ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS IN PALESTINE

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ABSTRACT

Objectives: To evaluate the antimicrobial activity of aqueous, ethanolic, methanolic and phenolic extracts from three Palestinian folkloric medicinal plants in addition to their commercial oils against ten pathogenic microorganisms.

Methods: The plants studied were sage, thyme and parsley. Five concentrations of leaf extract of each of the three plants were prepared. The antimicrobial effect of each concentration was measured.

Results: Aqueous extracts of sage and thyme had action against most of the tested microorganisms. Phenolic extract of sage and thyme showed antibacterial activity against *Staph. aureus* and *Enterococcus* sp, respectively. On the other hand, *E. coli* was more affected by the ethanolic extract of parsley. While, that extract does not elicit pronounce effect on the tested Gram positive organisms. The results of commercial oils of sage, thyme and parsley displayed no antimicrobial activity against *E. coli*, *Proteus mirabilis* and *Salmonella typhi*.

Conclusion: The data obtained revealed that, among the 10 tested microorganisms, *Staph. aureus* was, in general, the most susceptible microbe to most extracts of the three plants studied.

KEY WORDS: Antimicrobial, Medicinal Plants, Sage, Thyme, Parsley.

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INTRODUCTION

The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. On the other hand, there is an increment use of herbal products all over the world; in USA, it reached 380% between 1990 and 1997[1].

The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. A large number of plants indeed were used to combat different diseases and known to possess antimicrobial activity[2].

Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds. These plants then

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emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus[3-7].

However, the antimicrobial activity of several extracts of different plants was reported. For example the crude methanolic extracts of neem plant have been shown to have strong antibacterial activity[8].

Water extract of garlic and clove possesses antimicrobial activity. Some bacteria showing resistance to certain antibiotics were sensitive to extracts of both garlic and clove[2]. On the other hand, water extract of Miswak (*Salvadora persica*) roots and stem contains potential antimicrobial anionic components such as chloride, sulfate, thiocyanate and nitrate[9]. The decoction of six of the studied Jordanian medicinal herbs (*Teucrium polium, Marjoram vulgare, Varthemia iphionoids, Anabasis syriaca, Cloeme droserifolia* and *Calendula officinalis*) displayed good antibacterial activity against *Psedomonous aeruginosa*[10].

A comparison of the inhibitory characteristics of twelve plant extracts (*Calendula officinalis, Commiphora molmol, Hamamaelis virgiana, Allium sativum, Berberis vulgaris, Hydrastis canadensis, Aloe vera, Populus candicans, Thymus vulgaris, Rosmarinus officinalis, Eucalyptus smithii* and *Melaleuca alternifolia*) on *Candida albicans* were carried out. *Thymus vulgarlis* and *Populus candicans* essential oils, which are not usually considered to be particularly antifungal, were found to be highly inhibitory at normal therapeutic concentration and at much higher dilutions[11].

The antimicrobial effect of the phenolic compounds extracted from olive cake on three species of bacteria (*E. coli* O78:H12, *Klebsiella pneumoniae* and *Bacillus cereus*) and two species of fungi (*Aspergillus flavus* NRRL3251 and *A. parasiticus* NRRL 5862) were studied[12]. The results indicated that the extracted phenolic compounds had inhibitory effects against the above mentioned organisms at concentrations ranging from 0.1 to 0.6 mg/ml.

There is a lack of information about the antimicrobial action of parsley, thyme and sage, which are medical plants of the Palestinian flora and widely used in Palestinian folk-medicine in treating certain disease. This study is an attempt to determine the antimicrobial activity of aqueous (anionic components), ethanolic and methanolic extracts of parsley, thyme and sage in addition to their commercial oils and phenolic compounds, on selected pathogenic microbes isolated from patients.

