13-Epimanool	
18	35.053	8576	818,435	28,923.7	0.241	0.56		
19	35.264	8635	923,476	32,506.4	0.271	0.63		
20	35.306	8647	898,087	23,733.4	0.198	0.46		
21	35.527	8709	835,348	22,681.0	0.189	0.44		
22	35.598	8729	1,003,076	46,031.2	0.384	0.90		
23	35.623	8736	818,245	22,513.7	0.188	0.44		
24	35.684	8753	1,045,752	39,566.4	0.330	0.77		
25	35.744	8770	1,037,163	35,688.7	0.297	0.70		
26	35.805	8787	845,994	25,096.7	0.209	0.49		
27	35.954	8829	845,378	46,747.2	0.390	0.91		
28	36.054	8857	846,110	34,694.8	0.289	0.68		
29	36.207	8900	792,154	23,141.3	0.193	0.45		
30	36.311	8929	772,891	25,561.0	0.213	0.50		
31	36.432	8963	935,794	38,270.3	0.319	0.75		
32	36.595	9009	699,950	22,064.6	0.184	0.43		
33	36.692	9036	817,243	21,247.4	0.177	0.41		
34	36.749	9052	756,533	23,533.8	0.196	0.46		
35	36.891	9092	729,375	20,990.1	0.175	0.41		
36	36.973	9115	811,007	48,134.8	0.401	0.94		
37	37.728	9327	700,446	24,530.2	0.204	0.48		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\11_akofor_neama_B.raw
Acquired: 12-Sep-13 07:46:59 PM Printed: 05-Mar-15 05:02 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 11



#	RT	Scan	Height	Area	Area %	Norm	Name
1	6.202	475	1,241,405	34,322.0	0.162	0.27	alfa-Thujene
2	6.640	598	1,150,666	37,007.3	0.174	0.29	Camphene
3	7.391	809	3,008,450	78,589.1	0.370	0.62	B-Thujene
4	7.737	906	1,529,623	43,343.1	0.204	0.34	B-Myrcene
5	8.927	1240	385,840,352	12,739,400.0	60.057	####	Eucalyptol
6	10.042	1553	707,083	28,075.5	0.132	0.22	trans-4-Thujanol
7	10.925	1801	965,077	38,398.5	0.181	0.30	
8	11.082	1845	4,238,290	133,126.5	0.628	1.04	3-Thujanone
9	11.410	1937	2,643,863	84,501.2	0.398	0.66	alfa-Thujone
10	12.232	2168	46,695,200	1,751,255.5	8.256	####	(+ /-)Camphor
11	12.631	2280	948,989	32,885.2	0.155	0.26	3-Pinanone
12	12.920	2361	3,818,360	147,471.1	0.695	1.16	Ocimenol
13	12.969	2375	2,505,167	72,046.6	0.340	0.57	
14	13.222	2446	3,204,034	114,670.6	0.541	0.90	L-4-terpineneol
15	13.636	2562	16,954,800	703,190.4	3.315	5.52	Alfa-terpieol
16	16.132	3263	611,199	20,881.7	0.098	0.16	L-Bornyl acetate

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

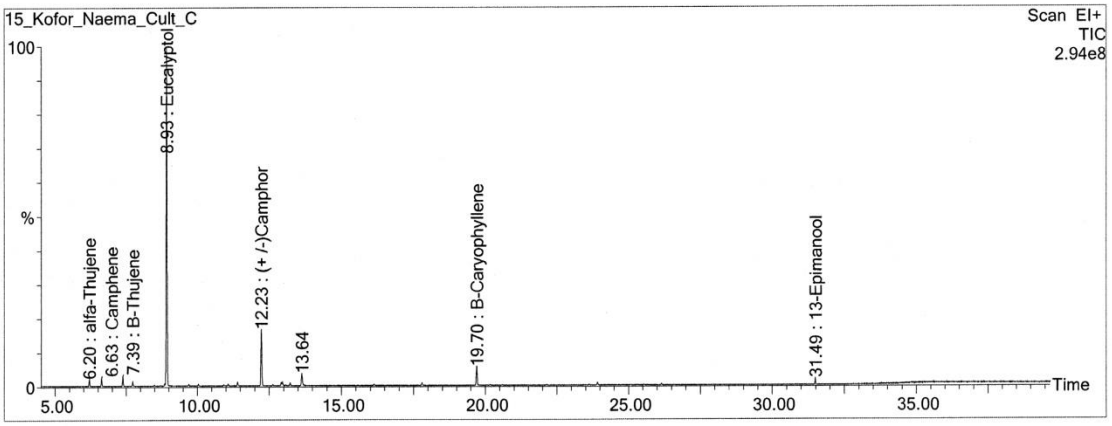
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\11_akofor_neama_B.raw
Acquired: 12-Sep-13 07:46:59 PM Printed: 05-Mar-15 05:02 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 11

