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Assessment of antimicrobial activity of ethyl acetate and *n*-butanol extracts from *Satureja graeca* L. growing in Algeria

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ABSTRACT

The present work focuses essentially on the phytochemical screening and evaluate the antimicrobial activity of Both extracts from Satureja graeca L. ethyl acetate and n-butanol using a diffusion method for the most common pathogenic bacterial strains: E.coli ATCC 2592, Pseudomonas aerogenosa ATCC2783, Staphylococcus aureus ATCC 252923, Salmonella sp. Klebseilla pneumonia and one fungi Candida albicans. The results revealed the presence of some chemical groups such as volatile oils, flavonoids, sterols or triterpenes, tannins, carotenoids and saponins. Two extracts showed a good antibacterial activity against Staphylococcus aureus ATCC 252923.

Key words: *Satureja graeca* L. , ethyl acetate extract, butanol extract, antimicrobial activity.

INTRODUCTION

The genus *Satureja* belonging to the family Lamiaceae, subfamily nepetoideae and tribe menteae, contains about 200 species of aromatic herbs and shrubs widely distributed in middle east, mediterranean region to Europe, west Asia, north Africa, the Canary Islands and south America [1]. The flora of Algeria has sixteen species of genus *Satureja* [2]. It is a medicinal herb and condiment can also be grown as an ornamental plant. It enjoys great popularity in Algeria and Morocco as a remedy against cough, indigestion and mild respiratory infections [3]. Recently, the other properties of *Satureja* sp. such as antibacterial, antifungal, antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory, expectorant and vasodilatory activities have been demonstrated [4]. The present work aims to determine a phytochemical screening and assessment of the antimicrobial activity of ethyl acetate and butanol extracts of leaves and flowers.

MATERIALS AND METHODS

Plant material

The leaves of *Satureja graeca* L. was collected in march 2012 (flowering stage) in Hama Bouziane - Constantine, Algeria. The plant was identified by Dr. Hallis youcef, Scientific and Technical Research Center for Arid Areas (CRSTRA), Biophysical Station, Touggourt, Algeria. A voucher specimen was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria (voucher number ZA 137).

Extraction and detection of chemical groups [5]
25g of each powdered dried plant were extracted with petroleum-ether in a continuous extraction apparatus soxhlet. The ether extracts were combined, filtered and concentrated up to 40 ml. The remaining dry vegetable product was extracted three times with methanol for 20 minutes. The vegetable product residue was then extracted with warm water for 20 minutes. The constituents were identified as follows:

•Identification of volatile oils
The ether extract was evaporated to dryness. The residue had a characteristic pleasant odor, thus the plant product contains volatile oils. The vegetable product was distilled with water in a Neo-Clevenger apparatus to extract the volatile oils.

•Identification of sterols and triterpenes
The residue of ether extract was dissolved in 0.5 mL acetic anhydride and then in 0.5 ml of chloroform. Then 1 ml of concentrated sulfuric acid is added (Libermann- Burchards reaction). At the contact zone of the two liquids a brownish red ring was formed denoting the presence of sterols and triterpenes.

•Identification of carotenoids
The ether extract was evaporated to dryness and 3 drops of saturated solution of antimony trichloride in chloroform were added (Carprice's reaction). The pigments are firstly blue and later became red, denoting the presence of carotenoids.

•Identification of flavonoids
The residue of methanolic extract was dissolved in 2 ml of methanol at 50 °C. Metallic magnesium and 5 drops of concentrated HCl were added. A red or orange color indicates the presence of flavones aglycones (Shibata's reaction).

•Identification of coumarins
The residue of methanolic extract or alcohol extract is dissolved after dryness in hot water. The solution is divided into two equal volumes: one of which contains the reference, and the second is made alkaline with 0.5 ml of 10 % ammonia solution. The appearance of an intense fluorescence under UV light indicates the presence of coumarins and derivatives.

•Identification of tannins:
The water extract (1ml) was diluted with water (2 ml) and a diluted solution of ferric chloride (3 drops) was added. The appearance of a blackish blue or blackish green color indicates the presence of tannins.

•Identification of saponins
The water extract (1ml) was diluted with water (2 ml) and the mixture was vigorously shaken for 2 minutes the formation of froth which persists for 10 minutes indicate the presence of sapnins.

Extraction
Air-dried powdered material from the leaves (100g) of *Satureja graeca* L. were extracted with 70% MeOH for 24 h, three times. The MeOH extract was evaporated to dryness. The residue was dissolved in boiling water. After filtration, the filtrate was concentrated and re-extracted several times with EtOAc and *n*-BuOH resulting in a residue of 0.52g from EtOAc extract and 0.93g from *n*-BuOH extract [6].

Antimicrobial activity
The Antimicrobial assay was carried out on two extracts using agar diffusion method [7], against five human pathogenic bacteria, including (standard strains) *Staphylococcus aureus ATCC252923and Pseudomonas aerugenosa ATCC 27853 E.coli ATCC 25922 (clinical strains) Klebsella pneumoniae Salmonella sp. and one fungi Candida albicans.*

The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 °C for 24 h prior to seeding on to the nutrient agar and the fungal strains at 30°C for 48 h.

