An – Najah National University
Faculty of graduate studies

Antimicrobial Activity of Palestinian Medicinal Plants Against *Propionibacterium acnes*, a Causative Agent of Acne

By

Anhar Ahmad Mohammed Al-Assali

Supervisor

Professor Dr. Mohammed S. Ali-Shtayeh

Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biological Sciences, Faculty of Graduate Studies, at An – Najah National University, Nablus, Palestine

May 2002

COMMITTEE DECISION

Antimicrobial Activity of Palestinian Medicinal Plants Against *Propionibacterium acnes*, a Causative Agent of Acne

By

Anhar Ahmad Al – Assali

This Thesis was Successfully Defended and Approved on 4 th May 2002.

By

Committee Members	***Signature***
1- Professor Dr . Mohammed S. Ali – Shtayeh (Superviser)	
Professor of Biological Sciences	
2- Dr. Mohammed Musmar (Internal Examiner)	
Assistant Prof. Pharmacology	
3- Dr. Hazem Th. Sawalha (External Examiner)	
Assistant Prof. Virology and Biotechnology	

DEDICATION

TO

MY DEAR PARENTS, BROTHERS

FOR THEIR ENCOURAGEMENT

WITH LOVE AND RESPECT

ACKNOWLEDGMENTS

I would like to express my sincere special thanks and gratitude to my supervisor, Professor Dr. Mohammed S. Ali-Shtayeh for his supervision, encouragement, guidance and help throughout this study.

I would like also to express my sincere gratitude to Dr Hussam K.hraim for his help

Also special thanks for my colleagues Suhail Abu-Ghdeib, Lubna Al-Khraz, Ranya Arafat for their help and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ANTIMICROBIAL ACTIVTY OF PALESTINIAN MEDICINAL PLANTS AGAINST PROPIONIBACTEIUM ACNES, A CAUSATIVE AGENT OF ACNE

Anhar Ahmad Al-Assali

Supervisor
Professor Dr. Mohammed S. Ali-Shtayeh

ABSTRSACT

Ethanolic 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 10 strains of *Propionibacterium acnes*, and five strains of aerobic bacteria, *Echerichia coli, Klebsiella pneumonia, Proteas vulgaris, Pseudomonas eruginosa,* and *Staphylococcus aureus*.

Two susceptibility tests were used in this work: the disk diffusion method for measuring the antimicrobial activity, and broth method for the determination of MIC, and MBC for the active plant extracts.

The results demonstrated that the studied plants differ significantly in their activity against the studied microorganisms. The

most active plants against bacterial strains were *Rhus coriaria, Ricinus communis,* and *Sarcopoterium spinosum.*

Test microorganisms differed significantly in relation to their susceptibility to different plant extracts used. The most susceptible test microorganism was *Propionibacterium acnes* (anaerobic bacteria), whereas the least susceptible microorganism was *Klebsiella pneumonia.* Generally, anaerobic bacteria were more susceptible to plant extract than aerobic bacteria. This was attributed to differences in modes of actions of plant extracts against both groups.

CHAPTER ONE

Introduction

1.1 Historical Review of Traditional Medicine

The screening of the Palestinian flora for pharmacological active compounds started in the sixties and was performed systematically by chemists, botanists and pharmacologists (Silva and Abraham, 1981).

The screening and ethnobotanical surveys of medicinal plants of the Palestinian area have been carried out in attempt to update knowledge of the current medicinal folk uses of plants by local healers (Ali–Shtayeh and Abu–Ghdaib, 1999; Ali–Shtayeh *et al.*, 1997, 1998, 2000; Essawi and Srour, 2000).

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Unesco, 1996).

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Unesco, 1998).

1.2 Medicinal Plants

Medicinal plants are integral component of research developments in the pharmaceutical industry (Gorman, 1992). Such research focuses on the isolation and direct use of active medicinal constituents or in the development of semi-synthetic drugs (Gorman, 1992).

Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defences against infection (Frank, 1996).

1.3 Antimicrobial Activity and Efficacy of Medicinal plants

In many parts of the world medicinal plants are used for their antibacterial, antifungal, and antiviral activities. Many plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders (Caceres *et al.*, 1995), and skin infections (Meyer *et al.*, 1996).

In the West Bank and Gaza Stip, natives use herbal medicine to treat various diseases including gastrointestinal diseases, urinary

tract infections, infertility and cutaneous abscesses (Essawi and Srour, 1999).

In recent years, several plants used in traditional medicine have been tested for antimicrobial (Boily and Vanpurelde, 1986), acaricidal (Vanpuyrelde *et al.*, 1985), antimycobacterial (Vanpuyrelde *et al.*, 1994).

Medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically to produce a combined effect that surpasses the total activity of individual constituents (Frank, 1999).

The combined action of these substances increases the activity of the main, medicinal constituent by speeding up or slowing down their assimilation in the body. Secondary substances might increase the stability of the active compounds, minimize the rate of undesired side effects and have an additive potentiating, or antagonistic effect. Secondary constituents might contribute to the overall effect (Frank, 1999).

Herbal medicines commonly are mixtures of several plants that might act synergistically (Frank, 1999). Herbal combinations are formulated in a precise way to increase or promote therapeutic effectiveness, alter the actions of substances, or reduce toxicity or side effects (Frank, 1999).

Two or more plant drugs with similar properties mutually reinforce therapeutic action in an additive or synergistic manner mixtures or formulations may act in different ways and on different parts of the body to yield an overall effect (Frank, 1999).

A plant may contain several natural compounds that cause a single biochemical effect. A chemical substance may affect some aspects of two diseases sharing a common biochemical mechanism, or a substance may possess two biochemical activities that contribute to itis effect in several therapeutic areas (Anjara, 1996).

Developed Countries, in recent times are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies. Herbal preparations are popular and are of significance in primary health care in Belgium, France, Germany and Netherlands (Gorman, 1992). Such popularity of health care plant–derived products

has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being (Akerele *et al.*, 1991).

1.4 Acne

Acne is a common skin disorder that caused by inflammation of the hair follicles and sebaceous glands. Almost every part of the body is covered with hairs, most of them virtually invisible. Each hair grows from a follicle, which is a tiny pit in the skin. Inside each follicle is a sebaceous gland that produces an oily substance called sebum that lubricated the skin. If the body produces too much of this oily substance and some of it becomes trapped in a follicle, bacteria can multiply in the blocked follicle and cause it to become inflamed. This causes a pimple, which may be a small white plug (white – head), a dark closed pore (black head), or a red lump that sometimes fills with pus. A simple or two is nothing to worry about (Plewige and Kligman, 1975).

Certain medications such as corticosteroids or antiepilepsy drugs, can also cause acne. Usually, however, acne is a propled that occurs during adolescence. It begins at puberty (when reproduction becomes possible) and usually clears up in the late teens or early twenties (kubo *et al.*, 1994).

Many adolescents have acne because the level of human hormones in the body rises when a boy or a girl reaches puberty, and this stimulates the sebaceous glands to increase their production of sebum (Wolff, 1975).

Pimples are usually concentrated on the face, but may appear on the back of the neck, the back, the chest, and in come cases the upper arms. An inflamed pimple may develop into a tender red lump with a white, pus–filled center. As individual acne pimples heal, other pimples appear. Each healed pimple leaves a purplish mark on the skin. Severely inflamed pimples may take many weeks to clear up and may leave scars (Downing *et al.*, 1982).

1.4.1 Epidemiology :

Acne is occasionally present at birth, however, it is not until puberty that it becomes a common problem. The disease may be an early manifestation of puberty, although in the very young patient the predominant lesions are comedones, and inflammatory lesions are rare (Allaker *et al.*, 1987).

The greatest number of cases is seen during the middle to late teenage period, then the incidence of the disease decreases, studies indicate that there is a slight prevalence in males (Allaker *et al.*, 1987).

Nodulocytic acne has been reported to be more common in white males that in black males (Wilkins and Voorhees, 1972).

1.4.2 Etiology and pathogenesis of acne

Acne is a multifactorial disease (Cunliffe and Shuster, 1987).

1.4.2.1 The sebaceous gland

The sebaceous glands are usually found in or near a hair follicle through which they secrete sebum, an oily substance that lubricates the skin and hair (Knuston, 1974) A follicle may become blocked, usually by a mixture of excess sebum and dead skin cells, then a white plug forms up in the blocked follicle (Wolff, 1975). The cellular depris and sebum becomes colonized by *Propinibacterium acnes,* causing inflammation, pus, and swelling, which is visible as an acne pimple (Cunliffe and Shuster, 1987).

1.4.2.2 Sebum

Sebum is made up of mature sebaceous cells, which have ruptured and of sebaceous lipids. Sebum first accumulates in the pilosebaceous unit which constitutes a follicular reservoir, and this is where the debris of corneocytes and of microonganisms become mixed in sebum (Kligman, 1974). Subsequently, the sebum is secreted to the surface of the skin where it mixes with lipids of epidermal origin (Marsden *et al.*, 1987).

Patients with acne produce more sebum than do normal persons, and those patients with severe acne secretes more sebum than do those with mild acne (Downing *et al.*, 1982). Nevertheless, there is considerable variation in sebum production within the group of patients with acne. Many other factors indicate that sebum plays a role in the overall pathogenesis of the disease (Marsden *et al.*, 1987).

1.4.2.3 Hormonal control of sebum secretion

The development and activity of the sebaceous gland are affected by several hormones of differing importance (Verchoore, 1987). Androgenic stimulation at puberty induces sebaceous gland development. It has been shown that during early puberal development, plasma testosterone levels are higher in those with acne (Lee, 1976).

Urinary androgen metabolites are increased in young children if acne is present (Pochi, 1977). In female patients with acne, it has been shown that there may be increased peripheral tissue conversion of testosterone to androstenediol, and this is presumed to have occurred in the skin (Mauvais, 1973).

Acne also may by aggravated by the administration of hormones such as testosterone, anabolic agents, gonadotopins, corticosteroids, and ACTH. These later two agents are the cause of steroid acne (Verschoore, 1987).

Because of the influence of hormones on acne, the sudden appearance of acne in an adult is as a result of the pituitary gonadal or pituitary – adrenal hormones. Furthermore, emotional stress may aggravate acne (Verschoore, 1987).

1.4.2.4 Microorganisms

Three genera of resident microorganisms occur on the trunk and face of the adult: *Staphyllococus aureus*, Pityrosporum, and *Propionibacterium acnes* (Elbaze and Ortonne, 1987).

Staphyllococus aureus

This aerobic – pathogens is found mainly on the surface of the skin, in the openings of the sweat glands, it does not play any role in acne (Elbaze and Ortonne, 1987).

Pityrosporum :-

Pityrosporum ovale and Pityrosporum orbiculare are both found on the surface of the skin but they do not have any effect on the pathogenesis of acne (Elbaze and Ortonne, 1987).

Propionibacterium acne

The propionibacteria are anaerobic but aerotolerant diphtheroid bacteria of which three species are known *propionibacterium acnes*, *Propionbacterium granulosum* and *Propionibacterium avidum*. The latter is found mainly in humid areas such as axilla and anal region, whereas the two other are found only in areas rich in sebum (Kubo *et al.*, 1996).

Very large numbers of *Propionibacterium* acnes are regularly found in the sebaceous follicles. Their number increases at puberty at the same time as will as sebum secretion (Kubo *et al.*, 1994).

Propionibacterium acne is the source of the inflammatory reaction which leades to the formation of the acne lesion (Webster *et al.*, 1980).