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	17.796	3730	842,784	29,657.1	0.140	0.23	Terpinyl acetate	
18	19.702	4265	9,406,216	341,655.1	1.611	2.68	B-Caryophyllene	
19	20.592	4515	1,436,039	53,705.2	0.253	0.42	alfa-Caryophyllene	
20	23.499	5331	938,854	33,360.9	0.157	0.26		
21	23.627	5367	1,659,609	57,321.3	0.270	0.45		
22	23.916	5448	8,865,264	320,663.5	1.512	2.52	Epiglobulol	
23	31.485	7573	4,251,908	94,454.2	0.445	0.74	13-Epimanool	
24	35.322	8650	739,497	30,770.2	0.145	0.24		
25	35.492	8698	750,958	25,492.1	0.120	0.20		
26	35.599	8728	835,076	19,726.2	0.093	0.15		
27	35.756	8772	747,646	26,686.8	0.126	0.21		
28	35.813	8788	787,995	26,223.0	0.124	0.21		
29	35.852	8799	742,590	19,417.6	0.092	0.15		
30	36.148	8882	745,459	27,576.3	0.130	0.22		
31	36.198	8896	884,991	22,273.9	0.105	0.17		
32	36.333	8934	698,221	23,074.0	0.109	0.18		
33	36.461	8970	631,075	18,429.0	0.087	0.14		
34	36.518	8986	730,993	17,601.8	0.083	0.14		
35	36.792	9063	642,967	18,108.6	0.085	0.14		
36	37.515	9266	655,705	21,892.7	0.103	0.17		
37	37.736	9328	564,845	18,542.0	0.087	0.15		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\15_Kofor_Naema_Cult_C.raw
Acquired: 13-Sep-13 05:23:20 AM Printed: 05-Mar-15 05:06 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 15



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.202	475	6,232,884	185,448.1	0.880	1.86	alfa-Thujene	
2	6.633	596	7,374,628	213,898.5	1.016	2.15	Camphene	
3	7.388	808	8,900,697	272,629.3	1.294	2.74	B-Thujene	
4	7.733	905	3,811,602	117,218.9	0.557	1.18	B-Myrcene	
5	8.859	1221	2,261,317	62,900.1	0.299	0.63		
6	8.927	1240	293,505,728	9,958,221.0	47.281	####	Eucalyptol	
7	9.707	1459	1,428,332	47,470.1	0.225	0.48	Gama-Terpinen	
8	10.042	1553	1,426,848	48,594.7	0.231	0.49	trans-4-Thujanol	
9	10.932	1803	1,096,209	43,210.6	0.205	0.43		
10	11.078	1844	1,794,114	60,484.5	0.287	0.61	3-Thujanone	
11	11.410	1937	3,316,771	112,676.1	0.535	1.13	alfa-Thujone	
12	12.229	2167	48,929,188	1,865,858.6	8.859	####	(+ /-)Camphor	
13	12.638	2282	1,149,348	40,949.3	0.194	0.41	3-Pinanone	
14	12.923	2362	2,793,026	105,366.3	0.500	1.06	Ocimenol	
15	12.963	2373	2,925,596	92,454.5	0.439	0.93		
16	13.230	2448	2,431,415	93,013.9	0.442	0.93	L-4-terpineneol	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

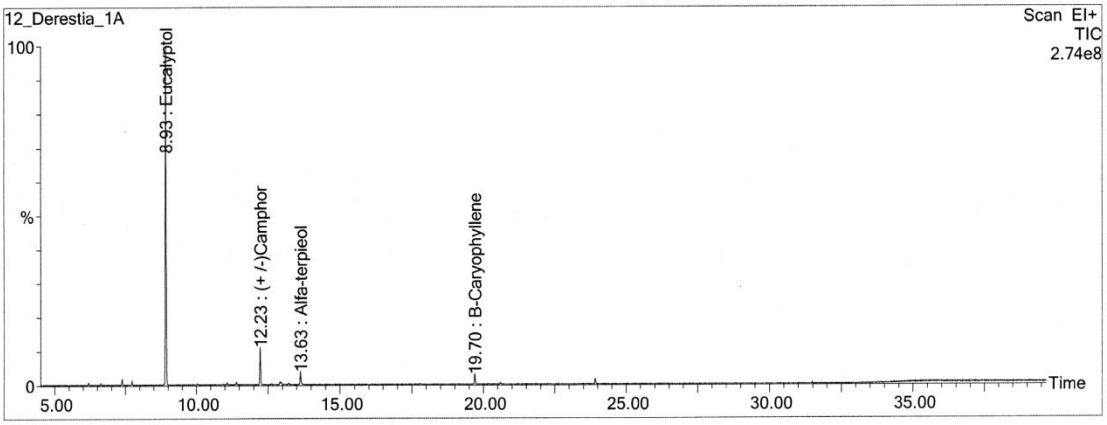
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\15_Kofor_Naema_Cult_C.raw
Acquired: 13-Sep-13 05:23:20 AM Printed: 05-Mar-15 05:06 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 15