MATERIALS AND METHODS

Extraction of plant materials:

Three medicinal plants of Palestinian flora were used in this study. Leaves of sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and parsley (*Petroselinum sativum*) were shade dried and crushed into powder using crushing machine. Phenolic compounds were extracted from one kg plant materials with 80% ethanol (1.5L) in the presence of 5 ml metasulphite (2%), agitated at 4 C for 20 minute and filtrated[13]. Water extracts were prepared by transfer of 20 grams of the powder to sterile wide-mouthed screw-capped bottles of 200 ml volume. 100 ml of sterile de-ionized distilled water was added to the powder samples which were allowed to soak for 24 hours at 4 C. The mixture were then centrifuged at 2000 rpm for 10 minute at 4 C. The supernatants were filtered through a 0.45 µm membrane and freeze dried[9]. However, ethanolic extract was done by maceration of the dried plant materials with 96% ethanol (the plant material to solvent ratio was 1:5)[14]. In addition, the methanolic extract was prepared by Soxhlet extraction of 50g of the powder with methanol for about 10 hours. The solvent was removed under reduced pressure below 50 C to give a crude extract. The crude extract was further dried in a vacuum dessicator over anhydrous copper sulphate to give a dry solid of the extract for bioassay[8]. On the other hand, commercial oil of parsley and sage were purchased from the local market manufactured by El Mahrosa Company for flavors and fragrance, Egypt. While, thyme oil was purchased from Al-Sanabel factory, Nablus, Palestine.

Microbiological tests of plant extracts:

Ten different pathogenic microorganisms of undesignated strain or serotype were isolated from ten infected patients in Khan Younis hospital (Gaza strip, Palestine). Where, *E. coli, Proteus mirabilis, Klebsiella pneumoniae, Enterobacter cloacae, Psedomonous aeruginosa, Acinetobacter haemolyticus, Enterococcus* sp. and *Candida albicans* were isolated from patients with UTI, while, *Salmonella typhi* and *Staph. aureus* from the stool of food poisoning patients. The nature of the work followed in the present study was fully explained to all participants and the study was conducted with their informed consent. The isolates were identified according to published guidelines[15].

Five concentrations of each extract (2.5, 5, 10, 20 and 40 mg/ml) were prepared. The antimicrobial effect of each concentration was measured. All media plates were 9 cm in diameter, prepared according to the manufacture recommendations (Sanofi Diagnostic Pasteur) and stored at 2-8°C for one week. The hole-plate diffusion technique was used[16,17]. Muller Hinton agar plates were swabbed with a suspension of each bacterial species, which contain 10^5 cfu/ml of the pathogenic bacteria, using sterile cotton swab. Five plugs were removed from each agar plate using a sterile cork borer to produce 8mm-diameter hole. To each hole, 100µl from different concentration of each extract was added and allowed to diffuse at room temperature for 20 min. The plates were incubated aerobically overnight at 37°C. Each extract was tested against each organism in triplicate. The antimicrobial activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeters.

The statistical analysis was conducted by using t- test on a statistical software package (SPSS).

RESULTS

Sage, thyme and parsley are widespread medicinal plants in Palestine and widely used in folkloric medicine in treating different disease symptoms. Aqueous, ethanolic, methanolic and phenolic compound extracts from the leaf of these plants in addition to their commercial oils were investigated for their antimicrobial activity against ten pathogenic microorganisms. The "hole plate" diffusion method was used in testing various concentrations of these extracts.

The results depicted in Table-I indicate that the high concentrations of sage aqueous extract (≥20 mg/ml) had inhibitory effects against most tested microorganisms. However, this extract showed inhibition action even at minimal concentration (2.5mg/ml) used against Gram positive bacteria (*Staph. aureus* and *Enterococcus* sp.). The inhibitory ability of sage aqueous extract was more pronounced against *Staph. aureus*, whereas it showed no activity against *Proteus mirabilis*. The sage aqueous extract (40 mg/ml) showed highly significant action ($p<0.01$) on *Enterococcus* sp. when compared to the action on *Candida albicans*.

It is obvious from the data in Table-II that the different concentrations of aqueous extract of thyme, in general, had inhibitory action on *E. coli, Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter haemolyticus* and *Staph. aureus,* but no effect on *Candida albicans, Proteus mirabilis* and *Salmonella typhi* was observed. Both *E. coli* and *Enterobacter cloacae* showed equal susceptibility to all the tested concentrations. However, *Staph. aureus* was the most susceptible organism to the different concentrations of the aqueous extracts of thyme.