The isolated compounds were mounted on sterile filter paper discs (6 mm in diameter) with the following concentrations (mg/ml) (8, 4, 2, 1 and 0.5). The discs were placed on the inoculated agar media. The treated Petri discs were kept at 4 ◦C for 1 h, and incubated at 37 ◦C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

Phytochemical screening

The present work is focused essentially on the phytochemical and antimicrobial screening of *Satureja graeca* L. This species have been screened for several chemical groups. It is worth noting the absence of coumarins, and alkaloids, nevertheless, the flavonoids, sterols or triterpenes, volatile oils, saponins, tannins, carotenoids are present and have not previously been reported in the literature (Table 1).

Table 1. Phytochemical screening from aerial parts of *Satureja graeca* L.

Extract	Chemical groups	tests
***n*-Hexane**	Volatil oils	+
	Sterols or triterpenes	++
	Carotenoids	+
Methanol	Coumarins	-
	Alkaloids	-
	Flavonoids	++
Aqueous	Tanins	++
	Saponins	+

Antimicrobial activity

The results summarized were in Table 2 and table 3, which showed that both extracts from *Satureja graeca* L. ethyl acetate and *n*-butanol were prevented the growth of all the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The results show that the two extracts exhibited variable degrees of antimicrobial activity.

Generally the obtained inhibition on bacteria strains varied from 06.83 in *E. coli* ATCC25922 to 16.0 mm *S. aureus* ATCC25923 with a highest inhibition zone at 8 mg/ml (ethyl acetate and butanolic extract extract successively), However no activity against *Salmonella sp* at low concentration 0.5mg/ml from ethyl acetate extract and from all concentration with butanolic extraction except at 8 mg/ml. Also no activity against *C.albicans* at 0.5, 1 and 2 mg/ml.

Table 2: antimicrobial activity of ethyl acetate extract from *Satureja graeca* L.

Strains microorganisms	Diameters of inhibition zones (mm)				
	Concentrations				
	08mg/ml	04mg/ml	02mg/ml	01mg/ml	0.5mg/ml
E. coli ATCC25922	09.17±0.76	08.17±0.28	07.50±0.50	-	-
S. aureus ATCC25923	**15.00±2.00**	**13.83±0.76**	**13.50±0.50**	**12.50±0.50**	**12.17±0.76**
P. aeruginosa ATCC27853	09.50±0.50	08.83±0.29	08.67±0.29	08.00±0.00	07.83±0.29
K.pneumoneae	12.33±0.29	11.50±0.50	09.83±0.76	08.00±0.00	07.67±0.29
Salmonella sp	11.17±0.76	09.67±0.58	09.00±0.87	07.50±0.50	-
C. albicans	12.17±0.76	09.67±0.58	-	-	-

- *(-): No inhibition (or ≤ 06mm)*

Our results showed that ethyl acetate and *n*-butanol exhibited improved antimicrobial activities compared with some studies. Our data agree with available literature. Antimicrobial activity of several extracts has been previously reported. Methanol and hexane extracts of *Satureja hortensis* L. was found to exhibit an inhibitory activity against 55 bacterial species, and 31 isolates of 1 yeast and 4 fungi species were tested by using disc diffusion assay [8].
Bougandoura and Bendimerad [3] have reported the antifungal activity of methanolic and aqueous extract of *Satureja calamintha* ssp.(Nepeta) briq growing in Algeria .

Table 3: antimicrobial activity of *n*-butanol extract from *Satureja graeca* L.

Strains microorganisms	Diameters of inhibition zones (mm)				
	Concentrations				
	08mg/ml	04mg/ml	02mg/ml	01mg/ml	0.5mg/ml
E. coli ATCC25922	09.83±0.29	07.83±0.29	06.83±0.29	-	-
S. aureus ATCC25923	**16.00±1.32**	**15.17±0.76**	**14.17±0.76**	**13.50±0.50**	**12.17±0.76**
P. aeruginosa ATCC27853	10.00±0.50	08.67±0.29	08.67±0.29	08.33±0.58	08.00±0.00
K.pneumoneae	09.67±0.58	08.67±0.58	08.17±0.29	07.83±0.29	07.59±0.00
Salmonella sp	09.33±0.58	-	-	-	-
C. albicans	10.17±0.76	09.83±0.76	-	-	-

- *(-):No inhibition (or ≤ 06mm).*

Amanloua *et al.*, [9] found that methanolic extract of *S. khuzistanica* had a large inhibition against *Staphylococcus aureus* , and *Candida albicans*, also Gordana *et al.* [10] supported our results particulary by ethyl acetate against

S.aureus and found that petroleum ether, chloroform and ethyl acetate extracts of *Satureja montana* L. subsp. *Kitaibelii* expressed a wide range of inhibiting activity against both gram positive and gram negative bacteria, Adiguzel *et al.* [11] showed that essential oils and methanolic extract of *Satureja hortensis* exhibited the activity against 25 bacteria, 8 fungi, and a yeast. Moreover Bensouici *et al*. [11] found that he essential oil of Algerian *Satureja calamintha ssp. sylvatica* exhibited the best antibacterial activity against 10 gram+ and gram – strains bacteria.

CONCLUSION

The effectiveness demonstrated by ethyl acetate and *n*-butanol against some pathogenic microorganisms of worldwide human health interest, can be considered important. Moreover, the activity against ***Staphylococcus aureus* ATCC25923** strain could lead to the use, as selective natural drug.

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