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Original Article

Antimicrobial Activity of *Plectranthus Tenuiflorus* Extracts

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Abstract:

The antimicrobial activity of different extracts of *Plectranthus tenuiflorus*, on Gram +ve (*Staphylococcus aureus*) and Gram -ve (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, *Candida albicans, Aspergillus fumigatus*, and *A. niger* revealed that essential oil extract of the plant (85% thymol) showed higher antimicrobial activity on all the tested organisms than pure thymol. Aqueous and organic solvents extracts showed antimicrobial activity on the tested organisms, but not comparable to the high activity obtained by essential oil extract. Aqueous extracts using cold and boiling water were more efficient in inhibiting the growth of *P. aeruginosa* and the two moulds, on the other hand, organic solvents, polar and non polar (ethyl alcohol and diethyl ether), extracts showed stronger antimicrobial activity on *E.coli, S. aureus* and *C. albicans* than aqueous extracts.

Key words: *Plectranthus tenuiflorus, Antimicrobial activity, Aqueous extraction, Organic solvents extraction.*

Introduction

Plectranthus tenuiflorus (Labiatae) plant is a small downy, very leafy herb, with stems about 60 cm long and small lilac-blue flowers. It is widely distributed in Saudi Arabia [1]. The plant is commonly used in folk medicine to treat different diseases, e.g. respiratory system infections, inflammation of ear and throat and abdominal disorders[2]. Several substances were extracted and identified from *Plectranthus* species. These materials were mainly thymol and different types of diterpenoids [3-6].

The antimicrobial activity of *Plectranthus* species extracts was studied against bacteria, fungi and viruses[3-5,7-10]. On the other hand, some workers revealed that oily extract of *P. deloliatus* leaves had no antimicrobial activity against bacterial flora of milk[11].

Therefore, the present work aimed to study the antimicrobial activity of local *P. tenuiflorus* extracts on some pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus fumigatus* and *A. niger*)[12]. Also, to elucidate the most proper method for extraction of plant materials of high antimicrobial activity, using broth medium technique.

Material and Methods

Plant material:

P. tenuiflorus (Labiatae) was collected from the garden of King Abdulaziz University. Its identification was clarified by the staff member of taxonomy at the Faculty of Science, Biological Sciences Department, King-Abdulaziz University, according to Collenette[1], after comparison with the herbarium samples at the department.

Extraction of plant materials:

About 5kg of fresh leaves were collected at the same time from one place, chopped into pieces and homogenously mixed and used for extraction. Different methods of extraction [13] were used, as follows:

1-Extraction of essential oil:

Fresh leaves (3kg) of *P. tenuiflorus* were cut into pieces and subjected to steam distillation. The distillate was then extracted with petroleum ether (40-60°C). The resulting extract was dried on anhydrous sodium sulphate. Petroleum ether was removed using a rotary evaporator and the essential oil was obtained [14]. Essential oil was dissolved in petroleum ether to prepare the different concentrations to test their antimicrobial activity.

2-Aqueous extraction:

a)Cold water

Fifty grams of chopped leaves were blended in a blender with 100 ml distilled water for 15min and centrifuged at 4000 rpm for 10 min. The filtrate was completed to 100 ml using distilled water and sterilized using bacterial filters.

b) Boiling water:

Fresh chopped leaves (50g) pieces were boiled in a distillatory flask for 15 min in 100 ml distilled water.

After cooling, the filtrate was separated by centrifugation at 4000 rpm for 10 min, completed to 100 ml, and sterilized using bacterial filters.

3-Extraction using volatile organic solvents:

Extraction using polar (ethyl alcohol) and non-polar (diethyl-ether) volatile organic solvents was carried out.

a) Alcohol extraction:

Fifty grams of *P. tenuiflorus* leaves pieces were extracted by boiling 96% ethyl alcohol (100ml) in distillatory flask for 15 min. The alcoholic extract was separated by filtration and completed to 100 ml volume with 96% ethyl alcohol and sterilized through bacterial filter.

b) Diethyl ether extraction:

Instead of 96% ethyl alcohol, diethyl – ether was used for extraction by the same method. The extracts were kept in a refrigerator at 10°C until used.

Microbial cultures growth conditions:

Test microorganisms included Gram +ve cocci: *Staphylococcus aureus* (ATCC 118592), and Gram -ve bacilli: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 10145). The yeast *Candida albicans* (ATCC 90028) and both *Aspergillus fumigatus* and *Aspergillus niger* were also tested.

