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Antimicrobial Activity of Some Palestinian Medical Plant Extracts: Effect of Crude Extracts and Some of their Subfractions

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Abstract: This study aimed to determine the antimicrobial activity of crude extracts of three Palestinian folkloric medicinal plants and some of their subfractions in addition to their commercial oils on certain pathogenic microbes. Leaves of sage, thyme and parsley were collected, dried and extracted with different solvents to yield eight extracts. Three concentrations of each plant extracts were prepared. Disk diffusion methods were used to evaluate the antimicrobial activities of the extracts against ten different pathogenic microorganisms. The aqueous extracts of sage were, generally, of broad action against most of the tested microorganisms. All other extracts, showed some activities against one or more microorganisms. Sage, thyme and parsley extracts showed no antimicrobial effect on *E. coli*. In general, thyme extracts showed low effect on the tested Gram negative bacteria. However, parsley extract showed low effect on both Gram positive and negative bacteria. Among the ten tested microorganisms, *Enterococcus* sp. was the most susceptible microbe to extracts from sage and thyme plants. It is recommended that sage and thyme extracts may be used for food preservation, as well as, pharmaceutical and natural plant based products.

Key words: Antimicrobial, sage, thyme, parsley extracts

INTRODUCTION

Palestinian climate favors a great array of plant species many of which have varied medicinal and antimicrobial potentials. A huge number of plants that have medicinal and antimicrobial activities in Palestine and some of their active ingredients have been identified and used in folkloric medicine.

Due to problems like adverse effects, limited lifespan, misuse of traditional antibiotics, the increasing prevalence of multidrug resistant strains of bacteria and appearance of strains with reduced susceptibility to antibiotics raise specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[1].

Plants produce many substances to protect themselves from microbial infection and deterioration, including, peptides, unsaturated long chain aldehydes, alkaloids, some essential oils, phenols. These compounds have potential significant therapeutical application against human pathogens, including bacteria, fungi or virus[2-6]. The compounds may be found in a particular part of the plant or all over its body and they are often localized in glands[7].

Presently there is an increasing interest in the use of plant microbicides because of the necessity of finding safer agents and the need for preventing environmental degradation[8]. Use of herbal products all over the world has been reported and in USA reached 380% between 1990 and 1997[9], also, the antimicrobial activity of several extracts of different plants was studied. Arora and Kaur[10], found that water extract of garlic and clove possess antimicrobial activity. Darout *et al.*[11] indicated that water extract of Miswak (*Salvadora persica*) roots and stem contains potential antimicrobial anionic components such as chloride, sulfate, thiocyanate and nitrate. The decoction of six of studied Jordanian medicinal herbs (*Teucrium polium*, *Marjoram vulgare*, *Varthemia iphionoids*, *Anabasis syriaca*, *Cloeme droserifolia* and *Calendula officinalis*) displayed significant antibacterial activity against *Psedomonas aeruginosa*[12].

The antibacterial activity of several extracts from the leaves of *Ocimum gratissimum* was tested by Adebolu and Oladimeji[13] against *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi* and *Salmonella typhimurium*. They found that only steam distillation extract has inhibitory effects on the selected bacteria.

Velickvic *et al.*[14] investigated the chemical composition and the antimicrobial action of ethanol 96% extract of *Salivia pratensis L., S. glutinosa L.* and *S. aethiopis* L. leaves. They stated that the antimicrobial action was only noticed for some extracts on the mold

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Aspergillus niger. While, the extracts of *S. aethiopis* had the best action on *Staph. aureus*. However, they had the weakest action on *Candida albicans*. On the other hand, leaves and flowers essential oils extracted from *Leonotis leonurus* and *L. ocymifolia* exhibited a broad spectrum antibacterial activity against Gram-positive (*Bacillus subtilis, B. cereus, Micrococcus kiristinae, Staph. aureus, Staph. epidermidis*) and Gram-negative (*E. coli, Pseudomonas aeruginosa, Shigella sonnei*) bacteria[15].

On the other hand, Zhu *et al.*[16] extracted phenolic compounds from artichoke leaves. They found that, then-butanol extract exhibited the most significant antimicrobial activities[16].

