An-Najah National University

Faculty of Graduate Studies

Antioxidant and antimicrobial activity of *Mandragora autumnalis* Bertol extracts

By

Noor Bashar Ehsan Jodallah

Supervisor

Prof. Dr. Mohammed S. Ali-Shtayeh

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Life Sciences (Biology), Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2013

Antioxidant and antimicrobial activity of *Mandragora autumnalis* Bertol extracts

By

Noor Bashar Ehsan Jodallah

This Thesis was defended successfully on 5 / 6 / 2013 and approved by:

Defense Committee members		**Signature**
– prof. Dr. Mohammed S. Ali-Shtayeh (Supervisor)		.....
– Dr. Yahya Faidy	(External Examiner)	.....
– Dr. Majdi Dwikat	(Internal Examiner)	Majdi Dwikat

Dedication

This thesis is especially dedicated to my family.

Acknowledgement

This study was carried out at the Department of Biotechnology, at An-Najah National University, Nablus, Palestine. I wish to express my deepest gratitude to my principal supervisor, Prof. Dr. Mohammed S. Ali-Shtayeh, for his guidness and encouragement to start and complete this work.

I also would like to thank my Dr. Ahmad Ibrahim Ahmad Husein for his technical help and Dr. Rana M. Jamous for her help and encouragement.

Special thanks are also due to my family, my school Tla' - Al amal, and my friends for their encouragement.

إقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان :

Antioxidant and antimicrobial activity of *Mandragora autumnalis* Bertol extracts

اقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص ، وان هذه الرسالة ككل أو أي جزء منها لم يقدم من قبل لنيل أية درجه علميه أو بحث علمي أو بحثي لدى أية مؤسسه تعليمية أو بحثيه أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالبة :**

Signature: **التوقيع:**

Date: **التـــــاريخ:**

Table of Contents

List of Tables

List of Figurs

List of Abbreviations

BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene
DPPH	1,1–Diphenyl-2-picrylhydrazyl
FCR	Folinciocalteus phenol reagent
GC-MS	Perkin Elmer clarus 500 Gas chromatography" and "Perkin Elmer clarus 560D mass spectrometer
LC-MS	Liquid chromatography–mass spectrometry detector
LC-NMR	Liquid chromatography– nuclear magnetic resonance spectroscopy
LC-PDA	Liquid chromatography–photo-diode-array detector
PEs	Pyrocatechol equivalents
ROS	Reactive oxygen species
TAPHM	Traditional Arabic Palestinian Herbal Medicine
TIC	Total ionic concentration values
TOC	Tocopherol
WHO	World Health Organization

Antioxidant and antimicrobial activity of *Mandragora autumnalis* Bertol extracts

By
Noor Bashar Jodallah
Supervisor
Prof . Dr . Mohammed S. Ali-Shtayeh

Abstract

Background: Mandragora was one of the most important medicinal plants and a herb of great cultural value. The plant is still widely used in Traditional Arabic Palestinian Herbal Medicine, TAPHM, for the treatment of many and varied ailments, including pain, insomnia, eye diseases, inflammation, and ulcers.

Objectives : Evaluate the antioxidant activity of different parts of *Mandragora autumnalis* using DPPH, β –carotene linoleic acid assays, and hydrogen peroxide scavenging activity assays . Evaluate the antimicrobial activity of all parts of *M.autumnalis*, using a modified poisoned food technique for dermatophytes and disc diffusion method for bacterial strains**.**

Methods: The antioxidant activity of *M. autumnalis* was determined using different methods, including: the DPPH Assay, β-Carotene-Linoleic acid assay, and Hydrogen peroxide scavenging activity, while the antimicrobial activity was determined by using the disc diffusion and modified poisoned food techniques. The GC-MS spectrophotometer was used for the identification of active constituents from all parts of mandragora.

Results: From our results it was shown that roots and leaves of mandragora have antioxidant activity using the DPPH Assay, While non of the extracts

have shown antioxidant activity using the β-Carotene-Linoleic acid assay, while all parts of mandragora have antioxidant activity using the Hydrogen peroxide scavenging activity. The Antimicrobial activity of different plant parts were investigated in this study. From our study, it was shown that only the ethanolic extracts of mandragora roots have antibacterial activity against the 6 bacterial strains. On the other hand, non of the extracts have shown antifungal activity.

Studying the chemical composition of the components of the plant using GC-MS test results have shown that different plant parts contains about 219 chemicals. Comparison between the chemical content of the various parts of the plant, it was found that coumarins material to be present in all parts of this plant, while anthraquinones and tannins were found in all plant parts with the exception of roots, and that glycosides was found only in ripe fruits.

Chapter One

General Introduction

1. General Introduction

1.1 Traditional medicine

For thousands of years, man used various natural materials as a remedy for the treatment of various diseases. In the past few decades, most natural products were replaced with synthetic drugs that were based on modern chemistry and biotechnology. However, over 20000 medicinal herbs were recently inventoried by the World Health Organization (WHO, 2003), and about 250 species had been intensively studied. In particular, the herbal medicine market has exploded and became prosperous in pharmacies and many stores. For example, there was a seven-fold increase in the number of people using herbal medicines between 1990 and 1997 in U.S.A (Eisenberg et al, 1998) With this increasing interest in natural medicine, more individuals will explore the possibility of using natural medicines to complement conventional therapy, as is already the case in certain minority cultures (Berman et al. 1999; Hunt et al, .2000). Furthermore, natural products are still a major source of new drug discoveries: for example, 65% of the drugs that were approved for marketing between the years 1983 and 1994 were based on natural sources (Cragg et al,. 1997; Soejarto, 1996). Ethno pharmacological research is considered crucial in the development and discovery of new drugs from natural sources (Soejarto & Fransworth , 1989 ; Fransworth & Soejarto, 1985).

1.1.1 Traditional Medicine in Palestine

The Palestinian Mountains are rich in plant species. About 2600 species are found on this small Mediterranean area, of which more than 700 are

mentioned in published ethnobotanical data (Dafni et al., 1984; Friedman et al., 1986; Palevitch and Yaniv, 1991; Shtayeh and Hamad, 1995; Ali-Shtayeh and Jamous, 2006).

Utilizing the healing properties of plants is an ancient practice. People in all continents have long used hundreds, if not thousands, of indigenous plants for treatment of various ailments dating back to prehistory. These plants are still widely used in ethnomedicine around the world. Herbal medicine is considered an integral part of the Palestinian culture and plays a pivotal and indispensable role in the current public healthcare. The modern use of plant medicine in Palestine has historical roots in an Ancient Arabic medicine, which itself was influenced by the ancient medicinal practices of Mesopotamia, Greece, Rome, Persia, and India (Abu- Rabia, 2005; Bailey & Danin,1981). The use of traditional medicine, particularly herbal medicine, is widespread throughout the contemporary Middle East, including Palestine (Abu-Rabia, 1999; Ali- Shtayeh et al., 2000; Abu-Rabia, 2005; Ali-Shtayeh and Jamous, 2006, Ali-Shtayeh et al., 2011; Ali-Shtayeh et al., 2012).

1.2 Antioxidants

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil et al., 2006). Antioxidant-based drug formulations are used for the prevention and

treatment of complex diseases like atherosclerosis , stroke, diabetes, Alzheimer's disease and cancer (Devasagayma et al., 2004; Khalaf et al., 2008).

Free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species are implicated in numerous diseases such as inflammation, metabolic disorders, repulsion damage, atherosclerosis and carcinogenesis (Robak et al., 1988; Ames et al., 1993). The consumption of plant foods, such as antioxidant supplements or antioxidant-containing foods may be used to protect against various diseases, including cancer, cardio and cerebrovascular diseases. They also help the human body to reduce oxidative damage or protect oxidative deterioration (Elmastas et al., 2007). Natural antioxidants can be phenolic compounds (α-tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid, whereas synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution (Hall & Cuppet, 1997). Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity and cause an increased interest towards natural antioxidant substances (Ames 1983; Madhavi & Salunkhe , 1995). Antioxidant compounds add to food products, especially to lipids and lipid containing foods and can increase shelf life by retarding the process of lipid peroxidation, which is one of the

major reasons for deterioration of food products during processing. An alternative natural and safe source of food antioxidant is found (Wanasundara & Shahidi, 1998).

This radical is also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide anion radicals (O2·) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils, and neutrophils and the production of O2· is an important factor in the killing of bacteria by phagocytes. In living organisms, O2· is removed by the enzymes called superoxide dismutases (Wettasinghe, & Shahidi, 2000).

Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death tissue damage. Oxidative damage plays a significantly pathological role in human disease. Cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage (Halliwell, & Gutteridge, 1985). Also, excessive generation of ROS, which is induced by various stimuli and exceeds the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Elmastas et al., 2006; Kourounakis et al., 1999).

Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutases and catalase or antioxidant compounds such as ascorbic acid, tocopherols, and glutathione (Niki et al., 1994). When the mechanism of antioxidant protection becomes unbalanced

by exogenous factors such as tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes, the result may be the above-mentioned diseases and accelerated aging. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage (Halliwell, & Gutteridge, 1985; Gülçin et al., 2002; Mau et al., 2001).

Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid per oxidation, which is one of the major reasons for deterioration of food.

Thus, the search for natural antioxidants, especially extracted from plant origin, has notably increased in recent years (Zainol et al., 2003).

Vegetables and fruits are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular diseases, especially atherosclerosis (Barros et al., 2007).

Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants (Salah et al 1995; Sabe et al., 1996). There is no information about the *in vitro* antioxidant activity of water or ethanol extracts of *Mandragora autamnalis*.

1.3 Antimicrobial activity

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniya et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin et al., 1986).

The abundance of plants has led to an increasing interest in the investigation of different plant extract as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004) and for new drugs for greater effective treatment of several diseases (Dimayuga & Garcia 1991). Antimicrobial activities of various herbs and spices in plant parts have been reported by many workers (Cowan, 1999; Mau et al., 2001). Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Jayaraman et al., 2008).

Antibacterial active principles isolated from higher plants appear to be one of the important alternative approaches to contain antibiotic resistance for the management of disease. It is believed that plant based drugs cause less

or no side effect when compared with other synthetic antibiotics (Sheriff et al., 2006; Satish et al., 2008). Chemical substances of plants produce physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavanoids, tannins and phenolic compounds. Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds (Jayaraman et al., 2008).

Fungal infections are often resistant and difficult to cure and there are only three classes of antifungal drugs available up to date. For example , Candida species have developed resistance against the traditionally used drugs, so new molecules with new modes of action would be needed (Fyhrquist, 2007). The interest in medicinal plants as a natural alternative to synthetic drugs (Doughari, 2006) is increasing because of the high rate of infections with antibiotic resistant micro-organisms and the side effects of some of synthetic antibiotics. Thus plant extracts can be used as an alternative way to control these pathogens.

1.4 Modern Phytochemistry

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases (Badugu, 2012). It was estimated that 61 % of the 877 small molecule new chemical entities introduced as drugs worldwide during 1981–2002 can be

traced back to or were developed from natural products (Satyajit et al., 2007). In some therapeutic areas, the contribution of natural products is even greater, e.g. about 78 % and 74 % of antibacterial and anticancer drug candidates respectively are natural products or structural analogues of natural products. Modern drug discovery approaches applying full automation and robotics, hundreds of molecules can be screened using several assays within a short time, and with very small amounts of compounds. Different techniques are used for the identification and analysis of chemical constituents including LC-PDA (liquid chromatography–photo-diode-array detector), LC-MS (liquid chromatography–mass spectrometry detector) and LC-NMR (liquid chromatography– nuclear magnetic resonance spectroscopy) (Husein, 2010). Recently the new and improved technologies related to separation, isolation and identification of natural products have improved remarkably. Natural products libraries have been established to preserve crude extracts, chromatographic fractions or semi-purified compounds. However, the best result can be obtained from a fully identified pure natural product library as it provides scientists with the opportunity to handle the 'lead' rapidly for further developmental work, e.g. total or partial synthesis, dealing with formulation factors, in vivo assays and clinical trials (Satyatit et al., 2007).

1.5 *Mandragora autamnalis*

Mandrake, the common name for members of the Mediterranean plant genus *Mandragora*, belongs to the nightshade family (Solanaceae). There are two species of *Mandragora, M. officinalis* var. *vernalis (M.*

officinarum) with white flowers and *M. officinalis* var. *autumnalis (M. autumnalis*) with purple flowers. *Mandragora autumnalis* is a perennial herb with thick tuberous roots native to the Middle East, southern Europe, and North Africa (Jackson & Berry, 1979). Mandragora fruit is the only edible part of the plant and is still consumed in Palestine especially in the rural areas.

Mandrake is one of the most ancient herbs known to mankind and yet remains one of the most misunderstood plants on earth (Vidal, 1982). The concept of this odd plant is surrounded with various myths and legends and recalls something mysterious, gloomy, and even horrible (Carter, 2003). Mandrake is among the long line of bewitching weeds, the most significant representative of magic power.

The intoxicating power of mandrake and its history goes back throughout the ages. Mandrake's fame struck all European civilizations. References to mandrake go back as far as the Holy Bible (Old Testament) and even further back in ancient eastern manuscripts. There was wide use of mandrake during the Greek and Roman ages, the medieval age as well as during the Renaissance period. This plant is known for its magic, aphrodisiac, healing, hallucinogenic, as well as poisonous properties. The fruits of the plant, also-called love apples, were believed to increase fertility. For many centuries, *Mandragora* was one of the most important medicinal plants and a herb of great cultural value. It had extraordinarily important value in ancient pharmacopoeias, and this has continued until recent times. Dioscorides wrote at length on the mandragora’s medicinal

qualities. The plant parts (the root, the fruit, and leaves) were used to treat many and varied ailments, including pain, insomnia, eye diseases, inflammation, and ulcers. The plant was used as an aphrodisiac in post-biblical times, and its use in folklore peaked during the middle Ages. Given the plant's popular use in different fields of medicine, one might assume much phytochemical and pharmacognostic work would have been carried out on it. In fact, very little work has been performed on any active compounds from *Mandragora* other than al-kaloids (Hanuš et al., 2005).

1.6 Objectives of the study:

The aims of the study were to :

- ✓ Evaluate the antioxidant activity of all parts of *Mandragora autumnalis* (leaf , root , ripe and unripe fruit) using DPPH, β – carotene linoleic acid assays, Hydrogen peroxide scavenging activity and determination phenolic and flavonoid compounds in all parts of the plant.
- ✓ Evaluate the antimicrobial activity of all parts of *Mandragora autumnalis* (leaves, roots, ripe and unripe fruits), using a modified poisoned food technique for dermatophytes and disc diffusion method for bacterial strains**.**
- ✓ Separation, identification and determination of active constituents from different parts of *Mandragora autamnalis*.

Chapter Two
Materials and Methods

2.1 Materials

2.1.1Chemicals

β-Carotene, linoleic acid, 1,1–Diphenyl-2-picrylhydrazyl (DPPH), buthylated hydroxytoluene (BHT), buthylated hydroxyanisol (BHA), and α-tocopherol were purchased from Sigma, (Sigma, Aldrich GmbH, Sternhheim, Germany).

While pyrocatechol, quercetin, Tween-40, Folinciocalteus phenol reagent (FCR), sodium carbonate, ethanol, chloroform and other chemicals and reagents were purchased from Merck (Darmstat, Germany), chloramphenicol, peptone, agar, dextrose, Muller–Hinton agar, all chemicals and reagents were of analytical grade.