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	13.639	2563	10,968,411	468,567.1	2.225	4.71	Alfa-terpieol	
18	16.126	3261	1,183,868	38,250.7	0.182	0.38	L-Bornyl acetate	
19	17.796	3730	2,199,872	88,401.3	0.420	0.89	Terpinyl acetate	
20	19.701	4265	16,423,974	657,014.3	3.119	6.60	B-Caryophyllene	
21	23.627	5367	788,084	33,525.9	0.159	0.34		
22	23.915	5448	2,333,392	88,555.8	0.420	0.89	Epiglobulol	
23	26.149	6075	1,345,075	54,145.5	0.257	0.54		
24	31.486	7573	5,982,784	131,723.5	0.625	1.32	13-Epimanool	
25	35.187	8612	970,835	62,580.6	0.297	0.63		
26	35.273	8636	1,006,517	37,486.0	0.178	0.38		
27	35.351	8658	1,038,481	36,701.3	0.174	0.37		
28	35.426	8679	910,323	36,992.7	0.176	0.37		
29	35.469	8691	1,084,779	34,445.8	0.164	0.35		
30	35.576	8721	1,198,434	36,219.1	0.172	0.36		
31	35.736	8766	1,044,114	43,729.6	0.208	0.44		
32	36.217	8901	926,151	38,377.9	0.182	0.39		
33	36.627	9016	873,969	31,595.1	0.150	0.32		
34	36.979	9115	921,626	39,551.7	0.188	0.40		
35	37.211	9180	848,423	34,837.1	0.165	0.35		
36	37.300	9205	878,886	35,622.8	0.169	0.36		
37	37.660	9306	814,893	31,400.4	0.149	0.32		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\12_Derestia_1A.raw
Acquired: 12-Sep-13 09:15:39 PM Printed: 05-Mar-15 05:03 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 12



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.198	474	2,107,794	58,828.5	0.367	0.62	alfa-Thujene	
2	6.629	595	1,696,445	46,913.6	0.293	0.49	Camphene	
3	7.383	807	4,553,800	131,461.6	0.820	1.38	B-Thujene	
4	7.733	905	3,054,446	80,442.5	0.502	0.85	B-Myrcene	
5	8.858	1221	493,852	15,560.0	0.097	0.16		
6	8.930	1241	273,938,496	9,495,314.0	59.262	####	Eucalyptol	
7	10.037	1552	1,041,830	34,671.9	0.216	0.37	trans-4-Thujanol	
8	10.928	1802	673,260	22,523.3	0.141	0.24		
9	11.077	1844	1,599,415	53,233.7	0.332	0.56	3-Thujanone	
10	11.401	1935	2,081,144	68,390.1	0.427	0.72	alfa-Thujone	
11	12.231	2168	30,049,178	1,055,420.4	6.587	####	(+ /-)Camphor	
12	12.641	2283	821,376	28,923.5	0.181	0.30	3-Pinanone	
13	12.922	2362	2,604,196	90,970.8	0.568	0.96	Ocimenol	
14	12.969	2375	1,803,780	56,609.1	0.353	0.60		
15	13.068	2403	661,828	23,283.1	0.145	0.25		
16	13.229	2448	1,274,257	44,214.6	0.276	0.47	L-4-terpineneol	

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

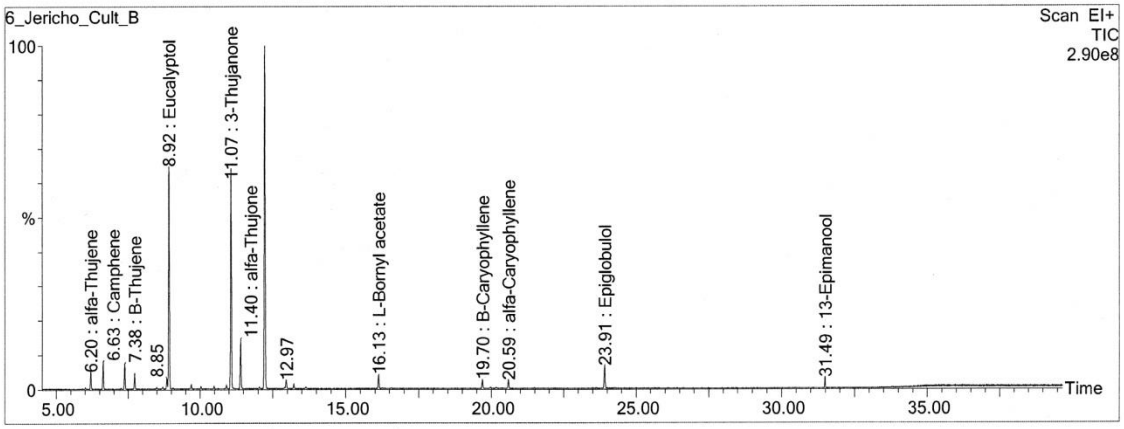
File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\12_Derestia_1A.raw
Acquired: 12-Sep-13 09:15:39 PM Printed: 05-Mar-15 05:03 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-CPage 2 of 2
Sample ID: Vial Number: 12

