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Antimicrobial Activity of Fifty-Four Plants Used in Folkloric Medicine in Palestine

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DEDICATION

TO

MY DEAR PARENTS, SISTERS FOR

THEIR ENCOURAGEMENT, WITH LOVE

AND RESPECT

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CMA	Corn Meal Agar
DMSO	Dimethyl Sulfoxide
FCCAU	Fungal Culture Collection of An-Najah University
L	Liter
mg	Milligram
MHA	Muller Hinton Agar
MHB	Muller Hinton Broth
MIC	Minimum Inhibitory Concentration
mm	Millimeter
No.	Number
SDA	Sabouraud Dextrose Agar
µg	Microgram

ANTIMICROBIAL ACTIVITY OF FIFTY-FOUR PLANTS USED IN FOLKLORIC MEDICINE IN PALESTINE

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ABSTRACT

Ethanolic and aqueous extracts of fifty four plant species used in folk medicine in Palestine for treatment of several infections and diseases were investigated for their antimicrobial activities against thirteen microbial isolates belonging to one yeast, *Candida albicans*; four gram negative bacteria, *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris* and *Pseudomonas aeroginosa*, and one gram positive bacterium, *Staphylococcus aureus*, and two isolates of dermatophytes, *Microsporum canis* and *M. gypseum*, and five isolates of plant pathogenic mycelial fungi, *Fusarium tricinictum, Pythium ultimum, P. aphanidermatum, P. middletonii,* and *Phytophthora citrophthora.*

Two susceptibility tests were used in this work, the disk diffusion method for measuring the antibacterial and anticandidal activity and the poisoned-food technique for measuring the antifungal activity.

The results demonstrated that the plans studied differ significantly in their activity against test microorganisms. The most active plants against both gram positive and gram negative bacteria include *Alcea setosa, Coridothymus capitatus* and *Satureja thymbra.*

For *C. albicans,* the most active plants include *Coridothymus capitatus, Satureja thymbra,* and *Quercus calliprinos.*

For dermatophytes, the most active plants include the ethanolic extracts of *Coridothymus capitatus, Micromeria nervosa,* and *Satureja thymbra,* and the aqueous extracts of *Anthemis tunictoria* and *Verbascum sinuatum.* For phytopathogenic *Pythium sp.*, the most active plants the ethanolic extracts of *Micromeria nervosa, Pinus halepensis* and *Satureja thymbra* and the aqueous extracts of *Rubia tenuifolia, Anthemis tunictoria* and *Coridothymus capitatus.* For phytopathogenic *Phytophthora citrophthora* the most active plants include the ethanolic extracts of *Pinus halepensis* and *Satureja thymbra.*

For phytopathogenic *Fusarium tricinictum,* the most active plants include the ethanolic extracts of *Salvia fruticosa* and *Satureja thymbra* and the aqueous extracts of *Anthemis tunictoria* and *Juglans regia.*

Ethanolic extracts were more active than aqueous extracts for antimicrobial activity.

Test bacterial strains differed significantly in relation to their susceptibility to different plant extracts used. The most susceptible test strain was *S. aureus* (gram positive bacteria), whereas the least susceptible strain was *K. pneumonia* (gram negative bacteria).

For mycelial fungi, the most susceptible phytopathogenic fungi was *P. aphanidermatum,* whereas the most susceptible dermatophyte was *M. gypseum* to both aqueous and ethanolic extracts.

CHAPTER ONE

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

1.1 Historical review of traditional medicine

Since the dawn of history, plants have played an important role in the treatment of human diseases and ailments; they were the main source for man's early primitive drugs (Mossa *et al.*, 1983).

The earliest records of ancient Babylonia, China, Greece, India, Rome, Assyrian and Egyptians are filled with references to medicinal plants (Plotkin *et al.*, 1992). Some of these records illustrate the great advances that had been made in the understanding of suitable drugs and pharmaceutical preparations (Chiej, 1984).

Hypocrates was the first Greek to regard medicine as a science, and his *Materia Medica* consisted essentially of herbal recipes (Sofowora, 1982).

The discovery of the New World in the year 1492, initiated the identification of several plants of considerable economic value based on observations of native people. This led to increasing emphasis on cultural significance of plants prior to the introduction of the term ethnobotany in 1895 and considering it as a field of academic study in the year 1900. The practical significance of understanding folk classification systems was highlighted during 1950-1970 (Cotton, 1996).

More recently, in 1990s, both postgraduate and undergraduate programs in ethnobotany become increasingly available, while many research projects focus on the practical applications of traditional plant knowledge (Cotton, 1996).

Arabs also have contributed to the development of herbal medicine during the middle ages. However, folk medicine still constitutes a significant part of heritage of many Arab countries including Palestine (Silva *et al.*, 1981; Ali-shtayeh *et al.*, 1997,1998; Ali-Shtayeh & Abu-Ghdeib, 1999; Ali-Shtayeh, Yaniv, *et al.*, 2000; Essawi & Srour, 2000), Jordan (Karim and Quraan, 1986, Alkofahi *et al.*, 1990), Egypt (Hanafy *et al.*, 1991), Qatar (Rizk, 1982), Saudi Arabia (Massa *et al.*, 1983), United Arab Emirates (UAE) (Tanira *et al.*, 1994), Sudan (Elsheikh *et al.*, 1982, Al-Magboul *et al.*, 1985) and Morocco (Haiji *et al.*, 1993).

1.2 Medicinal plants

A plant is said to be "medicinal' when at least one of its parts contains substances that can be used for therapeutic purposes (Sofowora, 1982; Bruneton, 1995). This definition includes plants used in galenical preparations (e.g., decoctions, infusions, etc...), in extraction of surgical dressings, in addition to some food spices and perfumery plants that are used medicinally.

The use of these plants in preventing, or eliminating physical, mental or social diseases is referred to as traditional or folk medicine. This medicine can

be described as the combination of knowledge and practice, relying on past experience and observation handed down from generation to generation (Sofowora, 1982). Folk medicine comprises numerous herbal and plant prescriptions for therapeutic purposes. These include healing of wounds, treatment of inflammation and skin ulcers (Karim and Quraan, 1986; Dafni *et al.*, 1994; Ghazanfer, 1994; Tanira *et al*, 1994), pneumonia and bullet wounds (Desta, 1993), dermatomucosal, skin and candidal infections (Caceres 1991, 1993).

The interest in studying the biological effects of traditional medicinal plants or isolating their active components for treatment of illness, has increased all over the world and comprehensive screening programs have been established (Boulos, 1983; Kottob, 1983; Azzam, 1984). A large proportion of the current research in ethnobotany remains focused on the American continent where up to 41 % of the studies are carried out (Cotton, 1996). In Palestine, the screening of the flora for pharmacological active compounds started in the late sixties (Silva *et al.*, 1981). The abundance of species (> 2600) condensed on a very small geographical area (about 25000 Km^2) is a major characteristic of the Palestinian flora. This richness is due to the diversity of habitats created by the soil and climatic conditions, in addition to the lack of medical care, and economics.

The remarkable diversity of environments and habitats stimulates also the process of genetic differentiation and thus the development of new

ecotypes finally leading to new species. Indeed, the splitting of some species in ecotypes or chemotypes is another characteristic feature of the Palestinian flora. Above all, passing knowledge from one generation to the next about medicinal plants and their use, is a part of the heritage in this area of the world (Boulos, 1983; Karim & Quraan, 1986).

Many plant species (> 700) have been used in folkloric medicine in Palestine to treat various ailments of man (Palevitch, 1991; Sezik *et al.*, 1991; Shtayeh & Hamad, 1995; Ali-Shtayeh, Yaniv, et al., 2000). Folk remedies used are prepared as powders, poultices, ointments, baths, decoctions, infusions and teas. Decoction is the most popular form of home remedy. Decoctions, infusions and teas are usually prepared just before application and filtered through a cloth or cotton wool. Most plants are stored for use in the dry state, which permits their utilization throughout the year, sometimes fresh plants are used (Sezik *et al.*, 1991). Fifty-four of these plants (Table 2.1) which are used to treat dermatomucosal infections and other ailments, were selected in the present work for antimicrobial activity testing. However, some of the selected plants have been tested for biological activities other than antibacterial or anticandidal activities such as antifungal activity (e.g. Amoros *et al.*, 1988; Cacers *et al.*, 1991; Bagchi *et al.*, 1999), hypoglycemic activity (e.g. Yaniv *et al.*, 1987; Gharaibeh *et al.*, 1988; Resher *et al.*, 1991; Glombitza *et al.*, 1994), antiulcerogenic, antihelminthic and hepatoprotective (e.g. Akhtar *et al.*, 1989; Naqvi *et al.*, 1991; Sultana *et al.*, 1995; Abreu *et al.*,

1999), analgesic, antipyretic and antirheumatic activities (e.g. Karim and Quraan, 1986; Al-Said *et al.*, 1990; Dafni *et al.*, 1994; Ali *et al.*, 1995), antileishmania and insecticidal activities (e.g. Abreu *et al.*, 1999; Chariandy *et al.*, 1999). To the best of our knowledge the remaining plants have not yet been studied for their antimicrobial activities. It is hoped that this study can focus the light on the antimicrobial activities of the selected plants (Table 2.1).

1.3 Antimicrobial activity and phytochemistry of medicinal plants

Antimicrobial agent may be defined as "a chemical substance derived from a living source (plants, animals or microbes) that in dilute solutions has the capacity to inhibit the growth of or destroy microorganisms' (Torota & Becker, 1978). Reports of antimicrobial activity of indigenous plants have been published from many regions of the world (Desta, 1993).

A multidisciplinary approach is being developed in some countries to organize a system for medicinal plants used for treatment of specific symptoms based on field ethnobotanic surveys (e.g. Silva *et al.*, 1981, 1996; Friedman *et al.*, 1986; Deans, 1989; Caceres *et al.*, 1993; Ahmad *et al.*, 1998).

In the last few years, research on medicinal plants has increased and their antimicrobial activity has been screened in a number of studies (Al-

Magboul *et al.*, 1985; Tomesi *et al.*, 1986; Rios *et al.*, 1987; Siddiqi & Husain, 1991, Tanira *et al.*, 1994; Silva *et al.*, 1996; Ahmad *et al.*, 1998; Sindambiwe *et al.*, 1999).

In the constant effort to improve the efficacy and ethics of modern medical practice, researchers are increasingly turning their attention to folk medicine as a source of new drugs (Haslam *et al.*, 1989). One aspect of the scientific approach to natural products is to chemically isolate, identify and screen the active ingredients from medicinal plants. However, the benefits of traditional remedies frequently result from combination of various plants and other ingredients (Bai, 1990).

Biologists prefer to work on pure crystalline soluble compounds. However, the fact that the stage of pure compounds isolation comes only after initial biological tests on crude extracts, provides justification for detailed phytochemical investigation and preliminary screening (Rizk, 1982; Mossa *et al.*, 1983). A revolution in the field of medicine has been brought about by chemical and pharmacological studies on the plants used in folk medicine, since a large number of active constituents isolated from medicinal plants have been introduced in the modern pharmacopoeias throughout the world (Rizk, 1982). Some of the phytochemical surveys have been conducted or specifically directed for the detection of a class of compounds like alkaloids, glycosides, essential oils, flavanoids, isothiocyanate glycosides, saponins, steroidal sapogenins and tannins (Rizk, 1982).

Chemical constituents of plants that are known to be antimicrobially active are classified into three groups. First group includes phenolics, shiokimates and acetates (e.g., phenols, phenolic acids, aryl alkanones, flavanoide flacanenes, anthocyanins, quinones and cocemarines) (Fernandez *et al.*, 1996; Hasan & Ahmad, 1996; Ortega *et al.*, 1996; Pedetzoglou, 1996; Raman & Faroque, 1996; Srivastava *et al.*, 1996; Swiader *et al.*, 1996; Tsuchiya *et al.*, 1996; Vaishnav, 1996). The second group includes terpenoids and steroids (e.g., monoterpenes, diterpenes, triterpenes, sesquiterpenes, sesquiterpenoid lactones, essential oils, steroids, saponins, flavanoid glycosides and tannins) (Ansari & Ali, 1996; De La Fuente *et al.*, 1996; Fereira *et al.*, 1996; Gruz *et al.*, 1996; Hasan & Ahmad, 1996; Jain & Sharma, 1996; Montanaro *et al.*, 1996; Raman & Farouque, 1996; Vaishnav *et al.*, 1996). The third group includes alkaloids (e.g., quinolizidine, isoquinolines, piperidine, pyrrolizidine, imidazole terpenoid alkaloid, tryptamines (Brunetone, 1995; De La Fuente *et al.*, 1996; Hasan & Rashid, 1996; Raman & Farouque, 1996; Touati *et al.*, 1996).

The need to evaluate phytochemical constituents and their biological activities is not only important for the development of new therapeutic agents, but the novel chemicals isolated from plants with some biological activity give a guideline to the chemist to synthesize very useful semi - synthetic drugs such as homatropine from atropine (Mossa *et al.*, 1983) and as

interesting tools that can be applied to a better understanding of biological processes (Farnsworth, 1984).

In the last few years, research on medicinal plants and the evaluation of their constituents and their antimicrobial activity has been screened in a number of studies in West Bank (e. g., Hussain, 1995; Ali-Shtayeh *et al.*, 1997, 1998; Ali-Shtayeh & Abu-Ghdeib, 1999; Ali-Shtayeh, Yaniv, *et al.*, 2000; Essawi & Srour, 2000).

1.4 Screening methods for antimicrobial activity of natural products

Antimicrobial activity screening of natural products is usually performed using the agar diffusion and dilution methods and poisoned food technique (Dikshit *et al.*, 1984; Rios *et al.*, 1988; Wood *et al.*, 1995; Silva, 1996). The recommended methods include the followings.