2.1.2 Plant Material

The plant in this study was collected in April – June 2010 from Nablus area and was identified by Prof. M. S. Ali-Shtayeh in the Department of Biology at An-Najah National University. Voucher specimens were preserved at BERC Herbarium,Til, Nablus.

2.1.3 Test Microorganisms

The antimicrobial activity of *Mandragora autamnalis* against six medically important bacterial strains and two dermatophytes was studied in this work.

2.1.3.1 Bacterial Strains:

The bacterial strains used in this study included: 2 isolates of *Escherichia coli* (ATCC 25922, and JM109), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 13315), and *Pseudomonas aeruginosa* (ATCC 27853).

2.1.3.2 Fungal Isolates:

The fungal species used in this study were, *Trichophyton rubrum* and Yeast.

The isolates have been maintained on SDA media at room temperature. Experimental cultures were kept on SDA media and subcultured monthly (Murray et al., 1995; Yaghmour, 1997).

2.1.4 Antibiotic:

Gentamicin, and econazole,

2.2 Methods

2.2.1 Extract preparation

From a toxicological point of view, ethanol and water, as solvents, are safer than other organic solvents, and therefore more suitable for the food and pharmaceutical industries. Thus water and ethanol extracts were used in the present study.

Fresh plant parts (roots, leaves, ripe and unripe fruits) were grinded and 500 g of each part was soaked separately in 500 ml of 95% ethanol and 500 ml water and the mixtures were incubated at room temperature for 72 hours. The extracts were then filtered through Whatman No. 4 filter paper and dried using rotary evaporator for ethanolic extracts and freeze drying for water extracts. Dried samples were stored at -20 °C for future use.

2.2.2 Biological Activities

2.2.2.1 Antioxidant activity

DPPH Assay (Free radical scavenging activity). The hydrogen atom or electron donation abilities of the corresponding extracts and some pure

compounds were measured from the bleaching of the purple- colored methanolic solution of 1,1-diphenly-2-picrylhydrazylhydrate (DPPH).This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits& Bucar, 2000 ; Cuendet et al ., 1997). Different concentrations of plant extracts were prepared using ethanol (25, 50, 100, and 250 µg/ml). One milliter of each concentration was added to 4 ml of various concentrations of methanolic solutions of DPPH (0.004%, 0.008%, 0.012%, and 0.016%). The mixtures were incubated for 30 minutes at room temperature, and the absorbance was read against the blank at 517 nm. The percent Inhibition I (%) of free radical by DPPH was calculated as follows:

$I\ (\%) = ((A_{blank} - A_{sample})/ A_{blank}) \times 100$.

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the plant extract), and A_{sample} is the absorbance of the tested plant extract. Extract concentrations providing 50% inhibition (I_C50) are calculated from the plot of inhibition (%) against extract concentration. Tests were carried out in triplicates.

β-Carotene-Linoleic acid assay. The antioxidant activity of the plant ethanolic extracts, based on coupled oxidation of β-carotene and Linoleic acid emulsion, was evaluated following a modified method of Gazzani and Miller (Gazzani et al., 1998 ; Miller , 1971). Briefly, 1mg of β- carotene was dissolved in 2 ml chloroform and 20 mg of linoleic acid, 200 mg of Tween 40 were added. Chloroform was completely evaporated using a rotary evaporator under reduced pressure at low temperature (less than 30°C), and 200 ml of distilled water saturated with oxygen were added to the flask with vigorous shaking for 30 minutes. Aliquots (5ml) of the

prepared emulsion were transferred to a series of tubes each containing 0.1ml of extract or tocopherol (2mg/ml). A control sample was prepared exactly as before but without adding antioxidants. Each type of sample was prepared in triplicate. The test systems were placed in a water bath at 50°C for 75 min. The absorbance of each sample was read spectrophotometrically at 470 nm, immediately after sample preparation and at 15-min interval periods until the end of the experiment (75 min). Antioxidant activities in β-carotene-linoleic acid model were measured by the changes in the absorbance at 470 nm.

Hydrogen peroxide scavenging activity. Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). 100 µl of different plant extracts (500µg/ml) was transferred into test tubes, the volumes were made up to 0.4 ml using the 50 mM phosphate buffer (pH 7.4), followed by the addition of of 600 µl of 2 mM hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank (Ruch et al .,1989). Plant extracts abilities to scavenge the hydrogen peroxide were calculated using the following equation:

Hydrogen peroxide scavenging activity = (1-absorbance of sample / absorbance of control) × 100.

2mM Hydrogen peroxide was used as a control.

2.2.2.2 Antimicrobial activities.

***Antibacterial Activity of Mandragora autumnalis*.** The dried plant extracts were dissolved in 95% ethanol to a final concentration of 100 µg/ml and sterilized by filtration through a 0.45 µm membrane filter. Antibacterial

tests were then carried out using the disc diffusion method (Murray et al., 1995). An inoculum containing 10^6 bacterial cells/ml was spread on Muller–Hinton agar plates (1 ml inoculum/plate). Discs of 6 mm diameter were impregnated with 2 ml of plant extract at a concentration of 100 µg/ml, placed on the inoculated agar and incubated at 37°C for 24 h. Paper discs impregnated with 95% were used as a negative control.

***Anti fungal testing Mandragora autumnalis*.**The plants extracts were tested at different concentrations for their antifungal activity against the test pathogens using a modified poisoned food technique (Sharvelle ,1961). Different amounts of each extract were incorporated in presterilized SDA medium to prepare a series of concentrations of the extract (25, 50, 100, and 250 µg/ml). A mycelial agar disk of 5 mm diameter was cut out of 12 days old culture of the test fungus and inoculated onto the freshly prepared agar plates econazole was used as a control in place of the tested plant extract. Three replicate plates were used for each treatment (concentration).The inoculated plates were incubated in the dark at 24°C and the observations were recorded after 10 days. Percentage of mycelial inhibition was calculated using the following formula:

% mycelial inhibition = (dc-ds/dc) x100%

Where:

dc: colony diameter of the control

ds: colony diameter of the sample**.**

2.2.2.3 Phytochemical screening

Phytochemical screening of leaves, roots, ripe and un ripe fruits of *Mandragora autamnalis* for the presence of anthraquinones, cardiac

glycosides, coumarins, saponins, phlobatannins, tannins, terpenoids phenolic compounds and flavonoids was carried out.

Test for saponins. The ability of saponins to produce emulsion with oil was used for the screening test (Harborne , 1973). Twenty mg of each part of the plant dried material was dissolved in 20 ml of distilled water and boiled in a water bath for 5 minutes. The solution was filtered through Whatman filter paper no. 4, then 10 ml of the filtrate was mixed with 5 ml of distilled water and shook vigorously until the formation of froth. Three drops of olive oil were mixed with the froth, and shook vigorously and the mixture was observed for emulsion development.

Test for terpenoids. The Presence of terpenoids in different plant parts was carried out by taking 5 ml (1 mg/ml) of each dried plant part extract and mixed with 2 ml of chloroform, followed by the addition of 3 ml of concentrated H_2SO_4. A reddish brown coloration of the interface confirmed the presence of terpenoids (Harborne, 1973).

Test for anthraquinones. Two hundred mg of each dried plant part was mixed with 6 ml of 1% HCl, the mixture was boiled and filtered as mentioned in section 2.1.1. The filtrate was mixed with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, violet or red color in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones (Trease & Evans ,1989) .

Cardiac glycosides determination. Five ml of each dried plant extract (10 mg/ml in methanol) was mixed with 2 ml of glacial acetic acid and one

drop of FeCl3 solution. One ml of concentrated H_2SO_4 was added to the obtained mixture. The presence of brown ring of the interface indicated deoxy sugar characteristic of cardiac glycosides (Trease & Evans, 1989).

Test for coumarins. 300 mg of each dried plant part was transferred to a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was boiled in water bath for few minutes. After removing the filter paper it was examined under UV light, yellow florescence indicated the presence of coumarins(Trease & Evans,1989) .

Test for phlobatannins. 80 mg of each dried plant extract was boiled in 1% aqueous hydrochloric acid; the deposition of a red precipitate indicated the presence of phlobatannins (Trease & Evans,1989) .

Test for tannins. 50 mg of each dried plant extract was boiled in 20 ml of distilled water and filtered. A few drops of 0.1% FeCl3 was added to the filtrate and observed for colour change; brownish green or ablue-black colouration was taken as evidence for the presence of tannins (Sofowara, 1993) .

Test for phenolic compounds. Total soluble phenolics in the ethanolic extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard using pyrocatechol as a standard (Taga et al., 1998; Slinkard & Singleton, 1977). Briefly, 1 ml from extract solution (2mg/ml) was transferred into a volumetric flask of 50 ml, and made up to 46ml with distilled water. Folin-Ciocalteu reagent (1 ml) was added and the contents of flask were mixed thoroughly. After 3 min, 3 ml of 2% aqueous solution of sodium carbonate (Na2CO3) was added, then the mixture was allowed

to stand for 2h with intermittent shaking. The absorbance was measured at 760 nm.

***Test for flavonoids*.** Flavonoid concentration was determined by diluting 1 ml of ethanolic. Extracts with 4.3 ml of 80% aqueous ethanol, 0.1 ml of 10% aluminum nitrate Al(NO3)3 and 0.1 ml of 1 M aqueous potassium acetate (CH3COOK) were added to the test tubes. The mixture was incubated for 40 min at room temperature, and the absorbance was determined spectrophotometerically at 417 nm.

Identification of the constituents of Mandragora autumnalis parts. Diluted samples (10 µl) of the ethanolic extracts of each plant part were injected in the GC-MS spectrophotometer. The device "Perkin Elmer clarus 500 Gas chromatography" and "Perkin Elmer clarus 560D mass spectrometer" were used in the analysis.

The gas, temperature of the injector = 280 ºC, carrier was 1ml/ min and total flow was 60ml/min. Column was Elite – 5MS, 30 meter 0.25 µm df. Max projection at temperature 360 ºC, and minimum bleed at 320 ºC.

The experimental conditions were as follows:

Temperature (ºC)	Time (min.)	Rate (10/min.)
60 (beginning)	15	10
110	5	10
200	5	10
280	5	

Chapter Three
Results

3.1 Antioxidant activity tests.

3.1.1 DPPH assay

Antioxidant potency was evaluated through free radical scavenging using ethanolic and water extracts of roots, leaves, ripe and unripe fruits of *Mandragora autumnalis* or the known antioxidants α-tocopherol and BHA. Figure 3.1 illustrates a decrease of DPPH radical due to the scavenging ability of ethanol extracts of root, leave, ripe, and unripe fruit and standards. BHA, and α-tocopherol were used as reference radical scavengers. The scavenging effect of root, leave, ripe, and unripe fruit and controls on the DPPH radical were in the following order: α-tocopherol > root > BHA > leave > un ripe > ripe fruit , with percentage scavenging values of 91.0%, 77.2%, 75%, 72.2%, 68.30%, and 5.7%, respectively, at the concentration of 250 µg/mL for exstracts. Root is the most active in DPPH radical scavenging. However, root does not have more DPPH radical scavenging ability than that of controls. The IC50 values of α-tocopherol , root , BHA , leave and un ripe fruit are 48.0, 37.5, 80.0,100,and 60.0, respectively (Figure 3.2). Free radical scavenging activity of these samples was also enhanced with increasing concentration.

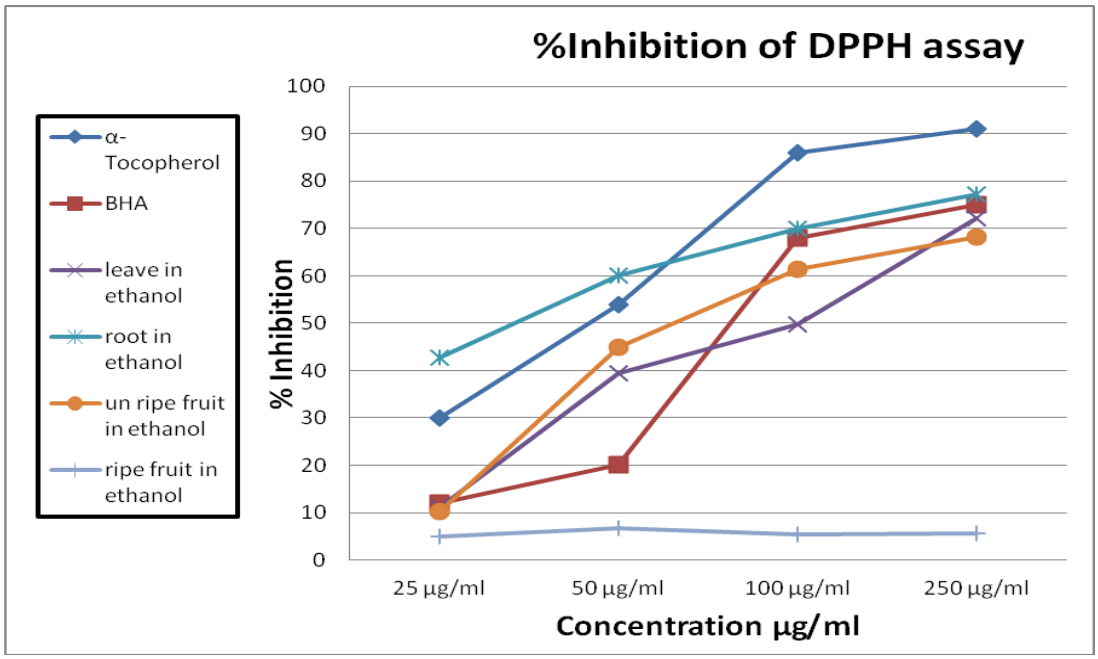

Figure:3.1 Free radical scavenging activity of different concentrations (25–250 µg/mL) of root, leave, ripe, unripe fruit, BHA, and TOC for1,1-diphenyl-2-picrylhydrazyl (DPPH).

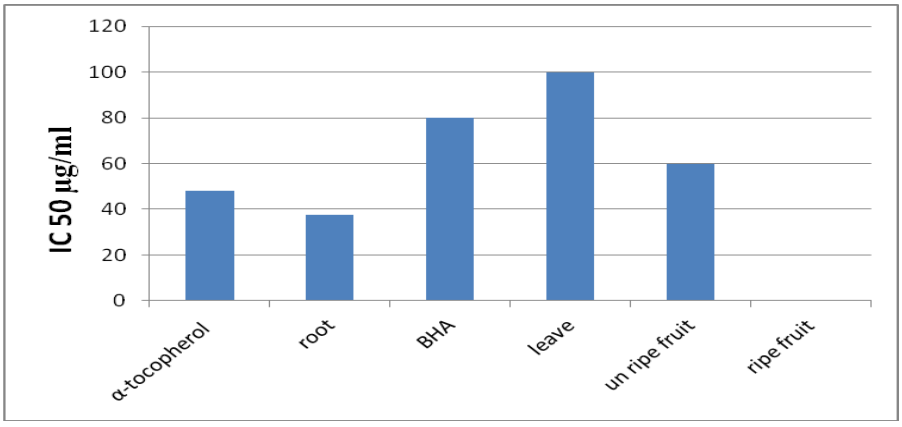

Figure 3.2 Free radical scavenging capacities IC50of ethanolic extracts

Additional result is also that when used different concentrations of DPPH 0.0004% , 0.0008% , 0.0012% , and 0.0016% and one concentration of ethanol and water extract 100 µg /ml .(Figure 3.3) illustrates that the percent inhibition values of the extract in ethanol was better than in water. It is found also that scavenging activity of free radicals is more in roots and leaves than the otherparts of Mandragora autamnalis fruit and ripe. The

concentration of 0.008 and 0.012 of DPPH are the most effective concentrations.