#	RT	Scan	Height	Area	Area %	Norm	Name	
17	13.635	2562	10,188,669	401,460.1	2.506	4.23	Alfa-terpieol	
18	16.128	3262	427,198	15,317.5	0.096	0.16	L-Bornyl acetate	
19	17.791	3729	620,178	24,361.9	0.152	0.26	Terpinyl acetate	
20	19.700	4265	7,983,738	289,766.0	1.808	3.05	B-Caryophyllene	
21	20.170	4397	484,214	18,240.1	0.114	0.19		
22	20.594	4516	1,265,254	47,576.1	0.297	0.50	alfa-Caryophyllene	
23	23.497	5331	502,496	16,700.6	0.104	0.18		
24	23.632	5369	474,063	15,918.7	0.099	0.17		
25	23.914	5448	4,592,187	159,138.0	0.993	1.68	Epiglobulol	
26	31.482	7573	1,241,348	28,796.9	0.180	0.30	13-Epimanool	
27	34.980	8555	670,119	15,877.6	0.099	0.17		
28	35.489	8698	615,209	15,981.6	0.100	0.17		
29	35.539	8712	634,129	17,010.3	0.106	0.18		
30	35.688	8754	566,063	17,321.7	0.108	0.18		
31	35.827	8793	672,987	20,624.1	0.129	0.22		
32	36.418	8959	502,767	17,022.1	0.106	0.18		
33	37.105	9152	573,666	21,911.0	0.137	0.23		
34	37.419	9240	683,798	20,359.0	0.127	0.21		
35	37.469	9254	637,938	15,953.0	0.100	0.17		
36	37.533	9272	579,527	19,499.6	0.122	0.21		
37	37.711	9322	502,515	16,028.8	0.100	0.17		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\6_Jericho_Cult_B.raw
Acquired: 12-Sep-13 02:35:17 AM Printed: 05-Mar-15 04:57 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 6



#	RT	Scan	Height	Area	Area %	Norm	Name	
1	6.013	422	1,345,836	36,369.0	0.103	0.34		
2	6.198	474	14,280,738	452,398.3	1.279	4.26	alfa-Thujene	
3	6.629	595	22,987,322	723,478.0	2.045	6.82	Camphene	
4	7.384	807	21,385,276	668,599.1	1.890	6.30	B-Thujene	
5	7.736	906	13,386,984	431,329.7	1.219	4.07	B-Myrcene	
6	8.498	1120	1,462,734	46,327.8	0.131	0.44		
7	8.716	1181	1,788,980	57,744.7	0.163	0.54		
8	8.851	1219	7,578,066	230,298.3	0.651	2.17		
9	8.922	1239	185,988,096	6,376,208.5	18.021	####	Eucalyptol	
10	9.702	1458	3,542,321	111,202.6	0.314	1.05	Gama-Terpinen	
11	10.030	1550	1,879,950	56,867.3	0.161	0.54	trans-4-Thujanol	
12	10.497	1681	2,230,642	70,194.7	0.198	0.66		
13	10.920	1800	2,779,219	96,764.8	0.273	0.91		
14	11.073	1843	185,740,448	6,448,522.0	18.225	####	3-Thujanone	
15	11.405	1936	41,590,584	1,410,472.5	3.986	####	alfa-Thujone	
16	12.042	2115	1,228,557	45,899.5	0.130	0.43		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