1.4.1 Antibacterial and anticandidal activity screening methods

1.4.1.1 Principal diffusion method

A technique, which does not require homogeneous dispersion in water, is the agar diffusion method (Murray *et al.*, 1995) using a disk, hole or cylinder as reservoir. The reservoir containing the sample to be tested is brought into contact with an inoculated medium and after incubation the

diameter of the clear zone is measured. The zone size is inversely proportional to the minimum inhibitory concentration (MIC) the least concentration of the extract that completely inhibits the growth of the test microorganism (Rios *et al.*, 1988; Wexler *et al.*, 1991; Woods & Washington, 1995).

The advantages of this method are the possibility of testing four or five compounds against a single microorganism on the same plate and the small size of the sample used in the screening.

1.4.1.2 Dilution methods

These techniques require homogeneous dispersion of the sample in water. They are used principally to determine the minimum concentration value (MIC) of an extract, essential oil or pure substance, and can be used in the preliminary screening of antimicrobial activity (Rios *et al.*, 1988).

In this method, turbidity is taken as an indication of bacterial or candidal density. The degree of inhibition is related to the turbidity of the medium and measured by spectrophotometer (Wood *et al.*, 1995).

The advantages of this method is the simplicity, speed and the possibility of using it in the antimicrobial study of water soluble or insoluble samples such as essential oils.

Dilution methods are the best for water-soluble or lipophilic samples and to determine the MIC of compounds (Rios *et al.*, 1988).

Dilution in liquid medium is the most complicated but also the most precise technique. This method is recommended for determination of MIC (Irobi & Daramala, 1994) of a pure sample and it is the only method for determination of minimum bacterial (MBC) or fungicidal concentration of the extract that does not permit any visible colony of microorganism to grow on the agar plate after the period of inoculation (Irobi & Daramala, 1994). It is determined, by subculturing of the tube that shows complete inhibition on an agar plate or in liquid medium (Rios *et al.,* 1988).

1.4.2. Antifungal activity screening method

1.4.2.1 Poisoned- food- technique

A technique in which the antimycotic activity is carried out by mixing the required amount of the dried plant extract or reference drug in requisite amount of pre-sterilized medium to give a specified final concentration. A mycelial disc, 6mm in diameter, cut from the periphery of old cultures, was aseptically inoculated onto the medium (Dikshit *et al.*, 1984). The advantages of this method are the rapidity and giving homogeneous dispersion of the extract in the test medium.

1.5 Extraction techniques

Antimicrobial activity of plant is usually assessed after extracting plant material with organic and inorganic solvents, in order to separate the chemical

constituents into groups of different polarities (Nadir *et al.*, 1985).

Many factors may affect the extractability and hence the biological activity of the chemical constituents of the plants. The pH of the extracting medium is one of these factors. For this reason, when working on medicinal plants it is recommended to apply various methods of extraction to ensure the recovery of the active components (Nadir *et al.*, 1985).

Decoction is one of the traditional extraction techniques. It is prepared by placing the plant drug in cold water, bringing it to boil for 15 minutes or longer (up to 1 hour), and then allowing the mixture to stand for a further 15 minutes, the extract (aqueous or organic) are decanted or filtered as and when required. This type of extraction may result in the alteration of many active constituents (e.g., some glycosides are decomposed during boiling).

Another technique of extraction is infusion which is carried out by pouring boiling water or organic solvent on a specific quantity of plant material and allowing the mixture to stand for 10-15 minutes or more (Sofowora, 1982).

1.6 Objectives of the present study

The development of resistance by a pathogen to many of the commonly used antibiotics including antimycotics, provide an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance and side effects of the currently

available antimicrobial agents. Hence this *in vitro* study was aimed at: Screening the selected Palestinian plants for their antimicrobial activity, evaluating their potential use in treating infections caused by bacteria and fungi, and determining whether their use in folkloric medicine to treat these diseases is justified.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

MATERIALS AND METHODS

2.1 Antimicrobial activity of plant extracts

2.1.1 Plant material

2.1.1.1 Collection

Fifty-four Palestinian plant species, used in folk medicine were selected in the current work (Table 2.1) to study their antimicrobial activity. Mature plants were collected from several sites in Nablus and Ramallah areas during May-June 1998. Collected plant material was either used fresh, or dried in the shade, ground using a seed mill and the powdered plant material stored in plastic labeled bags at room temperature until used.

2.1.1.2 Extraction

Crude extracts of plant parts were obtained using two different solvents: inorganic solvent (boiling distilled water, aqueous extract), and organic solvent (cold 95% ethanol, ethanolic extract) (Kandil *et al.*, 1994).

2.1.1.2.1 Ethanolic extracts

A 500 gm-portion of the powdered plant material was soaked in 2.5-3 L of 95% ethanol for 4-5 days at room temperature. The mixture was stirred daily for regular infusion. After a five-day period, the extract was filtered

Table 2.1 Selected plants used for antimicrobial susceptibility testing.

Species/ Family (Voucher Specimen No.)	Common name	Parts used	Popular uses	Ref. for folk Popular uses	Active constituents	Ref. for active constituents
Achillea fragrantissima (Forssk) Sch-Bip. (compositae) RZ100*	Sulphur coloured milfoil (Qaysoom)	AP, FL	Treatment of diabetes, digestive problem arthritis, fever reduction, severe cough, antidiuretic, stomach ailments, rheumatic pain, tumors and infections, antispasmodics.	55, 22, 40, 23, 47	Terpinen- 4-o1	9
Ajuga orientalis (L.) (Labiatae) RZ101	Eastern bugle (Oshbet Al-Qalb)	AP				
Alcea setosa (Boiss.) alef. (malvaceae) RZ102	Holly hock (Ward Al-jamal)	FL				
Anagallis arvensis (L.) (Primulaceae) 5408	Red Pimpernes (Ein El-jamal)	WP	Skin rash and ulcers, diaphoretic, diuretic, demulcent, emollient, antipyretic, antirheumatic, cholagogic, antitussive, vulnerary.	14, 24, 31	Triterpenoids, cucurbitacins, saponins, tannins, flavones, arvenins, cyclamine, crystalline, glycosides.	14, 17, 24, 44, 46
Anchusa strigosa Banks & sol (Boranginaceae) 5412	Alkanet (Hemhem)	RT, LF	Diaphoretic, diuretic, demulcent, emollient, antipyretic, treatment of gastriculcer.	18, 31	Cynoglossine, consolidine, alkaloids, mucilage, acids, tannins.	31
Anthemis palestina Reuter (Compositae) RZ103	Chamomile (Palestine chamomile)	AP, FL				
Anthemis tunictoria (L.) (Compositae) RZ104	Chamomile (Yellow chamomile)	FL				
Asphodelin lutea (L.) Rehb. (Liliaceae) 5413	Jacob's rod (otat)	WP	Antispasmodic, diuretic, nutritive, skin disorders.	3		

Table 2.1 / continue

Calycotome villosa (Poir.) Link (Papilionaceae) RZ105	Thorny broom (Kundail)	AP, YB				
Capparis spinosa (L.) (Capparidaceae) 5402	Caper bush (Qubbar)	RT, FL, FR	Treat earache, coughs, antihelmintic and for diabetes, diuretics, stimulant, vasoconstrictive, arteriosclerosis and for chills, reproduction enhancement, depurative, resolvent cataplasms for skin inflammation, antihepatotoxic, against painful menstruation.	10, 14, 17, 22, 23, 24, 44, 55	Choline, flavonoids, glucosinolates, rutoside, pectin, enzymes (myronases), saponin, capparenol, p-methoxy benzoic acid, capparidis.	24, 5, 14, 23
Ceratonia siliqua (L.) (Caesalpiniaceae) RZ106	Locust tree (Kharroob)	S	Treating diarrhoea, diabetes, syphilis and venereal diseases, epilepsy, clear the voice, astringent, antihelmintic.	10, 24, 31, 55	Tannins, alcohols, essential oil, mucilage, anthraquinone, glycoside.	24, 31
Cicorium pumilum Jacq. (Compositae) RZ107	Dwarfchicory (Hondoba')	AP, FL, LF	Antipyretic, antirheumatics, carminative, digestive.	35	Flavonoids	
Clematis cirrhosa (L.) (Rananculaceae) 4511	Clematis (Ghashia)	AP	Useful for impotency.	29, 39	Soponins, triterpenoids, oleanolic acid.	34, 49, 52
Companula rapunculus (L.) (Companulaceae) RZ108	Bell-Flower (Ward Al-jurus)	AP				
Coridothymus capitatus (L.) Reichb. (Labiatae) RZ109	Thyme (Zo'ameh)	AP,FL	Anti-inflammatory and antimicrobial activity, for eye infection, headache, disphoretic, stomach ache, carminative, whoopin cough, antihelmintic, antispasmodic, kidney disorders, antipyretic, emmenagogue.	30	Thymol, phenols, soponins, resins, flavonoids, fixed oils.	30
Crataegus aronia (L.) Bosc. ex.Dc. (Rosaceae) RZ110	Hawthorn (Za'roor)	LF	Cardiac sedative, hypotensive.	31	Simple flavonoids (hyperoside & rutin), oligomeric procyanidins.	33
Cyclamen persicum (Mill.) (Primulaceae) RZ111	Cyclamen (Za'matot)	R,ST	Antirheumatic, headache, cardiac sedative, treatment of goiter, anthelmintic, laxative.	7	Glycoside, cyclamen, cyclamenoside.	7, 37, 43

Table 2.1 / continue

Eryngium creticum Lam. (Umbilliferae, Apiaceae) 5416	Snake root (Qorsa'na)	LF,S, RT	For diabetes, fluid retention, diuretic, renal stones, skin diseases, calefacient, helminthiases.	31, 55	Saponins, tannins.	14
Euphorbia hierosolymitana Boiss. ex. Boiss. (Euphorbiaceae) RZ112	Spurge	AP	- Constipation.	22		
Foeniculum valgare (L.) Mill. (Apiaceae, Umbelliferae) RZ113	Fennel (Showmer)	AP, FL	Flavouring agent, bronchodilator, antitussive, lactagogue, galactagogic, analgesics, antispasmodics, treat skin diseases, carminative, used in infusions and tinctures, antiseptic, diuretic, expectorant, for abdominal colic, coughs, as toothbrush, liver complaint, stomach ache, flatulence, colic, pancreas complaint, dyspnoea, tonic.	10, 14, 7, 31, 24, 26, 22, 44	Essential oil, anethole, anisic acid, acids, pectin, fixed oil, fenchone, fatty oil, camphene, limonene, sugars, pinene.	7, 14, 24, 31, 44
Gagea chloranth (Bieb.) Schult. Fil (Liliaceae) RZ114	Gagea (Ze'tman)	R				
Inula viscosa (L.) Ait (Compositae) 5445	Inula (Erq Tayoon)	WP	Treatment of diabetes, antihelminitic, expectorant, diuretic, for lung and bronchial disorders, anti-inflammatory, reconstituant.	10, 31, 47, 55	Flavanoids, sesquiterpenoids, essential oils, (mainly thymol), inulavosin, ψ-taraxasterol acetate.	1, 12, 25, 36, 38, 57
Juglons regia (L.) (Juglandaceae) 5401	Walnut (Jouz)	LF, FR	Treat eczema, nervous problem, as food, for syphilis, anthelmintic astringent, stomachic, nerve tonic, treat scrofula, rickets, gastro- enteritis, vermifuge, as ahypoglycaemic agent, antidote poison, tonic, dental hygien, depurative, galacto-fuge, rubefacient, antiscrophulous.	10, 14, 24, 31, 56, 44	Inositol, juglane, carotein, vitamins A,B,C starch & proteins, juglandin, hydrojuglone , essential oil, tannin, resin, volatile oil, juglone, fatty oil, vitamin C, sesquiterpenes.	24, 29, 31, 44

Table 2.1 / continue

Lactuca serriola (L.) (Compositae) RZ115	Wild lettuce (Khas)	AP, LF	Sedative, snake bites, diuretic, laxative.	7, 44	Lactupicrine, latex.	7, 44
Lactuca tuberosa Jacq. (Compositae) RZ116	Lattuce (Khas Berry)	AP				
Lawsonia inermis (L.) (Lythraceae) RZ117	Henna (Henna)	LF	Fever, local anaesthetic, anti-inflammatory, mouth ulcers, antifungal, used in dermatology in leprosy and leucoderma, gums and skin disorders, antipyretic, analgesic.	2, 24, 31	Lawson, coumarins, luteolin, fats, resins, henna - tannin, ionones, mucilage, oxynaphtho - quinone.	2, 24, 31
Linum pubescens Banks & sol (Linaceae) RZ118	Pink flax (Kettan Zahry)	AP, FL				
Lupinus pilosus Murr. (Papilionaceae) RZ119	Lupine (Turmus Barry)	AP, FL, S			Isopenteny liso flavones.	28
Lycium europeum (L.) (Solanaceae) 4507	Box thorn (Awsaj)	WP	Treat skin infections, anesthetic for toothache pain, antiseptic, eye wash, treat cataract, analgesic, antistomachache.	16, 22, 39	Carvacrol, terpenes, sterols, alkaloids.	10, 16, 24, 26, 55
Micromeria fruticosa (L.) Druce (Labiatae) RZ120	(Za'tar Balat)	AP	Anti-inflammatory, for eye infections & headache.	45	Thymol, menthol, pulegone, flavanoid (narigenin & neoponcirin), essential oils.	11, 45
Micromeria nervosa (Desf) Benth (Labiatae) 5444	(Za'tar n'em)	LF	Anti-inflammatory effects, for infections & headache	3	Thymol, carvacrol, menthol, pulegone, flavanoids (narigenin & neoponcirin).	3, 11
Papaver rhoeas (L.) (Papaveraceae) RZ121	Common poppy (Khushkhash)	AP, LF	Poisonous, pectoral, expectorant, CNS and musculotropic depressant, cough, eye infections, sedative, measles, children's fever, antitussive, soporific, emollient.	10, 14, 17, 31	Rhoeadine, rhoeagenine, rhoearubine I & II, mucilage, anthocyanins.	7, 14, 44

