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Chemical composition, antimicrobial and antioxidant activities of the essential oils of *Santolina africana* flowers, endemic in Algeria

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ABSTRACT

The essential oils obtained by hydrodistillation of the flowers of *Santolina africana* Jord. et Four. were analyzed by GC/MS. The main constituents of the essential oil were acenaphtane (25.23 %), calarene (21.54 %), ocimene (17.44 %) and some other compounds that were presented only in minor amounts. In total, essential oils of *Santolina africana* Jord. et Four. were considered as a rich source of hydrocarbon mono and sesquiterpenes. Moreover, the antimicrobial activity of the essential oil against eight bacteria strains and three fungi was studied. It was found that the most powerful effect was against *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus flavus*. The essential oils were screened for their possible *in vitro* antioxidant activity by DPPH free radical-scavenging test. The findings showed that reduction percentage is 13.80 at 1M.

Key words: *Santolina africana*, Asteraceae, essential oil, flowers, antimicrobial activity; antioxidant activity

Abbreviations: GC/MS: Gas chromatography/ mass spectroscopy; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DMSO: Dimethyl sulfoxide; NIST: National Institute of Standards and Technology; NCCLS: National Committee for Clinical Laboratory Standards

Introduction

The use of aromatic plants and spices in phytotherapy is mostly related to different activities (such as antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, anticarcinogenic) of their essential oils. Many studies point to strong antioxidant activities of aromatic plants and their essential oils. Antioxidant activities are also confirmed for most of the phenolic compounds present in different spices and herbs (Blumenthal, 1999; Bruneton, 1999).

Genus *Santolina* is represented by more than 10 species widely distributed in Mediterranean area. This genus is constituted by a taxonomically complex group, whose classification is periodically revised. Some members of the *Santolina* genus are widely used in popular medicine (Ferrari et al., 2005).

Members of the *Santolina* genus have been of interest due to their excellent medicinal value. Different classes of natural products have been isolated from these species, including flavonoids, terpenoids, coumarins, and polyacetylenes (Silván et al., 1996; Barrero et al., 1994). Plants from this genus have been intensely investigated from a chemical and a pharmacological standpoint on account of their rich ethnopharmacology, which includes both medicinal

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(antispasmodic, antiseptic, anti-inflammatory, antihelmintic) and insecticidal uses (Fattorusso et al, 2004; Appendino et al., 2005).

The composition of essential oils from various species of *Santolina* has been investigated by many authors namey: *S. chamaecyparissus* (Lawrence, 1997; Garg et al., 2001), *S. oblongifolia* (De Pascual et al., 1983), *S. ligustica* (Flamini et al., 1999), *S. rosmarinifolia* (Palá Paúl et al., 1999, 2001) and *S. canescens* (Casado et al., 2001). All species produced monoterpene-rich oils and exhibited quite diverse compositions. Conversely, only three studies reported on the phytochemistry of *S. corsica*. A solvent extract from roots contained sesquiterpene hydrocarbons, triterpenes, furylthienylbutenynes and a spiroketalenol (Ferrari et al., 2005).

Ormenis is a smaller genus belonging to the Asteraceae family and comprised in Algeria three species, *Santolina africana* Jord. *et* Four.*(=Ormenis africana)*, *S. lonadioides* Coss., *S. nobilis* L. J. Gay. (Quezel et Santa, 1963).

The present study is aimed mainly to: (1) Determine the chemical composition of *S. africana* hydro-distilled essential oil by GC/ MS; (2) Investigate the antimicrobial activity of the essential oil of the plant by disc diffusion method against some pathogen bacteria and fungj; (3) Evaluate the antioxidant capacity of the plant essential oil.

Materials and Methods

Plant material

The flowers of *S. africana* were collected in May 2008 (flowering stage) in Constantine, Algeria. The plant was identified by Dr. Zellagui Amar, Constantine University. A voucher specimen was deposited at the Chemistry Department, University of Mentouri-Constantine under the code number ZA 116.

Extraction

Essential oils were obtained by hydrodistillation of 100 g of dried flowers using a Clevenger-type apparatus for 3 h. Diethyl ether (10 ml) was used as the collector solvent. After evaporation of the solvent, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at −20°C before analyses to afford 2.25% of crude oil. The oil sample was subsequently analyzed by GC-MS.

Gas chromatography/mass spectrometry (GC/MS)

The oil was analyzed by GC/MS using a Shimadzu gas chromatography model GC-17A coupled with Shimadzu mass spectroscopy model QP5050, equipped with a fused silica capillary DBX-5 column (30 m × 0.25 mm inner diameter, with 0.25 µm film thickness). Operating conditions were: carrier gas flow - 1.6 ml He/min, column pressure - 100 Kpa. The injector and detector temperatures were 220°C and 250°C, respectively. The column temperature was held at 60°C for 1 min, then raised from 60°C to 200°C at 10°C/min and held there for 5 min and from 200°C to 240°C at 10°C/min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV.