There is a little information about the antimicrobial action of crude extracts and their subfractions of sage, thyme and parsley, which are medical plants of the Palestinian flora and of traditional use in folk medicine in treating some diseases. This study is an attempt to determine the antimicrobial activity of aqueous (crude, cationic, anionic and neutral fractions), ethanolic 75% (crude, aqueous and butanolic soluble phenolic compounds) and ethanolic 96% extracts of these plants in addition to their commercial oils on certain pathogenic microbes.

MATERIALS AND METHODS

Extraction of plant materials: Leaves of three medicinal plants of Palestinian flora were brought from the local market on the season of 2004 and used in this study. Sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and parsley (*Petroselinum sativum*) leaves were dried in 40°C air- drying oven and crushed into powder using crushing machine and kept in sealed bags at room temperature for further extractions.

Phenolic compounds were extracted as described by Zhu *et al.*[16]. Dried leaf powder (100 g) was extracted with 75% ethanol (500 mL x 3). The solvent was evaporated at 50°C. The concentrated extract was partitioned successively with chloroform, ethyl acetate and n-butanol. However, water and butanolic soluble phenolic compound and crude extract of 75% ethanol fractions were tested for their antimicrobial activity. Fractions were evaporated to dryness using water bath at 50°C to determine the solid amounts in the extracted materials. Three concentrations of each extract (12.5, 25 and 50 mg mL^{-1}) were prepared.

Aqueous extracts were prepared as mentioned by Darout *et al.*[11]. One hundred grams of the dried powder were soaked in one-liter distilled water for 24 h at 4°C. The extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated by evaporation using water bath at 50°C[7]. The aqueous extracts were fractionated according to El-abbasi *et al.*[17] to cationic fraction (amino acids and other positively charged materials), anionic fraction (carboxylic acids and other negatively charged materials) and neutral fraction (sugars and other uncharged materials) using Dowex cation and anion exchange resin. Concentrations of 125, 250 and 500 mg mL^{-1} were prepared after evaporation of water at 50°C.

Ethanolic (96%) extract was done according to Velickvic *et al.*[14]. The dried material was macerated with 96% ethanol (the plant material to solvent ratio was 1:5). Extract concentrations of 12.5, 25 and 50 mg mL^{-1} were prepared after evaporation of ethanol using water bath at 50°C.

Commercial oils of parsley, thyme and sage were purchased from the local market manufacture by El Mahrosa Company for flavors and fragrance, Egypt.

Determination of total phenolic compounds: Phenolic compounds in the aqueous and 75% ethanolic (crude extracts, water and butanolic soluble materials fractions) were determined using Folin-Ciocalteu method[18]. The total phenolic compounds of the samples are expressed in mg per serving of Gallic acid equivalents. All extracts were prepared in three replication.

Microbiological tests of plant extracts: Ten different pathogenic microorganisms were isolated from ten infected patients in Khan Younis hospital (Gaza strip, Palestine) in October 2004. Where, *E. coil, Proteus mirabilis, Klebsiella pneumonia, Enterobacter cloacae, Pseudomonas 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 poisoned patients. The nature of the research performed 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[19] and kept at -20°C till use. The antimicrobial effect of each concentration was measured using disk diffusion method. 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. A loopful of inoculum was taken from the pure culture of the pathogenic organisms and inoculated in 10 mL thioglycolate broth (Oxoid). The broth suspension was then incubated at 37°C for 24 h. The growth so obtained was used as inoculum for the sensitivity assay. Mueller Hinton agar (Difco) was autoclaved and after cooling at 45°C, the media was dispensed into Petri dishes (each contains about 20 mL) and inoculated with 0.1 mL of the microbe's suspension (The inoculum concentration was

10^5 cfu mL^{-1}). Disks of Whatman No.1 filter paper (7 mm - diameter) containing 10 μL of the different concentrations of each extracts and the control standard antibiotic disks obtained from Oxoid (Cefalaxin, 30 mcg, Doxycyclin 30 mcg and Ciprofloxacin 5 mcg) were placed into the prepared Petri dishes. The plates were incubated at 37°C for 24 h. The inhibition zone of the growth were measured and reported. Each extract was tested against each organism in three replicates. The antimicrobial activity of plant extracts was recorded as the mean diameter of the inhibition zones measured in millimeters.