Serum levels of antibodies to *Propionibacterium acne* are markedly higher in persons with acne compared with those without acne (Webster *et al.*, 1980).

1.4.2.5 Mechanism of the inflammatory lesion

Inflammatory lesion may be produced at any stage of acne. *Propionibacterium acnes,* the comedo with its contents and the polymorphonuclear leukocyte chemotic factor (Puhvel and Sakomoto, 1978; Webster and Leyden, 1980), are the protagonists of the inflammatory process, *P. acnes* plays the most important role that possesses an enzymatic activity which can break down the triglycerides and release free fatty acids (Allaker *et al.*, 1987).

The chemotactic factor produced by *P. acnes* which has low molecular weight is capable of crossing the wall of the cyst (Puhvel and Sakomoto, 1978). This penetration of the cyst wall is responsible for initiation of the inflammatory lesion (Webster and Leyden, 1980).

The polymorphonuclear leukocyte chemotaic factor, attracted by various chemotactic factors, also act as enzymes by releasing lysosomal enzymes (Webster *et al.*, 1980). These enzymes attack the wall of acne cyst and break it down (Dahl and Macgibbon, 1979).

The inflammation caused by the influx of the polymorphonuclear leukocyte chemotaic factor is aggravated by a nonspecific inflammatory reaction manifested when the contents of the cyst – with the sebum, free fatty acids, hair cellular debris and *P. acnes* – come into direct contact with the dermis (Webster *et al.*, 1980).

1.4.2.6 Genetic factors

Acne is a common condition with a high familial incidence, up to 80 % of mononozygotic twins are affected (Fanta, 1978). The probability of acne developing is higher when both parents are affected. The mean age at which acne begins in patients with a positive family history is lower than the age of onset in acne patients without such a family history (Fanta, 1978).

1.4.2.7 Other factors

Dietary and professional factors, excessive sweating, change of climate, ultraviolet radiation, and stress (Kubo *et al.*,1994). While, it is

undeniable that any of these factors may affect the severity of acne, they do not play a role in the pathogenic process (Kubo *et al.*, 1994).

1.5 Screeing methods for antimicrobal activity of natural products

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

1.5.1 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, a hole or a 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 organism (Rios *et al.*, 1988; Wexler *et al.*, 1991; Woods and Washington, 1995).

The advantages of this method are the small size of the sample used in the screening and the possibility of testing five or six compounds against a single microorganism (Rios *et al.*, 1988).

1.5.2 Dilution methods

Dilution susceptibility testing methods are used to determine the minimal concentration of an antimicrobial agent required to inhibit or kill a microorganism. MIC determined by this dilution methods, is the lowest concentration that inhibits visible growth of an organism (Rios *et al.*, 1988).

Flexibility is a major advantage of dilution susceptibility testing methods. Further advantages include the ability of dilution methods to detect certain resistance patterns that may not be detected by disc diffusion methods (Rios *et al.*, 1988).

Another advantage of this method is that the only method for determination of minimum bactericidal concentration (MBC) which is defined as the lowest concentration of the extract that does not permit any visible colony of bacteria to grow on the agar plate after the period of incubation (Irobi and Daramala, 1994). It is determined, by sub

culturing of the tube that shows complete inhibition on an agar plate or in liquid medium (Rios *et al.*, 1988).

1.5.3 Extraction Techniques:

Antimicrobial activity of plant is usually assessed after extracting plant material with organic and inorganic solvents (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 (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 extracts are then decanted or filtered and kept in the freezer at – 20 °C until use .

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 provides and 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 selected Palestinian plants for their antimicrobial activity against *Propionibacterium acnes,* and determine whether their use in folkloric medicine to treat these infections is justified .

CHAPTER TWO

Materials and Methods

2 .1 Antimicrobial Activity of Plant Extracts

2.1.1. Plant Material :- Collection, Extraction, Preparation, and Sterilization

Collection. Plants used in folk medicine in Palestine for the treatment of acnes were selected for this work (Table 2.1). Mature plants were collected from several areas in the West Bank during the spring and summer seasons (2000 – 2001). Aerial parts of each medicinal plant were collected, dried in the shade, ground in to a powdered material using an appropriate seed mill, and then powdered plant material stored in labeled and sealed plastic bags. All collected plants were authenticated by Prof. Ali-Shtayeh. Voucher specimens of the plants were deposited at the Department of Biological Sciences, An – Najah University, West Bank.

Table 2 . 1 Selected plants used for antibacterial susceptibility testing

Species/ Family (Voucher Specimen No.)	Common name	Arabic name	Parts Used*	Popular uses	Ref. for folk popular uses
Achilla fragrantissima (Forssk) Sch. Bip. (Asteraceae, Compositae) (A1)	Yarrow	قيصوم	AP, FL	Treatment of diabetes, digestive problems, arthritis, fever reduction, severe cough, antidiuretics, stomach ailments, tumers & infections, antispasmotics, arthritis.	3, 7, 57, 79, 32, 58, 33, 70, 16
Ajuga orientalis (A2)		عشبة الدم	AP		
Allium sativum L. (Liliaceae) (A3)	Garlic	ثوم	LF	Skin & circulatory system (heart & blood vessels), antihelmintics.	7, 38
Arum dioscoridis Sibth.&Sm. (Araceae) (A4)	Spotted Arum	لوف	LF	Cancer	7
Asparagus aphyllus L. (Liliaceae) (A5)	Asparagus	الهليون	AP		
Capparis spinosa (L.) (Capparidaceae) (A6)	Caper bush	كبار	FR, FL	Antihepatotoxic, hepatitis, gastronomic, antihelmintic, resolvent cataplasms for skin inflammation,general pain.	2, 7, 57, 17, 22, 25, 32, 33, 35, 67, 79, 10, 14
Cardaria draba (L.) Desv (Cruciferae) (A7)	Hoary Pepperwort	قنيبره	AP		
Ceratonia siliqua (L.) (Caesalpiniaceae) (A8)	Carob	خروب	S	Treating warts, diarrhea, diabetes, syphilis, & venereal diseases, abdominal pain.	57, 17, 35, 45, 79, 52
Chrysanthemum coronarium (L.) (Chenopodiaceae) (A9)	Corn marigold	بسباس	FL		
Cicer arietenum (L.) (Leguminosae) (A10)	Checkbeans	حمص	AP		
Clematis cirrhosa (L.) (Rananculaceae) (A11)	Clematis	غاشية	AP	Treat the reproductive system.	57, 58, 42, 48, 73, 76

Companula rapunculus (L.) (Companulaceae) (A12)	Bell-Flower	ورد الجرس	AP		
Conium maculathum L. (Compositae, Asteraceae) (A13)	Poison hemlock	شوكرامة	LF, FL		
Convolvulus arvensis L. (Convolvulaceae) (A14)	Bind weed	لبلاب الحقول	AP	Treat recent wounds, wound healing & laxative.	12, 52
Coridothymus capitatus(L.) Reichb. (Labiatae) (A13)	Thyme	زعيمة	AP	Antihnflammatory, and antimicrobial activity, for eye infection, headache, digestive &urinary systems, inflammation.	57, 7, 44, 14
Crataegus aronia L. Bosc. Ex DC (Rosaceae) (A14)	Hawthom	زعرور	LF	Cardiac sedative, hypotensive Rheumatism, diabetes, digestive system, urinary system.	7, 45, 47
Daucus carota L.ssp. maximus. (Desf.)Ball (Umbelliferae, Apiaceae) (A15)	Wild carrot	جزر بري	LF, FL, RT	It is used as aphrodisiac.	29
Erodium malacoides (L.) LHer. (Geraniaceae) (A16)	Mallow stork's –Bill	ابرة العجوز	WP		
Eruca sativa Mill. (Cruciferae) (A17)	Gaden Rockets	جرجير	WP	Skin diseases.	7
Foeniculum vulgare (L.) Mill. (Umbelliferae) (A18)	Fennel	شومر	AP	Diarrhea, antiemetic, antispasmodic, treat skin diseases,analgesics, coughs, heart diseases.	7, 57, 17, 22, 13, 45, 35, 37, 32, 67, 14, 29
Gagea chloranthy (Bieb.) Schult. Fill (Liliaceae) (A19)	Gagea	زعيتمان			
Gundelia tournefortii L. (Compositae, Asteraceae) (A20)	Gundelia	عكوب	LF		
Lactuca tuberosa Jacq. (Compositae) (A21)	Lettuce	خس بري	AP		
Linum pubescens Banks & Sol. (Linnaceae) (A22)	Pink flax	كتان زهري	AP, FL	Skin disorders & prostate disorders, urine intermittence.	7

Lupinus pilosus Murr. (Papilionaceae) (A23)	Lupine	ترمس بري	AP, S		
Lycium europium (L). (Solonaceae) (A24)	Box thorn	عوسج	AP		16, 22, 39
Majorana syriaca (L.) Rafin. (Labiatae) (A25)	Thyme	زعتر بري	AP	Respiratory system.	7
Mandragora autumnalis Bertol. (Solanaceae) (A26)	Mandrake	تفاح مجن	FR	Resolvent for whitelows, pimples & phlegmons.	14
Mentha viridis L. (Labiateae) (A27)	Mint	نعنع	AP	Totreat diarrhea, digestive & to relieve menstrual pain, antispasmodic, jaundice.	14, 29
Notobasis syriaca (L.) Cass. (Compositae, Asteraceae) (A28)	Syrian thistle	خرفيش	YB		
Parietaria diffusa (Mert. & Koch.) (Urticaceae) (A29)		عشبة الدم	AP	To stop bleeding from fresh skin wounds, antitussive, hemorrhoid lentitive, resolvent for skin inflammation.	8, 25, 57
Paronychia argentea (Lam.) (Caryophylllaceae) (A30)	Silvery Whittle-wart	الماسة الفضية	AP	Treatment of diabetes, blindness, heart pains, kidney, stones, edema.	7, 79, 57
Petroselinum sativum Hoffm. (Umbelliferae) (A31)	Parsely	بقدونس	WP	Gastronomic use, digestive, hypotensive, urination, prostate cancer.	7, 14, 12
Phgnalon rupester (L.) DC. (Compositae) (A32)	African Fleabane	قديح	AP	To make deliberate burns, to treat asthma, to treat headache.	7, 57, 8, 32, 58, 77, 28
Pinus halepensis Mill. (Pinaceae) (A33)	Aleppo Pine	صنوبر	LF	Antirheumatic fever, skin abces, antiseptic, tuberculosis, for bronchitis, diuretics.	13, 17, 45, 52
Pistacia lentiscus (L.) (Anacardiaceae) (A34)	Mastic, Lentisk	سريس	LF	For stomachal pains, analgesic, protective covering for wounds, skin infections, cardiac stimulation, anti-inflammatory.	35, 22, 57, 7, 78, 63, 52, 43
Pyrus syriaca Boiss (Rosaceae) (35)	Pear	إجاص بري	LF, FL		