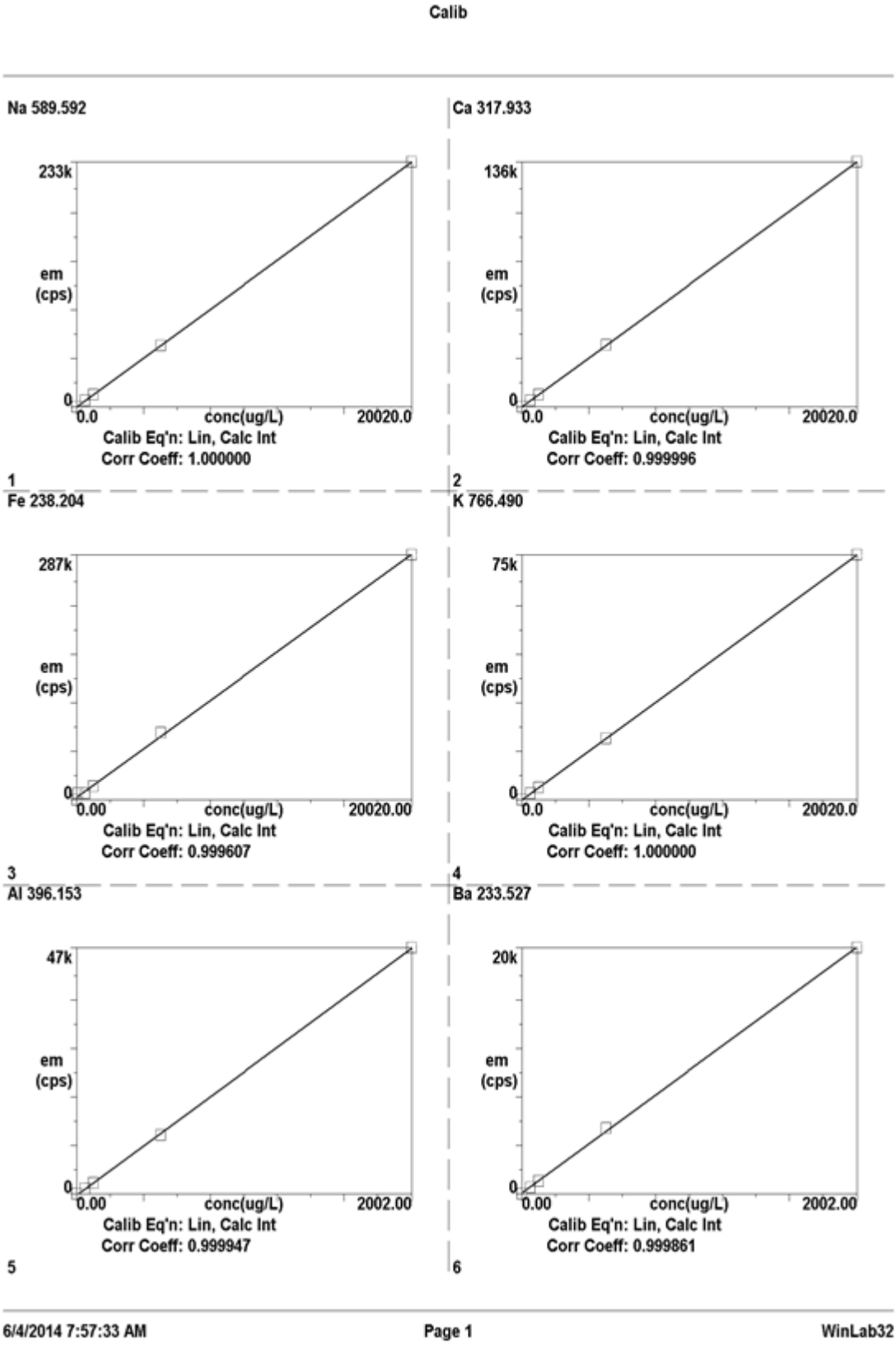
Qualitative Report

File: C:\TurboMass\FINAL TRANING 2904.PRO\Data\6_Jericho_Cult_B.raw
Acquired: 12-Sep-13 02:35:17 AM Printed: 05-Mar-15 04:57 PM
Description:
GC/MS Method: GC: KI Alakane C10-C40 17_4_2013.mth MS: KI Alkane C10-C
Sample ID: Vial Number: 6