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

RESULTS

The antimicrobial effect to ten pathogenic microorganisms was tested against various concentrations extracted from the leaves of sage, thyme and parsley, in addition to their commercial oils and three control bacterial antibiotics (Table 1-4).

Results in Table 1 indicate that, the different concentrations of sage aqueous extract and its subfractions (especially at concentration ≥ 250 mg mL^{-1}) had inhibitory effects against most tested microorganisms. The sage crude aqueous extract and its cationic subfraction showed inhibitory action, in general, even at minimal concentration (125 mg mL^{-1}) against *Acinetobacter haemolyticus*, *Enterococcus* sp. and *Candida albicans*. The inhibitory action of sage crude aqueous extract was more pronounced against *Enterococcus* sp., whereas, it showed no activity against *E. coli* and *Enterobacter cloacae*. The sage aqueous extract (500 mg mL^{-1}) showed high significant action ($p<0.05$) on *Enterococcus* sp. when compared to the action on *Pseudomonas aeruginosa*. On the other hand, the cationic and anionic fractions with high concentration of sage extracts showed inhibitory action on most of the tested organisms, whereas, the neutral fraction exhibited less effect.

The data in Table 2 clearly revealed that, the high concentrations of the crude aqueous extract of thyme, as well as its subfractions, had inhibitory action on *Enterococcus* sp., *Pseudomonas aeruginosa*, *Staph. aureus* and *Candida albicans,* but had no effect on the other tested microorganisms.

Concerning parsley, the results of aqueous extracts, generally, showed no inhibitory effect on the growth of most of the tested microorganisms (Table 3). However, the crude aqueous extract and the cationic subfraction produced inhibitory action on *Pseudomonas aeruginosa* and *Proteus mirabilis,* respectively.

The inhibition zones in Table 1 produced by the action of phenolic compounds that soluble in either water or n-butanol subfractions extracted from sage were more effective on most tested organisms when compared with the action of the crude phenloic extract (ethanol 75% fraction). Phenolic compounds extracted of thyme and parsley was generally not effective on the tested microorganisms (Table 2 and 3). The antimicrobial action of phenolic extract subfractions of sage was, approximately, resembled to the inhibition zone resulted from Doxycyclin (Table 1 and 4).

The action of sage ethanol 96% extract was significantly pronounced compared to that of parsley extract. However the ethanol 96% extract of thyme and parsley, in general, did not produce any action on *Candida albicans*, vice versa to that noticed for sage extract (Table 1-3). The effect of sage ethanol 96% extract on *Staph. aureus* and *Enterococcus* sp. was approximately, similar to the inhibition zone of Doxycyclin (Table 1 and 4).

Table 5 indicated that phenolic compounds content in thyme and sage extracts (either aqueous, n-butanol or ethanol 75% fractions) was significantly higher than that in parsley extracts. It was noticed that remaining water after the successively partitioning with chloroform, ethyl acetate and n-butanol still containing high amount of phenolic compounds, possessing antimicrobial action on some organisms.

The results of commercial oils of sage, thyme and parsley displayed a significant antimicrobial activity against *Salmonella typhi* (Table 1-3). On the other hand, no antimicrobial activity of these oils against, *Klebsiella pneumonia* and *Enterococcus* sp. was detected (Table 1).

DISCUSSION

Sage, thyme and parsley are widespread medicinal plants in Palestine and widely used in folkloric medicine in treating different disease symptoms. The present study was designed to obtain preliminary information on the antimicrobial activity of three Palestinian medicinal plants on certain pathogenic microorganisms.

The results showed that, sage aqueous extracts has a distinguishable antimicrobial activity against most of the tested organisms. The broad antimicrobial action of the aqueous extract 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[11]. Whereas, the parsley extracts showed lower antimicrobial

Table 1: Inhibition zone (mm) of sage extracts at various concentrations on some microorganisms