Table 2.1 / continue

Parietaria diffusa (Mert. & Koch.) (Urticaceae) 5432	(Oshbet-Dam)	AP	To stop bleeding from fresh skin wounds, vulnerary, diuretic and depurative, vermifuge, antitussive, sedative incases of intestinal colic, hemorrhoid lentitive, antiecchymotic, resolvent for skin inflammation.	3, 17, 39	Tannins.	14, 16, 19, 29, 39, 55
Paronychia argentea (Lam.) (Caryophyllaceae) RZ122	Silvery whitlow wort (Rejl Al-hamameh)	AP, F1	Treatment of diabetes.	55		
Phagnalon rupstre (L.) Dc. (Compositae) 5405	Rock phagnalon (Qadeeh)	WP, AP	To make deliberate burns, to treat asthma, anesthetic for toothache, to treat headach, to induce burns and as tinder.	3, 22, 39	Thymol, carvacrol, quinones.	19, 53
Pinus halepensis Mill. (Pinaceae) RZ123	Aleppo Pine (Snowber)	LF	Expectorant, diuretic, antiseptic, for wounds, antirheumatic fever, antidiabetic, for bronchitis, tuberculosis, skin abces.	7, 10, 31	Urpentine, coniferin, tannic acid, resin, pinite, pinene, vitamin C.	15, 31
Pistacia lentiscus (L.) (Anacardiaceae) 5430	Mastic, lentisk (Sarrees, Butm)	LF, YB	For fever, protective covering for wounds, breeth freshener, treat chest pain, expectorant, skin infections, hair-care, for diarrhea in children, could be masticated to sweeten breath, stimulant, diuretic, swelling, for gastro-intestinal disorders, anti-inflammatory, aid to minstruation, magic, cardiac stimulant, astringent.	14, 24	Masticadienoic acid, tucallol. Essential oil, resin, turpentines, thymol, triterpenoids, tannin.	14, 24, 31, 42, 54
Quercus calliprinos Webb (Fagaceae) RZ124	Holly Oak (Ballout)	R	Urination decrease, skin disorders, as astringent, homeostatic agent.	22, 24	Glycosides, tannin.	28
Retema raetam (Fossk.) webb. (Papilionaceae) 5422	Ratame (Retem)	LF, S, YB	Insect repellant, soothing inflamed eye and sour throat, anti-inflammatory, treat inflamed eyes, antirheumatic, treat infertility, treat paralysis, analgesic, treat stomach-ache back ache, gale abortive, toxic, skin diseases, antipruritic.	4, 10, 16, 22, 29, 39, 31, 55	Essential oil.	31

Table 2.1 / continue

Rhus coriaria (L.) (Anacardiaceae) RZ125	Sicilian sumach (Summag)	AP, LF	Astringent, anti-dysentery, stops bleeding, spice, treat gastric ulcer, for mouth ulcers, for burns.	7, 31, 56	Myricetin, tannin, oxyquercetin, vitamin C, flavanoids, carotenes.	7, 31
Rubia tenuifolia D'urv. (Rubiaceae) RZ126	Wild Madder (Faweh)	AP	Diurefic activity.	47		
Ruscus aculeatus (L.) (Liliaceae) 5403	Butcher's Broom (Safander) (Ajrum)	RT	Diuretic, stop bleeding, depurative, anti-arthritic, vasoconstrictive.	7, 14	Flavanoids, glycosides, euparone sterol mixture, saponins, resins, uscogenine, bitter substances.	7, 14, 20, 21, 32, 41
Ruta chalepensis (L.) (Rutaceae) 5420	Rue (Faijen)	LF, RT, WP	Anti-rheumatic and against abdominal colic, for snake bites, aphrodisiac for headaches and wounds, anti-spasmodic, diuretic, sedative, analgesic, anti-inflammatory, diarrhea, dysentery, colic, stomach pains, constipation, emetic, laxative gastritis, enterocolitis.	6, 24, 31, 13	Alkaloids, coumarins, rutin, caprinic acid, essential oil, tannins, saponins, sterols, triterpenes.	24, 31, 51, 6, 50
Salvia fruticosa (L.) Mill. (Labiatae) RZ127	White sage (Mariamia)	LF	Anti-inflammatory gargle, antiseptic, anti-tussive, anti-haemorrhoids pain, anti-rheumatic.	39, 55	Thymol, carvacrol, flavanoids, rosmarinic acid, saponins, monterpenoids, 1-8-cineol, essential oils, flavone aglycones, flacanoid glycoside.	14, 15, 16, 29, 39, 55
Sarcopoterium spinosum (L.) Sp (Rosaaceae) 5404	Thorny Burnet (Bullan)	AP	Treatment of diabetes, diuretic, useful in renal calculi, anti-inflammatory, for haemorrhoids.	22, 31, 55	Tannin, triterpenoids, glycosides.	31
Satureja thymbra (L.) (Labiatae) RZ128	(Nadeg Al-Pasasteen)	AP,FL	Fungicide.	45	Essential oils, tocopherols.	28, 45

Table 2.1 / continue

Scrophularia rubricaulis Boiss. (Scrophulariaceae)	(Khanazeeryeh)	AP			Glycosides.	28
Solanum nigrum (L.) (Solanaceae) 5406	Black night-shade (Enob El tha'lab)	LF, FR	As an expectorant, for fevers, gonorrhoea, kidney and bladder inflammation, stomach ache, skin ulcers, for eye inflammation, poisonous, treat cramps and epilepsy, narcotic, emollient, sedative, antispasmodic, antirheumatic.	14, 24, 31, 44	Solasodine, glycosides, solasonin, solana grine, sugars, vitamin C, carotenes, saponin, solanine, rutin, tannin, linoleic acids, palmitic acids.	14, 24, 31, 44
Teucrium polium (L.) (Labiatae) RZ129	Germander (Jadah)	AP	Treating abscesses, abdominal pain, jaundice, malarial fever, insect bites, diabetes, treatment of intestinal and cardiac disorders, anti-inflammatory for stomach and intestine, antipyretic, stimulate glands for piles, used for respiratory system disorders, blood - cleansing, used to treat skin diseases, stomach ache, liver pain, febrifuge, against chill, oedema, for toothache and against rheumatism.	10, 22, 24, 31, 48, 56, 55	Diterpenoids, sapogenin, hederagenin, alkanes, picropoline, B-sitosterol, stigmasterol, campesterol, brassicasterol, clerosterol, glucose, fructose, raffinose, rhamnose.	24, 48
Varthemia iphionoides Boiss & Blanche (Compositae) RZ130	Common varthemia (Kateeleh)	AP	Stomach ache.	22		
Verbascum sinuatum (L.) (Scrophulariaceae) RZ131	Mullein (Alboseer) (Lubaidah) (Awroor)	LF, FL, RT, YB	Used for neuralgic pain, gastric disturbance and bronchitis, emollient, anti-inflammatory, soothing inflamed eye, antirheumatismal, for opthalmic infections.	10, 31	Verbascoside aucubin, mucilage, saponin, colourin matter.	31
Viscum cruciatum sieber et. Boiss. (Loranthacesae) RZ132	Mistletoe (Hadal)	LF	Tumor inhibition, anti-spasmodic, anti-hypertensive, diuretic.	31	Viscotoxin, arginine, choline, tyramine, mucilage.	31

Table 2.1 / continue

Vitex agnus-castus (L.) (Verbenaceae) RZ133	Chaste tree (Kuf Muryam)	LF	For eye diseases, colic and gastric disturbance, toothache and sedation, treatment of cyclical mastodynia, calefacient.	10, 27, 31	Essential oil, tannin.	31
Ziziphus spina-christi (L.) Willd (Desf) (Rhamnaceae) 5417	Syrian christ thorn (Doum, seder)	LF, S, YB, FR, WP	Treat blisters, bruises, chest pains, dandruff, fractures, headache and mouth and gum problems, laxative, pectoral, nutritive, to cure toothache, astringent, used as anti-diarrhoetics, fermifuges, anti-inflammatory (eye wash) analgesic, pectoral, anti-rheumatic, purgative, for stomach pain, antihelmintic, back ache, arthritis, gums, joints, skin disorders.	110, 22, 24, 27, 31, 39	B-sitosterol, B sitosterol B- D-glucoside, octacosanol, octacosanyl behenate, N-nanocosane, betulic acid, ceanothic acid, tannins, sugars, ziziphic acid, mucilage, saponin, flavanoids, triterpenoids, essential oil.	16, 24, 31, 39, 55, 58

Parts used: AP, aerial parts; FL, flowers; WP, whole plants; RT, roots; YB, young branches; S, seeds; ST, stem; LF, leaves.

[1] Abu Zarga *et al.*, 1998; [2] Ali *et al.*, 1995; [3] Ali-Shtayeh *et al.*, 1997; [4] Ali-Shtayeh *et al.*, 1998; [5] Al-Said *et al.*, 1988; [6] Al-Said *et al.*, 1990; [7] Al-Wareh *et al.*, 1993; [8] Amoros *et al.*, 1988; [9] Barel & yashphe, 1991; [10] Bellakhdar *et al.*, 1991; [11] Bellino & Marceno, 1981; [12] Benayache *et al.*, 1991, [13] Caceres *et al.*, 1990; [14] Chieji, 1984; [15] Conigueral *et al.*, 1989; [16] Dafni & Yaniv, 1994; [17] Defeo *et al.*, 1991; [18] Disi *et al.*, 1998; [19] El-Damy *et al.*, 1994; [20] Elsholy *et al.*, 1975; [21] Facino *et al.*, 1995; [22] Friedman *et al.*, 1986; [23] Gadgoli & Mishra, 1999; [24] Chazanfar, 1994; [25] Grande *et al.*, 1992; [26] Gribanovski-sassu *et al.*, 1969; [27] Halaska *et al.*, 1998; [28] Haykel & Omar 1988; [29] Hussain, 1995; [30] Kandil *et al.*, 1994; [31] Karim & Quraan, 1986; [32] Karting *et al.*, 1991; [33] Kinghorn & Balandrin, 1993; [34] Kizu *et al.*, 1995; [35] Manadhar, 1991; [36] Manez *et al.*, 1999; [37] Murata & Takahashi, 1984; [38] Okzuz, 1976; [39] Palevitch & Yaniv, 1991, [40] Qureshi *et al.*, 1991; [41] Rauwald & Grunwid, 1991; [42] Rios *et al.*, 1987; [43] Sakai *et al.*, 1992; [44] Schauenberg, 1990; [45] Shimoni *et al.*, 1993; [46] Shoji, *et al.*, 1994; [47] Silva & Abraham, 1981; [48] Suleiman *et al.*, 1988; [49], Thapliyal & Bahuguna, 1993, [50] Ulubelen *et al.*, 1994; [51] Ulubelen *et al.*, 1988; [52] Uniyal & Sato, 1992; [53] Viollon & Chaumant, 1994; [54] Wyllie *et al.*, 1990; [55] Yaniv *et al.*, 1987; [56] Yesilada *et al.*, 1993; [57] Yoshida *et al.*, 1995; [58] Yuan *et al.*, 1987.

*Plants were collected and identified under the supervision of Prof. M. S. Ali-Shtayeh by Suheil Abu -Ghdeib, Reem Yaghmour, Rabee Zayed, Rana jamous, Abdl Rahman Salameh, Kamal Kamel, and Tayseer Khalid, An-Najah National University, Nablus.

using Whatman filter paper no. 1 and 4. The filtrate was then dried using a rotary evaporator at 60 °C. The final dried extract was stored in labeled sterile glass bottles and kept at - 20 °C (Kandil *et al.*, 1994).

2.1.1.2.2. Aqueous extracts

Five hundred gram of powdered plant material was infused in boiled distilled water until complete exhaustion, usually for 4-5 days. Extract was then filtered using Whatman filter paper no. 1 and 4 and then lyophilized using a freeze dryer (Model: 25 SL, Manufacturer: Vir Tis Company, New York USA). The final dried extracts were stored in labeled sterile bottles and kept in the freezer at -20 °C (Kandil *et al.*, 1994).

2.1.1.3 Sterilization of plant extracts

One gram of the powdered dry extract was dissolved in 2.5 ml of solvent (water for aqueous extract or 95% ethanol for ethanolic extract) in order to obtain a final concentration of 400 mg / ml. Aqueous and ethanolic extracts were sterilized using sterile 0.45 µm membrane filter. Sterile filtrates were stored in sterile vials in the refrigerator at 5 °C until use.

2.1.1.4 Application of extracts on sterile discs

Discs of 6-mm diameter were cut out of Whatman filter paper no. 3 and sterilized by autoclaving. Twenty-five microliters of the test extract were

added to each sterile disc. The discs were then dried under a laminar flow sterile bench. The final content of each sterile disc was 10 mg of extract.

2.1.2 Test microorganisms

Five strains of human pathogenic bacteria, one isolate of *Candida*, two isolates of dermatophytes, and five isolates of plant pathogenic fungi, were employed in this study (Table 2.2).

Table 2.2 Test microorganisms and source of collection

Microorganism	Number	Source / Researcher
Bacteria		
Escherichia coli	ATCC* 25922	
Klebsiella pneumonia	ATCC 13883	
Proteus vulgaris	ATCC 13315	
Pseudomonas aeroginosa	ATCC 27853	
Staphylococcus aureus	ATCC 5923	
Yeast		
Candida albicans (Robin) Berkhout	FCCAU** R10	Foot swab, patient / Mr. Suheil Abu-Ghdieb
Dermatophytes		
Microsporum gypseum (Bodin) Guiart and Grigorakis	FCCAU S15	Tinea capitis clinical specimens in Nablus area, patient / Mr. Suheil Abu-Ghdieb
Microsporum canis Bodin	FCCAU S14	Tinea capitis clinical specimens in Nablus area, patient / Mr. Suheil Abu-Ghdieb
Phytopathogenic fungi		
Fusarium tricinctum (Corda) Sacc.	FCCAU M10	Water pool in Nablus / Mr. Tayseer Khalid
Pythium ultimum var *ultimum* Trow	FCCAU H5R3	Soil samples in Nablus area / Prof. M. S. Ali-Shtayeh
Pythium aphanidermatum (Edson) Fitzp.	FCCAU H739	Soil samples in Nablus area / Prof. M. S. Ali-Shtayeh
Pythium middletonii Sparrow	FCCAU PH 122	Soil samples in Nablus area / Prof. M. S. Ali-Shtayeh
Phytophthora citrophthora (R. E. Smith & E. H. Smith)	FCCAU AIE 2005	Soil samples in Nablus area / Dr. Abdul Hadi Hamdan

*ATCC: American Type Culture Collection

** FCCAU: Fungal Culture Collection of An-Najah University.

2.1.3 Preparation of inocula

2.1.3.1 Bacterial and candidal inocula

Part of an isolated bacterial or *C. albicans* colony was transferred into a 5-ml Muller-Hinton broth tube and the tube was incubated for 4-18 hours at 37 °C. The growth turbidity in Muller-Hinton broth was adjusted by further incubation or dilution with sterile physiological saline, after comparison with that of a MacFarland nephelometer tube no. 0.5 (10^8 cfu/ml) using a spectrophotometer at 625 nm (optical density 0.08-0.1). An inoculum of 10^6 cfu/ml of bacterial suspension was prepared by diluting 0.1 ml of the prepared bacterial broth culture with 9.9 ml sterile saline. *Candida* specimens were used undiluted (10^8 cfu/ml) (Murray *et al.*, 1995) (Appendix C).