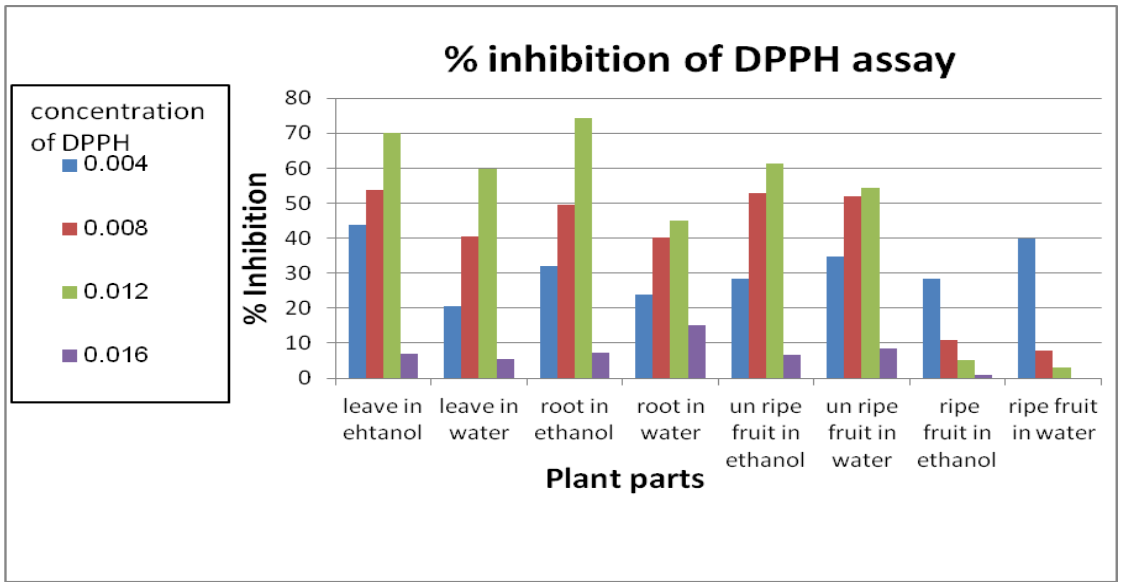

Figure 3.3 Free radical scavenging activity of water and ethanol extracts of root, leave, ripe and unripe fruit for different concentrations (0.004 – 0.016µg/ml) of 1,1-diphenyl-2-picrylhydrazyl (DPPH).

3.1.2 β-Carotene Linoleic acid Assay

Heat-induced oxidation of an aqueous emulsion system of β-carotenelinoleicacid was employed as another antioxidant test reaction. The test is based on the fact that β-carotene loses its color in the absence of antioxidant (Miller,1971). During oxidation, an atom of hydrogen is abstracted from the active methylene group of linoleic acid located on carbon-11 between two double bonds (Frankel,1998). The pentadienyl free radical so formed then attacks highly unsaturated β-carotene molecules in an effort to reacquire ahydrogen atom. As the β-carotene molecules lose their conjugation, the carotenoids lose their characteristic orange color. This process can be monitored spectrophotometrically . All ethanolic and water extracts of all parts of M. autumnalis revealed lower antioxidant

efficiency compared with water (control) and the synthetic antioxidant α-tocopherol which gave the lowest β-carotene color degradation (i.e. highest antioxidant efficiency) (Figure 3.4).

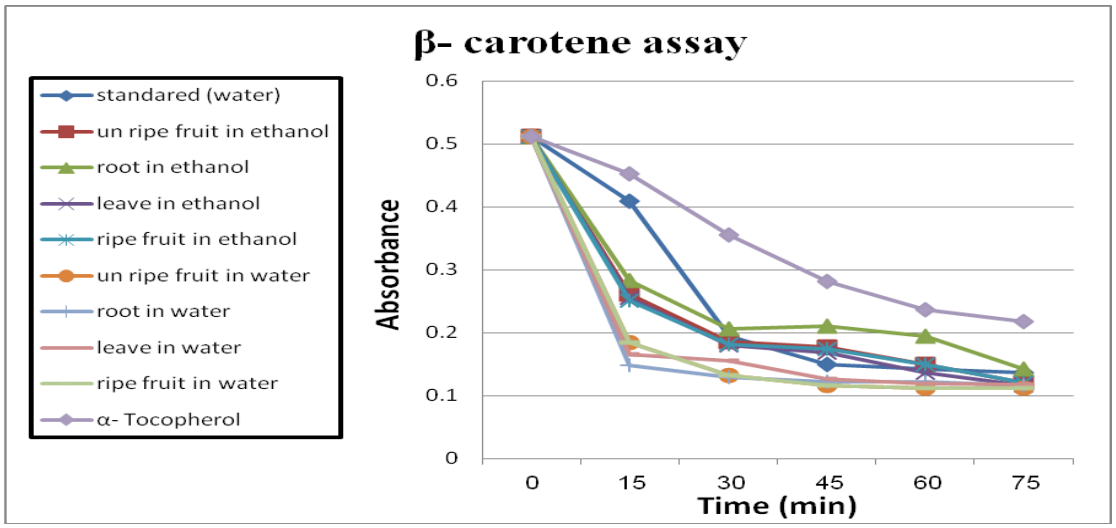

Figure: 3.4. Antioxidant activities of ethanolic and water extracts of all parts of Mandragora autumnalis (root,leafe,ripeandunripefruit),and and α- tocopherol, as assessed by β-carotene-linoleic acid assay over 75 minute.

3.1.3 Hydrogen Peroxide Scavenging Capacity.

The scavenging ability of ethanolic extracts of *Mandragora autumnalis* (leaves ,roots, ripe and un ripe fruits) on hydrogen peroxide are presented in Figure 3.5, and compared with α-tocopherol as standard. The mandragora extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. 500 µg of ethanol extracts of the plant parts exhibited 49.3-50% scavenging activity on hydrogen peroxide.On the other hand, using the same amounts, α-tocopherol exhibited 65% hydrogen peroxide scavenging activity. Results show that the scavenging activity values on hydrogen peroxide of 500 µg of the extracts of Mandragora parts

is less than that of α-tocopherol. in the order of α- tocopherol (65%) > un ripe extract (50%) > root leave (49.55%) > un ripe extract (49.3%). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Thus, the removing of H2O2 is very important for antioxidant defense in cell or food systems.

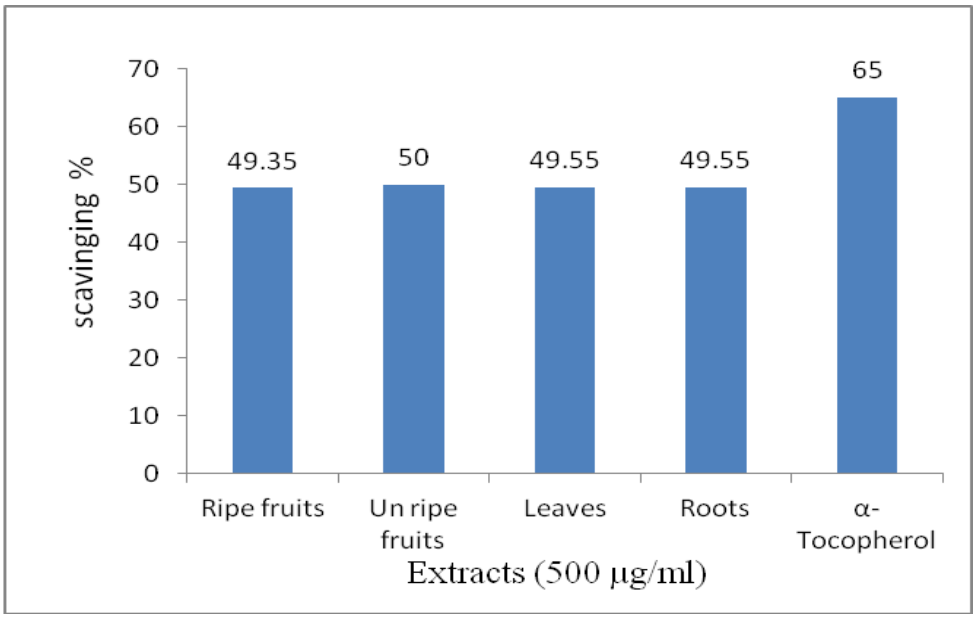

Figure: 3.5.Antioxidant activities of ethanolic and water extracts of all parts of Mandragora autumnalis ,and and α- tocopherol, as assessed by Hydrogen peroxide scavenging capacity.

3.2 antimicrobial activity

3.2.1 Antibacterial activity

The results of antibacterial activity of ethanolic and water extracts from *Mandragora autumnalis* plant parts are presented in Table 3.1. From our results, it was clear that the ethanolic root extract has shown to posses antibacterial activity against all tested bacterial strains at concentration 250 µg/ml, the other plant parts extracts did not show any activity against the

selected bacterial strains compared with the reference antibiotic gentamycin which was used as a positive control .

Table 3.1Antimicrobial activity of plant extracts against bacteria.

No	Plant extrac -ts	Inhibition zone diameter (mm)a Micro-organisms					
		p. vulgaris	E.coli. JM109	E. coli	p. aeruginosa	S. aureus	k. pneumo-niae
1	Genta m-icin	15.8±0.8	26±0.7	30.8±0.8	31.3±1.3	33.8±0.8	34.8±0.8
3	leaf in ethanol	0.0	0.0	0.0	0.0	0.0	0.0
4	leaf in water	0.0	0.0	0.0	0.0	0.0	0.0
5	un ripe in ethanol	0.0	0.0	0.0	0.0	0.0	0.0
6	Un ripe in water	0.0	0.0	0.0	0.0	0.0	0.0
7	root in ethanol	3.95±0.8	6.5±0.7	7.7±0.8	7.8±1.3	8.45±0.8	8.7±0.8
8	root in water	0.0	0.0	0.0	0.0	0.0	0.0
9	ripe in ethanol	0.0	0.0	0.0	0.0	0.0	0.0
10	Ripe in water	0.0	0.0	0.0	0.0	0.0	0.0

3.2.2 Antifungal activity

Different plant parts (root, leaf, unripe fruit, ripe fruit) were tested for three types of dermatophytes (yeast and rubrum) using poisoned technique method at different concentrations (25, 50, 100 and250 μg/ml) . None of the above parts of the plant showed any significant activity at the suggested concentrations.

3.3 Phytochemical screening of Mandragora autumnalis.

Phytochemical screening of all parts (leaves, roots, ripe and un ripe fruits) of *M. autumnalis* demonstrated the presence of anthraquinones, cardiac glycosides,coumarins, saponins, phlobatannins, tannins and terpenoids (Table 3.2) .

Table 3.2 Phytochemical screening of all parts of Mandragora autumnalis.

Chemical	Ripe fruits	Unripe fruits	Leaves	Roots
Saponins	-	+	-	+
Terpenoids	+	-	+	+
Anthraquinones	+	+	+	-
Glycosides	+	-	-	-
Coumarins	+	+	+	+
Phlobatannins	-	-	+	-
Tannins	+	+	+	-

3.3.1 Total flavonoid and phenolic compounds.

The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compound.

Estimation of total flavonoid content in all plant parts of *M. autumnalis*. Flavonoids are a large class of benzo-pyrone, total flavonoid concentration was calculated using quercetin as standard (Ljungvall et al., 2008) (figure 3.6).

Absorbance = 0.005358 μg quercetin – 0.0984 (R2: 0.9994)

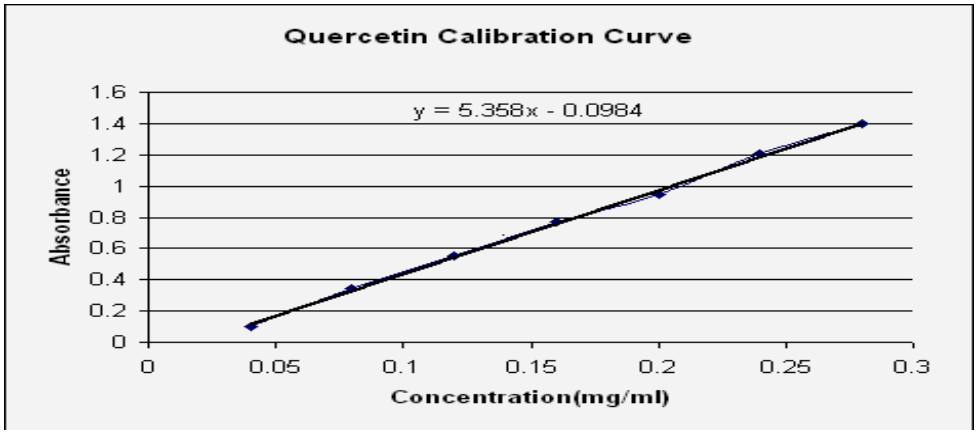

Figure: 3.6 Standard quercetin graph.

Total flavonoid concentrations were calculated for both ethanolic and water extracts and according to the equation (Absorbance = 5.1 µg quercetin – 0.083) (Gazzani et al., 1998). Unripe fruit ethanol extract had the highest concentration of flavonoid and also the concentrations of flavonoid in all ethanol extract higher than water extract (Table 3.3).The antiradical property of flavonoids is directed mostly toward hydroxyl, superoxide as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of freeradicals), their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron.

Table 3:3 Total flavonoid compounds in the ethanolic and water extracts determined as µg/mg of quercetin equivalents.

No.	Name of part of plant	Water extract (µg/mg)	Ethanolic extract(µg/mg)
1	Ripefruit	20	21
2	Root	19	22
3	Leave	19	21
4	un ripe fruit	21	23

The concentration of total phenolic compounds in the ethanolic extracts was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol calibration curve (figure 3.7) is given

as: Absorbance = 0.00 4757 µg pyrocatechol + 0.0022 (R2: 0.9997)

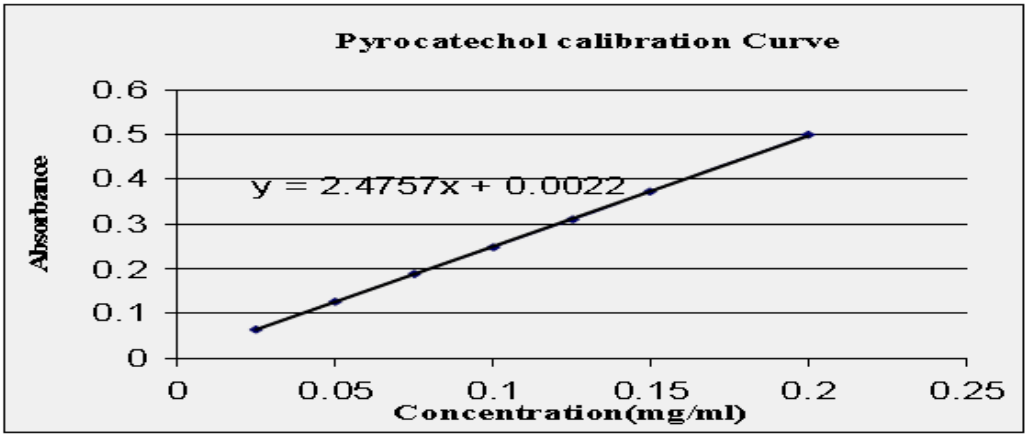

Figure: 3.7 standard pyrocatechol calibration graph

Estimation of total phenolic content in all parts of *Mandragra autumnalis* (root,leave,ripe and un ripe fruit) .Since the phenolic compounds are very important constituents of plants and known as powerful chain-breaking antioxidants (Shahidi &Wanasundara,1992) total phenolic content of the extracts was investigated and expressed as micrograms of pyrocatechol equivalents (PEs)per milligram of extract, as shown in (Table 3.4) ethanol extracts had higher phenolic contents than the water extracts also root and leave had the highest concentration of total phenolic compounds.

