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Antibacterial activity and chemical composition of essential oil of *Santolina rosmarinifolia* L. (Asteraceae) from Algeria

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ABSTRACT

The genus Santolina is represented by more than 10 species widely distributed in the Mediterranean area. Species of this genus are used in folk medicine as anthelmintic, antispasmodic and antinfectious. In the current study of the essential oil of Santolina rosmarinifolia L., 31 compounds were determined in the GC-MS, representing 93.40 % of the essential oil with the prevalence of germacrene-D (30.20%), □-myrcene (12.00%), tricosane□ (10.60%), □-pinene (10.10%), sabinene (7.00%) and pentacosane (6.70%). The essential oil exhibited a good antibacterial activity against almost food-borne pathogens specially Escherichia coli ATCC 25922, Escherichia coli, and Enterobacter aerogenes with 25 mm, 20 mm, 20 mm, inhibition zone diameters, respectively. Minimum inhibitory concentration (MIC) values for all the bacteria were ranged between 0.16 mg/ml and 0.64 mg/ml.

Keywords: *Santolina rosmarinifolia* L., Asteraceae, Essential oil, Antibacterial.

INTRODUCTION

Santolina L. is a small genus of the large subfamily Asteraceae and is native to the Mediterranean region. Species of this genus are used in folk medicine as anthelmintic, antispasmodic, antifectious [1, 2] and antimicrobial [3]. In continuation of our work on Asteraceae [4-16], We report here the chemical composition and antibacterial activity of the essential oil of *Santolina rosmarinifolia* L. In previous studies, *S. rosmarinifolia* ssp. *rosmarinifolia* and ssp. *canescens* growing in Spain have been investigated for their volatile components [17, 18]. The major components were monoterpenes (50%, 65%). Capillene has been identified as the main component of the essential oil of the ssp. *Rosmarinifolia* [19].

MATERIALS AND METHODS

Plant material

The flowering aerial parts of *Santolina rosmarinifolia* L. [20] were collected in June 2010 from Batna-Aures (North Eastern Algerian). A voucher specimen was deposited at the herbarium of the Laboratory of Therapeutic Substances, Faculty of Sciences, University of Constantine 1, Algeria (LOST.Sr.06.10).

Essential Oil extraction

The hydrodistillation of the fresh aerial parts (100 g) of S. *rosmarinifolia* L., for 3 h in a Clevenger-type apparatus,yielded 1.4 % (w/w) of a yellow essential oil which was stored at +4 °C until analysed GC-MS and tested.

Gas chromatography-mass spectrometry (GC/MS) analysis
The essential oil was analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard MS model 5871, equipped with a DB5 MS column (30m X 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium as carrier gas (1.0 ml/min); injection in split mode (1: 30); injector and detector temperature, 250 and 280°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

GC/FID
The essential oil was analyzed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30m X 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Hydrogen as carrier gas (1.0 mL/min); injection in split mode (1: 60); injector and detector temperature, 280°C and 300°C respectively. The essential oil was diluted in hexane: 1/30.

Identification of components
The compounds assayed by GC were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances [21, 22].

Microorganisms
Using the disk diffusion method [27]. A collection of 08 test microorganisms including Gram-positive and Gram-negative bacterial strains was used.

The groups included five organisms of American Type Culture Collection (ATCC): *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 43300, *and Escherichia coli* ATCC 25922. The reference strains were obtained from the Pasteur Institute (Algiers), five clinical organisms *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Morganella morganii* were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation).

RESULTS AND DISCUSSION

Chemical composition of the essential oil
Thirty one compounds were determined in the hydrodistilled essential oil of *Santolina rosmarinifolia* L., representing 93.40 % of total oil content. The main constituents were found to be germacrene-D (30.20%), □-myrcene (12.00%), tricosane□ (10.60%), □-pinene (10.10%), sabinene (7.00%) and pentacosane (6.70%) (Table 1). The present essential oil is also monterpenes-rich; that comes close to almost reported *Santolina* oils. It's the first time that germacrene-D, tricosane and pentacosane are found as main components in *Santolina* oil. Nevertheless, germacrene-D has been found in traces in *Santolina rosmarinifolia* L. ssp. *rosmarinifolia* [23]. Sabinene (5.5%) was found as a main component in *S. rosmarinifolia* ssp *rosmarinifolia* [23], highly represented by capillene (35.2 %). Other major compounds, such as □-pinene (7.8%, 6.6%, 9.9%, 4.5%) and □-myrcene (13.1%, 17%, 11.8%, 34.6%, 5.4%) [24,3,25,26], were reported from essential oils of *S. rosmarinifolia* ssp *rosmarinifolia* L., *S. insularis*, *S. etrusca*, *S. corsica* and *S. oblongifolia* Boiss., respectively. The highest percentages of 1,8-cineole (3.7%, 4.6%) were reported from *S. insularis* and *S. etrusca* oils, respectively [3, 25].