2.1.3.2 Mycelial fungi inocula

A 6-mm diameter agar plug with mycelium was aseptically cut out of an actively growing fungal (1-4 day old) culture on either SDA or CMA medium. The disc was then transferred onto the center of the test medium.

2.1.4 Antimicrobial activity screening methods

2.1.4.1 Antibacterial and anticandidal activity screening test

2.1.4.1.1 Disk diffusion method

Using a sterile cotton applicator, 10^8 cfu / ml of bacterial suspension or *C. albicans* culture was swabbed on the surface of Muller-Hinton agar (MHA) as follows (Murray *et al.*, 1995):

1. A sterile cotton applicator was dipped into the bacterial or *C. albicans* suspension, rotated several times and pressed against the inside wall of the tube to remove excess inoculum.
2. An agar plate was then swabbed in all directions and around the agar margin to ensure even distribution of the inoculum.
3. The plate was left to dry in a laminar flow bench for 4-5 minutes.
4. Using sterile forceps the selected extract discs were then distributed evenly on the surface of the seeded agar plate.
5. The specific reference antibiotics, water and ethanolic discs were placed onto the agar plate beside the extract discs (Table 2.3).
6. Three replicate plates were used for each test.
7. The plates were incubated upside down at 37 °C for 18 hours.
8. The inhibition zone around each disc was then measured using transparent ruler (Murray *et al.*, 1995).

Selection of reference antibiotics was made according to the species to be tested or the origin of the specimen (blood, urine, skin, plant, etc.). The selection of the antibiotic also took into consideration drugs that are epidemiologically useful (Jawetz *et al.*, 1995).

Table 2.3 Reference antibiotics used in susceptibility test for tested microorganisms.

No.	Test microorganism	Reference Antibiotic	Concentration
	Gram negative bacteria		
1.	*Escherichia coli*	Apmicillin	10 mg/disc
2.	*Proteus vulgaris*	Gentamicin	10 mg/disc
3.	*Pseudomonas aeroginosa*	Gentamicin	10 mg/disc
4.	*Klebsiella pneumonia*	Ciprofloxacin	10 mg/disc
	Gram positive bacteria		
5.	*Staphylococcus aureus*	Penicillin G	10 mg/disc
	Yeast		
6.	*Candida albicans*	Nystatin	10 mg/disc
	Dermatophytes		
7.	*Microsporum gypseum*	Nystatin	5 mg/ml
8.	*Microsporum canis*	Griseofluvin	0.6 µg/ml
	Phytopathogenic fungi		
9.	*Fusarium tricinctum*	Nystatin	5 mg/ml
10.	*Pythium ultimum*	Hymexazol	25 µ g /ml
11.	*Pythium aphanidermatum*	Hymexazol	25 µ g /ml
12.	*Pythium middletonii*	Hymexazol	25 µ g /ml
13.	*Phytophthora citrophthora*	Metalaxyl	10 µ g /ml

2.1.4.2 Screening for antifungal activities

2.1.4.2.1 Poisoned-food technique method

In this method all test isolates were inoculated onto SDA or CMA plates and incubated at 25 °C for 1-4 days to obtain young, actively growing cultures consisting of mycelia and conidia. The required amount of the dried plant extract or reference antimycotic drug was dissolved in 2 ml sterile distilled water or 10 % aqueous dimethyl sulfoxide (DMSO), sterilized by filtration through a 0.45 µm membrane filter, and then mixed in requisite amount of pre-sterilized SDA or CMA medium to give a final concentration of

1.5mg/ml. A mycelial disc of 6-mm diameter, cut out from the periphery of 1-4 day old cultures, was aseptically inoculated onto the medium. In controls, sterile DMSO or distilled water was used in place of plant extract as negative control and reference antibiotics as a positive control (Georgii *et al.*, 1991; McCutcheon *et al.*, 1994). The inoculated plates were then incubated at 25 °C and colony diameter measured and recorded after 7 days for dermatophytes and keratinophilic fungi, and one day for *Pythium* and *Phytophthora* species. Percentage of mycelial inhibition was calculated as follows:

$$\% \text{ mycelial inhibition} = \frac{dc-dt}{dc} \times 100$$

Where: dc, colony diameter in control (-ve); dt, colony diameter in treatment. Three replicate plates were used for each treatment (Dikshit *et al.*, 1984).

2.2 Statistical analysis

Data were analyzed and treatments compared using analysis of variance with Duncan multiple range test ($P < 0.05$).

CHAPTER THREE

RESULTS

CHAPTER THREE

RESULTS

3.1 Antibacterial and anticandidal activity of ethanolic extracts

Results of antibacterial and anticandidal activity *in vitro* testing of 37 ethanolic extracts of 37 plants (Table 2.1) against five bacterial species and one yeast *(C. albicans)* are presented in Table 3.1 and Figure 3.1.

3.1.1 Antibacterial activity of ethanolic extracts against gram negative bacteria (*E. coli, P. vulgaris, P. aeroginosa, K. pneumonia*)

All plants studied showed antibacterial activity against the test strains with the exception of *Cichorium pumilum, Cyclamen persicum, Foeniculum vulgare, Gagea chloranth, Lactuca serriola, Paronychia argentea* and *Rubia tenuifolia* (Table 3.1). The plants differ significantly in their activity ($F= 15.806$, $DF = 36$, $P < 0.01$). The most active plants (30% of the plant extracts) were: *Alcea setosa, Coridothymus capitatus, Satureja thymbra, Lactuca tuberosa, Rhus coriaria, Quercus calliprinos, Verbascum sinuatum, Achillea fragrantissima* and *Lupinus pilosus* with inhibition zone means ranging from 19.4-8.6 mm. The least active plants (30% of the plant extracts) were *Pistacia lentiscus, Vitex agnus-castus, Euphorbia hierosolymitana, Linum Pubescens, Papaver rhoeas, Scrophularia rubricaulis, Lycium europeum, Capparis*

spinosa and *Companula rapunculus* with inhibition zone means ranging from 7-6.6 mm (Table 3.1). Other plants (40% of the plant extracts) showed moderate antibacterial activity with a range of inhibition zone diameter mean 8.3-7.2 mm (Table 3.1)

3.1.2 Antibacterial activity of ethanolic extracts against gram positive bacteria

All plants studied showed antibacterial activity against the test strain *(S. aureus)* with the exception of *Cichorium pumilum, Cyclamen persicum, Foeniculum vulgare, Gagea chloranth, Lactuca serriola, Lactuca tuberosa, Paronychia argentea, Rubia tenuifolia, Teucrium polium* and *Vitex agnus-castus* (Tale 3.1). The plants differ significantly in their activity ($F= 79.475$, $DF= 36$, $P<0.01$). The most active plants (30% of the plant extracts) were *Satureja thymbra, Calycotome villosa, Coridothymus capitatus, Quercus calliprinos* and *Pinus halepensis* with inhibition zone means ranging from 24.9-16 mm. The least active plants (30% of the plants) were *Lupinus pilosus, Euphorbia hierosolymitana, Papaver rhoeas, Varthemia iphionoides, Scrophularia rubricaulis, Lycium europeum, Capparis spinosa* and *Companula rapunculus* with inhibition zone means ranging from 12.8- 9 mm (Table 3.1). Other plants (40% of the plants) showed moderate antibacterial activity with a range of inhibition zone diameter mean 15.7-12.9 mm (Table 3.1).

Table 3.1 Antimicrobial activity of ethanolic extracts against bacteria and *Candida albicans*

		Means* of % mycelial inhibition ± SE								
No	Microorganisms / Plants	*E. coli*	*P. vulgaris*	*P. aeroginosa*	*K. pneumonia*	Total 1*	*S. aureus*	Total 2*	*C. albicans*	Total 3*
1	*Achillea fragrantissima*	10.2 ± 0.22 e**	15.1 ± 1.06 cd	6 ± 0 h	6 ± 0 g	9.3 ± 1.15 fghi	14.6 ± 1.35 fgh	10.4 ± 1.09 de	12.9 ± 1.66 e	10.8 ± 0.96 cd
2	*Ajuga orientalis*	6*** ± 0 h	13.4 ± 0.48 de	6 ± 0 h	6 ± 0 g	7.9 ± 0.97 fghijk	13.3 ± 0.19 hi	8.9 ± 0.97 defg	11.6 ± 0.79 f	9.4 ± 0.84 cdef
3	*Alcea setosa*	18 ± 1.02 a	18 ± 0 b	20.4 ± 0.44 a	21 ± 0.57 b	19.4 ± 0.49 b	19.8 ± 0.96 d	19.4 ± 0.43 b	6 ± 0 i	17.2 ± 1.26 b
4	*Anthemis palestina*	6 ± 0 h	14.2 ± 0.55 de	6 ± 0 h	6 ± 0 g	8.1 ± 1.07 fghijk	15.3 ± 0.33 efg	9.5 ± 1.15 def	10.3 ± 0.19 g	9.6 ± 0.96 cde
5	*Anthemis tunictoria*	6 ± 0 h	15 ± 0.50 cd	6 ± 0 h	6 ± 0 g	8.2 ± 1.17 fghijk	15.7 ± 1.38 efg	9.7 ± 1.24 def	14.3 ± 0.50 d	10.5 ± 1.11 cde
6	*Calycotome villosa*	6 ± 0 h	10.8 ± 0.11 ghi	6 ± 0 h	6 ± 0 g	7.2 ± 0.62 hijk	21.8 ± 0.77 c	10.1 ± 1.64 de	6 ± 0 i	9.4 ± 1.40 cdef
7	*Capparis spinosa*	6 ± 0 h	8.6 ± 0.39 l	6 ± 0 h	6 ± 0 g	6.6 ± 0.34 jk	9.3 ± 0.33 i	7.2 ± 0.40 fg	6 ± 0 i	7 ± 0.34 fg
8	*Ceratonia siliqua*	6 ± 0 h	10.9 ± 0.11 ghi	6 ± 0 h	6 ± 0 g	7.2 ± 0.63 hijk	19.3 ± 0.50 d	9.6 ± 1.39 def	9.9 ± 0.11 g	9.7 ± 1.5 cde
9	*Cichorium pumilum*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
10	*Companula rapunculus*	6 ± 0 h	8.6 ± 0.11 l	6 ± 0 h	6 ± 0 g	6.6 ± 0.33 jk	9 ± 0.51 j	7.1 ± 0.37 fg	6 ± 0 i	6.9 ± 0.32 fg
11	*Coridothymus capitatus*	16.6 ± 0.86 b	17.9 ± 0.22 b	9.2 ± 0.58 g	15.2 ± 0.11 c	14.7 ± 1.02 c	20.7 ± 0.66 cd	15.9 ± 1.03 c	34.3 ± 0.19 a	19 ± 1.87 b
12	*Crataegus aronia*	6 ± 0 h	10.7 ± 0.19 hi	6 ± 0 h	6 ± 0 g	7.2 ± 0.61hijk	14 ± 0.57 ghi	8.5 ± 0.88 defg	6 ± 0 i	8.1 ± 0.76 defg
13	*Cyclamen persicum*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
14	*Euphorbia hierosolymitana*	6 ± 0 h	9.8 ± 0.22 ijkl	6 ± 0 h	6 ± 0 g	6.9 ± 0.49 ijk	12.4 ± 0.44 i	8 ± 0.71 efg	6 ± 0 i	7.7 ± 0.61 efg
15	*Foeniculum vulgare*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
16	*Gagea chloranth*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
17	*Lactuca serriola*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
18	*Lactuca tuberosa*	13 ± 0.19 c	9.3 ± 0.50 jkl	12.8 ± 0.86 d	12 ± 0.66 e	11.8 ± 0.51de	6 ± 0 k	10.6 ± 0.73 de	6 ± 0 i	9.8 ± 0.74 cde
19	*Linum pubescens*	6 ± 0 h	9.3 ± 0.66 jkl	6 ± 0 h	6 ± 0 g	6.8 ± 0.45 jk	13.4 ± 0.29 hi	8.2 ± 0.79 efg	6 ± 0 i	7.8 ± 0.68 efg
20	*Lupinus pilosus*	6 ± 0 h	12 ± 0.38 fg	10.3 ± 0.19 f	6 ± 0 g	8.6 ± 0.80 fghij	12.8 ± 1.05 hi	9.4 ± 0.79 def	6 ± 0 i	8.9 ± 0.73 defg
21	*Lycium europeum*	6 ± 0 h	8.7 ± 0.19 kl	6 ± 0 h	6 ± 0 g	6.7 ± 0.35 jk	9.7 ± 0.38 j	7.3 ± 0.43 fg	6 ± 0 i	7.1 ± 0.37 fg
22	*Micromeria fruticosa*	6 ± 0 h	12.4 ± 0.29ef	6 ± 0 h	6 ± 0 g	7.6 ± 0.84 ghijk	12.9 ± 0.77 hi	8.7 ± 0.88 defg	6 ± 0 i	8.2 ± 0.77 defg
23	*Papaver rhoeas*	6 ± 0 h	8.8 ± 0.44 kl	6 ± 0 h	6 ± 0 g	6.7 ± 0.37 jk	10.7 ± 0.83	7.5 ± 0.53 fg	8.4 ± 0.11 h	7.6 ± 0.45 efg
24	*Paronychia argentea*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
25	*Pinus halepensis*	6 ± 0 h	10.4 ± 0.55 hij	6 ± 0 h	6 ± 0 g	7.1 ± 0.59 hijk	16 ± 0.69 ef	8.9 ± 1.06 defg	6 ± 0 i	8.4 ± 0.92 defg
26	*Pistacia lentiscus*	6 ± 0 h	10 ± 0 ijk	6 ± 0 h	6 ± 0 g	7 ± 0.52 hijk	13.2 ± 0.98 hi	8.2 ± 0.80 efg	6 ± 0 i	7.9 ± 0.69 efg
27	*Quercus calliprinos*	6 ± 0 h	12.4 ± 0.44 ef	15.1 ± 0.44 c	6 ± 0 g	9.9 ± 1.21 efg	17 ± 0.57 e	11.3 ± 1.23 d	15.2 ± 0.40 d	12 ± 1.08 c
28	*Rhus coriaria*	6 ± 0 h	13.4 ± 0.29 de	14.6 ± 0.11 c	6 ± 0 g	10 ± 1.21 ef	15.7 ± 0.19 efg	11.1 ± 1.13 d	6 ± 0 i	10.3 ± 1.05 cde
29	*Rubia tenuifolia*	6 ± 0 h	6 ± 0 m	6 ± 0 h	6 ± 0 g	6 ± 0 k	6 ± 0 k	6 ± 0 g	6 ± 0 i	6 ± 0 g
30	*Sarcopoterium spinosum*	6 ± 0 h	13.8 ± 0.61 d	6 ± 0 h	6 ± 0 g	7.9 ± 1.02 fghijk	14.3 ± 0.38 fghi	9.2 ± 1.06 def	12.8 ± 0.11 e	9.8 ± 0.93 cde
31	*Satureja thymbra*	11.3 ± 0.50 d	15.4 ± 0.40 c	12 ± 0.19 e	13.3 ± 0 d	13 ± 0.49 cd	24.9 ± 0.39 b	15.4 ± 1.32 c	6 ± 0 i	16.8 ± 1.34 b
32	*Scrophularia rubricaulis*	6 ± 0 h	8.7 ± 0.33 kl	6 ± 0 h	6 ± 0 g	6.7 ± 0.35 jk	9.8 ± 0.48 j	7.3 ± 0.44 fg	6 ± 0 i	7.1 ± 0.38 fg
33	*Teucrium polium*	6 ± 0 h	11.6 ± 0.90 fgh	6 ± 0 h	6 ± 0 g	7.4 ± 0.75 hijk	6 ± 0 k	7.1 ± 0.61 fg	6 ± 0 i	6.9 ± 0.51 fg
34	*Varthemia iphionoides*	9.4 ± 0.67 f	9.2 ± 0.22 jkl	6 ± 0 h	6 ± 0 g	7.7 ± 0.52 fghijk	9.9 ± 0.22 j	8.1 ± 0.48 efg	6 ± 0 i	7.8 ± 0.44 efg
35	*Verbascum sinuatum*	8.4 ± 0.44 g	9.2 ± 0.11 jkl	10.4 ± 0.11 f	9.4 ± 0.67 f	9.4 ± 0.27 fgh	15.7 ± 0.38 efg	10.6 ± 0.70 de	6 ± 0 i	9.9 ± 0.72 cde
36	*Viscum cruciatum*	6 ± 0 h	15.3 ± 0.19 cd	6 ± 0 h	6 ± 0 g	8.3 ± 1.21 fghijk	18.9 ± 0.80 d	10.4 ± 1.49 de	6 ± 0 i	9.7 ± 1.29 cde
37	*Vitex agnus-castus*	6 ± 0 h	10 ± 0 ijk	6 ± 0 h	6 ± 0 g	7 ± 0.52 hijk	6 ± 0 k	6.8 ± 0.42 fg	6 ± 0 i	6.7 ± 0.36 fg
38	Control (+ve)	16.6 ± 0.44 b	24.1 ± 0.78 a	18.9 ± 0.48 b	26.3 ± 0.50 a	21.5 ± 1.20 a	33.8 ± 0.29 a	23.9 ± 1.62 a	19.3 ± 0.19 c	23.3 ± 1.41 a