Reseda alba (L.) (Resedaceae) (A36)	Mignonette	حصادة	WP		
Rhus coriaria (L.) (Anacariaceae) (A37)	Sumac	سماق	AP	Wounds, burns, bronchitis, treat gastric ulcers.	13, 45, 80, 57, 14, 52
Ricinus communis (L.) (Euphorbiaceae) (A38)	Castor Comunis	خروع	AP	For intestinal obstruction due to constipation, feverish, headache, skin diseases.	7, 61, 52, 4
Rosa centifolia (L.) (Rosaceae) (A39)	Rose	جوري	FL		
Rubia tenuifolia Durv. (Rubiaceae) (A40)	Wild Madder	فوة	AP	Diuretic activity.	71
Ruta chalepensis (L.) (Rutaceae) (A41)	Rue	فيجن	AP, FL	Anti-rheumatic, abdominal pain, analgesic, wounds, anti-inflammatory, skin disorders, kidney stones.	7, 57, 11, 35, 45, 21, 74, 75, 3, 83, 68
Saccharum ravennae (L.) Murray (Labiatae, Lamiaceae) (A42)	Wild cane	قصاب	FL		
Salvia fruiticosa (L.) (Labiatae) (A43)	White sage	مريمية	LF	Against bronchial infections, headache, diabetes, anti-inflammatory, ulcer pains, colds & couphs.	58, 7, 57, 79, 23, 14
Salvia hierosolymitana Boiss. (Labiatae) (A44)	Jerusalem Sage	لسينية	LF		
Sarcopoterium spinosum (L.) Sp. (Rosaceae) (A45)	Shruppy barnet	نتش	AP	Anti-inflammatory, diabetes, abdominal pain, haemorrhoids, external inflammation.	7, 57, 79, 45, 32
Satureja thymbra (L.) (Labiatae) (46)		ندغ البساتين	AP, FL	Fungicide, abdominal pain, heart pains, edema, open wounds, stress, paralysis, dizziness.	69, 57, 40
Scabiosa prolifera (L.) (Dipsacaceae) (A47)	Morning bride	ركاب جمال	AP		
Sinapis arvensis (L.) (Scrophulariaceae) (A48)	Mustard	خردل	WP		
Sonchus olecarcea (L.) (Solanaceae) (A49)	Sow thistle	علك خيل	WP		
Styrax officinalis (L.)	Snow bell	عبهر	YB		

(Styracaceae) (A50)					
Trigonella foenugraecum (L.) (Papilionaceae) (A51)	Fenugreek seed	حلبة	AP	Stomach disorders, dysentery, boils, hypertension, diapetes, abdominal pains.	7, 29, 14, 57
Varthemia iphionoides Boiss & Blanche (Compositae) (A52)	Common varthemia	كتيله	AP	Stomach ache, eye ailments, edema.	32, 57
Vicia faba (L.) (Papilionaceae) (A53)	Broad Bean	فول	AP	Hypertension, heart failure, renal failure, liver cirrhosis, prostate disorders.	27, 7
Viscum cruciatum Sieber et. Boiss. (Linaceae) (A54)	Mistletoe	هدال	LF	Tumer inhibition, anti-spasmodic, anti-hypertensive, dieretics.	7, 45, 64

AP, aerial parts; FL, flowers; WP, whole plants; RT, roots; LF, leaves; B, bulb; YB, young branches; S,seeds.
[1]Abu Zarqa *et al.*, 1998; [2]Ageel *et al.*, 1989; [3]Ali & Grever,1998; [4]Ali *et al.*, 1995; [5]Ali-Shtayeh *et al.*, 2000; [6]Ali-Shtayeh *et al.*,1997; [7]Ali-Shtayeh *et al.*, 1998; [8]Al-Said *et al.*, 1988; [9]Al-Said *et al.*, 1990; [10]Alkofahi *et al.*, 1997; [11]Al-Wareh *et al.*, 1993; [12]Amico & Sorce, 1997; [13]Amoros et al., 1988; [14]Barel & Yashphe, 1991; [15]Belakhadar *et al.*, 1991; [16]Bellino & Marceno, 1981; [17]Benayache *et al.*, 1991; [18]Bhat & Jacobs, 1995; [19]Caceres *et al.*, 1990; [20]Chieji,1984; [21]Conigueral *et al.*, 1989; [22]Dafni & Yanive, 1994; [23]Defeo *et al.*, 1991; [24]Disi *et al.*, 1998; [25]Dutta & Nath, 1998; [26]El-Damy *et al.*, 1994; [27]El-Kamali & Khalid, 1998; [28]Elsholy *et al.*, 1975; [29]Facino *et al.*, 1995; [30]Friedman *et al.*, 1986; [31]Gadgoli & Mishra, 1999; [32]Garcia *et al.*, 1999; [33]Ghazanfar,1994; [34]Grande *et al.*, 1992; [35]Gribanovski sassu *et al.*, 1969; [36]Guarrera,1999; [37]Halaska *et al.*, 1998; [38]Haykel & Omar, 1988; [39]Huq *et al.*, 1998; [40]Hussain, 1995; [41]Hussain,1997; [42]Kandil *et al.*, 1994; [43]Karim &quraan, 1986; [44]Karting *et al.*, 1991; [45]Kinghorn &Balandrin, 1993; [46]Kizu *et al.*, 1995; [47]Lisciani *et al.*, 1984; [49]Manadhar, 1991; [50]Manez *et al.*, 1999; [51]Merzouki & Ed-Derfoufi,1997; [52]Mostaqul Huq *et al.*, 1999; [53]Murata & Takahashi, 1984; [54]Okzuz, 1976; [55]Ong & Norzalina, 1999; [56]Palevitch *et al.*, 1984; [57]Palevitch & Yaniv, 1991; [58]Qureshi *et al.*, 1991; [59]Rauwald & grunwid, 1991; [60]Reddy *et al.*, 1998; [61]Rimbau *et al.*, 1999; [62]Rios *et al.*, 1987; [63]Sanez *et al.*, 1997; [64]Sakai *et al.*, 1992; [65]Seaworth *et al.*, 1998; [66]Schauenberg, 1990; [67]Shah *et al.*, 1991; [68]Shimoni *et al.*, 1993; [69]Shoji *et al.*, 1994; [70]Silva & Abraham, 1981; [71]Sulieman *et al.*, 1988; [72]Thapliyal & Bahuguna, 1993; [73]Ulubelen *et al.*, 1994; [74]Ulubelen *et al.*, 1988; [75]Uniyal & Sato, 1992; [76]Viollon & Chaumant, 1994; [77]Wyllie *et al.*, 1990; [78]Yanive *et al.*, 1987; [79]Yesilada *et al.*, 1993; [80]Yoshida et al., 1995; [88]Yuan *et al.*, 1987.

Extraction. A total weight of 100 g of dry powdered plant or fresh plants were infused in 70% ethanol until complete exhaustion (usually 1 : 5 W/V ratio) for 72 hr at room temperature with periodic shaking. The extract was filtered twice using Whatman filter paper No .1 . The filterate was then dried using a rotary evaporater sterile bottles and kept frozen at – 20 °C (Ali–Shtayeh *et al.*, 1997; Ali–Shtayeh & Abu – Ghdeib, 1998; Kandil *et al.*, 1994).

Preparation and Sterilization of Plant Extracts

Two grams from each dry extract were dissolved in 10 % dimethel sulfoxide (DMSO) to give a final concentration of 200 mg/ml. Sterilization was then carried out using Millipore filtration (0.45um Millipore filters) using an autoclaved sterile glass filter holder. Sterile filterates were stored in screw capped sterile tubes in the refrigerator at 5 °C until use.

2.1.2 Antimicrobial Activity Screening Methods of Test Microorganisms

2.1.2.1 Test microorganism

Test microorganisms include bacterial strains obtained from the American Type Culture Collection, ATCC, In addition to eight isolates

of *Propionibacterium acnes* recovered from infected persons and identified by gram stain, catalase reactivity and indole test (Innovative Diagnostics, Atlanta, GA, USA) (Table 2.2).

Table 2 .2 Test Micoorganisms

Reference Strains	NO
Propionibacterium acnes	ATCC 6919 ATCC 6921 A1* A2 A3 A4 A5 A6 A7 A8
Echerichia coli	ATCC 25922
Proteus vulgaris	ATCC 13315
Klebsiella pneumonia	ATCC 13883
Pseudomonas aerogenosa	ATCC 27853
Staphyllo coccus aureus	ATCC 25923

* Recovered from acne patients

2.1.2.2 Disk Diffusion Method

Application of extracts on sterile discs. Discs of 6 mm diameter were prepared from Whatman filter paper No.1, placed in a glass Petri dishes and autoclaved for 15 minute. Twenty-five microliters of the required extract were added to each sterile disc, and the discs were dried under a laminar flow sterile bench. The final content of each disc was 5 mg of extract (Ali-Shtayeh *et al.*, 1997; Murray *et al.*, 1995).

Preparation of Inocula. Part of an isolated bacterial colony is transferred into a 5 ml Muller – Hinton broth (MHB) tube for aerobic bacteria. Another of the bacterial colony was transferred into 25 ml liquid Reinforced Clostridium Medium (RCM) tube for *Propionibacterium acnes*, and the tubes were incubated for 4 – 8 hours for aerobic bacteria, and anaerobically for 48 hours for anaerobic bacteria, at 37 °C.

The growth turbidity in the broth is justified by further incubation or dilution with sterile physiological saline, after comparison with that of a Macfarland nephelometer tube No. 0.5, 10 colony forming unit

(Colony Forming Unit, CFU / ml) using a spectrophotometer at 625 nm (optical density 0.08 – 0.1). An inoculum of 10 CFU / ml of bacterial suspension was prepared by diluting 0.1 ml of the prepared bacterial broth culture with 9.9 ml sterile saline.

Susceptibility test. A sterile cotton applicator, 10 CFU / ml of bacterial suspension was swabbed on the surface of Muller –Hinton Agar (MHA) plates for aerobic bacteria, or blood agar (with sheep blood 5 – 7 %) (BASB) medium plates for anaerobic bacteria (*Propionibacterium acnes*) as follows :

1. The cotton applicator was dipped into the bacterial suspension, rotated several times and pressed against the internal wall of the tube to remove excess inoculum.
2. The agar plate was then streaked in three different directions and around the agar margin to obtain an even distribution of the inoculum.
3. The plates were left to dry for 3 – 5 minutes.
4. Using sterile forceps, the selected extract discs were then distributed evenly on the surface of the agar plates.
5. On each plate, an appropriate reference antibiotics discs (10 mg/discs) were placed onto the agar plate beside the extract discs. This was considered as a positive control for strain sensitivity and accuracy of the procedure (Jawets *et al.*, 1995).

6. Sterile discs soaked in each solvent were also applied to the surface of the agar as a negative control.

Table 2.3 Reference antibiotics used in susceptibility tests

Test microorganism	Reference Antibiotics
Propionibacterium acnes	Chloramphenicol
Echericia coli	Ampicillin
Proteus vulgaris	Gentamicin
Pseudomonas aeroginosa	Gentamicin
Klebsiella pneumonia	Ciprofloxacin
Staphyllococcus aureus	Penecillin G

7. Each test was done in triplicate.

8. The MHA plates were incubated upside down at 37 °C for 18 hours. The BASB plates were incubated upside down anaerobically at 37 °C for 48 hours.

9. The inhibition zone around each disc was then measured using a transparent ruler (Murray *et al.*, 1995).

Preparation of Media. For each strain 10 tubes, each with 0.6 ml MHB for aerobic bacteria and 0.8 ml RCM for an aerobic were prepared and autoclaved.

Preparation of the Extract Dilution. Several dilutions of the stock solution were prepared as shown in (Table 2 .4) (Rippon, 1988).

Incorporation of Active Extracts into Media. For the incorporation of the active extracts into the media, 10 broth tubes, containing 0.8 ml broth were prepared. Into broth tube 1, 0.2 ml aliquot of stock solution was added. Into broth tube 2, 0.2 ml aliquot of dilution 2 was added. The procedure was repeated for the remaining dilutions. For positive control, 0.2 ml of the reference antibiotic were added, while for the negative two tubes were prepared, 0.2 ml of thesolvent were added to the first tube, while 0.2 ml of sterile water were added to the second.