Identification of components

Identification of oil components was achieved on the basis of their retention indices (RI), determined with reference to a homologous series of normal alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor.

Antimicrobial activity

Microorganism strains

All bacteria (standard strains: *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Escherichia coli* ATCC25922, *Pseudomonas aereginosa* ATCC27853, *Proteus vulgaris* ATCC6897; clinical strains*: Klebsella pneumonia*) and fungi (*Aspergillus flavus* ATCC10239, *Aspergillus niger* and *Candida albicans*) were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U), Laboratory of microbial engineering and applications (University Mentouri Constantine).

Antimicrobial assay

The anti-microbial assay was carried out on essential oil using agar diffusion method [NCCLS], against eight human pathogenic bacteria, including Gram-positive, Gram-negative and three fungi strains. 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 essential oil was mounted on sterile filter paper discs (6 mm in diameter) with the following concentrations 8000, 4000, 2000, 1000, 500, and 250 µg/ml. 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

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antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Each experiment was carried out in triplicates.

Antioxidant activity

DPPH radical-scavenging activity

The capacity of essential oil extracted from *S. africana* to reduce the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed using the method of Masuda et al. (1999). 15 µl of the essential oil at different concentrations was added to 15 µl of a DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of essential oil was evaluated according to the formula:

DPPH scavenging effect (%) = $[(A_0 - A_1)/A0] \times 100$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample after 30 min. All samples were analyzed in three replications.

Results and Discussion

The composition and percentage of the compounds are summarized in Table 1. They are listed by order of their retention times. The oil yield was 2.25 % (w/ w) based on the dried weight which means that the organs are a potential oil source. Twenty six compounds were identified in the essential oils, representing 99.98% of the total oil. The essential oils were dominated by a large amount of monoterpene hydrocarbons (27.56%) and sesquiterpenes hydrocarbons (26.89%), while the oxygenated monoterpenes (6.42%) and oxygenated sesquiterpenes (0.86%) contents were very low. Furthermore, aromatic compounds were represented by 25.23%.

The main constituents of the essential oil were acenaphtane (25.23%), calarene (21.54%), ocimene 17.44% and some other compounds were present only in minor amounts. In total, essential oil composition of *S. africana* was considered as a rich source of hydrocarbon mono- and sesquiterpenes (Table 2).

A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols.

Table 1. *Composition of the flowers essential oils of S. africana.*

Compounds	**RI**	**%**
Cyclooctane	920	0.10
L- α –Pinene	932	4.48
β –Myrcene	988	4.18
P-Cymene	1024	0.06
Limonene	1027	0.17
α -cis-Ocimene	1038	0.09
Eucalyptol	1039	2.67
Ocimene	1055	17.44
Linalool	1095	0.15
Cucumber alcohol	1167	0.05
4-Thujanol	1171	0.03
Cis-Geraniol	1255	2.25
Limonene dioxide	1294	1.27
Dihydrocarveol acetate	1344	0.47
Carvomenthyl acetate	1347	0.90
Calarene	1385	21.54
Acenaphthene	1429	25.23
α –Farnesene	1500	5.35
Nerolidol	1531	0.78
Tricyclo 5,1,0,0,2,4 octane-5-carboxylic acid, 3,3,8,8, tetramethyl-,methylester	1580	8.17
Capillin	1637	1.54
Total		**96.92**

Table 2. *Classification of the constituents of the S. africana.*

Component	Peak area, %
Esters	9.26
Aromatic compounds	25.23
Oxygenated monoterpenes	6.42
Monoterpene hydrocarbons	27.56
Cyclic hydrocarbon	0.10
Oxygenated sesquiterpenes	0.86
Hydrocarbons Sesquiterpene	26.89
Nitrogen containing compounds	0.37
Others	0.24

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The lipophilic character of the hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components (Griffin et al., 1999). Due to these data we were interested to study antimicrobial activity of the essential oil. The results were summarized in Table 3, which showed that essential oil extracted from *Santolina africana* prevented the growth of all tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The obtained inhibition on bacteria strains varied from 7.0 to 20.15 mm with a highest inhibition zone recorded for *Bacillus subtilis* ATCC 6633 at 8.10^3 µg/ml, and a considerable inhibition effect with the same concentration at *Aspergillus flavus* ATCC10239. It should be mentioned that there are no background antibacterial studies on *Santolina africana*, while in genus *Santolina* some studies have been reported as 6.6 mm for *E. coli*, 14.7 mm for *Staphylococcus aureous*, and 6 mm for *Pseudomonas aerogenosa*. These data are for crude essential oil in 10% of DMSO extracted from *S. corsica (*Liu et al., 2007).

The antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Moreover, essential oils being also able of scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline (Miguel, 2010). Free radical scavenging was measured by using DPPH. The scavenging activity of the essential oil was tested at concentrations of 10^{-1} M, 10^{-2} M, 10^{-3} M and 10^{-4} M. Vitamin C was used as a standard at the final concentration of 1 mg/ml. The highest DPPH radical scavenging activity (%) was shown by essential oil at 10^{-1} M (13.80 %) (Figure 1), which was lower than the antioxidant activity of the standard vitamin C (Figure 2). Similar results were reported in a study of antioxidant effect of essential oils of *Santolina canescens* aerial parts (Utrilla et al., 1995).

Conclusion

The chemical analyses by GC/MS allowed identification of ~99.98% of the total volatile products for *S. africana* and 26 volatile compounds. A major constituent in flowers was acenaphtane (25.23%) and the yield of essential oils was 2.25%. These extracts reveal *in vitro* antibacterial activity on some bacterial strains, confirmed by the inhibition zone diameter ranging from 6.5 mm to 20.15 mm. Moreover this study concludes that the essential oils from flowers of *S. africana* possess antioxidant activities that might be a natural potential resource for use in food and other allied industries.

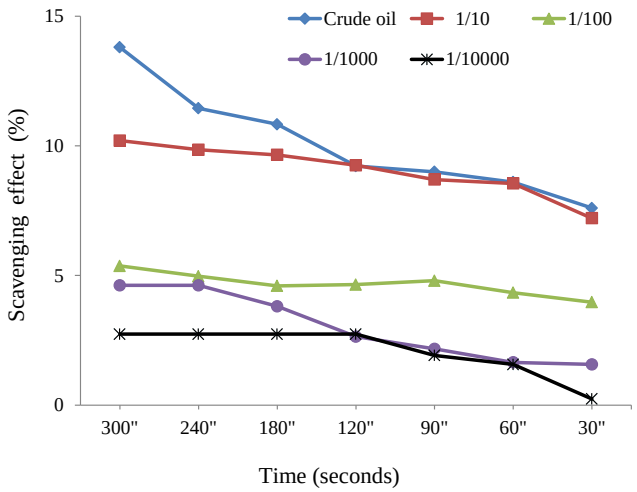

Figure.1 *DPPH radical scavenging activity of essential oil at different times.*

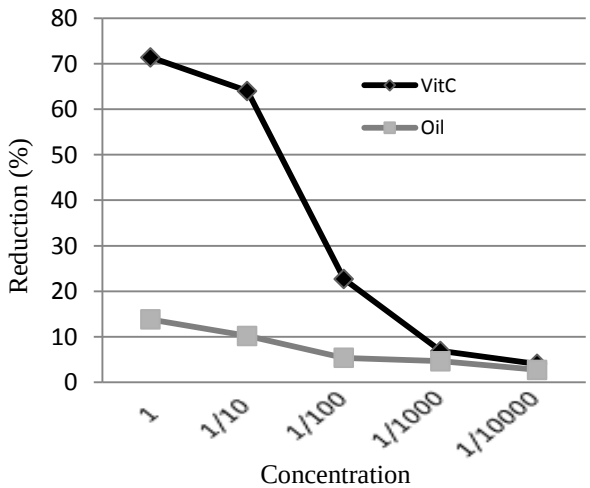

Figure 2. *DPPH radical scavenging activity of essential oil and Vitamin C.*

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Table 3. *Antimicrobial activity of essential oil of S. africana.*

Microorganisms	Oil concentration (µg/ml)					
	250	500	1000	2000	4000	8000
Gram-positive						
Bacillus subtilis ATCC 6633	-	7.0±0.40	8.50±0.25	14.50±0.50	17.0±0.70	20.15±1.10
Enterococcus faecalis ATCC29212	-	-	-	08.0±0.66	11.30±0.45	15.0±0.55
Staphylococcus aureus ATCC25923	-	-	6.50±0.30	13.0±0.50	16.50±0.65	19.50±0.70
Staphylococcus epidermidis ATCC12228	-	-	-	08.0±0.30	09.60±0.40	11.30±0.35
Gram-negative						
Escherichia coli ATCC25922	-	-	-	-	-	7.20±0.50
Klebsiela pneumoniae	-	-	-	-	0.80±0.33	09.00±0.63
Pseudomonas aereginosa ATCC27853	-	-	-	-	-	6.50±0.20
Proteus vulgaris ATCC6897	-	-	-	-	-	7.50±0.95
Fungi						
Aspergillus flavus ATCC10239	-	-	7.0±0.33	8.0±0.20	12.0±0.25	16.50±1.00
Aspergillus niger	-	-	-	07.0±0.45	8.50±0.60	10.50±0.70
Candida albicans	-	-	-	-	-	08.50±0.27

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