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Research Article

ANTIMICROBIAL ACTIVITY OF *TAVERNIERA LAPPACEA* FORSSK. EXTRACT AFFECTED BY ITS ACTIVE CONSTITUENTS

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Research Center, El-Mataria, Cairo, Egypt.**Abstract:**

Aerial parts successive extraction of *Taverniera lappacea* demonstrated that the 70% methanol was the highest value (5.66%). It has positive activity on all tested bacteria (*Staphylococcus aureus*, *Streptococcus* spp as bacterial gram positive bacteria), (*Escherichia coli*, *Klebsiella pneumonia*, *Acinetobacter* spp. as gram negative bacteria) and on fungal strain (*Candida albicans*). Investigation of the active extract (70% methanol) by using HPLC analysis revealed the presence of 22 flavonoid compounds at the aerial parts of the plant, with the major compounds; Apiginin-6- glucose -8- rhamnose (227.59 mg/100g), luteolin-6- arabinose -8- glucose (103.91 mg/100g) and Kampferol-3,7-dirhamoside (57.776 mg/100g).

Key Words: *Taverniera lappacea*, flavonoids, antimicrobial activity, HPLC.

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INTRODUCTION:

Infectious diseases can be the result of the colonization of the body by various microbes. There are many similar disease states that can arise from different causes, i.e., pneumonia can be caused by viruses, many types of bacteria, protozoa, and even fungi. The number of microorganisms living on and in us is about ten times higher than the number of cells that make up our entire body. Disease-causing microorganisms, however, are another matter entirely. They use simple tricks to enter our bodies so they can cause disease. Hence we must search for new methods for combat these micro-organism. Medicinal plants represent a rich source of antimicrobial agents. In different countries, plants are used medicinally and are the source of several effective and powerful drugs [1,2]. Even though hundreds of plant species have been tested for antimicrobial activities, the enormous mass of them have not been adequately evaluated [3,1]. The antimicrobial agent contained in plants usually extracted using different solvents and the antimicrobial properties of the extracts may vary [4,5]. Ethanol and methanol extracts of *Taverniera abyssinica* A. Rich. showed better antimicrobial activity against *S. aureus*, *E. faecalis* and *C. albicans* while *E. coli* and *A. flavus* were the most resistant microorganisms to this medicinal plant [6]. While The extracts of *T. cuneifolia* root, exhibited promising anti-inflammatory, anti-tumor, anti germination tube formation (in *Candida albicans*), protection from mutagen toxicity and cytotoxic activities comparable to that of *G. glabra*. In general, the results suggest that *T. cuneifolia* could be used as substitute of *G. glabra* [7].

Taverniera lappacea Forssk. belong to family Leguminosae. The genus of *Taverniera* in Egypt includes two species (*Taverniera lappacea* and *Taverniera aegyptiaca*) [8]. Preliminary phytochemical screening of *Taverniera lappacea* revealed that it contained steroids, terpenoids, saponins, coumarins, flavonoids and phenolics and glycosides and/or carbohydrates. It was detected ten compounds of the flavonoids, two compounds of phenolic acids and one compound of coumarin in the plant [9]. Meanwhile, primary metabolite, saponins and diterpens of *Taverniera lappacea* were investigated [10].

MATERIALS AND METHODS:

Plant material

Taverniera lappacea aerial parts were collected from Ras Mohamed protected area, South Sinai, Egypt during spring season (2011). Samples were identified [11] and deposited with the plant protection collection at the Desert Research Center at Cairo, Egypt. The aerial parts of *Taverniera lappacea* were cleaned, dried in an oven at 40°C, ground to fine powder and for different analysis.

Extraction using different organic solvents:

Successive extraction technique:

About 300 gram of the aerial parts of *Taverniera lappacea* were subjected to extraction with successive selective organic solvents using soxhlet apparatus, in order of increasing polarity including benzene, diethyl ether, chloroform, 95% methanol and 70% methanol. The obtained residue from each solvent was dried and weight.

Antimicrobial screening:

The effect of different successive-selective extracts of the aerial parts of *Taverniera lappacea* plant using (100 µg/ml) of the following solvents; benzene, chloroform, 95% methanol and 70% methanol on some pollutant micro-organisms were achieved.

Microorganisms:

The following five bacterial and one fungal strains were tested: Bacterial strains of gram positive bacteria (*Staphylococcus aureus*, *Streptococcus* spp). and gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Acinetobacter* spp.). Fungal strain was *Candida albicans*.

The microorganisms were obtained from Plant Pathology and Microbiology Department, the National Research Center, Cairo, Egypt. It was checked for purity and identity and regenerated to obtain active microorganisms. The cultures were stored in refrigerator at 5°C and reactivated on the media suitable for each microorganism.