		Diameter of inhibition zone (mm)									
Fractions	Conc.mg mL^{-1}	EC	PM	ENC	KP	ST	PA	AH	SA	ES	CA
Aqueous extract											
Crude extract	500	-	15	-	14	15	12	15	14	16	14
	250	-	12	-	11	11	9	12	12	13	12
	125	-	9	-	8	-	-	9	10	10	9
Cationic fraction	500	-	12	-	12	13	-	14	13	13	13
	250	-	-	-	8	10	-	12	11	11	10
	125	-	-	-	-	-	-	10	9	8	8
Anionic fraction	500	-	12	-	13	9	11	11	10	12	13
	250	-	8	-	11	-	9	9	-	9	10
	125	-	-	-	9	-	-	-	-	-	-
Neutral fraction	500	-	-	-	12	-	-	12	9	9	13
	250	-	-	-	10	-	-	8	8	8	10
	125	-	-	-	-	-	-	-	-	-	8
Ethanol 75% extract (Phenolic compounds)											
Crude extract	50	-	14	-	-	-	-	-	17	18	15
	25	-	10	-	-	-	-	-	12	13	12
	12.5	-	-	-	-	-	-	-	9	11	10
Butanol soluble	50	-	12	8	10	8	8	13	18	20	12
	25	-	10	-	-	-	-	10	14	16	9
	12.5	-	8	-	-	-	-	8	10	10	-
Water soluble	50	-	9	8	15	-	14	12	15	25	9
	25	-	-	-	10	-	9	9	12	19	-
	12.5	-	-	-	-	-	-	8	10	15	-
Ethanol 96% ext.											
Crude extract	50	-	14	-	-	-	-	8	18	24	24
	25	-	10	-	-	-	-	-	16	18	14
	12.5	-	-	-	-	-	-	-	12	14	9
Commercial oil	10.0µL	-	-	10	-	17	-	10	-	-	15

*All values expressed as mean of three replicates. * (-) = no activity * EC: *Escherichai coli*, PM: *Proteus mirabilis*, KP: *Klebsiella pneumonia*, ENC: *Enterobacter cloacae*, ST: *Salmonella typhi*, PA: *Pseudomonas aeruginosa*, AH: *Acinetobacter haemolyticus*, SA: *Staphylococcus aureus*, ES, *Enterococcus* sp. CA: *Candida albicans*

Table 2: Inhibition zone (mm) of thyme extracts at various concentrations on some microorganisms

		Diameter of inhibition zone (mm)									
Fractions	Conc.mg mL^{-1}	EC	PM	ENC	KP	ST	PA	AH	SA	ES	CA
Aqueous extract											
Crude extract	500	-	-	-	-	-	10	-	9	17	15
	250	-	-	-	-	-	-	-	-	13	11
	125	-	-	-	-	-	-	-	-	9	9
Cationic fraction	500	-	-	-	-	-	-	-	-	11	11
	250	-	-	-	-	-	-	-	-	9	9
	125	-	-	-	-	-	-	-	-	-	-
Anionic fraction	500	-	-	-	-	-	-	-	9	9	9
	250	-	-	-	-	-	-	-	8	8	-
	125	-	-	-	-	-	-	-	-	-	-
Neutral fraction	500	-	-	-	-	-	12	-	-	15	13
	250	-	-	-	-	-	-	-	-	12	11
	125	-	-	-	-	-	-	-	-	9	-
Ethanol 75% extract (Phenolic compounds)											
Crude extract	50	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Butanol soluble	50	-	-	-	-	-	-	-	11	11	9
	25	-	-	-	-	-	-	-	8	9	-
	12.5	-	-	-	-	-	-	-	-	-	-
Water soluble	50	-	-	-	-	10	-	-	10	10	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Ethanol 96% ext.											
Crude extract	50	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Commercial oil	10.0µL	12	10	10	-	9	-	10	-	-	-

All values expressed as mean of three replicates. (-) = no activity

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

Table 3: Inhibition zone (mm) of parsley extracts at various concentrations on some microorganisms