Determination of Minimum Inhibitory Concentration (MIC)

All tubes were inoculated with 10 ml of the test suspension (10 CFU/ml). The tubes were then incubated for 24 hours at 37°C for the aerobic bacteria and 48 hours at 37 °C in Gas Pak Jars for the anaerobic bacteria (Murry *et al.*, 1995; Yaghmour, 1997).

Table 2.4 Preparation of dilutions of the extracts

Tube No.	Stock Solution (200 mg/ml)	Emulsifier (DMSO)	Final Concentration (Mg /ml)
1	0.2	——	40
2	0.1	0.1	20
3	0.075	0.125	15
4	0.05	0.15	10
5	0.04	0.16	8
6	0.03	0.17	6
7	0.02	0.18	4

Minimum Bactericidal Concentration (MBC)

Subcultures were made from the visually clear tubes of inoculum with antimicrobial agent (active plants) on MHA plate for aerobic bacteria, and on BASB for anaerobic bacteria. MBC was interpreted to be at a tube that showed no growth on agar plate (Irobi *et al.*, 1994).

2 .2 Statistical Analysis

Data of the Susceptibility test were analysed by three way analysis of variance (ANOVA) with 95 % confidence ($P < 0.05$).

Determination of relative antimicrobial activity = [(inhibition zone diameter mean of active plant)^2/ (inhibition zone diameter mean of reference antibiotics)^2] × 100%

CHAPTER THREE

Results

3.1 Antimicrobial activity of 56 plants

Antimicrobial activity of 56 plant extracts (Table 2.1) has been evaluated *in vitro*, against two type ATCC strains of *Propionibacterium acnes* (anaerobic bacteria), 8 *P. acnes* strains recovered from acne patients (Table A.1), and five ATCC strains of aerobic bacteria (Table A.2).

All plants studied in this work showed antimicrobial activity against the test microorganisms with the exception of *Arum dioscoridis and Ceratonia siliqua* which showed no activity. These plants differ significantly in their activity against the test microorganism ($F = 51.317$, $DF = 839$, $P < 0.05$). All studied plants showed antibacterial activity against *P. acnes* with the exception of *Arum dioscoridis, Cardaria draba, Ceratonia siliqua, Daucus carota and Vicia faba.* The most active plants (25% of all plant extracts) were *Ajuga orientalis, Clematis cirrhosa, Majorana syriaca, Lycium europium, Phagnalon rupester, Pinus halepensis, Rhus coriaria, Ricinus communis, Salvia fruiticosa, Sarcopoterium spinosum, Sinapis arvensis, Sonchus oleraceus,*and *Viscum cruciatum* with inhibition zone means ranging

from 10 – 24.67 mm. The least active plants (27% of all plant extracts) were *Capparis spinosa, Conium maculathum, Crataegus aronia, Eruca sativa, Foeniculum vulgare, Gagea chnoranth, Gundelia tourmeforti, Mandragora autummalis, Notobasis syriaca, Paronychia argentea, Pistacia lentiscus, Reseda alba, Rubia tenuifolia, Saccharum ravennae, Trigonella foenumgraecum and Varethemia iphionoides* with inhibition zone means ranging from 6.1 – 8 mm. Other plants (39% of the plant extracts) showed moderate antibacterial activity with a range of inhibition zone means of 8.1 – 9.9 mm.

For aerobic bacteria 11% of plant extracts had no activity, 48 % were the least active with inhibition zone of 6.1-8 mm, other plants 27% had a moderate activity with inhibition zone of 8.1-10 mm, 14% represents high activity with inhibition zone diameter of 10-18.87 mm, as shown in (Table A.2).

Table 3.1 Relative antimicrobial activity * of plant extracts against ten strains of *Propionibacterium acnes*

Strain No. / Plant extract	*Propionibacterium acnes*									
	P. acnes 6919	*P.acnes 6921*	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
Achillea fragrantissima	14	14	14	14	14	14	14	14	14	14
Ajuga orientalis	25	27	32	32	32	25	32	32	32	32
Allium sativum	17	17	14	15	14	17	17	14	14	14
Arum dioscoridis	8	8	8	8	8	8	8	8	8	8
Asparagus aphyllus	14	14	15	14	14	14	14	14	14	14
Capparis spinosa	11	11	10	11	9	9	11	10	11	11
Cardaria draba	8	8	8	8	8	8	8	8	8	8
Ceratonia siliqua	8	8	8	8	8	8	8	8	8	8
Chrysanthemum coronarium	15	15	15	14	15	15	15	15	15	14
Cicer arietenum	15	15	15	14	15	15	15	15	15	15
Clematis cirrhosa	24	24	27	27	27	25	25	27	27	27
Companula rapunculus	17	17	17	17	20	17	17	17	17	20
Conium maculathum	11	11	11	11	11	11	11	11	11	11
Convolvulus arvensis	14	14	17	14	14	14	14	17	14	14
Coridothymus capitatus	15	15	14	15	14	15	15	14	15	15
Crataegus aronia	14	14	14	14	14	14	14	14	14	14
Daucus carota	8	8	8	8	8	8	8	8	8	8

Table 3.1/ continue

Erodium malacoides	22	20	22	20	22	19	19	22	20	19
Eruca sativa	14	14	14	13	14	14	14	14	13	13
Foeniculum vulgare	12	12	11	12	11	12	12	11	12	11
Gagea chloranthy	11	11	11	9	10	11	11	11	9	9
Gundelia tournefortii	13	13	12	13	13	13	13	13	12	14
Lactuca tuberosa	37	37	32	32	32	37	37	32	32	32
Linum pubescens	18	18	17	18	17	18	18	18	18	18
Lupinus pilosus	15	15	15	14	15	15	15	15	14	15
Lyscium europeum	32	32	32	32	32	32	32	32	32	32
Majorana syriaca	27	27	24	22	24	27	27	24	22	24
Mandragora autumnalis	11	11	11	10	11	11	11	11	10	10
Mentha viridis	17	17	15	14	14	17	17	15	14	14
Notobasis syriaca	14	14	14	14	14	14	14	14	14	14
Parietaria diffusa	19	19	19	19	18	19	19	19	19	19
Paronychia argentea	8	8	8	8	8	8	8	8	8	8
Petroselinum sativum	18	18	18	17	18	18	18	18	18	18
Phagnalon rupester	32	32	28	28	28	32	32	28	28	32
Pinus halepensis	59	59	45	45	45	59	56	45	45	45
Pistacia lentiscus	12	14	11	12	12	12	13	11	12	11
Pyrus syriaca	18	18	18	18	18	18	18	18	18	18
Reseda alba	9	8	8	8	8	8	9	14	14	14
Rhus coriaria	153	153	134	134	134	130	100	134	134	130
Ricinus communis	127	127	127	127	127	107	107	107	107	107

Table 3.1/ continue

Rosa centifolia	19	19	19	19	19	19	19	19	19	19
Rubia tenuifolia	12	12	11	12	12	12	12	11	12	12
Ruta chalepensis	11	11	10	11	11	11	11	10	11	10
Saccharum ravennae	11	11	11	10	10	11	11	11	10	11
Salvia fruiticosa	42	42	32	32	32	30	28	32	32	32
Salvia hierosolymitana	18	18	16	17	17	18	18	15	17	15
Sarcopoterium spinosum	120	120	127	127	127	103	100	107	107	107
Satureja thymbra	18	18	19	19	19	17	15	19	18	19
Scabiosa prolifera	21	21	18	17	17	17	14	18	17	17
Sinapis arvensis	100	106	97	97	97	88	97	97	88	97
Sonchus oleraceus	43	43	43	43	43	43	43	43	43	43
Styrax officinalis	18	18	17	18	18	18	18	17	18	17
Trigonella foenumgraecum	12	12	11	10	10	11	11	11	10	10
Varethemia iphionoides	15	15	12	12	12	13	14	12	12	12
Vicia faba	8	8	8	8	8	8	8	8	8	8
Viscum cruciatum	27	27	27	25	25	22	24	25	25	25

* Relative antimicrobial activity = [(inhibition zone diameter mean of active plant)^2/ (inhibition zone diameter mean of reference antibiotics)^2] × 100%

Table 3.2 Relative antimicrobial activity * of plant extracts against aerobic bacteria

Strain No.* / Plant extract	1	2	3	4	5
Achillea fragrantissima	7	26	6	8	4
Ajuga orientalis	7	26	10	7	4
Allium sativum	8	33	13	13	8
Arum dioscoridis	4	16	6	6	4
Asparagus aphyllus	4	16	6	6	4
Capparis spinosa	29	42	10	14	9
Cardaria draba	5	28	7	6	5
Ceratonia siliqua	4	16	6	6	4
Chrysanthemum coronarium	7	16	12	10	4
Cicer arietenum	5	16	8	9	7
Clematis cirrhosa	7	28	10	10	7
Companula rapunculus	4	16	6	6	4
Conium maculathum	10	33	6	9	6
Convolvulus arvensis	8	36	12	10	7
Coridothymus capitatus	9	36	12	13	8
Crataegus aronia	15	44	23	16	11
Daucus carota	6	22	13	13	5
Erodium malacoides	6	33	14	13	9

Table 3.2/ continue

Eruca sativa	7	28	8	6	4
Foeniculum vulgare	6	16	10	9	7
Gagea chloranth	4	22	10	10	4
Gundelia tournefortii	4	16	8	10	7
Lactuca tuberosa	15	42	16	6	4
Linum pubescens	6	22	10	10	4
Lupinus pilosus	7	28	11	12	7
Lycium europium	5	16	6	6	4
Majorana syriaca	15	54	22	19	18
Mandragora autumnalis	5	22	6	6	5
Mentha viridis	7	24	10	9	7
Notobasis syriaca	7	31	8	6	5
Parietaria diffusa	7	26	10	9	5
Paronychia argentea	4	16	6	6	4
Petroselinum sativum	5	18	6	6	4
Phagnalon rupester	9	31	13	12	9
Pinus halepensis	8	16	14	6	4
Pistacia lentiscus	8	28	11	10	7
Pyrus syriaca	8	33	14	14	10
Reseda alba	4	16	6	6	4
Rhus coriaria	58	215	77	30	19
Ricinus communis	42	144	52	6	21
Rosa centifolia	9	31	12	14	10

Table 3.2/ continue

Rubia tenuifolia	9	28	8	12	6
Ruta chalepensis	4	16	6	10	5
Saccharum ravennae	7	28	10	6	5
Salvia fruiticosa	8	28	10	10	7
Salvia hierosolymitana	4	16	6	6	4
Sarcopoterium spinosum	40	53	18	19	16
Satureja thymbra	14	64	23	23	16
Scabiosa prolifera	4	16	6	8	7
Sinapis arvensis	13	42	21	14	10
Sonchus oleraceus	7	28	10	10	5
Styrax officinalis	7	28	10	10	5
Trigonella foenumgraecum	8	39	6	6	4
Varethemia iphionoides	7	26	10	9	5
Vicia faba	12	44	16	15	11
Viscum cruciatum	36	149	23	8	9

**1, Staphyllococus aureus; 2, Echerichia coli; 3, Pseudomonas eruginosa; 4, Proteus vulgaris; 5, Klebsiella pneumonia*

3.2 Susceptibility of test bacterial strains to plant extracts

Test strains differed significantly in their susceptibility to the different plant extracts used ($F = 2.078$, $DF = 839$, $P < 0.05$).