Cultures of bacteria were grown in nutrient broth (Difco) at 37°C and maintained on slopes of nutrient agar (Difco) at 4°C. While, the yeast and mould fungi were grown in Czapek's broth (Difco) at 28°C and maintained on slopes of Czapek's agar (Difco) at 4°C.

Antimicrobial activity assay:

The different extracts of *P. tenuiflorus* were tested for their antimicrobial activity using test tubes method, each containing 8 ml of the sterilized broth medium (nutrient for bacteria and Czapek for fungi). Each test tube was inoculated with one ml containing 10^6 bacterial cells or 10^4 yeast cells/ml or 10^3 mould spores / ml. Each tube accepted one ml of the tested extract containing (0, 5, 10, 20 mg) prior to microorganism inoculation. The tubes were vortexed and incubated at 37°C for 24h for bacteria and at 28°C for 72h for fungi.

The inocula were freshly prepared; 24h old cultures of bacteria in nutrient broth, 72h old yeast cultures in Czapek's broth, and 96h old fungal cultures.

After incubation, one ml from each tube, after vortexing, was mixed with cool (45°C) sterilized specific medium (nutrient agar or Czapek's agar) in Petri-dish, incubated for 48h for bacteria at 37°C and at 28°C for fungi for 4 days. Thereafter, CFU of the tested organisms were estimated [15]. Three replicats from each concentration were made and the recorded data were the arithmetic mean.

Identification of major constituents of essential oil:

The steam distilled from *P.tenuiflorus* leaves was examined using a Carlo Erba 800 gas chromatograph fitted with a Carlo Erba MD800 mass spectrometer (GC.MS)[16,17]. .

Statistical Analysis:

With respect to observed antimicrobial activities, the values of difference between two means that would just achieve significance (least significance differences) were calculated [18].

Results

Chemical composition of the essential oil:

The essential oil, steam distilled from leaves of *P.tenuiflorus*, was analyzed by GC. MS. The main component of the oil was identified as thymol (85%), beside minor components of diterpenoids.

Antimicrobial activity of essential oil:

The antimicrobial activity of the steam distillate (essential oil) from the leaves of *P. tenuiflorus* (consists of 85% thymol) as compared with pure thymol (SIGMA) on the test organisms (Table I) revealed that the essential oil was more efficient in inhibiting the growth of the different tested organisms at concentrations of 5, 10mg/ 100ml. On the other hand, 20mg / 100ml was lethal (no growth) to the tested organisms. However, 10mg/100ml of both thymol and essential oil were also lethal to the tested mould fungi; *A. fumigatus* and *A. niger* as well as to *S. aureus* bacterium, while only essential oil was lethal to *Candida albicans* in concentration of 10mg/100ml.

Table (1). Antimicrobial activity of essential oil extract of *Plectranthus tenuiflorus* on colony forming unit (CFU) of the test organisms, as compared with pure thymol

Test material	Conc. (mg/100ml)	CFU of tested organisms					
		E.coli X 10^4	*P.aeruginosa* X 10^4	*S. aureus* X 10^4	*C.albicans* X 10^3	*A.fumigatus* X 10^2	*A.niger* X 10^2
None (cntrol)*	0.0	45.3 ± 2.35	43.7 ± 4.60	39.5 ± 3.96	31.3 ± 3.52	49.1 ± 3.65	59.2 ± 3.17
Thymol	5	34.1 ± 2.19	39.5 ± 2.48	9.1 ± 1.71	17.5 ± 1.94	18.4 ± 2.49	25.6 ± 2.13
	10	18.7 ± 4.14	29.8 ± 4.39	0.0	7.5 ± 1.28	0.0	0.0
	20	0.0	0.0	0.0	0.0	0.0	0.0
Essential oil extract	5	29.5 ± 2.36	40.6 ± 3.75	4.5 ± 0.34	13.7 ± 1.17	15.2 ± 1.90	20.4 ± 1.67
	10	11.9 ± 1.62	25.5 ±1.32	0.0	0.0	0.0	0.0
	20	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D at 5%		5.71	2.37	3.92	2.15	6.10	4.08

*One ml of petroleum-ether.

Antimicrobial activity of aqueous extracts:

The antimicrobial activity of aqueous extracts (cold and boiling water) of *P. tenuiflorus* leaves (Table II). Results were recorded at the different tested extracts concentrations. Thus, boiling water extract resulted in inhibition percentages of 82.6, 33.4, 67.1 and 79.6 for *P.aeruginosa, C.albicans, A.fumigatus* and *A.niger*, respectively, as compared to 36.9, 6.8, 11.3 and 39% inhibition for the same organisms using cold water extract.