*Means of three replicate plates for each isolate of each species.

**Values in the same column followed by the same letter were not significantly different based on Duncan's multiple -range test ($p < 0.05$).

***Disk diameter, 6 mm.

1*: means for gram negative bacteria.

2*: means for both gram positive and gram negative.

3*: means for all bacteria and *C. albicans*.

3.1.3 Anticandidal activity of ethanolic extracts

Among all the plants studied, only *Coridothymus capitatus, Satureja thymbra, Quercus calliprinos, Anthemis tunictoria, Achillea fragrantissima, Sarcopoterium spinosum, Ajuga orientalis, Anthemis palestina, Ceratonia siliqua* and *Papaver rhoeas* showed anticandidal activity with inhibition zone means ranging from 34.3-8.4mm (Table 3.1). The plants differ significantly in their activity ($F=304.481$, $DF= 36$, $P <0.01$).

3.2 Susceptibility of test bacterial strains and *C. albicans* to ethanolic extracts

Test strains differed significantly in relation to their susceptibility to the different plants extracts used ($F= 30.648$, $DF= 5$, $P<0.01$). The most sensitive test microorganism was *S. aureus* (gram positive) with inhibition zone diameter mean 12.4 mm. Whereas the least sensitive test microorganism was *K. pneumonia* (gram negative) with inhibition zone diameter mean 7.1mm. The most sensitive gram negative strain was *P. vulgaris* with inhibition zone diameter mean 10.7 mm (Table 3.2 & Fig. 3.1).

3.3 Antifungal activity of ethanolic extracts

Results of antifungal activity *in vitro* testing of 48 ethanolic extracts of 48 plants (Table 2.1) against seven isolates of mycelial fungi are presented in Table 3.3 and Figure 3.2.

Table 3.2 Susceptibility of test bacteria and *C. albicans* to plant extracts and reference antibiotics as shown by inhibition zone diameter (mm).

Inhibition zone diameter (mean + SE)			
Microorganism		**Reference antibiotic**	
C. albicans	8.5 ± 0.55	Nystatin	19.3 ± 0.19
E. coli	7.2 ± 0.28	Ampicillin	16.6 ± 0.44
K.. pneumonia	7.1 ± 0.30	Ciprofloxacin	26.3 ± 0.50
P. vulgaris	10.7 + 0.32	Gentamicin	24.1 ± 0.77
P. aeroginosa	7.5 ± 0.32	Gentamicin	18.9 ± 0.48
S. aureus	12.4 ± 0.50	Penicillin G	33.8 ± 0.29

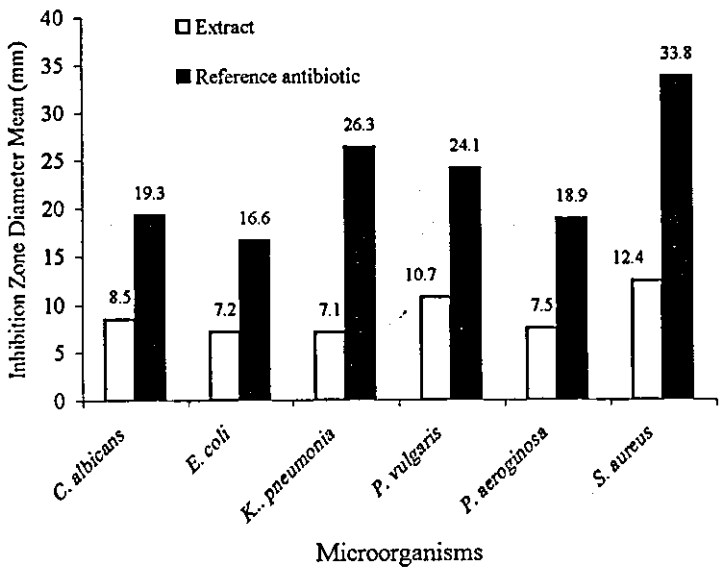

Figure 3.1 Susceptibility of *Candida albicans* and bacterial strains to ethanolic plant extracts.

3.3.1 Antifungal activity of ethanolic extracts against human and animal pathogenic dermatophtes (*M. canis* and *M. gypseum*)

The inhibitory effect of plants extracts against *M. canis* and *M. gypseum* varied (about 9% to 100% inhibition) significantly between plants ($F= 34.457$, $DF= 47$, $P < 0.01$). Extracts of *Coridothymus capitatus*, *Micromeria nervosa* and *Satureja thymbra* completely prevented growth of *M. canis* and *M. gypseum* (Table 3.3).

Extracts of *Cichorium pumilum, Coridothymus capitatus, Micromeria nervosa, Pinus halepensis, Salvia fruticosa, Satureja thymbra* and *Viscum cruciatum* were the most activie (100% inhibition) against *M. canis*. Extracts of *Ceratonia siliqua, Alcea setosa, Gagea chloranth, Eryngium creticum, Capparis spinosa, Varthemia iphionoides* and *Crataegus aronia* were on the other hand, least active (< 20% inhibition).

Extracts of *Rhus coriaria, Satureja thymbra, Inula viscosa, Ruta chalepensis, Micromeria nervosa, Lawsonia inermis, Coridothymus capitatus* and *Anthemis tunictoria* were the most active (80%-100% inhibition) against *M. gypseum*. Extracts of *Juglans regia, Ceratonia siliqua, Alcea setosa* and *Ziziphus spina-christi* were on the other hand, least active (<20% inhibition).

Table 3.3 Antifungal activity of ethanolic extr

No.	Plants \ Fungi	*M. canis*	*hthora*	Total 3**	Total 4**
1	*Achillea fragrantissima*	36.4 ± 2.77	.32 fg	27.7 ± 7.39 cdef	53.4 ± 10 def
2	*Ajuga orientalis*	33.6 ± 0.52	.34 gh	45.7 ± 3.39 efghijklm	45.2 ± 4.9 defghij
3	*Alcea setosa*	10.3 ± 0.6	16 kl	39.5 ± 5.81 ghijklmnop	31.9 ± 9 jklmno
4	*Anthemis palestina*	23.6 + 1.04	0 d	48.2 ± 4.31 efghijk	42.9 ± 6.7 efghij
5	*Anthemis tunictoria*	61.8 + 1.0	)	68.7 ± 8.71 bc	69.6 ± 10.7 bc
6	*Asphodelin lutea*	47.3 + 1.0	)	44.6 ± 7.72 fghijklmn	50 ± 10.6 defg
7	*Calycotome villosa*	47.3 + 3.1	)	54.7 ± 8.90 defghi	53.5 ± 10.7 def
8	*Capparis spinosa*	17.6 + 4.9	)	61.5 ± 9.94 cde	50.3 ± 14.2 defg
9	*Ceratonia siliqua*	1.8 + 0	.14 d	20.7 ± 4.12 rstu	17.3 ± 5.9 p
10	*Cichorium pumilum*	100 + 0	)	39.3 ± 6.91 ghijklmnop	51.5 ± 12.9 defg
11	*Clematis cirrhosa*	28.5 + 2.18	)	19.4 ± 2.05 stu	21.2 ± 2.7 nop
12	*Coridothymus capitatus*	100 + 0	)	79.6 ± 5.32 ab	86.4 ± 7.7 a
13	*Crataegus aronia*	18.8 + 1.21	.34 n	18.3 ± 2.30 stu	20.5 ± 3.5 op
14	*Cyclamen persicum*	56.4 + 5.2	2.66 d	48.5 ± 1.25 efghij	51.3 ± 2.2 defg
15	*Eryngium creticum*	17.3 + 0.5	)	27.1 ± 7.03 nopqrstu	26.9 ± 8.8 klmnop
16	*Euphorbia hierosolymitana*	20.6 + 0.61	0 m	32.2 ± 4.01 klmnopqrst	29 ± 5.6 klmnop
17	*Gagea chloranth*	16.4 + 00	m	25.6 ± 4.12 nopqrstu	24 ± 5.5 lmnop
18	*Inula viscosa*	73.9 ± 0.6	)	66.5 ± 2.04 bcd	73.3 ± 5.9 b
19	*Juglans regia*	26.7 ± 0.60	)	23.3 ± 5.02 pqrstu	23.2 ± 6.1 nop
20	*Lactuca serriola*	27.3 ± 0 n	0 ij	36.1 ± 6.08 ijklmnopqr	33.6 ± 8 ijklmn
21	*Lactuca tuberosa*	44.5 ± 2.6	.34 n	47.9 ± 6.25 efghijkl	46.5 ± 8.1 defghi
22	*Lawsonia inermis*	37.9 ± 5.6	)	30.3 ± 3.81 lmnopqrstu	43.2 ± 12.3 efghij
23	*Linum pubescens*	30.3 ± 0.61	.16 l	36 ± 4.73 ijklmnopqrs	34.7 ± 6.2 ijklmn
24	*Lupinus pilosus*	42.7 ± 2.6	0 e	50.5 ± 2.29 efghij	49.4 ± 2.8 defgh
25	*Lycium europeum*	56.4 ± 0	16 hij	33.4 ± 1.76 jklmnopqrst	40.4 ± 5 fghijk
26	*Micromeria fruticosa*	53.9 ± 1.2	.32 jk	34.3 ± 2.04 ijklmnopqrs	40.1 ± 4.4 fghijk
27	*Micromeria nervosa*	100 ± 0	)	91.9 ± 4.23 a	94.6 ± 5.4 a
28	*Papaver rhoeas*	43.6 ± 1.0	0 l	22.1 ± 2.56 qrstu	27.8 ± 4.9 klmnop
29	*Parietaria diffusa*	33.9 ± 1.2	)	12.6 ± 2.86 v	18.6 ± 5.2 op
30	*Paronychia argentea*	22.7 ± 1.57	14 jk	40.9 ± 8.23 ghijklmnop	35.4 ± 11.2 ijklm
31	*Pinus halepensis*	100 ± 0	0 a	88.8 ± 5.96 a	88.5 ± 8.2 a
32	*Pistacia lentiscus*	27.3 + 1.04	.34 n	37.4 ± 4.98 ijklmnopqr	34.9 ± 6.6 ijklmn
33	*Quercus calliprinos*	72.7 ± 1.0	0 c	71.2 ± 4.27 bc	69.8 ± 5.8 bc
34	*Retema raetam*	58.2 ± 1.8	)	34.1 ± 3.40 ijklmnopqrst	37.9 ± 5.6 ghijkl
35	*Rhus coriaria*	78.3 ± 0	hi	60 ± 4.74 cde	68.3 ± 8.5 bc
36	*Rubia tenuifolia*	30.9 ± 1.0	)	16 ± 1.70 u	20.9 ± 3.7 nop
37	*Ruscus acculeatus*	63.6 ± 1.8	)	25 ± 3.89 opqrstu	32.1 ± 7.9 jklmno
38	*Ruta chalepensis*	67.3 ± 0	)	37.7 ± 3.72 hijklmnopq	53 ± 11.5 def
39	*Salvia fruticosa*	100 ± 0	)	70.4 ± 6.16 bc	73.9 ± 9.2 b
40	*Sarcopoterium spinosum*	71.8 ± 1.5	0 ef	54.9 ± 6.35 cdefgh	57.5 ± 8.5 cd
41	*Satureja thymbra*	100 ± 0	0 ef	94.2 ± 3.10 a	95.9 ± 4.1 a
42	*Solanum nigrum*	50.9 ± 1.6	)	28.3 ± 4.47 lmnopqrstu	34.5 ± 6.7 ijklmn
43	*Teucrium polium*	30.3 ± 2.1	.16 l	27.7 ± 4.03 mnopqrstu	28.7 ± 5.2 klmnop
44	*Varthemia iphionoides*	18.8 ± 0.61	.34 hij	42.6 ± 4.16 fghijklmno	36 ± 6.8 hijklm
45	*Verbascum sinuatum*	41.8 ± 1.8	)	28.1 ± 3.34 mnoppqrstu	32.1 ± 4.8 jklmno
46	*Viscum cruciatum*	100 ± 0	1.34 e	67.4 ± 7.51 bc	71.8 ± 10.8 b
47	*Vitex agnus-castus*	37 ± 0.6	0 m	38.7 ± 5.81 ghijklmnopq	37.7 ± 7.5 ghijkl
48	*Ziziphus spina-christi*	21.8 ± 1.04	)	17 ± 3.45 tu	16.8 ± 4.3 p
	+ ve control (reference antibiotic) ***	63.6 ± 0	0 b	55.7 ± 0 cdefg	54.6 ± 3.40 de