By the use of the disc diffusion method, the essential oil exhibited the best antibacterial activity against *Escherichia coli ATCC 25922*, *Escherichia coli*, and *Enterobacter aerogenes* with 25 mm, 20 mm and 20 mm, inhibition zone diameters, respectively (Table 2). This good activity may be due to the combined effect of main components such as □-myrcene (12.00%), □-pinene and sabinene which have been already reported for their antibacterial activity [28-30].

Table 1: Chemical composition of the essential oil of *Santolina rosmarinifolia* L.

Pic	Compounds[a]	RI[b]	%
1	Santolina triene	909	0.20
2	α-Pinene	939	0.70
3	Sabinene	975	**7.10**
4	β-Pinene	979	**10.10**
5	β-Myrcene	991	**12.00**
6	Limonene	1029	0.10
7	1,8-Cineole	1031	4.60
8	*cis*-Sabinene hydrate	1070	0.10
9	*p-trans*-menth-2-en-1-ol	1141	0.20

10	Camphor	1146	0.10
11	*cis*-Chrysanthenol	1164	0.90
12	Borneol	1169	0.30
13	Terpinen-4-ol	1177	0.10
14	α-Terpineol	1189	0.30
15	α-Copaene	1377	0.10
16	β-Elemene	1391	2.90
17	*trans*-Caryophyllene	1419	0.30
18	α-Gurjunene	1477	0.10
19	γ-Curcumene	1483	1.20
20	Germacrene -D	1485	**30.20**
21	Valencene	1496	0.20
22	Bicyclogermacrene	1500	0.40
23	β-Cadinene	1523	0.20
24	γ-*E*-Bisabolene	1531	1.70
25	Germacrene -B	1550	0.20
26	Elemol	1561	0.30
27	Spathulenol	1578	0.20
28	β-Eudesmol	1654	0.90
29	α-Bisabolol	1686	0.40
30	Tricosane	2300	**10.60**
31	Pentacosane	2500	**6.70**
	Identified compounds	**Total**	**93.40%**

[a]*RI = retention indices as determined on DB-5MS column using homologous series of n alkanes.*

Table 2: Antibacterial activity of the essential oil of *Santolina rosmarinifolia* L.

Microorganism	Inhibition zone (mm)		MIC (µg/ml)	
	Ampicillin (10 µg/ml)	Essential oil (128 µg/ml)	Ampicillin (10 µg/ml)	Essential oil (128 µg/ml)
Escherichia coli ATCC 25922	18	25	10	16
Escherichia coli		20		16
Staphylococcus aureus ATCC 43300	30	10	5	64
Staphylococcus aureus		8		64
Pseudomonas aeruginosa ATCC 27853	-	18	-	32
Pseudomonas aeruginosa		15		32
Enterobacter aerogenes	-	20	-	16
Klebsiella pneumoniae	14	18	32	32
Morganella morganii	-	16	-	32

CONCLUSION

Thirty one compounds were determined in the essential oil of *Santolina rosmarinifolia* L., mainly represented by germacrene-D (30.20%), β-myrcene (12.00%), tricosaneβ (10.60%), β-pinene (10.10%) and sabinene (7.00%) and pentacosane (6.70%). To the best of our knowledge, germacrene-D, tricosane and pentacosane are found, for the first time, as main components in *Santolina* essential oil. The present essential oil inhibited remarkably the growth of bacterial strains namely *Escherichia coli* ATCC 25922, *Escherichia coli,* and *Enterobacter aerogenes.* This good antibacterial activity may be due to the synergic effect of main components such as β-myrcene, β-pinene and sabinene.

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