Table 3.4 Total phenolic compounds in the ethanolic and water extracts determined as µg/mg of Pyrocatechol as standard curve.

No.	Name of parts of plant	water extract (µg/mg)	ethanol extract (µg/mg)
1	Ripe fruit	19	21
2	Root	20	24
3	Leave	18	22
4	Un ripe fruit	9	10

3.3.2 GC-MS comparative studies

The compounds of each plant part were identified by GC-MS depending on the total ionic concentration values (TIC) and mass spectrum for each. 319 compounds were identified by GC–MS of ethanol extracts of unripe, ripe fruits, leaf and root of mandragora. The most important compounds found in these parts are listed in (Tables A1, A2, A3 and A4) in Appendix A. The chemical composition of different extracts were compared . To facilitate comparison, only the major components identified in each fraction were listed. Compounds shared by all plant parts are listed in Table 3.5.

Table 3.5 compounds shared by all plant parts of *Mandragora autumnalis*

10-Bromodecanoic acid, ethyl ester
9,12,15-OctaDecatrienoic Acid,Ethyl ester,(z,z,z)-
Dodecanoic acid
L-(+)-Ascorbic acid 2,6-Dihexadecanoate
N-decanoic acid
Nonanoic acid
Nonanoic acid, 9-bromo-, ethyl ester
Undecanoic acid
Pentadecanoic acid, ethyl ester

Compounds shared between (leaves and roots) ,(leave and un ripe fruit) and(unripe and root) as shown in Tables 3.6.,3.7,3.8 respectively

Table 3.6 compounds shared between the leaves and roots

10-Undecenoic Acid, Ethyl Ester
2-ChloroEthyl Oleate
HeptaCosanoic Acid, 25-Methyl -, Methyl Ester
Megastigma TriEnone
Tetra Decanoic Acid, 10,13-diDimethyl-,Methyl ester

Table 3.7 compounds shared between the leaves and unripe fruit

11,14,17-EicosaTriEnoic Acid, Methyl Ester
1-PentaTriAcontanol
2-PentaCosanone
3,7,11,15-TetraMethyl-2-Hexadecen-1-OL
4-Hexen-1-OL,5-Methyl-2-(1-MethylEthenyl)-,Acetate,(R)-
Androstan-17-One, 3-Ethyl-3-Hydroxy-,(5.Alpha.)-
Butanoic acid,3-Methyl-,3,7-DiMethyl-6-Octenyl ester
Caryophyllene
Humulen-(v1)
CycloHexanol,5-Methyl-2-(1-MethylEthyl)-,(1.Alpha.,2.Beta.,5.Alpha)
Z,Z-6,28-HeptaTriActontadien-2-one
Z-25-TetratriAconten-2-One
Bicyclo[5.2.0]Nonane,2-Methylene-4,8,8-Trimethyl-4-vinyl-

Table 3.8 compounds shared between the root and ripe fruit

2-Methoxy-4-Vinylphenol
3-phenyl-1-AZA-Bicyclo[1.1.0]butane
4-Hydroxy-2-Methylacetophenone
Metoprolol Di-TMS Derivative
Pentadecanoic acid, 14-Methyl -, Methyl ester
Tetradecanoic acid, ethyl ester
Trans-Cinnamic acid
Octanoic aAcid,2-Hexyl-

From our results, we found that some compounds were common in all plant parts of the *Mandragorta* except in the un ripe fruit such as Octadecanoic acid, 11-Methyl -, methyl ester and Tetra Cosanoic Acid. On

the otherhand, (Z)-14-Tricosenyl formate was found in all plant parts except the roots, and Hexadecanoic acid,ethyl ester was found in all plant parts except the leaves. Because the un ripe fruits of *Mandragora* caused madness as previously mentioned in the introduction, we isolate existing compounds that exist only in unripe fruit and does not exist in other parts of the plant as shown in table 3.9

Table 3.9 compounds found only in unripe fruits

Cyclohexane, 1Methyl-4-(1-MethylEthenyl)-,CIS-
CIS-8-Methyl-EXO-TriCyclo[5.2.1.0(2.6)]Decane
2(1H)-Benzocyclooctenone,Decahydro-10A-Methyl-,Trans-
2-Naphthalenemethanol,Decahydro-5-methylene-8-vinyl-
Bicyclo[5.2.0]Nonane,4-Methylene-2,8,8-Trimethyl-2-vinyl-
Bicyclo[7.2.0]Undec-4-ENE,4,11,11-Trimethyl-8-Methylene-
Butanoic acid,3-Methyl-,3,7-DiMethyl-6-Octenyl ester
9-OctaDecen-1-OL,(E)-
Oleyl Alcohol
Hexadecanoic acid,ethyl ester
9,12,15-OctaDecatrienoic Acid,Methyl ester,(z,z,z)-
Androstan-17-One, 3-Ethyl-3-Hydroxy-,(5.Alpha.)-
2-PentaCosanone
2-HeptaCosanone
1-PentaTriAcontanol
4-Methoxy-6-Methyl-6,7-DiHydro-4H-Furo[3,2-C]Pyran

Chapter Four
Discussion

4. Discussion

For many centuries, mandragora was one of the most important medicinal plants and a herb of great cultural value. Dioscorides wrote at length on the mandragora's medicinal qualities, mentioning the root, the fruit, and the leaves as remedies for many and varied ailments, including pain, insomnia, eye diseases, inflammation, and ulcers (Zohary,1982). In the present study, antioxidant and antimicrobial activities of ethanolic and water extracts from different parts of *Mandragora autamnalis* (leaf,root,ripe and unripe fruit) were assessed by using various in vitro testing systems.

In the present study, antibacterial activity of various extracts was assayed in vitro by disc diffusion method against six different bacterial strains. Among the parts screened,ethanol root extracts only showed antibacterial activity against six type of bacteria shown table (3.1)but other study showed that water leave extract had antibacterial activity against *S. aurues* strain (Obeidat et al., 2012) and another study also showed that ethanolic fruit extract had antibacterial activity against *E.coli* (Obeidat, 2011). This can be explained that they have used highest concentration of extracts than that has been used in our study.

Plant phenolics constitute one of the major groups of compounds acting as a primary antioxidant or free radical terminators. Antioxidant activity of phenolic compounds is attributed to their ability to donate hydrogen atoms to free radicals (Zin et al., 2006). In addition, they possess the structural properties of free radical scavengers which enable them to serve as potential antioxidants (Jayathilakan et al.,2007; Norshazila et al., 2010).

Total phenolic content of plants was evaluated using Folin-Ciocalteu method which measured the redox properties of polyphenols (Okonogi et al., 2007). Our results suggested that ethanolic extracts of mandragora leaves and roots contained highest amount of phenolic compounds. The antioxidant activity of the extracts obtained in this study was determined by using DPPH method.

DPPH is a useful reagent for investigating the free radical scavenging activities of phenolic compounds and a substrate to evaluate the antioxidative activity of antioxidants (Duh et al. , 1999) . Decreased DPPH absorption is an indication of the capacity of the extracts to scavenge free radicals, independent of any enzymatic activity. In this method, it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. In the radical form DPPH, this molecule had an absorbance at 517 nm that disappears after the acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus ,2002) .

The extracts obtained in this study showed a varied level of scavenging activities, highest activity being indicated by ethanol extract of leave and root . Similar to the total phenolic content, significant relationship could be established between antioxidant activity and phenolic content in this study.

The β-Carotene Linoleic acid Assay and Hydrogen Peroxide Scavenging Capacity were used also in this study for the analysis of antioxidant activity of Mandragora. Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However,

it may be toxic if converted to hydroxyl radical in the cell (Gulcin et al.,2003). Scavenging of H2O2 by the plant extracts may be attributed to their phenolics, which donate electron to H2O2, thus reducing it to water. The all extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner .

Plants with antioxidant activities have been reported to possess free radical scavenging activity (Das& Pereira,1990). Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Parr &Bolwell ,2000).

The β-Carotene Linoleic acid Assay showed a negative result for all parts of the plant. The difference in the antioxidant results using the three different antioxidant analysis tests might be attributed to the difference of the pathways for each analysis test.

The phytochemical analysis conducted on *Mandragora autamnalis* extract revealed the presence of tannins, flavonoids, steroids and saponins. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Ruch et al.,1989 ; Motar et al.,1985). Thus, *M. autamnalis* containing this compound may serve as a potential source of bioactive compounds in the treatment of cancer.Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li et al.,2003) and this property may explain the mechanisms of antioxidative action of

M. autamnalis. Flavonoids serve as health promoting compound as a results of its anion radicals (Hausteen,1983). These observations support the usefulness of this plant in folklore remedies in the treatment of pain, insomnia, eye diseases, inflammation, and ulcers as previously mentioned.Also, the plant extract was revealed to contain saponins, known to produce inhibitory effect on inflammation (Just ,1998) and are major ingredients in traditional Chinese medicine and thus responsible for most of the observed biological effects (Liu & Henkel,2002) and this tend to justify the use of *M.autmnalis* in traditional medicine. The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone (Okwu ,2001). (Azaizeh et al., 2006) said that Roots of *Mandragora autamnalis* have shape similar to human body and fruits that resemble human testis are used traditionally for stimulating sexual desire or treating sexual weakness. All parts of *M.autamnalis* contain Coumarin which constitutes one of the major classes of naturally occurring compounds, and interest in its chemistry continues unabated because of its usefulness as biologically active agents. It also represents the core structure of several molecules of pharmaceutical importance. Coumarin has been reported to serve as antibacterial (Ukhov et al.,2001 ; Liu et al .2008), anti-oxidant (Trapkov et al.,1996 ; Vukovic et al., 2010), anti-inflammatory (Emmanuel-Giota et al., 2001; Hamdi et al., 2007), anticoagulant (Hamdi et al., 2007) and antitumour (Wang et al.,2001; Marchenko et al.,2006) agents. Another study also proved the existence of derivatives of Cumarins in all parts of

mandragora (Al-Khalil & Alkofahi,1996). In GC-MS we isolate 219 compound from all parts of *Mandragora* most of these compounds is volatile compounds and Fatty Acid many study isolate the similar compounds from ripe and unripe fruits only such as (Baser et al.,1998) who isolate 100 volatile compound from fresh ripe fruit and (Hanuš et al., 2005) Compare between compounds which isolated by GC-MS from ripe and un ripe fruits but in this study we compare between compounds which isolate from (roots, leaves, ripe and unripe fruits).

References

- Abu-Rabia, A. (2005). ***Herbs as food and medicine source in Palestine***. Asian Pacific Journal of Cancer Prevention, 6, 404-407.
- Abu-Rabia A(1999). ***Herbs as a Food and Medicine Source in Palestine***. Bedouin's Traditional Medicine, Tel-Aviv:Mod Publishing (in Hebrew).
- Ali-Shtayeh, M. S., & Jamous, R. M., (2006). **Ethnobotany of Palestinian herbal medicine in the Northern West Bank and Gaza Strip: Review and a comprehensive field study**. BERC, Palestine, Nablus, Til, No. 4.
- Ali-Shtayeh, M. S., Yaniv, Z., & Mahajna, J. (2000). **Ethnobotanical survey in the Palestinian area: A classification of the healing potential of medicinal plants**. Journal of Ethnopharmacology, 73, 221-232.
- Ali-Shtayeh, M. S., Jamous, Rana M., Jamous, R. M. (2011). ***Complementary and Alternative Medicine use amongst Palestinian diabetic patients***. Complementary Therapies in Clinical Practice, doi:10.1016/j.ctcp.2011.09.001
- Ali-Shtayeh, M. S., Jamous, Rana M., Jamous, R. M. (2012***). Herbal preparation use by patients suffering from cancer in Palestine***. Complementary Therapies in Clinical Practice, 17 (04), 235-240. http://dx.doi.org/10.1016/j.ctcp.2011.06.002
- Al-Khalil, S., Alkofahi, A., (1996). **The chemical constituents of Mandragora autumnalis**. Alexandria J. Pharm. Sci. 10, 135,138.–

- Ames BN, Shigenaga MK, Hagen TM.(1993). **Oxidants, antioxidants and the degenerative diseases of aging**. Proceedings of the National Academy of Sciences USA:; 90: 7915-22.
- Ames BN.(1983). **Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases**. Science; 221: 1256-63.
- Aqil F, Ahmed I, Mehmood Z. (2006). ***Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants***. Turk J Biol 30: 177-183.
- Azaizeh. H., Saad, B., Khalil, Kh., & Said, O. (2006). **The State of the Art of Traditional Arab Herbal Medicine in the Eastern Region of the Mediterranean**: A Review. eCAM;3(2)229–235.
- Badugu , L. R. (2012). ***Phytochemical Screening, Quantitative Estimation Total Phenolics and Total Flavonoids, Anti Microbial Evaluation of Cyamopsis tetragonoloba***. International Journal of Research in Pharmaceutical and Biomedical Sciences Vol. 3 (3): 1139-1142.
- Bailey, C., & Danin A. (1981). **Bedouin plant utilization in Sinai and the Negev**. Economic Botany,35,145–162.
- Balandrin, M.F., J.A. Klocke, E.S. Wurtele and W.H. Bollinger, (1985). **Natural plant chemicals**: Sources of Industrial and Medicinal materials. Science, 228: 1154-1160.
- Barros L; Joao Ferreira M; Queiros B; Ferreira IC; Baptista P (2007). ***Total phenol, ascorbic acid, β-carotene and lycopene in Portuguese***

wild edible mushroom and their antioxidant acitivities. Food Chem. 413-419.

- Baser, K.H.C., Kurkcuoglu, M., Demirci, B., Erdogmus, H., (1998) **Composition of the essential oil and the headspace sample of Mandragora autumnalis Bertol**. fruits. J. Ess. Oil Res. 10, 632–634.
- Berman BM, Swyers JP, Kaczmarczyk J. (1999). **Complementary and alternative medicine: herbal therapies for diabetes**. J Assoc Acad Mino Phys 10:10–14.
- Bonjar G.and Farrokhi P.R. (2004). ***Antibacillus activity of some plants used in traditional medicine of Iran***. Nigar. Journal of Natural Products and Medicine , 8: 34-39.
- Burits M., Bucar F. (2000). ***Antioxidant activity of Nigella sativa essential oil***. Phytother. Res., 14, 323–328.
- Carter, A.J.,(2003). **Myths and mandrakes**. J. Roy. Soc. Med. 96, 14_147.
- Cowan, M. M. (1999). **Plant products as antimicrobial agents**. Clinical Microbiology Reviews, 12:564-582.
- Cragg, GM, NewmanDJ, Snader KM. (1997). ***Natural products in drug discovery and development***. Journal of Natural products 60, 52-62.
- Cuendet M., Hostettmann K., Potterat O. (1997). ***Iridoid glucosides with free radical scavenging properties from Fagraea blumei, Helv***. Chem. Ac., 80, 1144-1152.