The most sensitive test microorganism was *Propionibacterium acnes 6919* with inhibition zone diameter mean of 9.79 mm. Whereas the least sensitive microorganism was A7 (*P. acnes* recovered from acne patient) with inhibition zone diameter mean of 9.48 mm as shown in (Figure 3.1).

The most sensitive species of aerobic bacteria was *Staph. aureus* with inhibition zone diameter of 9.19 mm. Whereas the least sensitive test microorganism was *Klebsiella pneumonia* with inhibition zone diameter of 7.67 mm as shown in (Figure 3.1).

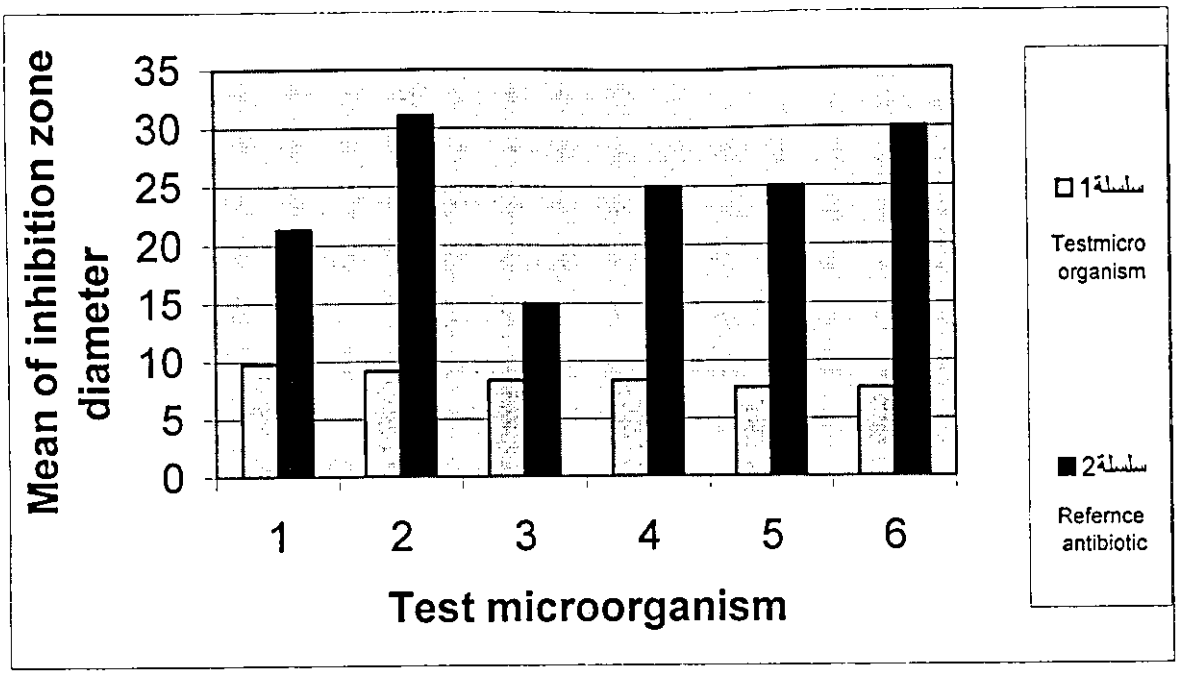

Figure 3.1 Susceptibility of test microorganism to plant extracts (*1, P. acnes; 2, S. aureus; 3, E. coli; 4, P. aeruginosa; 5, P. vulgaris, 6, K. pneumonia*).

3.3 MIC and MBC of the active plant extracts

Minimum inhibitory concentration (MIC) and MBC of active plant extracts were determined against test microorganisms. For *P. acnes* (anaerobic bacteria), the most susceptible microorganisms was *P. acnes (6919, 6921)* with MIC raging from 6 – 28 mg/ml and MBC 6 – 30 mg/ml as shown in (Tables 3.2, 3.3). *Rhus coriaria* had the lowest MIC 6 mg/ml, MBC 6 mg/ml. Whereas *Viscum cruciatum* had the highest MIC 28 mg/ ml, MBC 30 mg/ml.

For aerobic bacteria, the most susceptible microorganism was *Staph aureus* with MIC ranging 4 – 18.33 mg/ml and MBC 6 – 20 mg/ml, and the least susceptible microorganism was *Klebsiella pneumonia. Rhus coriaria* had the lowest MIC, while *Sinapis arvensis* and *Vicia faba* had the highest MIC as shown in (Table 3.4, 3.5).

Table 3.3 Mean of MIC (mg/ml) for active plant extracts against ten strains of *Propionibacterium acnes*

Strain No. / Plant Extract	*Propionibacterium acnes*									
	P acne 6919	*P acne* 6921	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
Ajuga orientalis	20 ± 0*	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0
Clematis cirrhosa	25 ±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0
Lactuca tuberose	20 ±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0
Lycium europeum	20 ±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0	20±0
Majorana syriaca	25 ±0	25±033	30±0	30±0	30±0	30±0	30±0	30±0	30±0	30±0
Phagnalon rupester	20 ±0	20±0	26±0.33	26±0	26±0	26±0	26±0	26±0	26±0	26±0
Pinus halepensis	10 ±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Rhus coriaria	6 ±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Ricinus communis	6 ±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Salvia fruiticosa	16±0.33	16±0	17±0.67	16±0	16±0	16±0	16±0	17±0.67	16±0	16±0
Sarcopoterium spinosum	6 ±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Sinapis arvensis	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0	10 ±0
Sonchus oleraceus	10 ±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Viscum cruciatum	28 ±0	28±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0

* Standard error (SE).

Table 3.4 Mean of MBC (mg/ml) plant extracts against ten strains of *Propionibacterium acnes*

Strain No. / Plant Extract	*Propionibacterium acnes*									
	P acne 6919	*P acne* 6921	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
Ajuga orientalis	30±0*	30±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0
Clematis cirrhosa	30±0	30±0	28±0	28±0	28±0	28±0	28±0	28±0	28±0	28±0
Lactuca tuberose	25±0	25±0	28±0	28±0	28±0	28±0	28±0	28±0	28±0	28±0
Lycium europeum	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0	25±0
Majorana syriaca	30±0	30±0	32±0	32±0	32±0	32±0	32±0	32±0	32±0	32±0
Phagnalon rupester	20±0	20±0	25±0.33	25±0	25±0	25±0	25±0	25±0.33	25±0	25±0
Pinus halepensis	12±0	12±0	16±0	16±0	16±0	16±0	16±0	16±0	16±0	16±0
Rhus coriaria	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Ricinus communis	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Salvia fruiticosa	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0
Sarcopoterium spinosum	20±0	20±0	22±0	22±0	22±0	22±0	22±0	22±0	22±0	22±0
Sinapis arvensis	23±0	23±0	23±0	23±0	23±0	23±0	23±0	23±0	23±0	23±0
Sonchus oleraceus	16±0	16±0	16±0	16±0	16±0	16±0	16±0	16±0	16±0	16±0
Viscum cruciatum	30±0	30±0	32±0	32±0	32±0	32±0	32±0	32±0	32±0	32±0

* Standard error (SE)

Table 3.5 Mean of MIC (mg/ml) for active plant extracts against aerobic bacteria

Microorganism / Plant Extract	*S. aureus*	*E. coli*	*Pseudomonas eruginosa*	*Proteus vulgaris*	*Klebsiella pneumonia*
Crataegus aronia	15±0*	20±0	20±0.33	20±0.33	20±0
Lactuca tuberose	20±0	25±0	20±0.33	> 25	> 25
Majorana syriaca	15±0	14±0.33	14±0	12±0	15±0.67
Rhus coriaria	4±0	6±0	4±0	15±0	20±0
Ricinus communis	8±0	14±0.67	14±0	> 25	12±0.67
Sarcopoterium spinosum	8±0	19±0.67	17±0.67	18±0	18±0
Satureja thymbra	11±0.33	12±0	12±0.67	12±0	11±0.33
Sinapis arvensis	18±0.33	20±0.33	20±0	20±0	21±0.33
Vicia faba	18±0.33	20±0	20±0	20±0	20±0
Viscum cruciatum	11±0.67	8±0	20±0	> 25	25±0

* Standard error (SE)

Table 3.6 Mean of MBC (mg/ml) for active plant extracts against aerobic bacteria

Microorganism / Plant Extract	*S. aureus*	*E. coli*	*Pseudomonas eruginosa*	*Proteus vulgaris*	*Klebsiella pneumonia*
Crataegus aronia	18±0*	25±0	25±0.33	25±0	25±0
Lactuca tuberose	20±0	30±0	25±0.33	> 30	>30
Majorana syriaca	21±0.67	21±0.67	20±0	20±0	20±0.33
Rhus coriaria	6±0	8±0	6±0.33	18±0	25±0
Ricinus communis	10±0	16±0	16±0	>30	15±0
Sarcopoterium spinosum	10±0	20±0	18±0	20±0	20±0
Satureja thymbra	16±0.33	16±0	17±0.67	16±0	16±0
Sinapis arvensis	20±0.33	25±0.33	25±0	25±0	26±0.67
Vicia faba	20±0	25±0	25±0	25±0	25±0
Viscum cruciatum	12±0	10±0	25±0	>30	30±0

* Standard error (SE)

CHAPTER FOUR

Discussion

4.1 Antimicrobial activity of selected medicinal plants

Traditional medicine is an important source of potentially usefull new compounds for the development of chemotherapeutic agents (Alonse *et al.*, 1995). The first step towards achieving this goal is screening of plants used in popular medicine. The search for new, safer and more effective antifungal and antibacterial agents has grown with the increasing incidence of microbial infections (Larhsini *et al.*, 1996) relying on plants used in folk medicine.

The above results show that most of the studied plants 54/56 (96%) are potentially a rich source of antimicrobial agents. Ninety one percent of the screened plants were active against *P. acne* (anaerobic bacteria), whereas 88 % of the screened plants were active against aerobic bacteria. However, it is noteworthy to point out that *P. acne* strains (anaerobic bacteria) were more susceptible than aerobic bacteria. This may be attributed to differences in modes of actions of plant extracts against aerobic and anaerobic bacteria.

The optimal effectiveness of a medicinal plant may not be due to one main active constituent but to the combined action of known and unknown compounds originally occurring in the plant (Bai, 1990). Hence, discrimination between the different medicinal plants in terms of their antimicrobial activity depends mainly on their content of chemically active compounds. This could obviously account for the significant differences in antimicrobial activities detected between the studied plants in this study.

Differences in antimicrobial activity of the test plants are obviously related to differences in their contents of active compounds as shown in (Table 4.1). The most active plants studied in this work seem to posses similar antimicrobially active compounds including essential oils, flavanoids and triterpenoids and other compounds of phenolic nature or with free hydroxyl group, which are classified as active antimicrobial compounds (Rojas *et al.*, 1992).