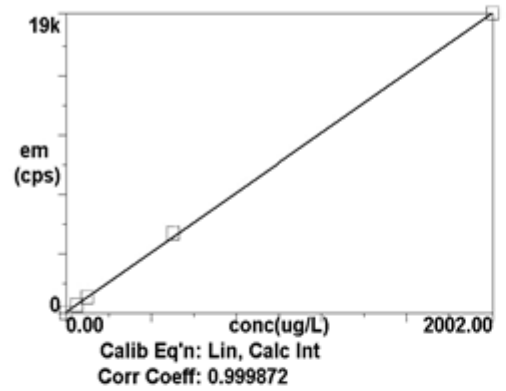
#	RT	Scan	Height	Area	Area %	Norm	Name	
17	12.227	2167	289,141,120	10,610,362.0	29.988	####	(+ /-)Camphor	
18	12.968	2375	7,053,839	313,467.3	0.886	2.95		
19	13.221	2446	3,527,238	118,871.2	0.336	1.12	L-4-terpineneol	
20	13.634	2562	1,577,347	63,987.3	0.181	0.60		
21	16.128	3262	11,704,692	427,492.0	1.208	4.03	L-Bornyl acetate	
22	19.697	4264	7,448,344	252,962.4	0.715	2.38	B-Caryophyllene	
23	19.975	4342	1,187,876	43,726.9	0.124	0.41		
24	20.164	4395	868,608	33,381.6	0.094	0.31		
25	20.591	4515	7,058,492	263,921.9	0.746	2.49	alfa-Caryophyllene	
26	23.910	5447	19,159,154	749,042.1	2.117	7.06	Epiglobulol	
27	31.485	7574	9,096,326	212,267.5	0.600	2.00	13-Epimanool	
28	35.065	8579	940,582	31,363.7	0.089	0.30		
29	35.129	8597	916,798	33,542.6	0.095	0.32		
30	35.257	8633	1,088,494	44,428.2	0.126	0.42		
31	35.734	8767	1,153,229	59,291.5	0.168	0.56		
32	35.777	8779	1,004,817	31,335.8	0.089	0.30		
33	35.937	8824	1,010,729	39,039.9	0.110	0.37		
34	35.973	8834	1,103,926	42,666.4	0.121	0.40		
35	36.204	8899	1,029,438	32,777.6	0.093	0.31		
36	36.290	8923	964,370	31,735.3	0.090	0.30		
37	36.482	8977	864,255	33,493.2	0.095	0.32		

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 50°C for 2 min, ramp 5°C/min to 180°C, hold 0 min, ramp 15°C/min to 280°C, hold 5 min, InjAauto=235°C, Volume=0 µL, Split=20:1, Carrier Gas=He, Solvent Delay=4.50 min, Transfer Temp=260°C, Source Temp=250°C, Scan: 50 to 480Da, Column 28.0m x 250µm