* Means of three replicate plates for eac

** Values in the same column followe test ($p < 0.05$)

*** Type and (concentration) of refe metalaxyle (10 µg/ml), for *F. tricinctu*

3.3.2 Antifungal activity of ethanolic extracts against phytopathogenic fungi (*P. ultimum, P. aphanidermatum, P. middletonii, Ph. citrophthora, F. tricinctum*)

The inhibitory effect against the five fungi varied (about 13% to 94% inhibition) significantly between plants ($F=16.066$, $DF = 47$, $P< 0.01$) (Table 3.3). Extracts of *Satureja thymbra, Micromeria nervosa* and *Pinus halepensis* were the most active (> 80% inhibition).

Extracts of *Clematis cirrhosa, Crataegus aronia, Ziziphus spina-christi, Rubia tenuifolia* and *Parietaria diffusa* were on the other hand, least active (<20% inhibition).

3.3.3 Antifungal activity of ethanolic extracts against phytopathogence *Pythium* species (*P. ultimum, P. aphanidermatum, P. middletonii*)

The inhibitory effect against the three fungi varied (8.1 % to 100% inhibition) significantly between plants ($F=18.474$, $DF= 47$, $P< 0.01$) (Table 3.3). Extracts of *Micromeria nervosa, Pinus halepensis* and *Satureja thymbra* completely prevented growth of *P. ultimum, P. aphanidermatum* and *P. middletonii.*

Extracts of *Achillea fragrantissima, Micromeria nervosa, Pinus halepensis, Quercus calliprinos, Satureja thymbra, Viscum cruciatum, Anthemis tunictoria* and *Coridothymus capitatus* were the most active (80%-

100% inhibition) against *P. ultimum.* Extracts of *Ceratonia siliqua* and *Parietaria diffusa* were on the other hand, least active (<10% inhibition).

Extracts of *Calycotome villosa, Capparis spinosa, Micromeria nervosa, Paronychia argentea, Pinus halepensis, Salvia fruticosa, Sarcopoterium spinosum, Satureja thymbra, Viscum cruciatum, Achillea fragrantissima, Anthemis tunictoria, Asphodelin lutea, Coridothymus capitatus* and *Rhus coriaria* were also most active (80%-100% inhibition) against *P. aphanidermatum,* whereas extracts of *Ruscus acculeatus* and *Ziziphus spina-christi* were the least active (< 10% inhibition).

Extracts of *Coridothymus capitatus, Micromeria nervosa, Pinus halepensis, Satureja thymbra* and *Anthemis tunictoria* were the most active (80%-100% inhibition) against *P. middletonii.* Extracts of *Parietaria diffusa, Eryngium creticum, Rubia tenuifolia* and *Juglans regia* were on the other hand, least active (<10% inhibition).

3.3.4 Antifungal activity of ethanolic extracts against phytopathogenic *Ph. citrophthora*

The inhibitory effect against these fungi varied (5.3% to 100% inhibition) significantly between plants ($F=256.138$, $DF = 26$, $P<0.01$) (Table 3.3).

Extracts of *Pinus halepensis* and *Satureja thymbra* were the most active (100% inhibition). Extracts of *Pistacia lentiscus, Lactuca tuberosa* and *Crataegus aronia* were on the other hand, least active (<10% inhibition).

3.3.5 Antifungal activity of ethanolic extracts against phytopathogenic *F. tricinctum*

The inhibitory effect against these fungi varied (11% to 72% inhibition) significantly between plants (F= 101.012, DF= 47, P< 0.01) (Table 3.3).

Extracts of *salvia fruticosa* and *Satureja thymbra* were the most active (>70% inhibition). Extracts of *Capparis spinosa, Papaver rhoeas, Rubia tenuifolia, Calycotome villosa* and *Anthemis tunictoria* were on the other hand, least active (< 20% inhibition).

3.4 Antifungal activity of aqueous extracts

Results of antifungal activity *in vitro* testing of 14 aqueous extracts of 14 plants (Table 2.1) against six isolates of mycelial fungi are presented in Table 3.4 and Figure 3.3.

3.4.1 Antifungal activity of aqueous extracts against human and animal pathogenic dermatophytes (*M. canis* and *M. gypseum*)

The inhibitory effect against the two dermatophytes varied (6% to 61% inhibition) significantly between plants (F=16.812, DF= 13, P< 0.01) (Table 3.4). Extracts of *Anthemis tunictoria* and *verbascum sinuatum* were the most active (>50% inhibition). Extracts of *Juglans regia, Phagnalon rupstre,*

Table 3.4 Antifungal activity of aqueous extracts of 14 plants

		Mean of % mycelial inhibition + SE									
No.	Fungi / Plants	*M. canis*	*M. gypseum*	Total 1*	*P. ultimum*	*P. aphanidermatum*	*P. middletonii*	Total 2*	*F. tricinctum*	Total 3*	Total 4*
1.	*Anchusa strigosa*	10.9 ± 1.04 g	14.6 ± 0 i	12.7 ± 0.94 efg	13.6 ± 0.87 i	37.3 ± 0.69 g	11.8 ± 0 g	20.9 ± 4.13 gh	26.9 ± 0 de	22.4 ± 3.14 e	19.2 ± 2.36 g
2	*Anthemis tunictoria*	63 ± 0.60 a	58.3 ± 0 a	60.7 ± 1.08 a	78.8 ± 0.87 b	100 ± 0 a	50 ± 0 a	76.3 ± 7.24 ab	53.2 ± 1.69 b	70.5 ± 6.15 a	67.2 ± 4.20 a
3.	*Calycotome villosa*	12.1 ± 1.20 g	27.1 ± 0 g	19.6 ± 3.39 def	43.9 ± 1.51 f	88.8 ± 0 b	36.8 ± 1.70 b	56.5 ± 8.16 bcd	8.3 ± 0.32 i	44.5 ± 8.71 bcd	36.2 ± 6.47 de
4.	*Capparis spinosa*	7.9 ± 0.61 h	36.8 ± 0.69 de	22.3 ± 6.48 de	10.1 ± 0.50 j	35.3 ± 0.40 g	9.3 ± 0.49 g	18.3 ± 4.27 h	26.1 ± 1.04 defg	20.2 ± 3.33 e	20.9 ± 2.99 fg
5.	*Coridothymus capitatus*	25.4 ± 0 d	46.9 ± 2.49 b	37.7 ± 5.58 c	100 ± 0 a	63 ± 1.45 d	36 ± 0.42 b	66.4 ± 9.28 abc	26.3 ± 0.84 def	56.3 ± 8.62 ab	50.1 ± 6.30 bc
6.	*Inula viscosa*	27.9 ± 0.60 c	31.3 ± 0 f	29.6 ± 0.80 cd	48.5 ± 0.87 e	37.4 ± 2.08 g	29.4 ± 3.06 c	38.4 ± 2.97efgh	27.2 ± 0.84 de	35.6 ± 2.64 cde	33.6 ± 1.88 def
7.	*Juglans regia*	1.8 ± 0 j	15.6 ± 0.60 hi	8.7 ± 3.10 fg	34.1 ± 1.31 g	2.4 ± 0 i	8.8 ± 0 g	15.1 ± 4.85 i	31.1 ± 1.15 c	19.1 ± 4.15 e	15.6 ± 3.12 g
8.	*Parietaria diffusa*	45 ± 0.52 I	7.3 ± 0.60 j	5.9 ± 0.71 g	56.1 ± 2.62 de	100 ± 0 a	30 ± 0.74 c	62.1 ± 10.22 bc	24.5 ± 0 efg	52.7 ± 9 abc	37.1 ± 7.97 de
9.	*Phagnalon rupstre*	1.8 ± 0 j	31.3 ± 1.20 f	16.5 ± 6.60 efg	42.4 ± 1.74 f	77.7 ± 0.34 c	27.9 ± 0 cd	49.4 ± 7.40 cde	24 ± 1.11 fg	43 ± 6.39 bcd	34.2 ± 5.57 def
10.	*Retema raetam*	21.2 ± 1.21 e	25 ± 0 g	23.1 ± 1 de	42.4 ± 0.87 f	48.2 ± 1.39 f	8.8 ± 0.85 g	33.1 ± 6.15 fgh	28.3 ± 0.84 d	31.9 ±4.59 de	28.9 ± 3.19 defg
11.	*Rubia tenuifolia*	29.1 ± 1.04 c	51.1 ± 1.80 b	40.1 ± 5 c	100 ± 0 a	100 ± 0 a	52.4 ± 0.98 a	84.1 ± 7.93 a	10.3 ± 0.8 i	65.7 ± 11.28 a	27.1 ± 8.12 ab
12.	*Salvia fruticosa*	7.3 ± 0 h	34.7 ± 0.69 e	21 ± 6.14 def	6.1 ± 0 k	24.7 ± 2.43 h	16.2 ± 0.84 f	15.6 ± 2.79 h	23.4 ± 0.32 g	17.6 ± 2.29 e	18.7 ± 2.47 g
13.	*Verbascum sinuatum*	54 ± 0 b	46.5 ± 1.39 c	50.5 ± 1.89 ab	33.8 ± 1.33 g	63.3 ± 2.43 d	24.3 ± 1.27 e	40.5 ± 5.93 defg	28.5 ± 1.39 cd	37.5 ± 4.65 bcde	41.8 ± 3.45 cd
14.	*Ziziphus spina-christi*	19.1 ± 0.52 f	18.1 ± 0.69 h	18.6 ± 0.45 def	21.7 ± 0.50 h	51.2 ± 1.04 f	11 ± 0.41 g	28 ± 6.01 fgh	19.2 ± 0.55h	25.8 ± 4.58 de	23.4 ± 3.12 efg
	+ve control (reference antibiotics)	63.6 ± 0 a	39.6 ± 0 d	51.6 ± 0 ab	60.6 ± 0 c	56.6 ± 0 e	25 ± 0 de	47.4 ± 0 def	64.4 ± 0 a	51.7 ± 0 abc	51.6 ± 3.52 bc

1*: Means for dermatophytes.
2*: Means for *Pythium* species.
3*: Means for all phytopathogenic fungi.
4*: Means for all fungi.

Parietaria diffusa, Salvia fruticosa and *Capparis spinosa* were on the other hand, least active (< 10% inhibition).

Extracts of *Rubia tenuifolia* and *Anthemis tunictoria* were the most active against *M. gypseum.* Extracts of *Parietaria diffusa, Anchusa strigosa, Juglans regia* and *Ziziphus spina-christi* were on the other hand, least active (< 20% inhibition).

3.4.2 Antifungal activity of aqueous extracts against phytopathogenic fungi (*P. ultimum, P. aphanidermatum, P. middletonii, F. tricinctum*)

The inhibitory effect against the four fungi varied (18% to 71 % inhibition) significantly between plants (F=7.722, DF= 13, P< 0.01). Extracts of *Anthemis tunictoria, Rubia tenuifolia, Coridothymus capitatus* and *Parietaria diffusa* were the most active (>50% inhibition). Extracts of *Juglans regia* and *Salvia fruticosa* were on the other hand, least active (<20% inhibition) (Table 3.4).