Preparation of the Spore Suspension [12]

Agar Diffusion Method [13]

Qualitative and quantitative analysis of highest antimicrobial activity extract from *Taverniera lappacea* by HPLC:

The highest antimicrobial activity extract of the aerial parts of *Taverniera lappacea* were analysis by HPLC. The analytical HPLC system employed consisted of HP 1090M Series II high performance liquid chromatography equipped with an HP 1090M Series II diode array and an eight-channel electrochemical colorimetric array detector (EC; Esa Inc., USA). The EC was operated using 100-800 mV potentials (100mV intervals). The detector array was housed in a temperature-regulated compartment at 35°C. Flavonoid separation was done by ODS-3 (4.0 × 150 nm, 3µm) column with a C-18 guard column, with temperature set at 35°C. The flow rate of the mobile phase was 0.7mL/min, and the injection volumes were 10µL of the standards and sample extracts. All flavonoids were quantified using the external standard method. Quantification was based on peak area (DAD) or beak height (EC) [14].

RESULTS AND DISCUSSIONS:**Extraction using different organic solvents:****Successive extraction:**

Data presented at table (1) showed that the 70% methanol was the highest extract residues (5.66 %) obtained from the aerial parts of *Taverniera lappacea*.

Table 1: Successive extraction residues of the aerial parts of *Taverniera lappacea*.

Plant extract	Residue percentage (%)
Benzene	1.21
Diethyl ether	1.67
Chloroform	0.87
95% Methanol	4.57
70% Methanol	5.66

Antimicrobial screening:

Data at table (2) showed that the most antibacterial and antifungal active extract was 70% methanol, which was effective on all tested bacterial and the fungal strain.

Table 2: Antimicrobial activity of the successive extraction of the aerial parts of *Taverniera lappacea*.

Plant extract	Inhibition zone (mm)					
	Gram (+ve) bacteria		Gram (-ve) bacteria			Fungi
	<i>Staphylococcus aureus</i>	<i>Streptococcus</i> spp.	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Acinetobacter</i> spp.	<i>Candida albicans</i>
Benzene	0	0	9	0	7	10
Diethyl ether	12	8	13	15	13	15
Chloroform	0	0	0	7	5	9
95% Methanol	16	13	17	18	14	17
70% Methanol	34	30	30	32	22	19

The highest antimicrobial activity extract was 70% methanol, this may be mostly related the presence of flavonoid glycosides. Which encourage us to analyzed it with HPLC.

Table 3: HPLC analysis of the 70% methanol extract of *Taverniera lappacea* aerial parts.

No.	Flavonoids	Mg/100g
1	Luteolin-6- arabinose-8- glucose	103.91
2	Luteolin-6- glucose -8- arabinose	45.112
3	Apigenin-6- arabinose -8- galactose	28.028
4	Apignin-6- rhamnose -8- glucose	5.379
5	Apignin-6- glucose -8- rhamnose	227.59
6	Luteolin-7- glucose	45.618
7	Narengin	47.942
8	Rutin	12.177
9	Hesperidin	31.957
10	Quercetin-3-O-glucoside	2.551
11	Rosmarinic	5.877
12	Apigenin-7-O- neohespiroside	44.304
13	Kampferol-3,7-dirhamoside	57.776
14	apigenin-7- glucose	10.213
15	Quercetrin	1.204
16	Quercetin	1.385
17	Naringenin	4.434
18	Hespirtin	2.187
19	Kampferol	0.199
20	Rhamnetin	0.393
21	Apignin	0.637
22	Acacetin	20.832

Qualitative and quantitative analysis of the 70% methanol extract of the aerial parts of *Taverniera lappacea* by HPLC:

Investigation of 70% methanol extracts by HPLC revealed the presence of 22 flavonoid compounds at the aerial parts of *Taverniera lappacea*, where the major compounds were Apiginin-6- glucose -8-rhamnose (227.59 mg/100g), luteolin-6- arabinose -8-glucose (103.91 mg/100g) and Kampferol-3,7-dirhamoside (57.776 mg/100g) as illustrated at (Table 3).

Antimicrobial activity of *T. lappacea* forssk. varied with different extract solvent. These results were in agreement with those obtained by [15], which showed that, the successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Ethanol and methanol extract of *T. abyssinica* showed better antimicrobial activity against tested microorganisms that generally increased with the increase in the concentration of the extract. It means that they are more efficient in cell walls degradation which has non-polar character and cause polyphenols to be released from cells [6]. In addition to this enzyme polyphenol oxidase are inactivated in methanol and ethanol extract [5]. This may be the reason why the antimicrobial activities of selected medicinal plant showed higher in water extract in our study.

CONCLUSION:

The objective of the present work focuses on antimicrobial activity of extracts of *Taverniera lappacea* Forssk., as affected by its active constituents, where 70% methanol extract was the most active antibacterial and antifungal effect. These results encourage us to identifying its natural chemicals constituents, which were responsible for phytotoxic effects against most bacterial and fungal strains, by using HPLC analysis. HPLC analysis revealed the presence of 22 flavonoid compounds at the aerial parts of *Taverniera lappacea*, where the major compounds were Apiginin-6- glucose -8-rhamnose (227.59 mg/100g), luteolin-6- arabinose -8-glucose (103.91 mg/100g) and Kampferol-3,7-dirhamoside (57.776 mg/100g).

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