- Dafni, A., Yaniv, Z., & Palevitch, D. (1984). ***Ethnopharmacological survey of medicinal plants in northern Israel***. Journal of Ethnopharmacology, 10, 295-310.
- Das NP, Pereira TA(1990). ***Effect of flavonoids on thermal auto-oxidation of palm oil: structure Activity relationship***. J Am oil Chem Soc,67:255-258.
- 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.
- Dimayuga R.E.and Garcia S.K. (1991). ***Antimicrobial, screening of medicinal plants from Baja California sur, Mexico***. Journal of Ethnopharmacology , 31:181-192.
- Doughari J.H. (2006). ***Antimicrobial activity of Tamarindus indica Linn***. Tropical Journal of pharmaceutical Research, 5: 597-603.
- Duh PD, Tu YY, Yen GC.(1999): ***Antioxidant activity of aqueous extract of harnjyur (Chrysanthemum morifolium Ramat)***. LWT Food Sci Technol;32:269–277.
- Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC.(1998). **Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey.JAMA**. Nov 11;280(18):1569–1575.

- Elmastas M, Isildak O, Turkekul I, Temur N.(2007). **Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms**. J Food Comp Anal; 20: 337-45.
- Elmastas, M., Gülçin, İ., Beydemir, Ş., Küfrevioğlu, & Aboul-Enein, Ö.İ., H.Y. (2006). **A study on the invitro antioxidant activity of juniper (*Juniperus communis L.*) seeds extracts, Anal**. Lett. 39, 47-65.
- Emmanuel-Giota, A.A.; Fylaktakidou, K.C.; Hadjipavlou-Litina , D.J.; Litinas, K.E. and Nicolaides, D.N. J.(2001). ***Heterocyclic*** Chem., 38, 717-722.
- Frankel E. N. (1998). ***Hydroperoxide formation. In Lipid oxidation***. Dundee:The Oily Press. (23-41).
- Fransworth NR, Soejarto DD, (1985). **Potentil consequences of plant extinction in the United States on the current and future availability of prescription drugs**. Economic Botany 39, 231 – 240.
- Friedman, J., Yaniv, Z., Dafni, A., & Palevitch, D. (1986). ***A preliminary classification of healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among Bedouins in the Negev desert, Israel***. Journal of Ethnophrmacology, 16, 275-287.
- Fyhrquist p. (2007). **Traditional medicinal uses and biological activities of some plant extracts of African Combretum Loefl., Terminalia L.and pteleopsis Engl**. Species (Combretacea). Helsinki University printing House. pp183.

- Gazzani G., Papetti A., Massolini G., Daglia M., (1998). ***Antioxidant and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment***, J. Agric. Food Chem., 46, 4118-22.
- Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI(2003). ***Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L) seed extracts***. Food Chem, 83:371-382.
- Gülçin, I., Oktay, M., Küfrevio˘glu, Ö.˙I., Aslan, A., (2002). ***Determination of antioxidant activity of lichen Cetraria islandica (L.) Ach***. Journal of Ethnopharmacology 79, 325–329.
- Hall CA, Cuppet SL.(1997**).Structure-activities of natural antioxidants. In: Aruoma OI, Cuppett SL, editors**. Antioxidant Methodology: in vivo and in vitro concepts. Champaign, IL: AOCS Press;. p. 2-29.
- Halliwell, B. (1991) **Reactive oxygen species in living systems: Source, biochemistry, and role in human disease**. American Journal of Medicine, 91, pp. 14-22.
- Halliwell, B., & Gutteridge, J.M.C. (1985). **Free Radicals in Biology and Medicine, Oxford**: Oxford University Press.
- Hamdi, N. and Dixneuf, P. H. (2007) .**Synthesis of Triazole and Coumarin Compounds**. Topics in Heterocyclic Chemistry, Springer-Verlag, Berlin Heidelberg,.

- Hanuš LO , Řezanka T , Spížek j, and Dembitsky V.M. (2005). **Substances isolated from *Mandragora* species**. Phytochemistry 66: 2408–2417.
- Harborne JB,(1973). ***Phytochemical methods. London: Chapman and Hall, Ltd;:pp. 49–pp. 188.Hausteen B: Flavonoids, a class of natural products of high pharmacological potency***. Biochem Pharm 1983, 32:1141-1148.
- Hunt LM, Arar NH, Akana LL. (2000). **Herbs, prayer, and insulin: use of medical and alternative treatments by a group of Mexican-American diabetic patients**. J Fam Pract 49:216–223.
- Husein, A. (2010***). Modification of biologically active compounds from selected medicinal plants in Palestine***. PhD thesis, Nablus, Palestine: An-Najah National University.
- Hausteen B:,(1983).**Flavonoids, a class of natural products of high pharmacological potency**. Biochem Pharm , 32:1141-1148.
- Jackson B.P, and Berry M.I,(1979). ***Mandragora Taxonomy and Chemistry of the European species***. Linnean Society Symposium Series, London, Academic Press, 39, 505.
- Jayaraman S., Manoharan M.S.and Illanchezian S. (2008). **In-vitro Antimicrobial and Antitumor Activities of Stevia Rebaudiana (Asteraceae)**
- Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS (2007).***Antioxidant potential of synthetic and natural antioxidants***

and its effect on warmedover- flavour in different species of meat. Food Chem., 105: 908–916.

- Just MJ, Recio MC, Giner RM, Cuellar MJ, Manez S, Bilia AR, Rios JL,(1998). ***Anti-Inflammatory activity of unusual lupine saponins from***. Bupleurum fruticescens, 64:404-407.
- Khalaf, N A., Shakya, A. K., Al-Othman, A., El-Agbar, Z., And Farah, H. (2008). **Antioxidant Activity of Some Common Plants**. Turk J Biol 32 51-55
- Kourounakis, A.P., Galanakis, D., & Tsiakitzis, K. (1999). **Drug Develop**. Res. 47.
- Li H, Wang Z, Liu Y,(2003). ***Review in the studies on tannins activity of cancer prevention and anticancer***. Zhong-Yao-Cai, 26(6):444-448.
- Liu J, Henkel T,(2002). ***Traditional Chineese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities?*** Curr Med Chem, 9:1483-1485.
- Liu, X.; Dong, M.; Chen, X.; Jiang, M.; Lv, X. and Zhou, J,(2008). ***Antimicrobial activity of an endophytic Xylaria sp.YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin***. Appl Microbiol Biotechnol., 78, 241–247.
- Ljungvall, K., Veeramachaneni, D. N. R., Hou, M., Hultén, F., & Magnusson, U. (2008). **Morphology and morphometry of the reproductive organs in prepubertal and postpubertal male pigs exposed to di(2-ethylhexyl) phthalate before puberty: Precocious development of bulbourethral glands**. *70 (6)*, 984-991.

- Madavi, D. L., & Salunkhe, D. K. (1995***). Toxicological aspects of***
- ***food antioxidant***. In D. L. Madhavi, S. S. Deshpande, &D. K. Salunkhe (Eds.), Food antioxidants (p. 267). New York Marcel Dekker.
- Marchenko, M. M.; Kopyl'chuk, G. P.; Shmarakov, I. A; Ketsa, O. V. and Kushnir, V. N,(2006). ***Synthesis and Biological Evaluation of Some Coumarin Derivatives***. Pharm.Chem. J., 40, 296-297.
- Matthäus B.(2002). **Antioxidant activity of extracts obtained from residues of different oilseeds**. J. Agric. Food Chem. ;50:3444–3452.
- Mau, J. L., Chao, G. R. and Wu, K. T. (2001). ***Antioxidant properties of methanolic extracts from several ear mushrooms***. J. Agric. Food Chem. 49, 5461-5467.
- Miller H. E. (1971). ***A simplified method for the evaluation of antioxidants***, J. Am. Oil Chem. Soc. ,48 , 92-97.
- Motar MLR, Thomas G, Barbosa Fillo JM,(1985). ***Effects of Anacardium occidentale stem bark extract on in vivo inflammatory models***. J Ethnopharm, 95(2-3):139-142.
- Murray P.R., Baron E.J., Pfaller M.A., Tenover F.C., Yolke R.H. (1995): **Manual of Clinical Microbiology**. 6th Ed. ASM, Washington.
- Niki, E., Shimaski, H., & Mino, M. (1994). ***Antioxidantisim-Free Radical and Biological Defense***. Tokyo: Gakkai Syuppan Center.
- Norshazila S, Syed ZI, Mustapha SK, Aisyah MR, Kamarul RK (2010). **Antioxidant Levels and Activities of Selected Seeds of Malaysian Tropical Fruits**. Mal. J. Nutr., 16(1): 149-159.

- Obeidat, M. (2011). ***Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens***. Journal of Medicinal Plants Research Vol. 5(16), pp. 3856-3860, 18 August, 2011.
- Obeidat, M., Shatnawi, M., Al-Alawi, M., Al-Zu'bi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J., & Otri, I. (2012). ***Antimicrobial activity of crude extracts of some plant leaves***. Research Journal of Microbiology, 7(1): 59-67.
- Okonogi S, Duangrat C, Anuchpreeda S, Tachakittirungrod S, Chowwanapoonpohn S (2007). ***Comparison of antioxidant capacities and cytotoxicities of certain fruit peels***. Food Chem., 103: 839–846.
- Okwu DE,(2001**). Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae**. Pak Vet J, 14:160-162.
- Palevitch, D., & Yaniv, Z. (1991). ***Medicinal plants of the Holy Land***. Tel-Aviv: Modan Publishing House. I: 24-29 (Hebrew).
- Parr A, Bolwell GP,(2000). ***Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet bymodifying the phenols content or profile***. J Sci Food Agric, 80:985-1012.
- Robak J, Shridi F, Wolbis M, Krolikowska M,(1988). **Screening of the influence of flavonoids on lipoxygenase and cyclooxygenase activity, as well as on non-enzymatic lipid oxidation**. Pol J Pharmacol Pharm; 40: 451-8.
- Ruch, R. J., Cheng, S. J. and Klaunig, J. E. (1989). **Prevention of cytotoxicity and inhibition of intercellular communication by**

antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10, 1003–1008.

- Sabe VA, Van Den Vijgh WJF, Bast F,(1996). **Structural aspects of antioxidant activity of flavonoids**. Free Rad Bio Med; 20 Suppl 3: 331–342.
- Salah N, Miller J, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C,(1995). **Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants**. Arch Biochem Biophys; 322 Suppl 2: 339–346.
- Satish S., Raghavendra M.P. and Raveesha K.A. (2008). **Evaluation of the Antibacterial Potential of some plants Against Human Pathogenic Bacteria** . Advances in Biological Research, 2: 44-48.
- Satyajit D. S., Lutfun N. (2007). **Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry, The Atrium, Southern Gate, Chichester, West Sussex, England** : John Wiley & Sons Ltd,
- Shahidi, F., and Wanasundara, P.K.J.P.D. (1992). ***Phenolic antioxidants***. Crit. Rev. Food Sci. 32, 67–103.
- Shariff N., M.S. Sudarshana S. Umesha and bioactive p. Hariprasad. (2006). ***Antimicrobial activity of Rauvolifa tetraphylla and physalis minima leafe and callus extracts***. African Journal of Biotechnology , 5:946-950.
- Sharvelle, E. G., (1961). **The nature and use of modern fungicides**. Burgess Publishing Co., Minnesota, USA, pp. 308.

- Shtayeh, M., S., Hamad, A. Kh., (1995). ***Protection of the Palestinian Environment***. Nablus, Alhasoub Alarabi (Arabic).
- Slinkard K., Singleton V.L. (1977). ***Total phenol analyses***: automation and comparison with manual methods. American J. Enolo. Viticul., 28,49-55.
- Soejarto DD, Fransworth NR. (1989). **Tropical rain forests**: Potential source of new drugs? Perspectives in Biology and Medicine. 32, 244-256.
- Soejarto DD. (1996). **Biodiversity prospecting and benefit sharing**: perspectives from the field. J. of Ethnopharmacology 51, 1 – 15.
- Sofowara AE,(1993). **Medicinal plants and traditional medicine in Africa**. 2nd edition.Ibadan, Nigeria: Spectrum books Ltd.;:289.
- Srivastava, J., J. Lambert and N. Vietmeyer,(1996). ***Medicinal plants: An expanding role in development***. World Bank Technical Paper. No. 320.
- Taga M. S., Miller E. E. , Pratt D. E. (1998***). Chia Seeds as a Source of Natural Lipid Oxidant***, J. Am. Oil Chem. Soc. ,61, 928-31.
- Trapkov, V. A.; ***Parfenov, E.A. and Smirnov***, L. D. Pharm. Chem. J. (1996), 30, 445-447.
- Trease GE, Evans WC: Pharmacognsy,(1989).. 11th edition. London: **Brailliar Tiridel Can Macmillian Publishers**;:60–75.
- Ukhov, S. V.; Kon'shin, M. E. and Odegova, T. F.(2001). ***Synthesis and antimicrobial activity of 2-iminocoumarin-3-carboxyli c acid amides***. Pharm. Chem. J., 35, 364-365.

- Uniyal, S.K., K.N. Singh, P. Jamwal and B. Lal, (2006). **Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan**. African . J. Ethnobiol. Ethnomed., 2: 1-14.
- Vidal, F.,(1982). **An anesthetic 19 centuries old: mandragora**. Chi Dent. Fr. 52, 21–26.
- Vukovic, N.; Sukdolak, S.; Solujic, S. and Niciforovic, N,(2010). **Synthesis and Biological Evaluation of Some Coumarin Derivatives**. Arch. Pharm. Res., 33, 5-15.
- Wanasundara UN, Shahidi F(1998).. **Antioxidant and pro-oxidant activity of green tea extract in marine oils**. Food Chem; 63: 335-42.
- Wang, M.; Wang, L.; Li, Y. and Li, Q.(2001). **Synthesis and Biological Evaluation of Some Coumarin Derivatives**.Trans. Met. Chem., 26, 307-310.
- Wettasinghe M., Shahidi F. (2000): ***Scavenging of reactive species and DPPH free radicals by extracts of borage and evening primrose meals***. Food Chemistry,70: 17–26.
- World Health Organization (WHO). (2003). **Guidelines on good agricultural and collection practices (GACP) for medicinal plants**.
- Yaghmour, R. (1997) **Antimicrobial Activity of Twenty Plants Used in Folkloric Medicine in Palestine. MSc Thesis**. Nablus, Palestine: An-Najah National University.
- Zohary,M., Plants of the Bible.(1982). **New York**: Cambridge University Press, Cambridge.

- Zainol MK, Abd-Hamid A, Yusof S, Muse R,(2003). ***Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of Centella asiatica (L.) urban***. Food Chem; 81: 575-81.
- Zin ZM, Hamid AA, Osman A, Saari N (2006). ***Antioxidative activities of chromatographic fractions obtained from root, fruit, and leaf of Mengkudu (Morinda citrifolia L.)***. Food Chem., 94: 169-178.