From our results, it was noted that plants belonging to the same family exhibited comparable antimicrobial activities. In the present work *Rhus coriaria* belonging to the family Anacardiaceae,which showed the highest activity against *P. acnes*. This is in agreement with the results of Kubo *et al* (1994), who also found that *Anacardium*

occidentale belonging to the same family to have potent antimicrobial activity against *P. acne.*

Table 4.1 Active antimicrobial constituents of active plant extracts

Plant	Active antimicrobial constituents	References
Clematis cirrhosa	Saponins, triterpenoids, oleanolic acid	12
Crataegus aronia	Simple flavinoids, oligomeric, procyaidins	13,16,17
Lycium europeum	Carvacrol, terpenes, sterols, alkaloids	2,5,7,8
Phagnalon rupester	Thymol, thymol derivatives, quinines	6,13
Pinus halepensis	Urpentine, coniferin, resin, tannic acid, pinite, pinene, vitamin C	4
Rhus coriaria	Myricetin, tannin, oxyquercetin, vitamin C, flavinoids, carotenes	1,11
Salvia fruiticosa	Thymol, carvacol, flavinoids, rosmarinic acid, saponins, monterpenoids, 1-8 cineol, essential oils, flavone aglycones, flacanoid, glycoside	3,4,5,10,14,19
Sarcopoterium spinosum	Tannin, triterepenoids, glycosides.	11
Satureja thymbra	Essential oils, tocopherols	9,15
Viscum cruciatum	Viscotoxin, arginine, choline, tyramin, mucilage.	11

[1] Al-Wareh *et al.*, 1993; [2] Bellakhdar *et al.*, 1991; [3] Chieji, 1984; [4] Conigueral *et al.*, 1989; [5] Dafni & Yaniv, 1994; [6] El-Damy *et al.*, 1994; [7] Chazanfar,1994; [8] Gribanovski-sassu *et al.*, 1969; [9] Haykel & Omar, 1988; [10] Hussain, 1995; [11] Karim & Quraan, 1986; [12] Kinghorn & Balandrin,1993; [13] Kizu *et al.*, 1995; [14] Palevitch & Yaniv, 1991; [15] Shimoni *et al.*, 1993; [16] Thapliyal & Bahuguna, 1993; [17] Uniyal & sato, 1992; [18] Viollon & Chaumant, 1994; [19] Yaniv *et al.*, 1987.

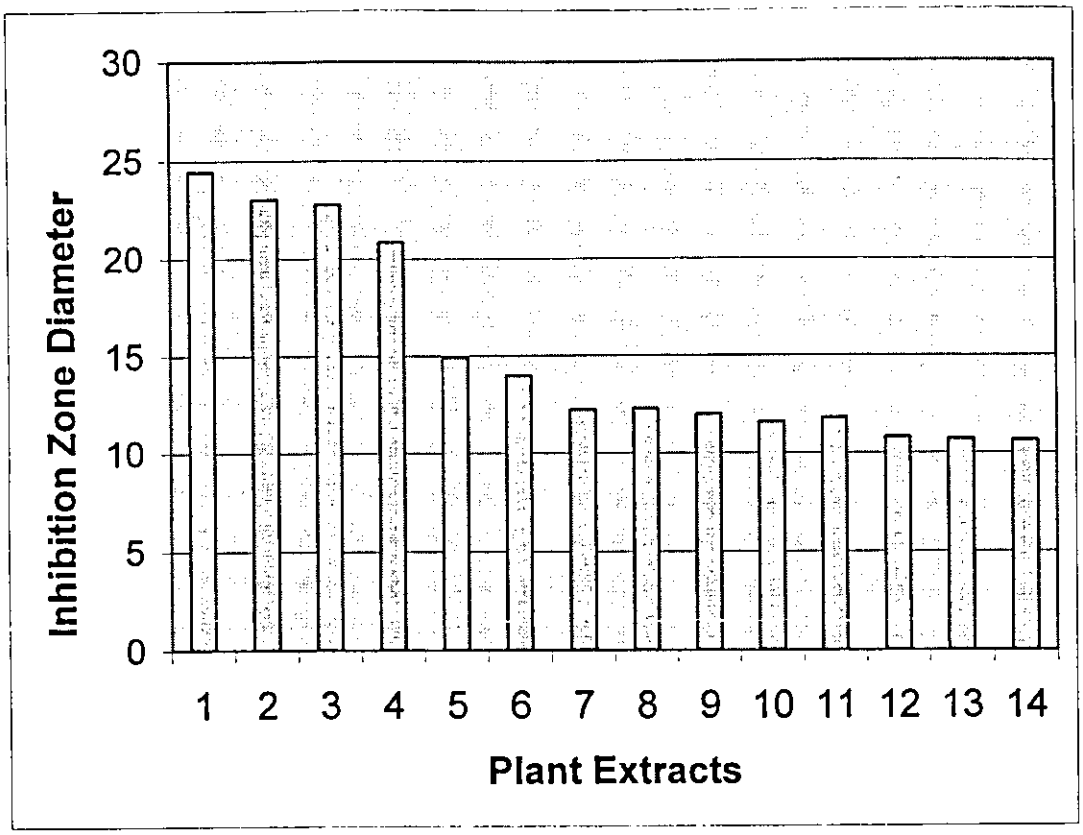

Figure 4.1 Susceptibility of *P. acnes* to active plant extracts *(1, Rhus coriaria; 2, Ricinus communis; 3, Sarcopoterium spinosum; 4, Sinapis arvensis; 5, Pinus halepensis; 6, Sonchus oleraceus; 7, Salvia fruiticosa; 8, Lactuca tuberosa;9, Lycium europium; 10, Phagnalon rupester; 11, Ajuga orientalis; 12, Clematis cirrhosa; 13, Viscum cruciatum; 14, Majoraha syriaca).*

Since some of the medicinal plants studied appear to have a broad antimicrobial spectrum of action, they could be useful in antiseptic and disinfectant formulations, and in chemotherapy (Olukoya *et al.*, 1993), taking into consideration the satisfaction of the same basic requirements

of other pharmaceutical products: quantity, safety and efficacy (Linnenbrink, 1990).

The antimicrobially active plants studies in the present work may be equally important for the treatment of the other infections (eg., urinary tract, gastrointestiestinal, eye, respiratory and wound infection) caused by these same test pathogens.

The present work has shown that most of the studied plants are potentially a rich source of antimicrobial agents and demonstrates the importance of such plants in medicine and in assisting primary health care in this part of the world.

Further studies are still needed and encouraged to test for the biological activities of wild plants and explore their potential as sources for antimicrobial agents and for other benefits to human health.

Conclusions

In vitro tests for screening the antimicrobial activity of 56 extracts against anaerobic bacteria *Propionibacterium acnes* and five aerobic bacteria species revealed the following:

1- The wealth of medicinal plants is one of the vital resources having important bearing on human health and the region's economy.

2- Ninety six percent of the plants studied were active against test microorganisms, which indicates that the use of these plants in folk medicine was justified. Only the use of two plants *Arum dioscoridis, and Ceratonia siliqua* was unjustified.

3- Rhus coriaria, Ricinus communis, Sarcopterium spinosum were the most active plants antimicrobially.

4- The most susceptible bacterial species to all studied plants was *P. acnes,* whereas the least active one was *K. pneumonia.*

Recommendations:

1- Since 96% of the plants studied have a broad antimicrobial spectrum of action, they could be useful in antiseptic and disinfectant formulation and in external chemotherapy, taking into consideration the satisfaction of the same basic requirements of other pharmaceutical products: quantity safety, and efficacy.

2- Some of the plant extracts examined (*Rhus coriaria, Ricinus communis, Sarcopoterium spinosum*) warrant further investigation using bioassay-guided fractionation to characterize the active constituent.

3- Further studies are needed to test the biological activities of wild plants and explore their potential as sources of antimicrobial agents and for other benefits to human health.

4-Both *in vitro* and *in vivo* works are needed to utilize the excellent antibacterial properties of these highly efficient and beneficial plant extracts.

REFERENCES

Ali-Shtayeh, M. S., and Abu-Ghdeib, S. I. (1999) Antifungal activity ofplant extracts against dermatophytes. *Mycoses* **42**, 665-672.

Ali-Shtayeh, M. S., Al-Nuri, M. A., Yaghmour, R. M. R., and Faidi, Y. R. (1997) Antimicrobial activity of *Micromeria nervosa* from the Palestinian area. *Ethnopharmacology* **58**, 143-147.

Ali-Shtayeh, M. S., Hamad, A. Kh. (1995) Protection of the Palestinian environment. Nablus. Authors. (Arabic).

Ali-Shtayeh, M. S., Yaghmour, R. M. R., Faidi, Y. R., Salem, K., and Al-Nuri, M. A. (1998) Antimicrobial activity of Twenty plants used in folkloric medicine in the Palestinian area. *Ethnopharmacology* **60**, 265-271.

Ali-Shtayeh, M. S., Yaniv, Z., and Mahajna, J. (2000) Ethnobotanical survey in the Palestinian area. *Ethnopharmacology* **73**, 221-232.

Allaker, R. P., Greenman, J., Osborne, R. H. (1987) The production of inflammatory compounds by *Propionibacterium acnes* and other skin organisms. *Dermatol* **117**, 175-183.

Al-Wareh, H. B., Al-Ghazal, R. K., & Mouchanta, A. H. (1993) Medicinal and aromatic plants. Aleppo university publications (Arabic).

Bellakhdr, J., Claisse, R., Fleurentic, J., & Younos, C. (1991) Repertory of standard herbal drugs in the Moroccan pharmacopoea. *Ethnopharmacology* **35**, 123-143.

Boily, Y., and Vanpuyveld, L. (1986) Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *Ethnopharmacology* **16**, 1-13.

Caceres, A., Cano, O., Samayoa, B., Aguilas, L. (1990) Plants used in Guatemala for treatment of gastrointestinal disorders. *Ethnopharmacology* **30**, 55-73.

Chieji, R. (1984) *The Macdonald encyclopedia of medicinal plants* London: Macdonald, Maxwell House.

Conigueral, S., Iglesias, J., Hamburger, M., & Hostettmann, K. (1989) Phenolic consituents of *Saliva lavandulifolia sp. Plant Medica* **55**, 502.

Dafni, A., & Yaniv, Z. (1994) *Solanceae* as medicinal plants in Israel. *Ethnopharmacology* **44**, 11-18.

Dahl, M. G. C., Mcgibbon, D. H. (1979) Complement C3 and immunoglobulin in inflammatory acne vulgaris. *Dermatol* **101**, 633-641.

Denys, G. A., Jerris, R. C., Swenson, J. M., and Thornsberry, C. (1993) Susceptibility of *Propionibacterium acnes* clinical isolates of 22 antimicrobial agents. *Antimicrobial Ag chemotheray* **22**, 335-337.

Downing, D. T., Strauss, J. S. (1982) The effect of accumulated lipids on measurements of sebum secretion in human skin. *Dermatol* **79**, 226-228.

El-Damy, S. I., Abdel-Aal, M., Abdel-fatah, H., & Fid, F. (1994) Yhymol derivatives from *Phagnalon sinaicum* Bornum & Kneuck. *Acta-Pharm-Hung* **64**, 115-116.

Essawi, T., and Srour, M. (2000) Screening of some Palestinian medicinal plants for antibacterial activity. *Ethnopharmacology* **70**, 343-349.

Frank, J. L. (1996) The efficacy, history, of medicinal plants. *Alternative Therapies in Health and Medicine* **4**, 36-41.

Gerri, S. H., Kathy, PR., David, M., J, A., Thomas, J., and Darlene, M. (1995) Minimum Bactericidal Concentration of *Propionibacterium acnes* isolates from cases of chronic Endophthalamitis. *Elsevier Science* **21**,187-190.

Glupcyznski, Y., Gordts, B., Vanodelinden, MP., Yourassowsky, E., and Butzler, J. P. (1985) Susceptibility f anaerobic bacteria to Sch-34343 and other antibiotics. *Antimicrobial chemother* **15**, 193-198.

Hamilton, J. B. (1970) Greater tendency to acne in white American than in Japanese population. *Clinical Endocrinology* Metabolites **24**, 267.