Regarding parsley, the results of aqueous extract showed no inhibitory effect on the growth of most of the microorganisms tested (Table-III). However, only the concentration 40mg/ml exerted on inhibition zone of 10 mm on *P. aeruginosa.*

Phenolic extract of sage and thyme, generally at most tested concentrations showed antibacterial activity against *Staph. aureus* and *Enterococcus* sp. The phenolic extract of the sage and parsley did not show any antifungal action. However, the concentrations (≥10 mg/ml) revealed an antibacterial action on some of Gram positive and negative bacteria (Tables I, II and III).

The inhibition zone produced by the action of aqueous extract of thyme (40 mg/ml) on *Escherichi coli* and *Enterobacter cloacae* was highly significant ($p<0.01$) when compared with the action of the same concentration of sage vice versa to that noticed on *Enterococcus* sp. (Tables I and II).

Sage ethanolic extract exhibited antibacterial action on *P. aeruginosa* and *Staph. aureus* (Table- I). However, the high concentration (40 mg/ml) of thyme ethanolic extract showed significant activity ($p<0.01$) against *Klebsiella pneumoniae*, *Staph. aureus* and *Enterococcus* sp. whereas the minimum concentration (2.5 mg/ml) was effective only on *Staph. aureus* (Table-II). On the other hand, *E. coli* was more affected by the ethanolic extract of parsley at $\geq$10 mg/ml. which did not elicit inhibitory effect on any of the tested Gram positive organisms (Table-III).

In general, methanolic extract of sage, thyme and parsley showed inhibitory activity against *P. aeruginosa* and *Staph. aureus*, but *Salmonella typhi* was affected by the high concentration of sage and parsley methanolic extract only.

The results of commercial oils of sage, thyme and parsley displayed a significant antimicrobial activity ($p<0.01$) (inhibition zone 10-21 mm) against *P. aeruginosa* and *Staph. aureus*. On the other hand, no antimicrobial activity of these oils against *E. coli*, *Proteus mirabilis* and *Salmonella typhi* was detected.

Data in Tables I, II and III clearly illustrates that aqueous extracts of sage and thyme at the different concentrations, generally, had a broad action against most of the tested micro-organisms. All of the other extracts at one or more tested concentrations showed some activity against one or more micro-organisms. On the other hand detailed study of the data revealed that among the 10 tested micro-organisms, *Staph. aureus* was, in general, the most susceptible microbe to most extracts from the three plants studied.

Table-I: Inhibition zone (mm)of sage extracts at various concentration on some microorganisms

Test	*Conc*	*Diameter of inhibition zone (mm)*									
extracts	*mg/ml*	*EC*	*PM*	*KP*	*ENC*	*ST*	*PA*	*AH*	*SA*	*ES*	*CA*
Aqueous	40	13.01±0.46	-	17.73±0.93	11.23±0.95	15.07±0.23	12.13±0.69	13.06±0.92	22.10±1.24	18.96±0.66	15.03±0.96
	20	10.00±0.55	-	17.30±0.85	-	12.10±0.38	10.07±0.64	10.36±0.57	19.10±0.78	16.06±0.45	12.10±0.66
	10	-	-	14.07±0.81	-	10.03±0.61	-	-	16.11±0.66	14.90±0.32	-
	5	-	-	13.20±0.65	-	-	-	-	14.9±0.545	13.03±1.12	-
	2.5	-	-	9.96±0.32	-	-	-	-	12.13±0.69	10.30±0.72	-
Phenolic	40	-	-	-	-	-	15.07±0.57	-	17.41±0.68	10.96±0.51	-
compound	20	-	-	-	-	-	13.03±0.31	-	15.07±0.12	-	-
	10	-	-	-	-	-	10.30±0.35	-	12.04±0.37	-	-
	5	-	-	-	-	-	-	-	10.79±0.36	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Ethanolic	40	-	-	-	-	-	17.20±0.75	-	18.03±0.26	-	-
	20	-	-	-	-	-	11.06±0.63	-	14.90±0.41	-	-
	10	-	-	-	-	-	-	-	12.22±0.19	-	-
	5	-	-	-	-	-	-	-	10.25±0.63	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Methanolic	40	-	-	-	-	13.13±0.13	16.15±0.49	-	15.07±0.23	-	-
	20	-	-	-	-	10.40±0.55	13.36±0.57	-	12.13±0.47	-	-
	10	-	-	-	-	-	-	-	11.34±0.41	-	-
	5	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Comm. Oil	100µl	-	-	-	-	-	12.90±0.32	-	9.67±0.32	13.07±0.52	-