Appendix A:
Compounds found in *Mandragora autumnalis* parts

Table A.1: Compounds found in ripe fruit

Compound Name	M.W.	Formula
Propennitrile,3-ethoxy-2-(2-Thienylmethylsulfonyl)-	257	C10H11O3NS2
Cyclohexane, 1-butenylidene-	136	C10H16
9-Methyl-11-OXO-1,6Diazatricyclo[7.2.0.0(6,8)] Undecane	180	C10H16ON2
(Z)-14-Tricosenyl formate	180	C10H16ON2
N-decanoic acid	172	C10H20O2
Cyclopropane, 1-Ethenyl-2-hexenyl-,[1.Alpha.,2.Beta.(E)]-(.+/-.)-	150	C11H18
1,4-Methanophthalazine, 1,4,4A,5,6,7,8,8A-Octahydro-9,9-Dimethyl-	178	C11H18N2
2-Cyclohexylpiperidine	167	C11H21N
Nonanoic acid, 9-bromo-, ethyl ester	264	C11H21O2Br
Undecanoic acid	186	C11H22O2
10-Bromodecanoic acid, ethyl ester	278	C12H23O2Br
Dodecanoic acid	200	C12H24O2
Dodecane, 1-Fluoro-	188	C12H25F
Octane, 1-(Butyl thio)-	202	C12H26S
Octanoic aAcid,2-Hexyl-	228	C14H28O2
Octanoic acid,Hexyl ester	228	C14H28O2
Butyl Caprate	228	C14H28O2
N-C apric acid Isobutyl ester	228	C14H28O2
Dodecanioc acid, ethyl ester	228	C14H28O2
Tetradecanoic acid	228	C14H28O2
14-pentadecenoic acid	240	C15H28O2
Pentadecanoic acid, 15-Bromo-	320	C15H29O2Br
Decanamide, N-Pentyl-	241	C15H31ON
Benzenesulfonohydrazide,N2-(2-Ethoxybenzylideno)-4-Methhyl	318	C16H18O3N2S
2-Furancarboxylic acid, Undecyl ester	266	C16H26O3
N-Butyl laurate	256	C16H32O2
Isobutyl laurate	256	C16H32O2
Dodecanioc acid, 1-Methylpropyl ester	256	C16H32O2

Tetradecanoic acid, ethyl ester	256	C16H32O2
Methyl 2,8-Dimethyl Tridecanoate	256	C16H32O2
Methyl 9-Methyl Tetradecanoate	256	C16H32O2
Octanoic acid, Nonyl ester	270	C17H34O2
Pentadecanoic acid, ethyl ester	270	C17H34O2
Tetradecanoic acid, 10,13-Dimethyl-,Methyl ester	270	C17H34O2
Pentadecanoic acid, 14-Methyl -, Methyl ester	270	C17H34O2
Hexadecanoic acid,ethyl ester	284	C18H36O2
.Beta.-1,5-O-Dibenzoyl-Ribofuranose	358	C19H18O7
1,3-Dioxolane,4,5-Dimethyl-2-Pentadecyl-	312	C20H40O2
Hexadecanoic acid, Butyl ester	312	C20H40O2
Octadecanoic acid, 11-Methyl -, methyl ester	312	C20H40O2
Metoprolol Di-TMS Derivative	411	C21H41O3NSi2
2-Furancarboxylic acid, Heptadecyl ester	350	C22H38O3
(Z)-14-Tricosenyl formate	366	C24H46O2
Tetracosanoic acid	368	C24H48O2
L-(+)-Ascorbic acid 2,6-Dihexadecanoate	652	C38H68O8
1,2-Pentanediol	104	C5H12O2
2-Thiophenemethanamine	113	C5H7NS
2-Furancaroboxaldehyde,5-(Hydroxymethyl)-	126	C6H6O3
2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-one	144	C6H8O4
Thiophene,2-Propyl-	126	C7H10S
Benzoic Acid	122	C7H6O2
2-Ethoxy-2-Cyclohexen-1-one	140	C8H12O2
1H-Imidazole,2-(Diethoxymethyl)-	170	C8H14O2N2
Octanoic acid	144	C8H16O2
Conhydrin	143	C8H17ON
3,4-Dimethyl-3-Hexanol	130	C8H18O
Phenylglyoxal	134	C8H6O2
2-Methoxy-4-Vinylphenol	150	C9H10O2
4-Hydroxy-2-Methylacetophenone	150	C9H10O2
4-Hydroxy-3-Methylacetophenone	150	C9H10O2
Cyclopentanone, 2-(1-Methyl propyl)-	140	C9H16O
3,6-Nonadien-1-OL,(E,Z)-	140	C9H16O
N-hexyl Acrylate	156	C9H16O2
N-(1-Methoxycarponyl-1-Methylethyl)-4-Methyl-2-AZA-1,3-Dioxane	203	C9H17O4N
Nonanoic acid	158	C9H18O2
Ribitol, 1,3:4,5-DI-O-(Ethylboranediyl)-2-Deoxy-	212	C9H18O4B

		2
Trans-Cinnamic acid	148	C9H8O2
(Z)- Cinnamic acid	148	C9H8O2
2-Propenoic acid, 3-phenyl-	148	C9H8O2
3-phenyl-1-AZA-Bicyclo[1.1.0]butane	131	C9H9N
2-Chloroethyl Benzoate	184	C9H9O2Cl

Table A.2 compounds found in unripe fruit

Compound Name	M.W.	Formula
Cyclohexane, 1Methyl-4-(1-MethylEthenyl)-, CIS-	138	C10H18
CycloHexanol,5-Methyl-2-(1-MethylEthyl)-, (1.Alpha.,2.Beta.,5.Alpha)	156	C10H20O
CycloHexanol,5-Methyl-2-(1-MethylEthyl)-,[1S-(1.Alpha.,2.Beta.,5.Beta)	156	C10H20O
N-decanoic acid	172	C10H20O2
CIS-8-Methyl-EXO-TriCyclo[5.2.1.0(2.6)]Decane	150	C11H18
Nonanoic acid, 9-bromo-, ethyl ester	264	C11H21O2Br
Undecanoic acid	186	C11H22O2
4-Hexen-1-OL,5-Methyl-2-(1-MethylEthenyl)-, Acetate,(R)-	196	C12H20O2
10-Bromodecanoic acid, ethyl ester	278	C12H23O2Br
Dodecanoic acid	200	C12H24O2
2(1H)-Benzocyclooctenone,Decahydro-10A-Methyl-,Trans-	194	C13H22O
2-Naphthalenemethanol,Decahydro-5-methylene-8-vinyl-	206	C14H22O
Bicyclo[5.2.0]Nonane,2-Methylene-4,8,8-Trimethyl-4-vinyl-	204	C15H24
Humulen-(v1)	204	C15H24
Bicyclo[5.2.0]Nonane,4-Methylene-2,8,8-Trimethyl-2-vinyl-	204	C15H24
Bicyclo[7.2.0]Undec-4-ENE,4,11,11-Trimethyl-8-Methylene-	204	C15H24
Caryophyllene	204	C15H24
Butanoic acid,3-Methyl-,3,7-DiMethyl-6-Octenyl ester	240	C15H28O2
Pentadecanoic acid, ethyl ester	270	C17H34O2
9-OctaDecen-1-OL,(E)-	268	C18H36O
Oleyl Alcohol	268	C18H36O
Hexadecanoic acid,ethyl ester	284	C18H36O2
9,12,15-OctaDecatrienoic Acid,Methyl ester,(z,z,z)-	292	C19H32O2
3,7,11,15-TetraMethyl-2-Hexadecen-1-OL	296	C20H40O
Androstan-17-One, 3-Ethyl-3-Hydroxy-, (5.Alpha.)-	318	C21H34O2

11,14,17-EicosaTriEnoic Acid,Methyl ester	320	C21H36O2
(Z)-14-TriCosenyl Formate	366	C24H46O2
2-PentaCosanone	366	C25H50O
2-HeptaCosanone	394	C27H54O
Z-25-TetraTriAconten-2-One	490	C34H66O
1-PentaTriAcontanol	508	C35H72O
Z,Z-6,28-HeptaTriActontadien-2-one	530	C37H70O
L-(+)-Ascorbic Acid 2,6-DiHexaDecanoate	652	C38H68O8
4-Methoxy-6-Methyl-6,7-DiHydro-4H-Furo[3,2-C]Pyran	168	C9H12O3
Nonanoic acid	158	C9H18O2

Table A.3 compounds found root

Compound Name	M.W.	Formula
Benzoic Acid, 2-(1-oxopropyl)-	178	C10H10O3
2,5-Dihydroxy-4-Isopropyl-2,4,6-Cycloheptatrien-1-One	180	C10H12O3
Cyclopentane, 1-Ethenyl-3-ethyl-2-methyl-	138	C10H18
N-decanoic acid	172	C10H20O2
2,2-DIEthyl-N-Ethylpyrrolidine	155	C10H21N
5-Decanol	158	C10H22O
Benzofuran, 2-Ethenyl-	144	C10H8O
1- Naphthalenol	144	C10H8O
Benzaldehyde, 3-(ChloroAcetoxy)-4-Methoxy-	228	C10H9O4Cl
Spiro[4.4]Nona-1,3-Diene, 1,2-Dimethyl-	148	C11H16
5,6-Undecadiene	152	C11H20
Nonanoic acid, 9-OXO-,Ethyl ester	200	C11H20O3
11-Bromo undecanoic Acid	264	C11H21O2Br
Nonanoic acid, 9-bromo-, ethyl ester	264	C11H21O2Br
11-bBromoUndecanoic Acid	264	C11H21O2Br
Undecanoic acid	186	C11H22O2
1-EthylAmino-1-propylcyclohexane	169	C11H23N
Diethyl Phthalate	222	C12H14O4
Trans,Cis-1,8-Dimethyl Spiro[4.5]Decane	166	C12H22
Trans,Trans-1,8-DiMethylSpiro[4.5]Decane	166	C12H22
Propionic Acid, 3-(2-MethylCycloHexyl)-, Ethyl ester	198	C12H22O2
10-Bromodecanoic acid, ethyl ester	278	C12H23O2Br
Dodecanoic acid	200	C12H24O2
N,N-DIEthyl-1-Cyclopropyl-pentanamine	183	C12H25N
9-AminoFluorene	181	C13H11N
2-(2-Phenyl Vinyl)Pyridine, Trans	181	C13H11N
Megastigma TriEnone	190	C13H18O
Tri Cyclo[6.3.0.0(1,5)]UnDEC-2-EN-4-ONE, 5,9-Dimethyl-	190	C13H18O
Bicyclo[6.3.0]Undeca-1(8),9-Diene,	176	C13H20

11,11-Dimethyl-		
Cis,Cis-1,9-DiMethylSpiro[5.5]Undecane	180	C13H24
Cis,Trans-2,9-DiMethylSpiro[5.5]Undecane	180	C13H24
10-Undecenoic Acid,Ethyl Ester	212	C13H24O2
Benzene, 1-Chloro-4-(4-MethoxyBenzyloxy)-3-Nitro-	293	C14H12O4NCl
Benzene, 1,1-[Oxybis(Methylene)]BIS-	198	C14H14O
Propane dinitrile, [3-(4-Methoxyphenyl)-1-Methylpropylidene]-	226	C14H14ON2
ChloroAcetic Acid, Dodec-9-ynyl ester	258	C14H23O2Cl
Octanoic Acid, 2-Hexyl-	228	C14H28O2
Tetra Decanoic Acid	228	C14H28O2
1-DiMethyl(ChloroMethyl)SilyloxyUndec-10-ENE	276	C14H29OClSi
Benzene, 1-TriFluoromethyl-4-(3-MethoxyBenzyloxy)-3-Nitro-	327	C15H12O4NF3
Benzene Propionic Acid, 4-Benzyloxy-	256	C16H16O3
Benzene Sulfono hydrazide, N2(2-EthoxyBenzylideno)-4-Methyl	318	C16H18O3N2S
1,2-Benzene DiCarboxylic Acid,Butyl 2-Methylpropyl ester	278	C16H22O4
1,E-6,Z-10-Hexadecatriene	220	C16H28
9-AZABICYCLO[6.1.0]Nonane, 9,9-Azobis-,[1.Alpha.,8.Alpha.,9[E(1R*,8S*)]]-	276	C16H28N4
11-Dodecenoic Acid, 2,4,6-TriMethyl-, Methyl ester, (R,R,R)-(-)-	254	C16H30O2
Tetra Decanoic Acid,Ethyl ester	256	C16H32O2
Pentadecanoic acid, Methyl ester	256	C16H32O2
Methyl 9-Methyl Tetradecanoate	256	C16H32O2
TetraDecanoic Acid, 12-Methyl-, Methyl ester,(s)-	256	C16H32O2
Tetra Decanoic Acid,Ethyl ester	256	C16H32O2
1,3-Dioxolane-2-Heptanenitrile, .Alpha.-Methyl-.Delta.-oxo-2-phenyl	287	C17H21O3N
Pentadecanoic acid, ethyl ester	270	C17H34O2
Tetra Decanoic Acid, 10,13-dDimethyl-,Methyl ester	270	C17H34O2

PentaDecanoic acid, 14-Methyl-,Methyl ester	270	C17H34O2
6-[4-Methoxybenzyloxy]-8-Nitrolepidine	324	C18H16O4N2
Hexadecanoic acid,ethyl ester	284	C18H36O2
Hexadecanoic acid, 15-Methyl-, Methyl ester	284	C18H36O2
Heptadecanoic Acid, Methyl ester	284	C18H36O2
Hexadecanoic acid, 14-Methyl-, Methyl ester	284	C18H36O2
9,12,15-OctaDecatrienoic Acid,Methyl ester,(z,z,z)-	292	C19H32O2
Di(1-Decynyl)Mercury	476	C20H34Hg
9,12,15-OctaDecatrienoic Acid,Ethyl ester,(z,z,z)-	306	C20H34O2
2-ChloroEthyl Oleate	344	C20H37O2Cl
Ethanol, 2-(9-Octadecenyloxy)-,(E)-	312	C20H40O2
OctaDecanoic Acid, 11-Methyl-, Methyl ester	312	C20H40O2
Metoprolol DI-TMS Derivative	411	C21H41O3NSi2
Heptacosanoic Acid, 25-Methyl-, Methyl ester	438	C29H58O2
9-HexaDecenoic Acid, 9-Octadecenyl ester,(z,z)-	504	C34H64O2
L-(+)-Ascorbic Acid 2,6-DiHexaDecanoate	652	C38H68O8
2,4(1H,3H)-Pyrimidinedione, 5-(TriFluoromethyl)-	180	C5H3O2N2F3
2-Pyridine Carbonitrile, 3-Nitro-	149	C6H3O2N3
Hydrazine, (PhenylMethyl)-	122	C7H10N2
1,2,4-TriAzabicyclo[2.2.2]Octan-3-One, 2-Acetyl-	169	C7H11O2N3
2-N-Propyl Thiolane, S,S-Dioxide	162	C7H14O2S
Cis-2,4-DiMethyl Thiane, S,S-Dioxide	162	C7H14O2S
Trans-2,4-DiMethyl Thiane, S,S-Dioxide	162	C7H14O2S
2-N-Hexyl Thiolane, S,S-Dioxide	162	C7H14O2S
Phenyl Ethyl Alcohol	122	C8H10O
2-Pentanone, 1,1-DiMethoxy-3-Methyl-	160	C8H16O
Benzaldehyde, 3-Hydroxy-4-Methoxy-	152	C8H8O3
Vanillin	152	C8H8O3
2-Methoxy-4-Vinylphenol	150	C9H10O2

4-Hydroxy-2-MethylAcetophenone	150	C9H10O2
4-Hydroxy-3-MethylAcetophenone	150	C9H10O2
3-Hydroxy-4-Methoxymandelic Acid	198	C9H10O5
Nonanoic acid	158	C9H18O2
Benzene Acetic Acid, .Alpha.-Methylene-	148	C9H8O2
Trans-Cinnamic Acid	148	C9H8O2
3-Phenyl-1-AZA-Bicyclo[1.1.0]Butane	131	C9H9N

Table A.4 compounds found in the leaves.