Irobi, O. N., and Daramala, S. O. (1994) Bactericidal properities of crude extracts of *Mitracarpus villous*. *Ethnopharmacology* **42**, 39-43.

Karim, F. M., & Quraan, S. A. (1986) *Medicinal plants of Jordan.* Published by Yarmouk University, Irbid, Jordan.

Kinghorn, T., Balandrin, M. F. (1993) *Human Medicinal Agents from Plants.* San Francisco American Chemical Society.

Kizu, H., Shimana, H., & Tomimori, T. (1995) Studies on the constituents of *Clematis* species. VI. The constituents of *Clematis stans* sieb. Etzucc. *Chem-Pharm-Bull-Tokyo* **43**, 2187-21

Kligman, A. M. (1974) An overview of acne, *Dermatol* **62**, 268-287.

Knuston, D. D. (1974) Ultrastructural observations in acne vulgaris: the normal sebaceous follicle and *acne lesions*. *Dermatol* **62**, 288-306.

Kubo, I., Muroi, H., Kubo, A. (1994) Natural occuring antiacne agents. *Natural Products* **57**, 9-17.

Lee, P. A. (1976) Acne and serum androgens during puberty. *Dermatol* **112**, 428.

Marsden, J. R., Middleton, B., Mills, C. (1987) Is remission of acne due to changes in sebum composition. *Dermatol* **12**, 18-20.

Meyer, J. J. M., Afolayan, A. J., Tayler, M. B., Engelbrecht, L. (1996) Inhibition of *herbes simplex* virus type1 by aqueous extracts from shoots of *Helichrysum aureonitens*. *Ethnopharmacology* **52**, 4143.

Mouvais, J. P. (1973) Simultaneous determination of urinary androstanoidal and testosterone as an evaluation of human androgenicity. *Clinical Endocrinology* **36**, 452-455.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., and Yolke, R. H. (1995) *Manual of Clnical Microbiology.* (6 th ed). USA: ASM Press.

Nadir, M. I., Dhahir, J., Abdul-Baqi, J., Al-Sarraj, B. M., and Hussein, W. A. (1985) The effect of different methods of extraction on the antimicrobial activity of medicinal plants. *Fitoterapia* **17**.

Palvetch, P. D., & Yaniv, Z. (1991) *Medicinal plants of the Hollyland,* Vol. **1** and **2**. Tel-Aviv: Tammuz Publisher Ltd.

Puhvel, S. M., Sakomoto, M. (1978) The chemoattractant properities of comedonal contents. *Dermatol* **71**, 324-329.

Pochi, P. E. (1977) Skin surface lipid composition, acne, pubertal development, and urinary excretion of testosterone in children. *Dermatol* **69**, 485-487.

Plewig, G., Christoptors, E., Braun-Falco, O. (1971) Cell transition in human sebaceous glands. *Dermatology* **51**, 423-428.

Raman, A., Weir, U., and Bloom field, S. F. (1995) Antimicrobial effects of tea-tree oil and itis major components on *Staphylococcus aureus,* and *Propionibacterium acnes. Lett Appl Microbiology* **21**, 242-245.

Rios, J. L., Recio, M. C., and Villar, A. (1988) Screning methods for natural products with antimicrobial activity. *Ethnopharmacology* **23**, 127-149..

Shimoni, M., Putievsky, E., Ravid, U., & Reuveni, R. (1993) Antifungal activity of volaile fractions of essential oils from four aromatic wild plants in Israel. *Chemical Ecology* **19**, 1129-1133.

Silva, F., and Abraham, A. (1981) The potentiality of the Israeli flora for medicinal purposes. *Fitotetapia* **195**-200.

Silva, O., Duarte, A., Cabrita, J., Pimentet, M., Dinize, A., and Gomes, E. (1996). Antimicrobial activity of Guinea-Bissau traditional remedies. *Ethnopharmacology*, 50, 55-59.

Sofowra, A . (1982). *Medicinal Plants and traditional medicine in Africa*. USA New York: *John Wiley & Sons Ltd.*

Thapliyal, R. P., & Bahuguna, R. P. (1993) Clemontanoside C, asaponin from *Clematis montana. Phytochemistry* **33**, 671-673.

UNESCO (1996) Culture and health. Paris, France, 129.

UNESCO (1998) *Terminal report. Promotion of ethnobotany and the sustainable use of plant resources in Africa*. Paris, France, 60.

Uniyal, S. K., & Sato, O. P. (1992) Triterpenoid saponins from roots of *Clematis grata. Phytochemistry* **31**, 1427-1428.

Vanpuyvelde, L., Geysen, D., Ayobangira, F. X., Hakizamungu, E., Nshimiyimana, A., Kalisa, A. (1985) Screening of medicinal plants of Rwanda for acaricidal activity. *Ethnopharmacology* **13**, 209-215.

Vanpuyvelde, L., Ntawukiliyayo, J. D., Portaels, L., Hakizamungu, E. (1994) **In vivo** inhibition of mycobacteria by Rwandese medicinal plants. *Phytotherapy Research* **8**, 65-69.

Verchoore, M. (1987) Aspects hormonax de acne. *Dermatol* **114**, 439-454.

Viollon, C., & Chaumant, J. P. (1994) Antifungal properities of essential oils and their main components upon *Cryptococcus neoformanss. Mycopathologia* **128**, 151-153.

Webster, G. F., Leyden, J. J. (1980) Characterization of serum-independent polymorphonuclear leukocyte chemotactic factors produced by *Propionibacterium acnes* inflammation. *Dermatol* **4**, 261-269.

Webster, G. F., Leyden, J. J., Tsai, C. C., Baehni, P., Mcarthur, W. P. (1980) Polymorphonuclear leucocyte lysosomal release in response to *Propionibacterium acnes* in vitro and itis enhancement by sera from patients with inflammatory acne. *Invest Dermatol* **74**, 398-401.

Wexler, H., and Finegold, S. (1991) Antibacterial susceptibiliy tests: Anaerobic bacteria, in manual of microbiology (Balows, A., Hauler, W., Herrmann, K., Isenberg, H., Shadomy, H.) USA: ASM. PP. 1133-1135.

Wilkins, JW. JR., and Voorhees, J. (1972). Prevalence of nodulocytic acne in white and Negro males. *Dermatol*, 102, 631.

Wolff, H. H., Plewig, G. E., Braun-Falco, O. (1975) Ultrastructure of human sebaceous follicles following treatment with vitamin A. *Dermatology* **74**, 99-110.

Woods, G., and Washington, J. (1995) Antibacterial susceptibility tests: dilution and disk diffusion methods, in manual of clinical microbiology (Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., and Yolken, R. H.) ASM Press, Washington, D. C. PP, 1337-1341.

Wrang, W L L., Everett, E. D., Johnson, M., and Dean, E. (1983) Susceptibility of *Propionibacterium acnes* to seventeen antibiotics. *Antimicrobial chemother* **11**, 171-173.

Yaniv, Z., Dafni, A., Friedman, J., & Palvitch, D. (1987) Plants used for the treatment of diabetes in Israel. *Ethnopharmacology* **19**, 145-151.

Appendix A: Susceptibility testing for anaerobic and aerobic bacteria using selected ethanolic plant extracts.

Table A.1 Means of inhibition zone diameter (mm) for ten strains of *Prpionibacterium acnes*

Strain No. / Plant extract	*Propionibacterium acnes*									
	P. acnes 6919	*P.acnes* 6921	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8
Achillea fragrantissima	* 8 ±0**	8 ±0	8 ±0	8 ±0	8±0	8±0	8±0	8±0	8±0	8±0
Ajuga orientalis	10.67±0.3	11±0	12±0	12±0	12±0	11.67±0.3	12±0.68	12±0	12±0	12±0
Allium sativum	8.87±0.3	8.87±0.3	8±0	8.33±0.3	8.330.3	8.67±0.3	8.I.7±0.3	8±0	8±0.3	8±0
Arum dioscoridis	6±0	6±0	6±0	6±0	6±0	6.0	6±0	6±0	6±0	6±0
Asparagus aphyllus	8±0	8±0	8.33±0.3	8±0	8±0	8±0	8±0	8±0	8±0.3	8±0.3
Capparis spinosa	7±0	7±0	6.67±0.3	7±0	7±0	6.33±0.3	7±0	6.17±0.3	7±0	6.6.7±0.3
Cardaria draba	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Ceratonia siliqua	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Chrysanthemum coronarium	8.3±0.3	8.3±0.3	8.3±0.3	8±0	8±0	8.3±0.3	8.3±0.3	8.3±0.3	8±0	8±0
Cicer arietenum	8.3±0.3	8.3±0.3	8.3±0.3	8±0	8±0	8.3±0.3	8.3±0.3	8.3±0.3	8.3±0.3	8.3±0.3
Clematis cirrhosa	10.3±0.3	10.3±0.3	11±0	11±0	11±0	10.7±0.3	10.7±0.3	11±0	11±0	10.3±0.3
Companula rapunculus	8.7±0.7	8.7±0.7	8.7±0.3	8.7±0.7	8.7±0.7	8.7±0.7	8.7±0.7	8.7±0.7	9.7±0.7	8.7±0.7
Conium maculathum	7±0	7±0	7±0	7±0	7±0	7±0	7±0	7±0	7±0	7±0
Convolvulus arvensis	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8.7±0.3	8±0	8±0
Coridothymus capitatus	8.3±0.3	8.3±0.3	8±0	8.3±0.3	8±08.3	8.3±0.3	8.3±0.3	8±0	8±0	8.3±0.3
Crataegus aronia	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0
Daucus carota	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0

Erodium malacoides	10±0	9.3±0.3	10±0	9.7±0.3	10±0	9.3±0.3	9.3±0.3	10±0	9.7±0.3	10±0
Eruca sativa	8±0	8±0	8±0	7.7±0.3	8±0	8±0	8±0	8±0	7.7±0.3	8±0
Foeniculum vulgare	7.3±0.3	7.3±0.3	7±0	7.3±0.3	7±0	7.3±0.3	7.3±0.3	7±0	7.3±0	7.3±0
Gagea chloranthy	7±0	7±0	7±0	6.3±0.3	6.7±0.3	7±0	7±0	7±0	6.3±0.3	6.3±0.3
Gundelia tournefortii	7.7±0.7	7.7±0.7	7.3±0.3	7.7±0.7	7.7±0.7	7.7±0.7	7.7±0.7	7.3±0.3	7.9±0.7	7.7±0.7
Lactuca tuberosa	13±0	13±0	12±0	12±0	12±0	13±0	13±0	12±0	12±0	12±0
Linum pubescens	9±0	9±0	8.7±0.3	9±0	8.7±0.3	9±0	9±0	9±0	9±0	9±0
Lupinus pilosus	8.3±0.3	8.3±0.3	8.3±0.3	8±0	8.3±0.3	8.3±0.3	8.3±0.3	8.3±0.3	8±0	8±0
Lyscium europeum	12±0	12±0	12±0	12±0	12±0	12±0	12±0	12±0	12±0	12±0
Majorana syriaca	11±0	11±0	10.3±0.3	10±0	10.3±0.3	11±0	11±0	10.3±0.3	10±0	10.3±0.3
Mandragora autumnalis	7±0	7±0	7±0	6.7±0.3	7±0	7±0	7±0	7±0	6.6±0	7±0
Mentha viridis	8.7±0.7	8.7±0.7	8.3±0.3	8±0	8±0	8.7±0.7	8.7±0.7	8.3±0.3	8±0	8.3±0.3
Notobasis syriaca	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0	8±0
Parietaria diffusa	9.3±0.3	9.3±0.3	9.3±0.3	9.7±0.3	9±0	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3
Paronychia argentea	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Petroselinum sativum	9±0	9±0	9±0	8.7±0.3	9±0	9±0	9±0	9±0	9±0	9±0
Phagnalon rupester	12±0	12±0	11.3±0.3	11.3±0.3	11.3±0.3	12±0	12±0	11.3±0.3	11.3±0.3	12±0
Pinus halepensis	16.4±0.3	16.4±0.3	14.3±0.3	4.3±0.3	14.3±0.3	13.7±0.3	16±0.6	14.3±0.3	14.3±0.3	16±0.6
Pistacia lentiscus	7.3±0.3	7.9±0.3	7±00	7.3±0.3	7.3±0.3	7.3±0.3	7.7±0.3	7±0	7.3±0.3	7±0
Pyrus syriaca	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0
Reseda alba	6.3±0.3	6±0	6±0	6±0	6±0	6.3±0.3	8±0	8±0	8±0	6±0
Rhus coriaria	26.3±0.3	26.3±0.3	24.7±0.7	24.7±0.7	24.7±0.7	24.7±0.7	24.7±0.7	24.7±0.7	24.7±0.7	24.7±0.7
Ricinus communis	24±0	24±0	24±0	24±0	24±0	22±0	22±0	22±0	22±0	22±0
Rosa centifolia	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3	9.3±0.3
Rubia tenuifolia	7.3±0.3	7.3±0.3	7±0	7.3±0.3	7.3±0.3	7.3±0.3	7.3±0.3	7±0	7.3±0.3	7±0
Ruta chalepensis	7±0	7±0	6.7±0.3	7±0	7±0	7±0	7±0	6.7±0.3	7±0	7±0
Saccharum ravennae	7±0	7±0	7±0	6.7±0.3	6.7±0.3	7±0	7±0	7±0	6.7±0.3	7±0