Antimicrobial activity of organic solvents extracts:

Two different volatile organic solvents, one polar (ethyl alcohol) and the other non – polar (diethyl-ether) were used to extract materials that may have antimicrobial activity against the tested organisms. The results (Table III) indicated that the polar solvent (ethyl alcohol) extracts substances with higher concentrations and / or not extracted by diethyl- ether that have high antimicrobial activity against *E.coli, S.aureus*, and *C.albicans* , while non- polar (diethyl-ether) extracts were more inhibitory against the growth of *P.aeruginosa* and both mould fungi than polar extracts. Thus, alcohol extracts resulted in inhibition percentages of 48.2, 57.5, and 51 for *E.coli, S.aures* and *C.albicans*, respectively. While, ether extracts gave inhibition percentages of 33.3, 51 and 26.5 for the same organisms, respectively. Both extracts gave comparable results for the inhibition of *A.fumigatus.* The results (Tables II,III) generally indicated that organic solvents extraction gave substances (quantity and / or quality) more inhibitory for the growth of *E.coli, S. aureus* and *C. albicans* than aqueous extracts (mean of inhibition percentages were 40.75, 54.25 and 38.75, respectively, as compared to 23.65, 38.55, and 20.1 for water extract), while aqueous treatments result in materials (quantity and / or quality) more efficient against the growth of *P. aeruginosa* and mould fungi than organic solvents extracts (mean of inhibition percentages were 59.75, 39.2, and 59.3,respectively, for aqueous treatment as compared to 25, 35.7, and 38.2 for organic solvents extraction for *P.aeruginosa, A.fumigatus* and *A.niger*, respectively) .

Table (II): Antimicrobial activity of aqueous extracts (cold and boiling water) of *Plectranthus tenuiflorus* on colony forming unit (CFU) of the tested organisms

Extract	Conc. (mg/100ml)	CFU of test organisms					
		E.coli X 10^4	*P.aeruginosa* X 10^4	*S. aureus* X 10^4	*C.albicans* X10^3	*A.fumigatus* X 10^2	*A.niger* X 10^2
Cold water	0.0	51.3± 4.13	49.3± 4.05	45.4± 3.46	51.5± 2.63	57.7± 3.28	59.3± 4.8
	5	47.9± 3.99	47.5±3.79	39.0±2.37	51.2±2.39	56.4±4.26	46.5±4.19
	10	28.5± 2.38	38.2± 3.56	31.2±2.03	49.0±2.58	52.9±3.31	39.1±2.56
	20	25.4±2.09	31.1± 2.31	24.6±1.28	48.0± 2.38	51.2± 3.02	36.2± 2.62
	L.S.D. at 5%	4.32	5.33	3.09	4.50	6.54	5.76
	% of inhibition*	50.5	36.9	45.8	6.8	11.3	39
Boiling water	0.0	51.3± 3.17	49.3± 2.92	45.4± 3.18	51.5± 3.38	57.7±3.89	59.3± 3.45
	5	51.0± 2.87	41.7± 2.67	44.7± 2.98	49.8± 3.51	42.9± 3.23	33.5± 2.01
	10	50.5± 3.04	23.4± 2.28	40.2± 2.71	41.7± 2.39	30.9± 2.44	31.2± 2.14
	20	49.8 ± 3.02	8.6±0.57	31.2± 1.75	34.3±2.59	19.0± 2.11	12.1±0.49
	L.S.D. at 5%	12.02	19.35	18.80	11.17	2.28	21.51
	% of inhibition*	2.9	82.6	31.3	33.4	67.1	79.6
	Mean of inhibition**	23.65	59.75	38.55	20.1	39.2	59.3

* % of inhibition= (CFU at 0.0 conc. - CFU at 20 mg/100ml) / CFU at 0.0 conc.