Compound Name	M.W.	Formula
CycloHexanol,5-Methyl-2-(1-MethylEthyl)-, (1.Alpha.,2.Beta.,5.Alpha)	156	C10H20O
CycloHexanol,5-Methyl-2-(1-MethylEthyl)-, [1S-(1.Alpha.,2.Beta.,5.Beta)	156	C10H20O
N-decanoic acid	172	C10H20O2
CIS-4-Amino-1,2,3,4-TetraHydro-2-Naphthoic Acid	191	C11H13O2N
Trans-2,6-DiMethyl-.Beta.-Methyl-.Beta.-Nitrostyrene	191	C11H13O2N
Nonanoic Acid, 9-Bromo-, Ethyl Ester	264	C11H21O2Br
Undecanoic acid	186	C11H22O2
5-BenzoCyclooctenol, 5,6,7,8-Tetrahydro-,(E)-	174	C12H14O
Coumarin, 3,4-Dihydro-4,4,7-TriMethyl-	190	C12H14O2
1H-Indene-4-Carboxylic Acid, 2,3-Dihydro-1,1-DiMethyl-	190	C12H14O2
CycloHexane, 1-Ethenyl-3-Methylene-5-(1-Propenylidene)-	160	C12H16
Cyclooctene, 4-Methylene-6-(1-propenylidene)-	160	C12H16
3-HydroxyMethyl-4-(1-Hydroxy-2-Methylprop-2-Enyl)Toluene	192	C12H16O2
Benzene, 1,4-Bis(1-MethylEthyl)-	162	C12H18
4-Hexen-1-OL,5-Methyl-2-(1-MethylEthenyl), Acetate,(R)-	196	C12H20O2
10-Bromodecanoic acid, ethyl ester	278	C12H23O2Br
Dodecanoic Acid	200	C12H24O2
1,4-MethanonAphthalen-9-OL, 1,2,3,4-Tetrahydro-,Acetate, Syn-	202	C13H14O2
2-(1,3-Butadienyl)-1,3,5-TriMethyl-	172	C13H16
NaphThalene, 1,2-Dihydro-1,5,8-TriMethyl-	172	C13H16
NaphThalene, 1,2-Dihydro-1,4,6-TriMethyl-	172	C13H16
NaphThalene, 1,2-Dihydro-4,6,8-TriMethyl-	172	C13H16
Benzene, 2-(1,3-Butadienyl)-1,3,5-TriMethyl-	172	C13H16
Benzene, 2-(1,3-Butadienyl)-1,3,5-TriMethyl-	172	C13H16
NaphThalene, 1,2-Dihydro-3,6,8-TriMethyl-	172	C13H16
1H-Inden-1-One, 2,3-DiHydroTetraMethyl-	188	C13H16O
1H-Inden-1-One, 2,3-DiHydro-3,3,4,6-TetraMethyl-	188	C13H16O

1H-Indene, 2,3-Dihydro-1,1,5,6-TetraMethyl-	174	C13H18
Naphthalene, 1,2,3,4-Tetrahydro-1,1,6-TriMethyl-	174	C13H18
Naphthalene, 1,2,3,4-Tetrahydro-1,5,8-TriMethyl-	174	C13H18
Naphthalene, 1,2,3,4-Tetrahydro-1,6,8-TriMethyl-	174	C13H18
Naphthalene, 1,2,3,4-Tetrahydro-1,4,6-TriMethyl-	174	C13H18
TetraCyclo[4.2.1.0(3,7).0(2,9)]Non-4-ENE, 4-butyl-	174	C13H18
Benzene, 1-CycloHexyl-3-Methyl-	174	C13H18
1H-Indene, 2,3-Dihydro-1,1,5,6-TetraMethyl-	174	C13H18
Benzene, 2-(2-Butenyl)-1,3,5-TriMethyl-	174	C13H18
Naphthalene, 1,2,3,4-TetraHydro-1,6,8-TriMethyl-	174	C13H18
2,5,8-TriMethyl-1,2,3,4-Tetrahydronaphthalene	174	C13H18
Benzene, 1-CyclopropylMethyl-4-(-1-MethylEthyl)-	174	C13H18
(1,4-DiMethylpent-2-Enyl)Benzene	174	C13H18
1-Hexanone, 5-Methyl-1-phenyl-	190	C13H18O
Megastigma TriEnone	190	C13H18O
3-(4-Isopropylphenyl)-2-Methylpropion aldehyde	190	C13H18O
Benzenepropanal, 4-(1,1-DiMethylEthyl)-	190	C13H18O
1,4-MethanoCycloOcta[D]pyridazine, 1,4,4A,5,6,9,10,10A-Octahydro-1	204	C13H20N2
10-Undecenoic Acid, Ethyl Ester	212	C13H24O2
Bicylo[4.1.0]Heptane, 7-Bicyclo[4.1.0]Hept-7-ylidene-	188	C14H20
1,4,6,7-Tetra Methyl 1,2,3,4-TetraHydroNaphthalene	188	C14H20
Naphthalene, 1,2,3,4-Tetrahydro-1-Methyl-8-(1-MethylEthyl)-	188	C14H20
1,3-CycloHexaDiene, 2,6,6-TriMethyl-1-(3-Methyl-1,3-Butadienyl)-	188	C14H20
5,6-Decadien-3-yne, 5,7-Diethyl-	190	C14H22
TetraDecanoic Acid	228	C14H28O2
N-Benzyl-2-PhenethylAmine	211	C15H17N
Caryophyllene	204	C15H24

BiCyclo[5.2.0]Nonane, 2-Methylene-4,8,8-TriMethyl-4-Vinyl-	204	C15H24
Humulen-(V1)	204	C15H24
1H-3A,7-Methanoazulene, Octa Hydro-1,4,9,9-TetraMethyl-	206	C15H26
1-Methylene-2B-HydroxyMethyl-3,3-DiMethyl-4B-(3-MethylBut-2-	222	C15H26O
1-Methylene-2B-HydroxyMethyl-3,3-DiMethyl-4B-(3-Methylbut-2-Enyl	222	C15H26O
Butanoic acid,3-Methyl-,3,7-DiMethyl-6-Octenyl ester	240	C15H28O2
Benzamide, 4-Benzoyl-N-(Immino)(Methyl Thio)Methyl-	298	C16H14O2N2S
TetraDecanoic Acid, Ethyl Ester	256	C16H32O2
Penta decanoic acid, ethyl ester	270	C17H34O2
Tetra decanoic Acid, 10,13-DiMethyl -, Methyl Ester	270	C17H34O2
Penta Decanoic Acid, 14-Methyl -, Methyl Ester	270	C17H34O2
HeptaDecanoic Acid	270	C17H34O2
6,6-Biquinoline	256	C18H12N2
Ethyl 9-HexaDecenoate	282	C18H34O2
17-OctaDecene-9,11-Diynoic Acid, 8-OXO-, Methyl ester	302	C19H26O3
9,12,15-OctaDecatrienoic Acid,Methyl ester,(z,z,z)-	292	C19H32O2
17-OctaDecen-14-Ynoic Acid, Methyl Ester	292	C19H32O2
9,12-Octadecadienoic Acid (z,z)-, Methyl Ester	294	C19H34O2
9,11-Octadecadienoic Acid, Methyl Ester, (E,E)-	294	C19H34O2
Bicyclo[10.1.0]TriDeca-4,8-Diene-13-Carboxylic Acid(2-Hydroxy-4-	356	C20H24O4N2
9,12,15-OctaDecatrienoic Acid,Ethyl ester,(z,z,z)-	306	C20H34O2
2-ChloroEthyl Linoleate	342	C20H35O2Cl
9,12-Octadecadienoic Acid, Ethyl Ester	308	C20H36O2
2-ChloroEthyl Oleate	344	C20H37O2Cl
3,7,11,15-TetraMethyl-2-Hexadecen-1-OL	296	C20H40O
Isophytol	296	C20H40O
OctaDecanoic Acid, 11-Methyl -, Methyl Ester	312	C20H40O2

Benzoic Acid, 2-Benzoyl-1-(phenylMethyl)Hydrazide	330	C21H18O2N2
Methyl Eicosa-5,8,11,14,17-pentaenoate	316	C21H32O2
Androstan-17-One,3-Ethyl-3-Hydroxy-, (5.Alpha.)-	318	C21H34O2
11,14,17-EicosaTriEnoic Acid, Methyl Ester	320	C21H36O2
CycloDecacyclo Tetra decene, 14,15-Didehydro-1,4,5,8,9,10,11,12,13,	296	C22H32
22-TriCosenoic Acid	352	C23H44O2
(Z)-14-TriCosenyl Formate	366	C24H46O2
TetraCosanoic Acid	368	C24H48O2
2-PentaCosanone	366	C25H50O
2-(3-Nitrophenyl)-4,4-Diphenyl-4H-Benz[D][1,3]Oxazine	406	C26H18O3N2
4H-3,1-Benzoxazine, 2-(4-Nitrophenyl)-4,4-Diphenyl-	406	C26H18O3N2
(E,E,E)-(5-phynyl Sulfonyl Geranyl)Geranoil	430	C26H38O3S
3-OxaTriCyclo[20.8.0.0(7,16)]TriAconta-1(22),7(16),9,13,23,29-Hexaene	406	C29H42O
HeptaCosanoic Acid, 25-Methyl -, Methyl Ester	438	C29H58O2
DiBenzo[A,H]CycloTetraDecene, 2,3,11,12-TetraEthyenyl-1,2,3,4,5,6,7,8	404	C30H40
1,30-TriAcontanedoil	454	C30H62O2
Z-25-TetratriAconten-2-One	490	C34H66O
Hexadecanoic acid, 2Hydroxy-1,3-propanEdiyl Ester	568	C35H68O5
1-pentaTriAcontanol	508	C35H72O
Z,Z-6,27-HeXaTriActontadien-2-one	516	C36H68O
Z,Z-6,28-HeptaTriActontadien-2-one	530	C37H70O
Z-28-HeptatriAconten-2-One	532	C37H72O
L-(+)-Ascorbic Acid 2,6-DiHexaDecanoate	652	C38H68O8
1,37-OctaTriAcontAdiene	530	C38H74
Tetra Contane-1,40-Diol	594	C40H82O2
Beta.-L-Arabinopyranose	150	C5H10O5
2H-Pyrrol-2-One, 1,5-DiHydro-4-Methoxy-	113	C5H7O2N
D-GlucoHexo DiAldose	178	C6H10O6
Hexanoic Acid	116	C6H12O2
Heptanoic Acid	130	C7H14O2
3,4-Methylpropyl Succinimide	155	C8H13O2N
Hexyl Ethylphosphono Fluoridate	196	C8H18O2FP

3-Methylpentyl Ethylphosphono Fluoridate	196	C8H18O2FP
Benzene ACetaldehyde	120	C8H8O
2-(4-Bromophenyl)-2-Hydroxy-N-Methyl-Acetamide	243	C9H10O2NBr
Benzene, Propyl-	120	C9H12
4-Methoxy-6,7-Dihydro-4H-Furo[3,2-C]pyran	168	C9H12O3
Ethanol, 2-[(phenylMethyl)Amino]-	151	C9H13ON
Nonanoic Acid	158	C9H18O2
1,3,4-OXADiazolium, 5-Mercapto-2-Methyl-3-phenyl-,Hydroxide,	192	C9H8ON2S