Calibration Curves for Minerals Analysis

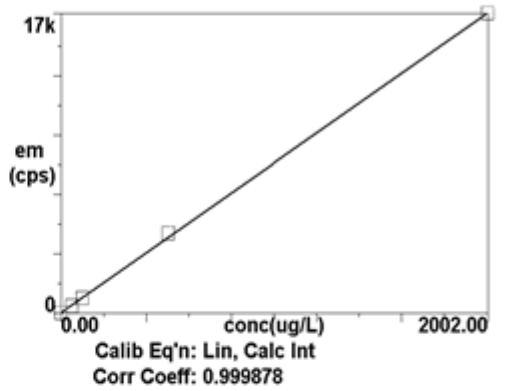


Cd 228.802



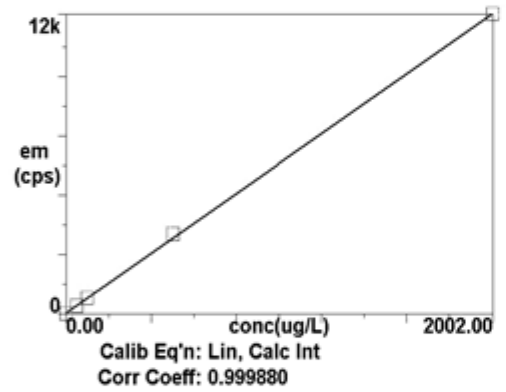
7

Cr 267.716



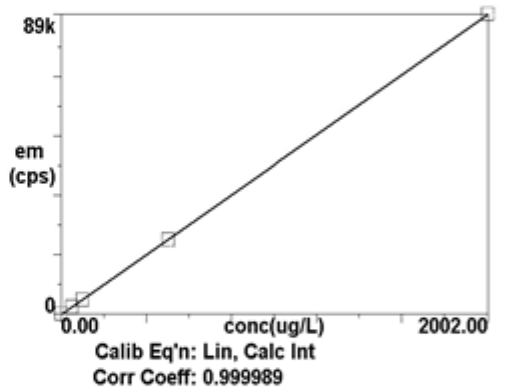
8

Co 228.616



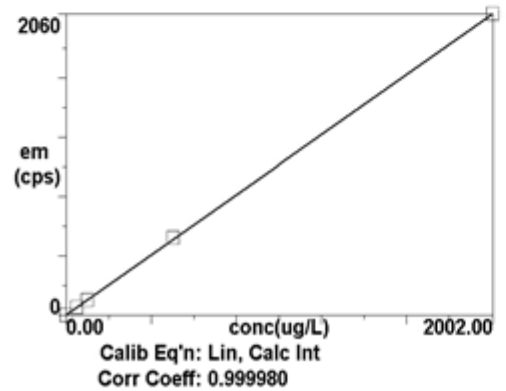
9

Cu 327.393



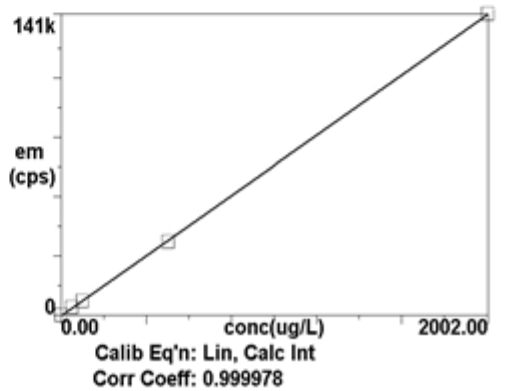
10

Pb 220.353



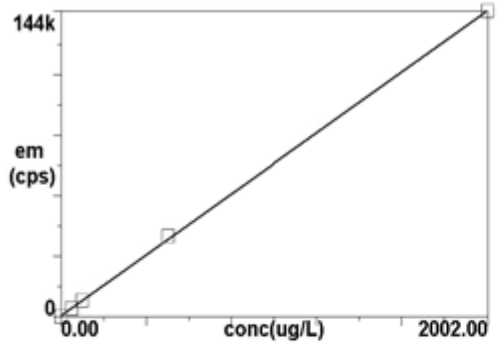
11

Mg 285.213



12

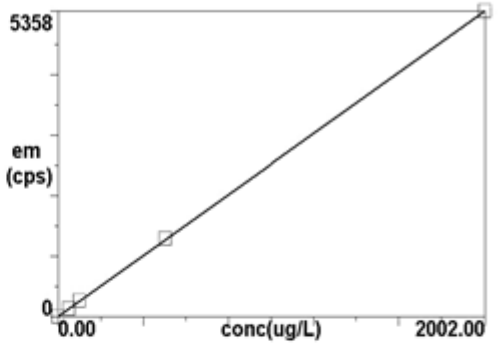
Mn 257.610



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999904

13

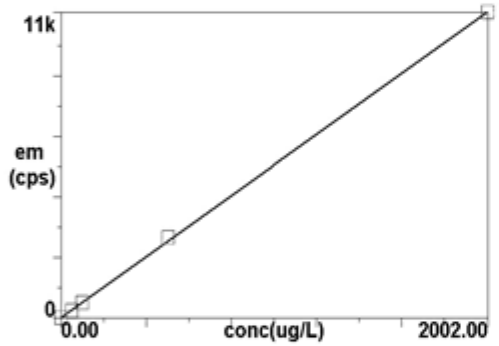
Mo 202.031



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999990

14

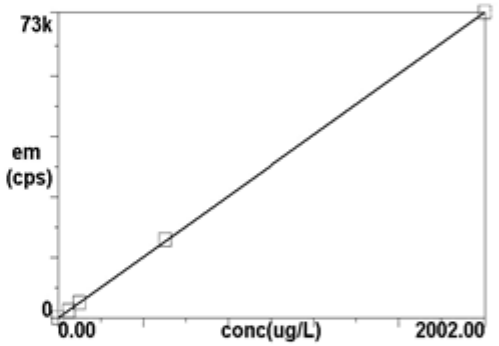
Ni 231.604



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999879

15

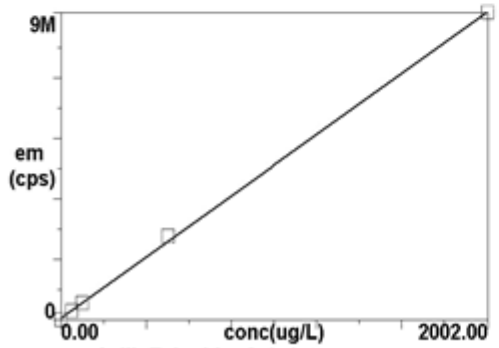
Ag 328.068



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999979

16

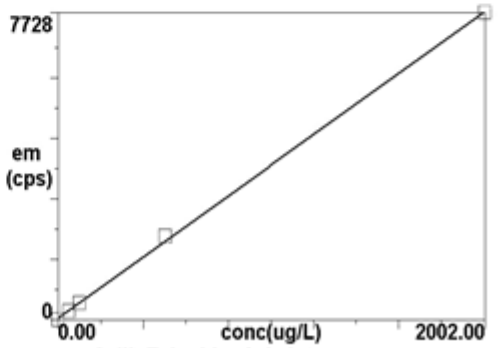
Sr 407.771



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999788

17

Zn 206.200



Calib Eq'n: Lin, Calc Int
Corr Coeff: 0.999749

18

تحليل المركبات الثانوية لأوراق المريمية البرية/ المروية بواسطة GC-MS ودراسة تأثيراتها المضادة للأكسدة والميكروبات