** Mean of inhibition = (Sum of inhibition % of cold and boiling water) / 2

Table (III): Antimicrobial activity of volatile organic solvents (ethyl alcohol and diethyl ether) extracts of *Plectranthus tenuiflorus* on colony forming unit (CFU) of the tested organisms

Extract	Conc. (mg/100ml)	CFU of test organisms					
		E.coli X 10^4	*P.aeruginosa* X 10^4	*S. aureus* X 10^4	*C.albicans* X 10^3	*A.fumigatus* X 10^2	*A.niger* X 10^2
Ethyl alcohol	0.0	49.6± 4.07	46.2± 3.93	41.6± 2.88	23.5± 1.51	45.9± 2.79	54.6± 3.83
	5	42.4± 2.95	44.7± 2.42	35.4± 2.28	22.6± 1.92	44.2± 2.68	49.3± 3.43
	10	34.1± 1.63	39.2± 2.03	21.3± 1.35	19.4± 1.16	34.8± 1.83	44.2± 3.18
	20	25.7±1.28	37.4± 1.47	17.7± 1.13	11.5± 0.84	30.2± 1.27	38.1± 1.59
	L.S.D. at 5%	15.26	11.71	17.96	9.34	10.45	7.32
	% of inhibition	48.2	19	57.5	51	34.2	30.2
Diethyl ether	0.0	44.4± 2.97	51.2± 2.92	42.7± 2.46	26.4± 1.71	49.7± 3.15	64.3± 3.77
	5	41.5± 2.74	42.8± 2.43	40.4± 2.69	26.3± 2.12	48.3± 3.10	62.7± 2.99
	10	37.2± 2.05	42.4± 2.43	35.1± 1.97	22.5± 1.18	42.6± 2.24	41.4± 2.40
	20	29.6± 1.57	35.4± 2.17	20.9± 1.41	19.4± 0.74	31.2± 1.82	34.6± 1.74
	L.S.D. at 5%	3.51	4.16	4.60	2.52	3.12	4.70
	% of inhibition	33.3	31	51	26.5	37.2	49
	Mean of inhibition	40.75	25	54.25	38.75	35.7	38.2

Discussion

It was reported that thymol was the main component of essential oils of *P. tenuiflorus*, harvested in Saudi Arabia [6,17]. Thymol as a phenolic alcohol inhibits the growth of several microorganisms and therefore, used in several antiseptics and pharmaceutical preparations[10].

The components of essential oil of *P. tenuiflorus,* (in addition to its 85% thymol content) have more lethal effect on the tested organisms than pure thymol at the different tested concentrations. The antimicrobial activity of essential oil extract of *Plectranthus* species against different micro-organisms was reported [10]. The plant species are rich in essential oils and diterpenoids which are the common secondary metabolites. Several diterpenoids were identified from the plant species, beside a group of long-chain alkylphenols and other miscellaneous constituents [6]. These components may reflect the highest antimicrobial activity of *P. tenuiflorus* essential oil (85% thymol beside minor components of diterpenoids) as compared with pure thymol.

Boiling water extraction was able to extract substances with more inhibitory action for the growth of *P. aeruginosa*, yeast and mould fungi than cold water treatment, i.e. boiling leads to extraction of more substances and / or higher amounts of the antimicrobial substances. However, cold water extract was more efficient in inhibiting *E.coli* and *S. aureus*. This may be attributed to the denaturation of substances due to boiling some materials that were more inhibitory to these bacteria.

The aqueous extract of plants used in traditional medicine was used to study its mutagenic properties, and antimicrobial activity [8,19]. It was reported that water is almost universally the solvent used to extract activity, either cold or boiling [20]. The validity of both aqueous and organic solvents for extraction of plant materials of antimicrobial activity was also reported [21].

The use of organic solvents to extract plant materials of antimicrobial activity was reported [7,9,10 &22].

In accordance with these findings, it was reported that in most cases organic solvent plant extracts have higher antimicrobial activity than aqueous extracts [10].

Conclusion

Plecturanthus tenuiflorus (Labiatae) plant is widely distributed in Saudi Arabia. The plant has several substances with antimicrobial activity against a group of disease causing microorganisms including bacteria, yeast and fungi. The essential oil of the plant (containing 85% thymol) has a complete bactericidal effect on the tested organisms at concentrations less than 20 mg /100ml with higher activity than pure thymol. Both aqueous and organic solvents extracts showed antimicrobial activity against the test organisms, but their efficiency can't be compared with the results of essential oil of the plant. Generally, aqueous extraction succeeded to extract substances from the stand point of quality and quantity more efficient to inhibit the growth of *P. aeruginosa*, *A. fumigatus* and *A. niger*, while organic solvents extracts were the best to inhibit the growth of *E.coli*, *S. aureus* and *C. albicans*.

The results revealed that both aqueous and organic solvents were efficient for extraction of antimicrobial substances from *P. tenuiflorus*. Their activity was depending on the tested organism. However, essential oil extracts showed promising bactericidal and fungicidal effect on the tested organisms.

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