Appendix B:
Figures for GC-MS spectrum for Ethanolic plant extract

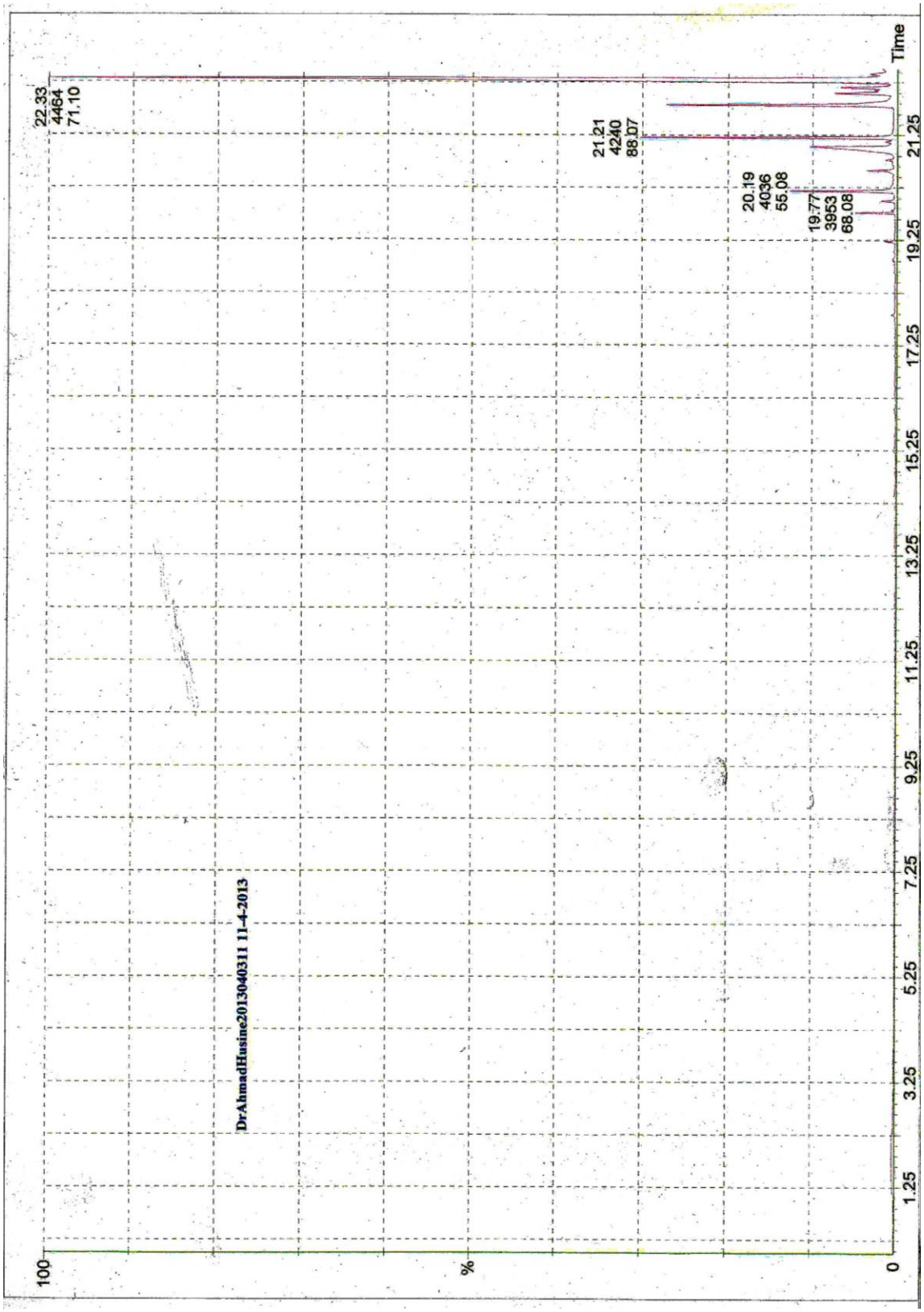

Figure B1: GC-MS spectrum for ethanolic extract of Unripe fruit

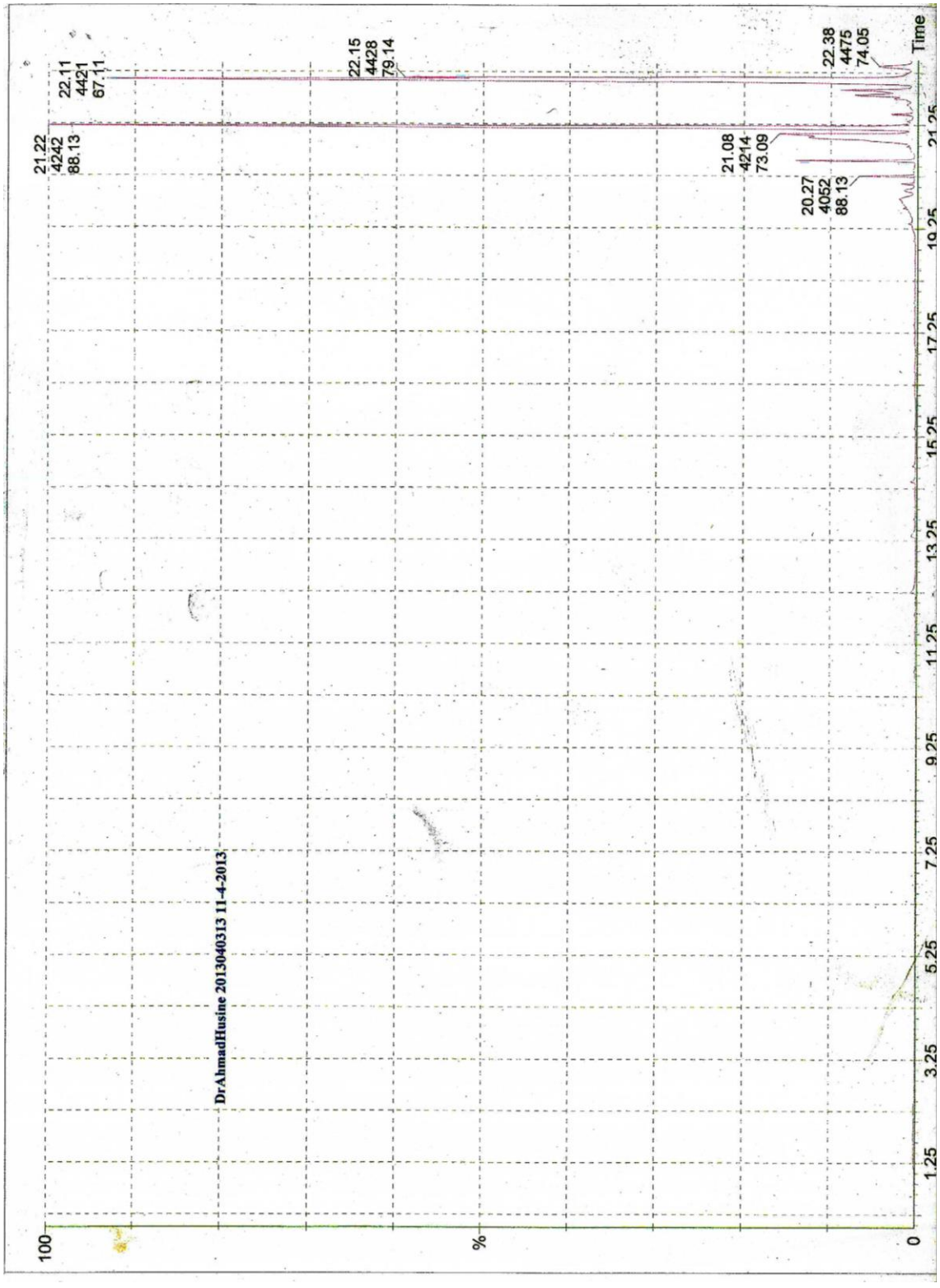

Figure B2: GC-MS spectrum for ethanolic extract of root

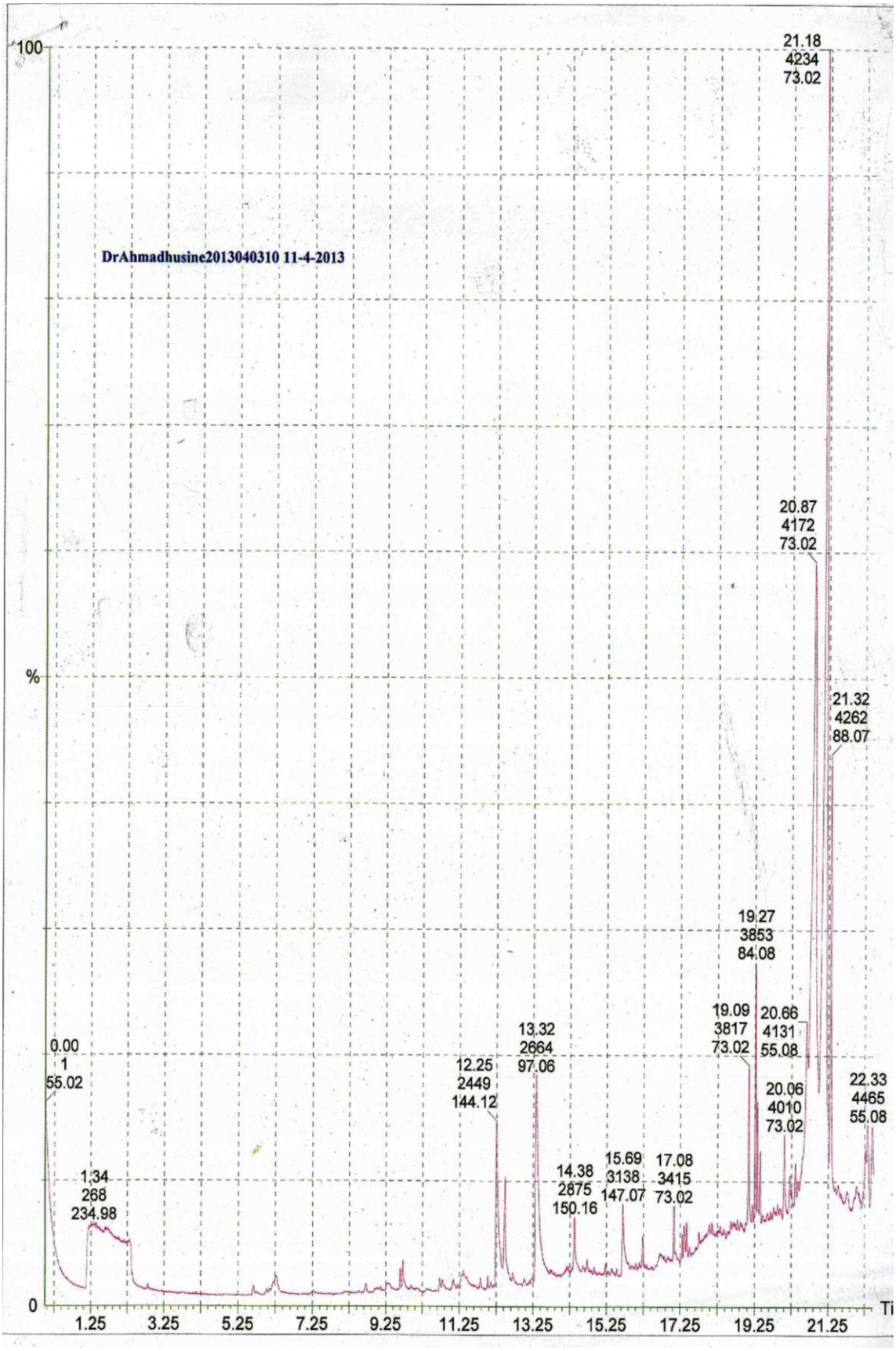

Figure B3: GC-MS spectrum for ethanolic extract of Ripe fruit

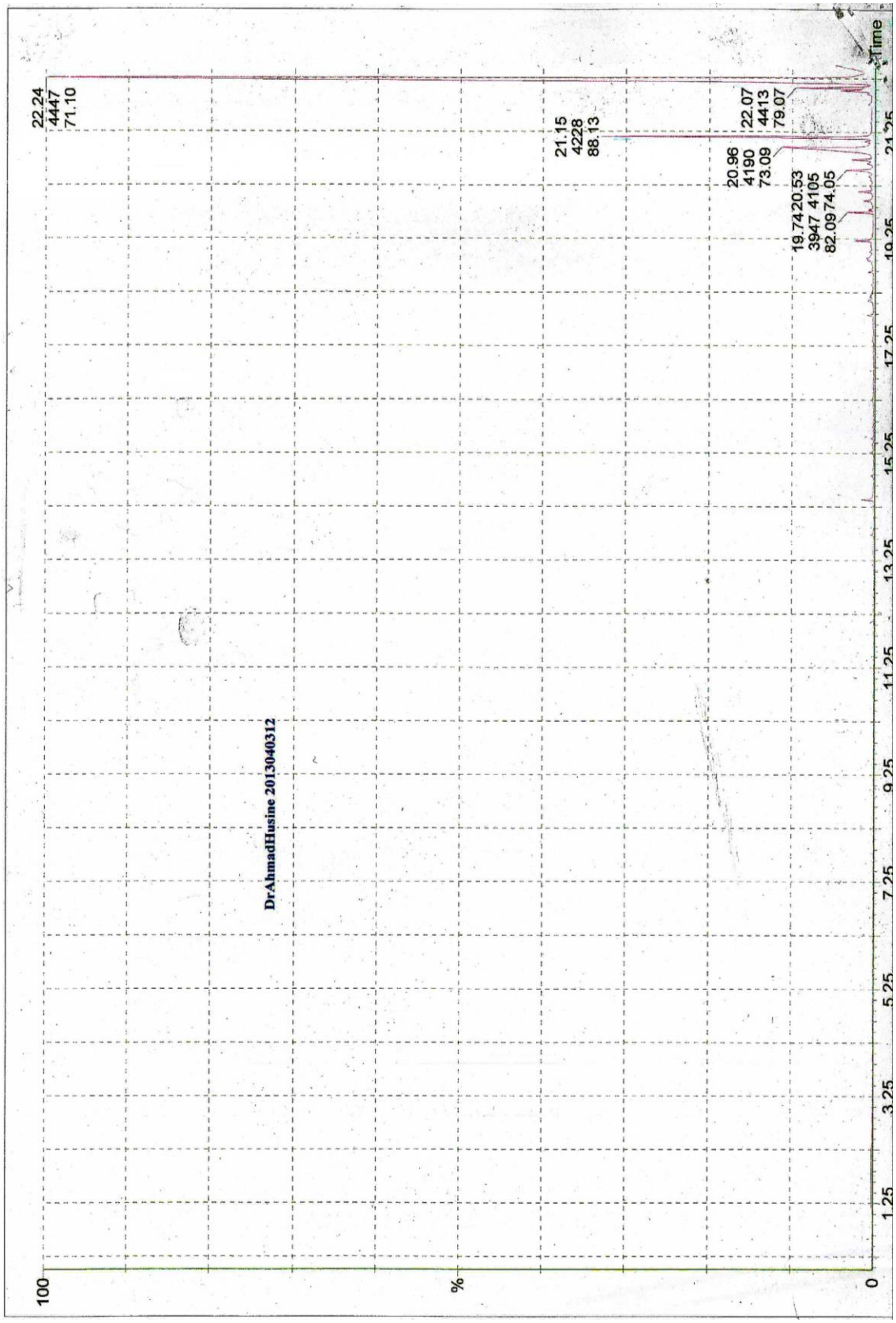

Figure B4: GC-MS spectrum for ethanolic extract of Leaf

جامعة النجاح الوطنية
كلية الدراسات العليا

المواد المضادة للبكتيريا والمضادة للأكسدة المأخوذه من نبات تفاح المحبة (*Mandragora autumnalis*)

إعداد
نور بشار احسان جاد الله

إشراف
أ.د. محمد سليم علي اشتية

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين .
2013

المواد المضادة للبكتيريا والمضادة للأكسدة المأخوذه من نبات تفاح المحبة (*Mandragora autumnalis*)

اعداد

نور بشار جاد الله

اشراف

أ.د. محمد سليم علي اشتية

الملخص

خلفية علمية: يعتبر تفاح المحبة mandragora أحد النباتات الطبية ذات الأهمية الطبية و ذو قيمة ثقافية كبيرة. لا يزال النبات يستخدم على نطاق واسع في الطب العربي الفلسطيني التقليدي، TAPHM، لعلاج أمراض كثيرة ومتنوعة، بما في ذلك الألم، والأرق، وأمراض العيون ، والالتهابات، والتقرحات.

أهداف الدراسة: تهدف هذه الدراسة إلى تقييم نشاط مضادات الأكسدة للأجزاء المختلفة من نبات تفاح المحبة باستخدام DPPH, β –carotene linoleic acid assays, hydrogen peroxide scavenging activity assays. وتقييم نشاط الأجزاء المختلفة للنبات كمضادات للميكروبات.

طرق البحث: تم تحديد نشاط مضادات الأكسدة للأجزاء المختلفة من نبات تفاح المحبة باستخدام DPPH, β –carotene linoleic acid assays, hydrogen peroxide scavenging activity assays، في حين تم تحديد نشاط أجزاء النبات كمضادات للميكروبات باستخدام تقنية Disc diffusion method لقياس فاعلية أجزاء النبات كمضاد للبكتيريا، وتقنية modified poisoned food technique لتحديد فاعلية النبات كمضاد للفطريات. كما تم استخدام مقياس الطيف الضوئي GC–MS لتحديد المكونات النشطة من جميع أجزاء النبات.

نتائج الدراسة: أظهرت نتائج الدراسة أن جذور وأوراق نبات تفاح المحبة لها نشاط مضاد للأكسدة باستخدام تقنية DPPH، في حين أن تقنية β –carotene linoleic acid assays , لم تظهر أي نشاط مضاد للأكسدة للأجزاء المختلفة للنبات على عكس تقنية hydrogen peroxide

scavenging activity assays والتي أظهرت أن جميع لأجزاء النبات تمتلك نشاط مضاد للأكسدة. كما أظهرت الدراسة أن المستخلص الكحولي لجذور النبات تمتلك نشاط مضاد للبكتيريا، في حين أن أجزاء النبات المختلفة لم تظهر أي نشاط مضاد للفطريات. أظهرت نتائج دراسة التركيب الكيميائي لمكونات النبات باستخدام تقنية GC–MS أن أجزاء النبات المختلفة تحتوي على نحو 219 مادة كيميائية. عند مقارنة المحتوى الكيميائي لأجزاء النبات المختلفة، وجد أن جميع أجزاء النبات تحتوي على مادة الكومارين، في حين توجد الأنثراكينونات والعفص في جميع أجزاء النبات باستثناء الجذور، كما وجد أن الجليكوسيدات توجد في الثمار الناضجة فقط.