3.4.3 Antifungal activity of aqueous extracts against phytopathogenic *Pythium* species (*P. ultimum, P. aphanidermatum, P. middletonii*)

The inhibitory effect against the three *Pythium* species varied (15% to 84% inhibition) significantly between plants (F=12.147, DF= 13, P< 0.01). Extracts of *Rubia tenuifolia, Anthemis tunictoria* and *Coridothymus capitatus*

were the most active. Extracts of *Capparis spinosa, Salvia fruticosa* and *Juglans regia* were on the other hand, least active (<20% inhibition). (Table 3.4).

Extracts of *Rubia tenuifolia, Coridothymus capitatus* and *Anthemis tunictoria* were the most active (70% to 100% inhibition) against *P. ultimum.* Extracts of *Anchusa strigosa, Capparis spinosa* and *Salvia fruticosa* were on the other hand, least active (<20% inhibition) (Table 3.4).

Extracts of *Anthemis tunictoria, Calycotome villosa, Parietaria diffusa* and *Rubia tenuifolia* were the most active (80% to 100% inhibition). Extract of *Juglans regia* was on the other hand, least active (<20% inhibition) against *P. aphanidermatum* (Table 3.4).

Extracts of *Anthemis tunictoria, Rubia tenuifolia, Calycotome villosa, Coridothymus capitatus* and *Parietaria diffusa* were the most active (30% to 50% inhibition) against *P.middletonii.* Extracts of *Anchusa strigosa, Capparis spinosa, Juglans regia, Retema raetam, Salvia fruticosa* and *Ziziphus spina-christi* were on the other hand, least active (<20% inhibition) (Table 3.4).

3.4.5 Antifungal activity of aqueous extracts against phytopathogenic *F. tricinctum*

The inhibitory effect against this fungus varied (8% to 53% inhibition) significantly between plants ($F=126.595$, $DF= 13$, $P< 0.01$). Extracts of *Anthemis tunictoria* and *Juglans regia* were the most active. Extracts of

Ziziphus spina-christi, Rubia tenuifolia and *Calycotome villosa* were on the other hand, least active (< 20% inhibition) (Table 3.4).

3.5 Susceptibility of test fungi to ethanolic plants extracts

Test fungi differed significantly (F=23.040, DF= 6, P< 0.01) in their susceptibility to antimycotic activity of plants extracts with the most susceptile fungus was *P. aphanidermatum* being completely inhibited by 9/48 (19%) of the extracts, whereas *Ph. citrophthora* was the least susceptible fungus being completely inhibited by 3/48 (6%) of the ethanolic extracts (Table 3.5, Figure 3.2).

3.6 Susceptibility of test fungi to aqueous plants extracts

Test fungi differed significantly (F=20.743, DF= 5, P< 0.01) in their susceptibility to antimycotic activity of plants extracts with the most susceptible fungus was *P. aphanidermatum* being completely inhibited by 3/14 (21%) of the extracts, whereas *M. canis* was the least susceptible fungus (Table 3.6, Figure 3.3).

Table 3.5 Susceptibility of test fungi to 48 ethanolic plants extracts as shown by % mycelial inhibition (mm).

% Mycelial Inhibition (mean + SE)			
Fungi		**Reference antibiotic**	
F. tricinctum	34.5 ± 1.22	Nystatin	64.4 ± 0
M. canis	47.6 ± 2.33	Griseofluvin	63.6 ± 0
M. gypseum	49.1 ± 2.22	Nystatin	39.6 ± 0
Ph. citrophthora	33.7 ± 2.68	Metalaxyl	72 ± 0
P. aphanidermatum	62.7 ± 2.33	Hymexazol	56.6 ± 0
P. middletonii	35.2 ± 2.22	Hymexazol	25 ± 0
P. ultimum	48.8 ± 2.31	Hymexazol	60.6 ± 0

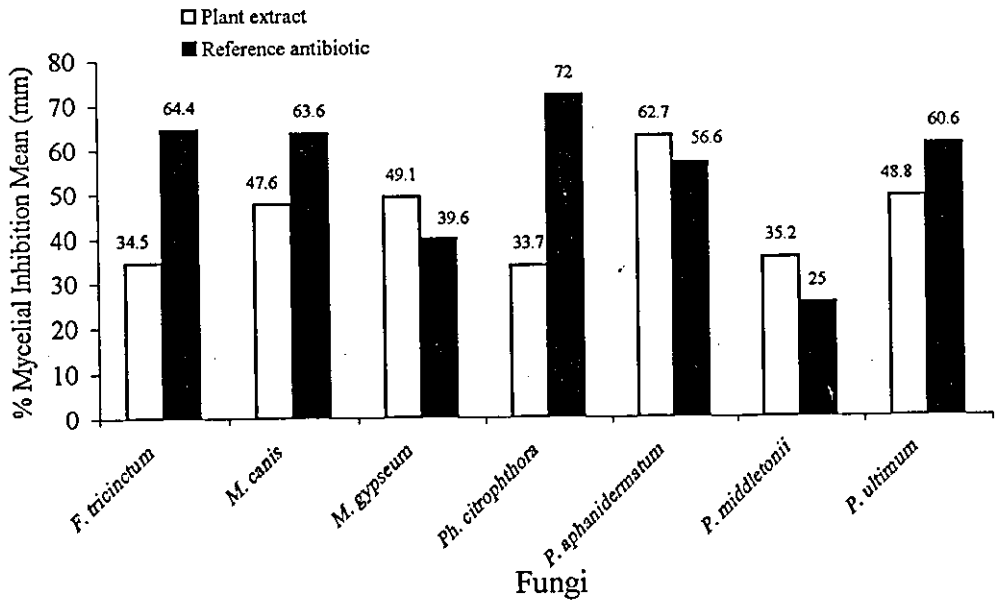

Figure 3.2 Susceptibility of test fungi to 48 ethanolic plants extracts as shown by % mycelial inhibition (mm).

Table 3.6 Susceptibility of test fungi to 14 aqueous plants extracts as shown by % mycelial inhibition (mm)

% Mycelial Inhibition (mean + SE)			
Fungi		**Reference antibiotic**	
F. tricinctum	25.5 ± 1.57	Nystatin	64.4 ± 0
M. canis	20.5 ± 2.82	Griseofluvin	63.6 ± 0
M. gypseum	32 ± 2.32	Nystatin	39.6 ± 0
P. aphanidermatum	59.2 ± 4.65	Hymexazol	56.6 ± 0
P. middletonii	25.2 ± 2.26	Hymexazol	25 ± 0
P. ultimum	45.1 ± 4.55	Hymexazol	60.6 ± 0

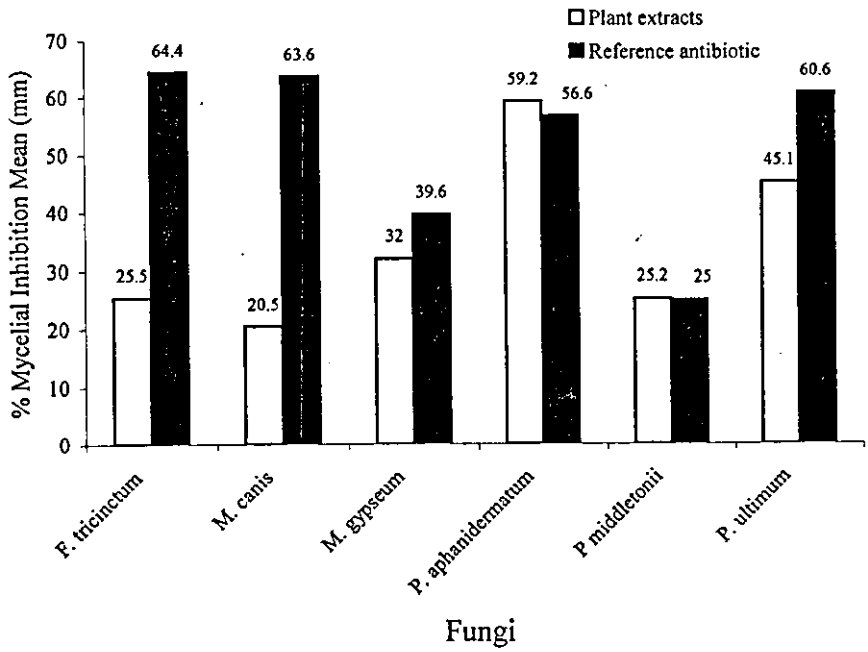

Figure 3.3 Susceptibility of test fungi to aqueous plants extracts.

CHAPTER FOUR

DISCUSSION

CHAPTER FOUR

DISCUSSION

4.1 Antibacterial and anticandidal activity of selected medicinal plants

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents (Alonso Paz *et al.*, 1995). The first step towards achieving this goal is the screening of plants used in popular medicine. The search for new, safer and more effective antimicrobial agents has grown with the increasing incidence of microbial infections (Larhsini *et al.*, 1996), relying on plants used in folk medicine. This folkloric use suggests that there may be a scientific basis for their utility in traditional medicine for the treatment of deferent infections (Irobi, 1992).

The results obtained, demonstrate that most of the studied plants 30/37 (82%) (Table 3.1) are potentially important sources of antibacterial agents. Seventy three percent and 81% of the screened plants were active against *S. aureus* (Gram +ve), and Gram -ve bacteria, respectively. The use of these plants in traditional medicine for treating various diseases

related to bacterial infections (Ali-Shtayeh *et al.,* 1998; Ali-Shtayeh, Yaniv *et al.,* 2000) is thus justified.

On the other hand, only 27% of the screened plants were active against *C. albicans.* This result is in agreement with that of (Alonso Paz *et al.,* 1995; Yaghmour, 1996 and Ali - Shtayeh *et al.,* 1998) Who also found that antibaterial agents to be more common in medicinal plants than anticandidal agents. This may be attributed to differences in structure between prokaryotic bacterial and eukaryotic candidal cells. Since antimicrobial agents should bind to sterols in eukaryotic membrane so as to exhibit their action, whereas this step is not needed for bacterial cells (Medoff & kayashi, 1993).

It is noteworthy to point out that G +ve bacteria were most susceptible to plant extracts than G -ve bacteria in the present work. This finding is therefore in conformity of those of Anesini & Perez (1993); Grosvenor *et al.,* (1995); Ali-Shtayeh *et al.* (1998) and Essawi & Srour (2000) Who also found that G +ve bacteria were more readily inhibited by plant extracts than G -ve bacteria. This may be attributed to the fact that cell wall in gram positive bacteria consist of a single layer, whereas gram negative cell wall is a multilayered structure and quite complex (Yao & Mollering, 1995).

4.2 Antifungal activity of selected medicinal plants

4.2.1 Antifungal assay of plants against dermatophytes

The fungitoxic effects of plant extracts of some plant species tested in the present work indicate the importance of many plant species as a natural source of antimycotic material (Table 3.3, 4) (major active constituents present are indicated in the Table 2.1). Antifungal activity of medicinal plants, e.g., *Juglans sp.* and, *Solanum sp.* extracts, against some dermatophytes including *M. canis* have also been reported by other workers (Ali-Shtayeh & Abu-Ghdeib, 1999). In the present study, among 48 locally available plant species tested *in vitro* against dermatophytes, the ethanolic extracts of *C. capitatus, M. nervosa* and *S. thymbra* were most active (100% inhibition), While the aqueous extracts of both *A. tunictoria* and *V. sinuatum* were most active (50-60% inhibition) against dermatophytes. The present results are therefore consistent with those of Vlietinck *et al.* (1995) Who showed that from 267 ethanolic crude extracts corresponding to 100 different plant species, 60% of the extracts have antidermatophytic activity including *M. canis*. Also Ali-Shtayeh & Abu-Ghdeib, (1999) found that among the 22 species they tested *in vitro* 27 - 81% of the aqueous extracts showed high antimycotic activity against one or more of the test dermatophytes including *M. canis.*

The present study indicates that the majority of the plants tested are an important source of antifungal compounds that may provide renewable sources of useful antifungal drugs against dermatophytic infections in humans. Among plant species tested, *A. tunictoria*, *C. capitatus*, *M. nervosa* and, *S. thymbra* were shown to have high antidermatophytic activity. This obviously justifies the use of many of these plants in traditional medicine to cure dermatophyte infections (Ali-Shtayeh & Abu-Ghdeib, 1999).

4.2.2 Antifungal assay of plants against phytopathogenic fungi

The importance of indigenous products for plant diseases control has been further investigated, and encouraging results on the subject have been reported by different workers (Akhtar *et al.*, 1986; Al-Abed *et al.*, 1993; Qasem & Abu-Blan, 1995).

In vitro assessment of the potential of plant extracts as fungitoxicants showed that plant species were different in their effects; some inhibited growth, others stimulated it or had no apparent effects (Al-Abed *et al.*, 1993). In the present study results indicated the presence of active fungistatic materials in the extract of certain plant species. Screening plant extracts for their antifungal effects in this work

demonstrates that plants are potential sources of fungitoxic compounds. Most active extracts among 48 ethanolic and 14 aqueous plant extracts tested *in vitro,* include the ethanolic extracts of *M. nervosa, P. halepensis* and *S. thymbra* against *Pythium sp.;* extracts of *P. halepensis* and *S. thymbra* against *Ph. citropthora;* and extracts of *S. fruticosa* and *S. thymbra* against *F. tricinctum.* These results are in consistent with those of Grosvenor *et al.* (1995) who showed that 20% of 114 plant species extracts inhibited the growth of *Fusarium oxysporum.* On the other hand among 14 locally available plant species tested *in vitro* the aqueous extracts of *R. tenuifolia, A. tunictoria* and *C. capitatus* were highly effective against *Pythium sp.,* while extracts of *A. tunictoria and J. regia* were highly effective against *F. tricinctum.* These findings substantiate those of Al-Abed *et al.* (1993) Who also found that from 40 weeds from Jordan, aqueous extracts of *I. viscosa* and *A. arvensis* were most active against the phytopathogenic fungi *Fusarium oxysporum* and *Helminthosporium sativum.*

The present ethanolic extracts were found to show higher antifungal activity than aqueous extracts ($F = 16.441$, $DF = 11$; $P < 0.01$). This may be attributed to differences in the nature and / or concentration and in the relative solubility in ethanol and water of chemical inhibitors in the different plant species (Al-Abed, 1992; Qasem & Abu-blan, 1995).