إعداد: ريم نمر محمد سبوبة

إشراف: الاستاذ الدكتور صالح أبو لافي

الملخص

إن التداوي بالأعشاب في فلسطين شائع بشكل كبير. ومن أكثر الأعشاب استخداما عشبة المريمية. إلا أن استخدامها مرتبط بتقاليد متوارثة أكثر من إعتماده على أسس وأبحاث علمية. لقد بات معروفا أن المريمية خاصة تحتوي على مركبات ثانوية لها استخدامات واسعة في الطب الشعبي و في صناعة الأغذية كمادة منكهة و حافظة. إن إنتاج و تراكم هذه المركبات الثانوية في المريمية يتأثر بالعديد من العوامل المختلفة التي قد تحدد تكوين ونسبة العائد منها. في الآونة الأخيرة أكتسبت هذة الزيوت اهمية خاصة لكونها مصدرا محتملا للمواد الفعالة حيويا ولكونها أكثر أمانا، خاصة بسبب ظهور مقاومة ميكروبية عند الجراثيم ضد المضادات الحيوية المتوفرة.

في هذا البحث العلمي، تم جمع أوراق المريمية (المروية والبرية) من سبع محافظات مختلفة في فلسطين، ثم استخرجت الزيوت العطرية من الأوراق المجففة بواسطة التقطير المجزأ Steam Distillation واما هوية ومكونات هذا الزيت فقد حددت ولأول مرة بواسطة GC-MS. وتمت دراسة القدرة المضادة للأكسدة لزيت المريمية باستخدام طريقة DPPH. اما بالنسبة لفعالية الزيوت ضد الميكروبات المختلفة فقد تم فحصها باستخدام القرص. ولتحديد محتوى الأوراق الجافة من المعادن فقد تم استخدام جهاز ICP-OES.

لقد تم تحديد هوية عشرين مركب أساسي (متطاير او شبه متطاير) في زيت المريمية ولقد كانت المكونات الرئيسية في جميع العينات المروية هي الأكاليبتول ثم الكافور باستثناء عينة أريحا التي كان

المكون الرئيسي فيها هو الكافور (30.65٪) و الذي لم تتجاوز نسبته (9.1٪) في العينات الأخرى كما أن عينة أريحا احتوت على مشتقات الثيوجون بنسبة (28.9٪) والتي لم تتعدى نسبة (2٪) في باقي العينات. أما بالنسبة للأوراق البرية كان الاكاليبتول هو المكون الرئيسي في جميع العينات البرية، وكان تركيزه أعلى منه في العينات المروية، في حين أن الاخيرة أحتوت على نسبة اعلى من الكافور.

لقد لوحظ ان التركيز اللازم من الزيت للوصول الى تثبيط الأكسدة بنسبة 50% (IC_{50}) هو 2.333ملغم/ مل بعد مرور 30 دقيقة، في حين انه بعد 90 دقيقة أنخفض ليصل الى 1.585 ملغم/ مل، وهذا يشير الى أن النشاط المضاد للأكسدة لزيت المريمية يزداد مع مرور الوقت و مع زيادة التركيز المستخدم من الزيت.

لوحظ عند دراسة التأثير المضاد للميكروبات ل 5 ميكرولتر من زيت المريمية أن التأثيرالمضاد لهذا الحجم من الزيت يفوق تأثير الجنتاميسين في حالة العنقوديات المكورة الذهبية بينما كان تقريبا يعادل تأثير الجنتاميسين ضد القولونية. علاوة على ذلك فأن تأثيره المضاد يفوق مرتين تأثير النيستاتين ضد المبيضات البيض.

إن أوراق المريمية غنية بالمعادن وخاصة البوتاسيوم ولكن العينة التي تم فحصها لوحظ انها تحتوي على كمية ملفتة للإنتباه من الألومنيوم و الذي يعرف بأنه يمكن أن يتراكم في الجسم و بالتالي فأنه قد يضر بالصحة. لذا، فإنه يوصى بمتابعة العمل على المعادن الموجودة في المريمية وذلك بدراسة مواقع أخرى إضافة إلى الموقع الذي تم دراسته (رام الله)، حيث أن الإعتقاد السائد بأن النباتات الطبية آمنة وخالية من السمية يمكن أن مضللا.

عمادة الدراسات العليا

جامعة القدس

تحليل المركبات الثانوية لأوراق المريمية البرية/ المروية بواسطة GC-MS ودراسة تأثيراتها المضادة للأكسدة والميكروبات

إعداد

ريم نمر محمد سبوبة

رسالة ماجستير

فلسطين-القدس

2015 م /1436 هـ

تحليل المركبات الثانوية لأوراق المريمية البرية/ المروية بواسطة GC-MS ودراسة تأثيراتها المضادة للأكسدة والميكروبات

إعداد

ريم نمر محمد سبوبة

بكالوريوس صيدلة ـ جامعة حلب, سوريا.

المشرف: الاستاذ الدكتور صالح أبو لافي

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الصيدلانية من كلية الدراسات العليا جامعة القدس ـ فلسطين.

2015 م /1436 هـ