(-) = no activity.

EC, *Escherichi coli*; PM, *Proteus mirabilis;* KP, *Klebsiella pneumoniae;* ENC, *Enterobacter cloacae;* ST, *Salmonella typhi;* PA, *Pseudomonas aeruginosa;* AH, *Acinetobacter haemolyticus;* SA, *Staphylococcus aureus;* ES, *Enterococcus* sp. and CA, *Candida albicans.*

Values = mean of 3 readings. All values expressed as mean ± SE

Table-II: Inhibition zone (mm)of thyme extracts at various concentration on some microorganisms

Test extracts	*Conc mg/ml*	*Diameter of inhibition zone (mm)*									
		EC	*PM*	*KP*	*ENC*	*ST*	*PA*	*AH*	*SA*	*ES*	*CA*
Aqueous	40	19.07±0.63	-	16.10±0.37	19.03±0.54	-	10.96±0.54	15.96±0.61	21.06±0.81	13.10±0.75	-
	20	17.20±0.69	-	15.03±0.20	17.06±0.46	-	-	14.90±0.49	19.21±0.75	-	-
	10	13.97±0.08	-	12.13±0.13	14.20±0.20	-	-	11.82±0.58	17.15±0.63	-	-
	5	12.00±0.41	-	10.03±0.38	12.11±0.44	-	-	9.90±0.15	15.03±0.09	-	-
	2.5	11.02±0.28	-	-	10.93±0.55	-	-	-	12.31±.055	-	-
Phenolic	40	-	11.21±0.20	-	-	-	12.11±0.31	-	13.30±0.35	18.40±0.66	14.95±0.43
compound	20	-	-	-	-	-	-	-	-	17.30±0.46	13.03±0.03
	10	-	-	-	-	-	-	-	-	14.13±0.38	9.87±0.233
	5	-	-	-	-	-	-	-	-	13.22±0.93	-
	2.5	-	-	-	-	-	-	-	-	11.07±0.22	-
Ethanolic	40	-	-	10.96±0.49	-	-	-	-	19.06±0.46	9.98±0.02	-
	20	-	-	-	-	-	-	-	16.29±0.33	-	-
	10	-	-	-	-	-	-	-	15.19±0.54	-	-
	5	-	-	-	-	-	-	-	12.89±0.93	-	-
	2.5	-	-	-	-	-	-	-	10.78±0.39	-	-
Methanolic	40	-	9.97±0.06	-	-	-	14.10±0.55	-	21.13±1.04	10.10±0.32	-
	20	-	-	-	-	-	10.97±0.55	-	18.93±0.41	-	-
	10	-	-	-	-	-	-	-	16.27±0.41	-	-
	5	-	-	-	-	-	-	-	14.37±.089	-	-
	2.5	-	-	-	-	-	-	-	12.88±0.94	-	-
Comm. Oil	100µl	-	-	13.10±.49	12.13±0.70	-	21.30±0.35	13.77±0.49	11.00±0.57	-	14.10±0.38

(-) = no activity.