The results also showed that dermatophytes were more susceptible to ethanolic extracts than phytopathogenic fungi ($F=5.808$, $DF= 1$; $P< 0.01$) while the later fungi were more susceptible to aqueous extracts than dermatophytes ($F = 15.095$, $DF = 1$; $P< 0.01$).

It is interesting to note that some of the ethanolic plant extracts (e.g., *C. capitatus* and *S. thymbra*) and some of the aqueous extracts (e.g., *C. capitatus*) found in the present work to be active against dermatophytes and plant pathogenic fungi, were also active against *C. albicans*. The present results are therefore in conformity of those of Ali-Shtayeh & Abu-Ghdeib, (1999) who also found that some of the aqueous plant extracts they tested to be highly active against dermatophytes. These extracts were also found to be active against *C. albicans* (Ali-Shtayeh *et al.*, 1998).

On the other hand, many of the plant species (e.g., ehtanolic extracts of *P. halepensis* (87.5%), *A. setosa* (8.7 %), and the aqueous extracts of *A. tunictoria* (60.7%), *C. spinosa* (22.3%) that showed high to low antifungal activity against dermatophytes, were found to be inactive against *C. albicans*. The present results are therefore in conformity of those of Vlietinck *et al.* (1995) Who also found that plants with high activity against dermatophytes, were inactive against *C. albicans*. This indicates that anticandidal compounds are less frequently encountered in the ethanolic and aqueous extracts of the test plants than other antifungal

compounds and also indicates the difference in the mode of action of these compounds. It is noteworthy to mention that anticandidal compounds (e.g., nystatin- a polyene macrolide antibiotic) are characterized by having a large lactone ring with a number of conjugated double bonds. They are membrane active agents which produce their effect by creating polar 'pores' by insertion alongside the phospholipid and sterol molecules (Gale *et al.,* 1981). On the other hand, antidermatophytic compounds like griseofluvin which affect only dermatophytes, where have chitin in their walls have no effect on yeast like *C. albicans*. Their mode of action is binding strongly to the proteins associated with the microtubules so they disrupt nuclear division molecules (Gale *et al.,* 1981).

The present study demonstrates that plants are an important source of fungitoxic compounds, and that they may provide a renewable source of useful pesticides that can be utilized in integrated pest control programs. Further studies are therefore needed on these plants in the search for new and more potent antifungal substances from natural sources.

4.3 Conclusions and recommendations

1. Since high percentage of the plants studied have a broad antimicrobial spectrum of action, they could be useful in antiseptic, disinfectant and

pesticide formulation and in external chemotherapy, taking into consideration the satisfaction of the same basic requirements of other pharmaceutical products: quantity, safety and efficacy.

2. Further studies are needed and encouraged to test for the biological activities of wild plants and explore their potential as sources of antimicrobial agents and for other benefits to human health.
3. Further studies are needed to find out active constituents of many plants.
4. For achieving better results, plant extracts would be more efficient if prepared using infusion extraction technique with suitable organic solvent such as ethanol.
5. The establishment of Palestinian research institute on medicinal plants and a horticultural station to grow and preserve endangered indigenous species of medicinal plants is recommended.
6. Further work is therefore needed on these plants to identify and study their active ingredients.

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APPENDIX A

MEDIA

Cornmeal Agar (CMA)

Cornmeal (Dehydrated infusion from corn)	17.0 g
Agar	15.0 g

Directions:

17.0g are suspended in one liter of distilled water with 15.0-g agar and heat to dissolve, sterilized by autoclaving at 121^{O}C for 15 minutes.

Muller-Hinton Agar (MHA) (OXOID)

Beef, dehydrated infusion from	300.0g
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	17.0 g

pH 7.4 ± 02

Directions:

38.0-g are suspended in one liter of distilled water and heated to dissolve, and sterilized by autoclaving at 121 ^{O}C for 15 minutes.

Muller-Hinton Broth (MHB) (OXOID)

Beef, dehydrated infusion from	300.0g
Casein hydrolysate	17.5 g
Starch	1.5 g

pH 7.4 ± 0.2

Directions:

21.0 g are suspended in one liter of distilled water and heated to dissolve, and sterilized by autoclaving at 121 ^{o}C for 15 minutes.

Sabouraud's Dextrose Agar (SDA) OXOID)

Dextrose	40.0 g
Peptone	10.0 g
Agar	15.0 g

Directions:

65.0-g medium is suspended in one liter of distilled water and heated to dissolve, and sterilized by autoclaving at 121 ^{o}C for 15 minutes.

APPENDIX B

ANOVA Tables

Source	D.F	Sum of Squares (SS)	Mean Squares (M.S)	F. Value	P. Value
Between Groups	K*-1	Between groups SS	MSTR	$F = \frac{MSTR}{MSE}$	≤ 1
Within Groups (Error)	N**-K	Within groups SS	MSE		
Total	N-1	Total SS			

* K = number of experimental groups.
** N = total number of data in the experiment.

Table B.1 Antibacterial activity of 37 ethanolic extracts against gram-negative bacteria (Table 3.1, Total 1*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	36	3235.723	89.881	15.806	0.000
Within Groups	407	2314.431	5.687		
Total	443	5550.154			

K = 37 plants extracts
N = 37 X 4 X 3
N = 444

Table B. 2 Antibacterial activity of 37 ethanolic extracts against *S. aureus* (gram-positive bacterium) (Table 3.1).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	36	2998.401	83.289	79.475	0.000
Within Groups	74	77.551	1.048		
Total	110	3075.952			

K = 37
N = 37 X 1 X 3
N = 111

Table B. 3 Susceptibility of test bacteria and *C. albicans* to 37 plants extracts as shown by inhibition zone diameter (mm) (Table 3.2).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	5	2625.138	525.028	30.648	0.000
Within Groups	660	11306.457	17.131		
Total	665	13931.595			

K = 6
N = 37 X 6 X 3
N = 666

Table B.4 Antifungal activity of 48 ethanolic extracts against human and animal pathogenic dermatophytes *(M. canis & M. gypseum)* (Table 3.3, Total 1*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	47	186861.88	3975.785	34.457	0.000
Within Groups	240	27692.018	115.383		
Total	287	214553.90			

K = 48
N = 48 X 2 X 3
N = 288

Table B.5 Antifungal activity of 48 ethanolic extracts against phytopathogenic fungi (Table 3.3, Total 3*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	47	270215.25	5749.261	16.066	0.000
Within Groups	609	217938.18	357.862		
Total	656	488153.43			

K = 48
N = 48 X 5 X 3 - 63
N = 657

Table B.6 Antifungal activity of 48 ethanolic extracts against phytopathogenic *Pythium* species as shown by means of % mycelial inhibition (Table 3.3, Total 2*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	47	262392.87	5582.827	18.474	0.000
Within Groups	384	116045.44	302.202		
Total	431	378438.31			

K = 48
N = 48 X 3 X 3
N = 432

Table B.7 Antifungal activity of 14 aqueous extracts against human and animal pathogenic dermatophytes *(M. canis & M. gypseum)* (Table 3.4, Total 1*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	13	19566.173	1505.090	16.812	0.000
Within Groups	70	6266.822	89.526		
Total	83	25832.996			

K = 14
N = 14 X 2 X 3
N = 84

Table B.8 Antifungal activity of 14 aqueous extracts against phytopathogenic fungi (Table 3.4, Total 3*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	13	47497.383	3653.645	7.722	0.000
Within Groups	154	72866.063	473.156		
Total	167	120363.45			

K = 14
N = 14 X 4 X 3
N = 168

Table B.9 Antifungal activity of 14 aqueous extracts against phytopathogenic *Pythium* species (Table 3.4, Total 2*).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	13	62180.923	4783.148	12.147	0.000
Within Groups	112	44102.900	393.776		
Total	125	106283.82			

K = 14
N = 14 X 3 X 3
N = 126

Table B.10 Susceptibility of test fungi to 48 ethanolic plants extracts (Table 3.5).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	6	90815.020	15135.837	23.040	0.000
Within Groups	938	616220.096	656.951		
Total	944	707035.116			

K = 7
N = 48 X 7 X 3 - 63
N = 945

Table B.11 Susceptibility of test fungi to 14 aqueous plants extracts (Table 3.6).

Source	D.F	Sum of Squares	Mean Squares	F. Value	P. Value
Between Groups	5	45975.883	9195.177	20.743	0.000
Within Groups	246	109047.729	443.283		
Total	251	155023.612			

K = 6
N = 14 X 6 X 3
N = 252

APPENDIX C

Preparation of McFarland nephelometer standards

Principle: A chemically induced precipitation reaction can be used to approximate the turbidity of a bacterial or candidal suspension.

Method:

1. Set up 10 test tubes or ampules of equal size and of good quality. Use new tubes that have been thoroughly cleaned and rinsed.
2. Prepare 1% chemically pure sulfuric acid.
3. Prepare a 1.175% aqueous solution of barium chloride ($Bacl_2$ - $2H_2O$).
4. Slowly, and with constant agitation, add the designated amounts of the two solutions to the tubes as shown in the table below to make a total of 10 ml per tube.
5. Seal the tubes or ampules.
6. Store the McFarland standard tubes in the dark at room temperature. They should be stable for 6 months.

McFarland nephelometer standards

	Tube number										
	0.5	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Sulfuric acid (ml)	9.95	9.9	9.8	9.7	9.6	9.5	9.4	9.3	9.2	9.1	9
Approximate cell density (X 10^8 / ml)	1.5	3	6	9	12	15	18	21	24	27	30

بسم الله الرحمن الرحيم

دراسة الفعالية المضادة للكائنات الدقيقة لمستخلصات أربع وخمسين من النباتات المستخدمة في الطب الشعبي في فلسطين

إعداد

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لقد تمت دراسة تأثير المستخلصات المائية والكحولية لأربع وخمسين من النباتات المستخدمة في الطب الشعبي في فلسطين في علاج الكثير من الأمراض ضد ثلاثة عشرة عزلة تابعة لنوع واحد من الخمائر(*Candida albicans*) وأربعة أنواع من البكتيريا السالبة الصبغة الجرامية وهي (*E. coli,* *K. pneumonia, P. vulgaris* and *P. aeroginosa*) ونوع واحد من البكتيريا موجبة الصبغة الجرامية وهي (*Staphylococcus aureus*) واثنتين من الفطريات الجلدية المسببة للقوباء الحلقية وهي (*Microsporum canis* and *M. gypseum*) وخمسة من الفطريات المسببة لأمراض النباتات وهي (*F. tricinictum, P. ultimum, P. aphanidermatum, P. middletonii,* and *Ph. citrophthora.*).

وقد استخدم في هذه الدراسة اختباران خاصان بقياس حساسية الكائنات الدقيقة للمواد ذات الأثر المضاد للميكروبات وهما: (Disk diffusion method) من أجل قياس النشاط المضاد ضد البكتيريا والخميرة والآخر (Poisoned-food technique) من أجل قياس النشاط المضاد ضد الفطريات.

وأظهرت الدراسة وجود اختلافات معنوية بين النباتات بالنسبة لتأثيراتها المضادة للكائنات الدقيقة. فكانت النباتات التالية وهي: ورد الجمل (*Alcea setosa*) والزعيمة (*Coridothymus capitatus*) وندغ البساتين (*S. thymbra*) هي الأعلى فعالية ضمن النباتات المدروسة ضد البكتيريا سالبة الجرام والبكتيريا موجبة الجرام.

كما أظهرت الدراسة أن النباتات التالية وهي: الزعيمة (*C. capitatus*) وندغ البساتين (*Satureja thymbra*) والبلوط (*Quercus calliprinos*) هي الأعلى فعالية ضمن النباتات المدروسة ضد *Candida albicans*.

أما بالنسبة للفطريات المسببة للقوباء الحلقية فكانت النباتات التالية وهي: المستخلصات الكحولية لكل من الزعيمة (*C. capitatus*) والزعتر الناعم (*Micromeria nervosa*) وندغ البساتين (*S. thymbra*) والمستخلصات المائية لكل من الأقحوان الأصفر (*Anthemis tunictoria*) والبوصير (*Verbascum sinuatum*) هي الأعلى فعالية ضمن النباتات المدروسة ضد هذه الفطريات.

كما أظهرت الدراسة أن المستخلصات الكحولية لكل من الزعتر الناعم (*M. nervosa*) والصنوبر (*Pinus halepensis*) وندغ البساتين (*S. thymbra*) والمستخلصات المائية لكل من الفوة (*Rubia tenuifolia*) والأقحوان الأصفر (*A. tunictoria*) والزعيمة (*C. capitatus*) هي الأعلى فعالية ضمن النباتات المدروسة ضد أنواع *Pythium* الثلاثة.

كما أظهرت الدراسة أن المستخلصات الكحولية لكل من الصنوبر (*P. halepensis*) وندغ البساتين (*S. thymbra*) هي الأعلى فعالية ضمن النباتات المدروسة ضد *Ph. citrophthora*.

كما أظهرت الدراسة أن المستخلصات الكحولية لكل من المريمية (*Salvia fruticosa*) ونـــدغ البساتين (*S. thymbra*) والمستخلصات المائية لكل من الأقحوان الأصفـــر (*A. tunictoria*) والجـــوز (*Juglans regia*) هي الأعلى فعالية ضد فطر *F. tricinictum*.

كما أظهرت الدراسة أن المستخلصات الكحولية كانت أكثر فاعلية من المستخلصات المائية.

كما أظهرت الدراسة أيضا وجود اختلافات معنوية بين الأنواع المختلفة للبكتيريا، فقد كـــانت بكتيريا (*S. aureus*) (إيجابية الصبغة الجرامية) هي أكثر الأنواع حساسية للمستخلصات النباتية بينمـــا كانت (*K. pneumonia*) (سالبة الصبغة الجرامية) هي النوع الأقل حساسية.

وقد بينت النتائج أيضا أن فطر *P. aphanidermatum* هي أكثر الفطريات المسببة أمراضـــا للنباتات حساسية، بينما كان فطر *M. gypseum* هو أكثر الفطريات المسببة للقوباء الحلقيـــة حساســـية للمستخلصات النباتية.