Salvia fruiticosa	13.9±0.3	13.9±0.3	12±0	12±0	12±0	11.7±0.3	11.3±0.3	12±0	12±0	12±0
Salvia hierosolymitana	9±0	9±0	8.3±0.3	8.7±0.3	8.7±0.3	9±0	9±0	8.3±0.3	8.7±0.3	8.3±0.3
Sarcopoterium spinosum	23.3±0.3	23.3±0.3	24±0	24±0	24±0	21.7±0.3	21.3±0.3	22±0	22±0	22±0
Satureja thymbra	9±0.7	9±0.7	9.3±0.3	9.3±0.3	9.3±0.3	8.7±0.3	8.3±0.3	9.3±0.3	9±0	9±0
Scabiosa prolifera	9.7±0.3	9.7±0.3	9±0	8.7±0.3	8.7±0.3	8.7±0.3	8±0	9±0	6.7±0.3	9±0
Sinapis arvensis	21.3±0.7	22±0.7	21±0.7	21±0.7	21±0.7	20±0.7	21±0.7	21.07	20.0.7	20±0.7
Sonchus oleraceus	14±0	14±0	14±0	14±0	14±0	14±0	14±0	14±0	14±0	14±0
Styrax officinalis	9±0.7	9±0.7	8.7±0.3	9±0	9±0.3	9±0.7	9±0.7	8.7±0.3	9±0	8.7±0.3
Trigonella foenumgraecum	7.3±0.3	7.3±0.3	7±0	6.7±0.3	6.7±0.3	7±0	7±0	7±0	6.7±0.3	6.7±0.3
Varethemia iphionoides	8.3±0.7	8.3±0.3	7.3±0.3	7.3±0.3	7.3±1	7.7±0.7	8±0.7	7.3±0.3	7.3±0.3	7.3±0.3
Vicia faba	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0	6±0
Viscum cruciatum	11±0	11±0	11±0	10.7±0.3	10.7±0.3	10±0	10.7±0.3	10.7±0.3	10.7±0.3	10.3±0.3
Reference antibiotic	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8	21.3±0.8

* dike diameter, 6 (mm)
** standard error (SE)

Table A.2 Means of inhibition zone diameter (mm) for aerobic bacteria

Strain No.* / Plant extract	1	2	3	4	5
Achillea f2ragrantissima	**8.3±0.3***	7.7±0	6±0	7±0	6±0
Ajuga orientalis	8±0	7.7±0.3	8±0	6.7±0.7	6±0
Allium sativum	8.7±0.3	8.7±0.3	9±0	9±0	8.7±0.3
Arum dioscoridis	6±0	6±0	6±0	6±0	6±0
Asparagus aphyllus	6±0	6±0	6±0	6±0	6±0
Capparis spinosa	16.7±0.3	9.7±0.3	8±0	9.3±0.3	9±0
Cardaria draba	7±0	8±0	6.7±0.3	6.3±0.3	6.7±0.3
Ceratonia siliqua	6±0	6±0	6±0	6±0	6±0
Chrysanthemum coronarium	6±0	6±0	8.7±0.3	8±0	6±0
Cicer arietenum	6.7±0.3	6±0	7±0	7.7±0.3	8±0
Clematis cirrhosa	8.3±0.3	8±0	8±0	8±0	8±0
Companula rapunculus	6±0	6±0	6±0	6±0	6±0
Conium maculathum	9.7±0.3	8.7±0.3	6±0	7.7±0.3	7.3±0.3
Convolvulus arvensis	9±0	9±0	8.7±0.3	8±0	8±0
Coridothymus capitatus	9.3±0.3	9±0	8.7±0.3	9±0	8.7±0.3
Crataegus aronia	12±0.3	10±0	12±0	10±	10±0
Daucus carota	7.7±0.3	7±0	9±0	9±0	7±0
Erodium malacoides	7.7±0.3	8.±0.9	9.3±0.3	9±0.7	9±0
Eruca sativa	8.3±0.3	8±0	7±0	6±0	6.3±0.3
Foeniculum vulgare	7.3±0.3	6±0	8±0	7.7±0.3	8±0

Gagea chloranth	6±0	7±0	8±0	8±0	6±0
Gundelia tournefortii	6.3±0.3	6±0	7±0	8±0	8±0
Lactuca tuberosa	12±0	9.3±0.3	10±0	6±0	6±0
Linum pubescens	7.7±0.3	7±0	8±0	8±0	6±0
Lupinus pilosus	8.3±0.3	8±0	8.3±0.3	8.7±0.3	8±0
Lycium europium	7±0	6±0	6±0	6±0	6±0
Majorana syriaca	12±0	11±0	11.7±0.3	11±0	12.7±0. 3
Mandragora autumnalis	7±0	7±0	6±0	6±0	7±0
Mentha viridis	8±0	7.33±0.3	8±0	7.33±0.3	8±0
Notobasis syriaca	8.3±0.3	8.3±0.3	7±0	6±0	7±0
Parietaria diffusa	8±0	7.7±0.3	8±0	7.7±0.3	7±0
Paronychia argentea	6±0	6±0	6±0	6±0	6±0
Petroselinum sativum	6.7±0.3	6.3±0.3	6.3±0.3	6.3±0.3	6.3±0.3
Phagnalon rupester	9.3±0.3	8.3±0.3	9±0	8.7±0.3	9±0
Pinus halepensis	8.7±0.7	6±0	9.3±0.3	6±0	6±0
Pistacia lentiscus	8.7±0.3	8±0	8.3±0.3	8±0	8±0
Pyrus syriaca	9±0	8.7±0.3	9.3±0.3	9.3±0.3	9.3±0.3
Reseda alba	6±0	6±0	6±0	6±0	6±0
Rhus coriaria	23.7±0.3	22±0	22±0	13.7±0.3	13±0
Ricinus communis	20±0	18±0	18±0.7	6±0	13.9±0.3
Rosa centifolia	9.3±0.3	8.3±0.3	9.7±0.3	9.3±0.3	9.3±0.3
Rubia tenuifolia	9.3±0.3	8±0	7±0	8.7±0.3	7.3±0.3
Ruta chalepensis	6±0	6±0	6±0	8±0	7±0
Saccharum ravennae	8±0.3	8±0	8±0	6±0	7±0
Salvia fruiticosa	8.7±0.3	8±0	8±0	8±0	7.7±0.3
Salvia hierosolymitana	6.3±0.3	6±0	6±0	6±0	6±0
Sarcopoterium spinosum	19.7±0.3	11±0	10.7±0.3	11±0	12±0

Satureja thymbra	11.7±0.3	12±0	12±0	12±0	12±0
Scabiosa prolifera	6.3±0.3	6±0	6±0	7±0	8±0
Sinapis arvensis	11.3±0.3	9.7±0.3	11.3±0.3	9.3±0.7	9.3±0.3
Sonchus oleraceus	8.3±0.3	8±0	8±0	8±0	7±0
Styrax officinalis	8.3±0.3	8±0	8±0	8±0	7±0
Trigonella foenumgraecum	9±00	9.3±0.3	6±0	6±0	6±0
Varethemia iphionoides	8±0	7.7±0.3	8±0	7.7±0.3	7±0
Vicia faba	10.7±0.3	10±0.6	10±0	9.7±0.3	10±0
Viscum cruciatum	18.7±0.3	18.3±0.3	12±0	7±0	9±0
Reference antibiotics	31±0.57	15±0	25±0	25±0	30±0

**1, Staphyllococus aureus; 2, Echerichia coli; 3, Pseudomonas eruginosa; 4, Proteus vulgaris; 5, Klebsiella pneumonia*

** disk diameter, 6 (mm)
*** Standard error (SE)

بسم الله الرحمن الرحيم

دراسة الفعالية المضادة للبكتيريا المسببة لحب الشباب للنباتات الطبية في فلسطين

إعداد

أنهار احمد محمد العسالي

بإشراف

أ. د محمد سليم اشتية

لقد تمت دراسة تأثير المستخلصات الكحولية لأربعة وخمسين من النباتات المستخدمة في الطب الشعبي في فلسطين في علاج كثير من الامراض ضد ثمانية عزلات من البكتيريا الهوائية المسببة لحب الشباب (*Propionibacterium acnes*) وخمسة انواع من البكتيريا الهوائية وهي (*Echerichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, and Staphylococcus aureus*).

وقـد اسـتخدم في هذه الدراسة اختباران خاصان بقياس حساسية الكائنات الدقيقة للمـواد ذات الأثر المضاد للميكروبات وهما: Disk diffusion method من اجل قياس النشـاط المضـاد ضد البكتيريا والأخر Broth dilution method من اجل تحديد أقل تركـيز من المادة الفعالة يمكن أن يمنع نمو الكائنات الدقيقة او يقضي عليها كلياً (MIC, and MBC).

وأظهـرت الدراسة وجود اختلافات معنوية بين النباتات بالنسبة لتأثيراتها المضادة للكائنات الدقيقة فكانت المستخلصات الكحولية للنباتات التالية وهي : *(Rhus coriaria, Ricinus communis,* and *Sarcopoterium spinosum)* الاعلـى فعالـية ضمن النباتات المدروسة وضد الميكروبات المذكورة. وكانت المستخلصات الكحولية التالية (*Arum dioscoridis, and Ceratonia siliqua*) هي الاقل فاعلية.

بالنسـبة لأنـواع الكائـنات الدقـيقة المسـتخدمة، فقـد كانـت بكتـيريا (*Propionibacterium acnes*) هـي أكـثر الانواع حساسية للمستخلصات النباتية بينما كانت (*Klebsiela pneumonia*) هي النوع الاقل حساسية.