		Diameter of inhibition zone (mm)									
Fractions	Conc.mg mL^{-1}	EC	PM	ENC	KP	ST	PA	AH	SA	ES	CA
Aqueous extract											
Crude extract	500	-	-	-	-	-	14	-	-	-	-
	250	-	-	-	-	-	12	-	-	-	-
	125	-	-	-	-	-	9	-	-	-	-
Cationic fraction	500	-	11	-	-	-	-	-	-	-	-
	250	-	9	-	-	-	-	-	-	-	-
	125	-	-	-	-	-	-	-	-	-	-
Anionic fraction	500	-	-	-	-	-	-	-	-	-	-
	250	-	-	-	-	-	-	-	-	-	-
	125	-	-	-	-	-	-	-	-	-	-
Neutral fraction	500	-	-	-	-	-	-	-	-	-	-
	250	-	-	-	-	-	-	-	-	-	-
	125	-	-	-	-	-	-	-	-	-	-
Ethanol 75% extract (Phenolic compounds)											
Crude extract	50	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Butanol soluble	50	-	-	8	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Water soluble	50	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-
	12.5	-	-	-	-	-	-	-	-	-	-
Ethanol 96% ext.											
Crude extract	50	-	-	-	-	-	-	-	14	13	-
	25	-	-	-	-	-	-	-	13	10	-
	12.5	-	-	-	-	-	-	-	11	8	-
Commercial oil	10.0µL	9	15	-	-	12	9	9	10	-	-

*All values expressed as mean of three replicates. * (-) = no activity

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

Table 4: Inhibition zone (mm) of some antibiotics on some microorganisms

	Diameter of inhibition zone (mm)									
Antibiotics	EC	PM	ENC	KP	ST	PA	AH	SA	ES	CA
Cefalaxin	17	35	17	34	22	-	-	32	-	-
Doxycyclin	13	22	17	17	12	27	10	22	20	-
Ciprofloxacin	44	20	35	17	34	30	-	40	17	-

*All values expressed as mean of three replicates. * (-)= no activity

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

Table 5: Concentrations of phenolic compounds in different extracts expressed as gallic acid

		mg/100 g dry mater		
Extracts		Thyme	Sage	Parsley
Aqueous extract	Crude extract	506.73±5.04	512.69±0.88	357.69±9.53
Ethanol 75% extract	Crude extract	1111.93±32.24	1340.23±18.10	449.62±16.15
	Butanol soluble	176.30±8.32	195.75±9.04	85.60±5.92
	Water soluble	265.29±4.00	259.03±5.90	218.84±10.51

action, which may be due to that plant extracts contain little active substances.

Phenolic compounds content in sage and thyme extracts (either aqueous, n-butanol or ethanol 75% fractions) were significantly higher than that in parsley extracts. That leads to higher antimicrobial effect of sage ethanol 75% extract and its subfractions compared to parsley.

The findings that *Staph. aureus* is susceptible to some extracts obtained from the three studied plants agreed with the susceptibility of that microbe to different plant extracts reported by several researchers[10,20-22]. It was also noticed that ethanol 96% extract of sage and parsley has antibacterial action against *Staph. aureus* and *Enterococcus* sp. generally at most of the tested concentrations, Gram positive bacteria were found to be more susceptible than Gram negative bacteria. These results are consistent with previous reports regarding Gram positive bacteria[23]. This could be explained according to 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

which may leads to easier penetration through Gram positive bacteria cell wall[24,25].

The observed resistance of *E. coli* to the prepared extracts of the three studied plants could be due to cell membrane permeability barrier, membrane accumulation mechanisms[26] or due to other genetic factors.

In general, the obtained data by the disc diffusion method revealed that, among the ten tested microorganisms, *Enterococcus* sp. was the most susceptible microbe to most extracts from the studied plants. That's may be due to cell membrane permeability, or to the poorly and debatable understood mechanisms by which microorganisms survive the action of antimicrobial agents[21].

The resistance of many microorganisms to the essential oils extracted by ethanol 96% may be due to the volatility of oils leading to the escape or evaporation of active components during the evaporation processes.

The most promising commercial oil plant is parsley. It has an antimicrobial effect on six of the tested microorganisms either Gram positive or Gram negative microbes. Whereas, sage and thyme commercial oils showed activities against only four and five microorganisms, respectively. However, *Enterococcus* sp. was not affected by the three commercial oil.

Present findings have validated that sage, thyme and parsley extracts could be used for the treatment of some microbial infections and diseases caused by these organisms, like UTI and bacterial food poisoning.

It seems Important to recommend that, further phytochemical studies using isolated individual chemical constituents instead of whole extract or subfractions are required in this field to determine the types of compounds responsible for the antimicrobial effects of these medicinal plants. Sage and thyme extracts may be an ideal candidate for further research into their uses for food preservation. Health foundations have to increase their care of these studies and researches to help in saving the lives of many people. This will, also, offer a great help in facing the increasing emergence of antimicrobial resistance.

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