EC, *Escherichi coli*; PM, *Proteus mirabilis;* KP, *Klebsiella pneumoniae;* ENC, *Enterobacter cloacae.;* ST, *Salmonella typhi;* PA, *Pseudomonas aeruginosa;* AH, *Acinetobacter haemolyticus;* SA, *Staphylococcus aureus;* ES, *Enterococcus* sp. and CA, *Candida albicans.*

Values = mean of 3 readings. All values expressed as mean ± SE

Table-III: Inhibition zone (mm)of parsley extracts at various concentration on some microorganisms

Test extracts	*Conc mg/ml*	*Diameter of inhibition zone (mm)*									
		EC	*PM*	*KP*	*ENC*	*ST*	*PA*	*AH*	*SA*	*ES*	*CA*
Aqueous	40	-	-	-	-	-	10.19±0.66	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Phenolic	40	13.30±0.79	9.90±0.10	-	-	13.07±0.18	12.39±0.68	-	-	-	-
compound	20	9.91±0.04	-	-	-	11.07±0.12	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Ethanolic	40	15.03±0.55	-	-	-	10.07±0.23	13.25±0.41	-	-	-	-
	20	12.28±0.33	-	-	-	-	9.83±0.49	-	-	-	-
	10	11.09±0.18	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Methanolic	40	-	-	-	-	17.07±0.41	15.11±0.34	-	23.10±0.66	10.13±0.10	-
	20	-	-	-	-	14.20±0.25	10.13±0.13	-	20.26±0.65	-	-
	10	-	-	-	-	13.10±1.06	-	-	18.90±0.32	-	-
	5	-	-	-	-	10.00±0.06	-	-	16.97±0.03	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
Comm. Oil	100µl	-	-	-	-	-	13.71±0.57	-	12.19±0.28	14.39±0.58	-

(-) = no activity.

EC, *Escherichi coli*; PM, *Proteus mirabilis;* KP, *Klebsiella pneumoniae;* ENC, *Enterobacter cloacae.;* ST, *Salmonella typhi;* PA, *Pseudomonas aeruginosa;* AH, *Acinetobacter haemolyticus;* SA, *Staphylococcus aureus;* ES, *Enterococcus* sp. and CA, *Candida albicans.*

Values = mean of 3 readings. All values expressed as mean ± SE

DISCUSSION

The present study was designed to obtain preliminary information on the antimicrobial effect of three Palestinian medicinal plants on certain pathogenic microorganisms. The hole plate diffusion method was preferred to be used in this study since it was found to be better than the disc diffusion method[18].

The broad antimicrobial action of the aqueous extract of all the tested plants could be ascribed to the anionic components such as thiocynate, nitrate, chloride and sulphates beside other water soluble components which are naturally occurring in most plant materials[9]. The other extracts showed lower action as antimicrobial agents. This may be due to little diffusion properties of these extracts in the agar or because fresh plants contain active substances which may be affected or disappeared by the steps of extraction methods.

In this study, the most promising commercial oil plant is thyme. Findings of this study are similar to those reported by Mcfadden, 1995. However, *Enterococcus* sp. was not affected by thyme commercial oil, while, it was inhibited by sage and parsley oils.

The findings that *Staph. aureus* is susceptible to a lot of extracts obtained from the three studied plants are also similar to the susceptibility of that microbe to different plant extracts reported by several researchers[2,8,19,20]. However, Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope[21,22].

E. coli showed no responce to each of methanolic, ethanolic and phenolic compound extracts in addition to the commercial oil of thyme and sage. It was also not affected by aqueous and methanolic extracts of parsley. The observed resistance of *E. coli* probably could be due to cell membrane permeability or due to other genetic factors.

The methanolic extract showed slightly better killing action than the ethanolic extract, which means that the methanolic extract could be used more. However, but it needs further investigations to distinguish its components and their individual antimicrobial effect. Our findings have validated the use of thyme, sage and parsley for the treatment of some microbial infections like UTIs and bacterial food poisoning.

In general, the mechanisms by which microorganisms survive the action of antimicrobial agents are poorly understood and remain debatable[8]. On the other hand, the chemical constituents of these extracts may have a causal role in protecting plants from microbial attack *in vivo*. Nevertheless, at least in part, if not all, they should be valuable in the multi-chemical defense against microbial attack.[23]

It seems important to recommend that, further studies using isolated constituents instead of whole extract must be done in this field. Health foundations have to increase their funding of these studies and research to help saving the lives of many peoples. This will also offer a great help in facing the emergence spread of antimicrobial resistance.

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