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Chemical composition and biological activities of Algerian *Santolina africana* essential oil

Lamia Boudjedjou[a,b], Messaoud Ramdani[a], Azzeddine Zeraib[c,d], Tarek Benmeddour[b,c], Azzedine Fercha[d,*]

[a] Laboratory of Natural Resources Valorization, Setif 1 University, 19000 Setif, Algeria
[b] Department of Life and Natural Sciences, Mohamed Khider University, 7000 Biskra, Algeria
[c] Laboratory of Genetics, Biotechnology and Valorization of Bio-resources, Mohamed Khider University, 7000 Biskra, Algeria
[d] Department of Biology, Abbes Laghrour University, 40000 Khenchela, Algeria

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ABSTRACT

The chemical composition and the biological activities of the essential oil (EO) extracted by hydrodistillation from the aerial parts of Algerian population of *Santolina africana* have been studied. *S. Africana* EO was chemically characterized by means of GC–MS. Forty-six compounds were identified representing ~92% of the total oil. β-pinene (11.65%), germacrene (10.33%), 1,8-Cineole (9.46%) and sabinene (7.18%) were the major components. The EO antioxidant activity was evaluated by DPPH assay. All EO concentrations showed considerable scavenging ability on DPPH radicals ($IC_{50} < 1.13\ mg.mL^{-1}$). The antibacterial screening of EO alone and in combination with three conventional antibiotics (ABs) was made by way of disc diffusion against four standard strains. The interactive effects between EO and ABs were evaluated using the One-way ANOVA analysis. The results showed a remarkable antibacterial activity of *S. africana* EO against *Staphylococcus aureus* (29 mm), *Escherichia coli* (29.27 mm) and *Bacillus subtilis* (15 mm). Excluding the antagonistic effect observed with cefazolin, the combined application of *S. africana* EO with the other two antibiotics led to synergistic and additive effects. These findings show the potential use of *S. africana* EO as an interesting source of potent antioxidant and antibiotic components useful for medicine.

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Introduction

The search for new natural products with promising biological activities continues to draw attention. Medicinal plants have long been a source of molecules with therapeutic potential, and nowadays still represent an important pool for the identification of new innovative drugs [3,37].

Abbreviations: EO, essential oil; AB, antibiotic; DPPH, 2,2-diphenyl-1-picrylhydrazyl; AsA, ascorbic acid; ANOVA, analysis of variance; RI, retention index; MHI, Mueller Hinton medium; ROS, reactive oxygen species; GEN, gentamicin; AMX, amoxicillin; CFZ, cefazolin.

* Corresponding author.
E-mail address: ferchazzed@yahoo.fr (A. Fercha).

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Oxidative stress refers to the imbalance between reactive oxygen species (ROS) production and the organism's ability to counteract the harmful effects of these species by antioxidants [32]. ROS may bind to vital components of cells, causing extensive damage to various cellular components including proteins, lipids, and DNA. ROS are involved in the pathogenesis of many diseases including cancer, Parkinson, Alzheimer, atherosclerosis, heart failure, myocardial infarction, vitiligo, and chronic fatigue syndrome (for review, see [14]). In recent years, several epidemiological studies have confirmed that intake of exogenous antioxidants is effective in preventing or suppressing such diseases [33], therefore, there is a growing interest in natural molecules with antioxidant activity that might help preventing or decreasing oxidative damages without exerting harmful side effects [26].

The increase of microbial resistance to antibiotics threatens public health worldwide as it reduces the effectiveness of treatments and increases morbidity, mortality, and costs for healthcare systems. Therefore, there is a pressing need to develop new and innovative antimicrobial agents [22]. Among the potential sources of new agents, plants have long been investigated. A major group of plant antimicrobial compounds is represented by essential oils (EOs), which are complex mixtures of volatile secondary metabolites [22]. Since the biological activities of EOs are composition-dependent [5], no particular resistance or adaptation to EOs has been described to date. Besides antimicrobial activity, EOs and their components can act in synergy with some antibiotics, enhancing their antimicrobial activity [23,26].

The genus *Santolina* (*Asteraceae*), widely represented in the Mediterranean and North Africa flora, is a taxonomically complex group of species used in traditional medicine (https://en.wikipedia.org/wiki/Santolina). Flowers and leaves of different *Santolina* species have been used in the treatment of liver and digestive disorders [34]. *Santolina africana* is an endemic shrub of North Africa (Algeria, Tunisia, and Morocco) that naturally grows in steppe or forest-steppe areas [28]. In folk medicine, *S. africana* has been used for its hypoglycemic effect as well as for the treatment of stomacal pain [7]. Traditionally the inflorescences of *S. africana* are mixed with honey and used for the treatment of the cardialgia ulcer and stomacal pains [8]. Although the various members of this genus have been widely studied [11,25,34], *S. africana* has not been hitherto investigated in detail since only a few studies have been concerned with the chemical and biological properties of this species [4,12,13,19,38]. Therefore, the purpose of the present study was to determine the chemical composition and to evaluate the biological activities of *S. africana* EO as new potential source of natural antibiotic and antioxidant components.

Materials and methods

Plant material and essential oil extraction

Fresh aerial parts of *Santolina africana* Jord. and Fourr., were collected at the flowering stage in May 2014 from Ichemoul (1365 m of altitude), province of Batna, located 350 km South-East of Algiers, with a supra-Mediterranean climate and precipitations superior to 450 mm. Voucher specimens were deposited in the Herbarium of the Natural Resources Valorization Laboratory, Setif 1 University.

The EO of *S. africana* was obtained by hydrodistillation with a Clevenger-type apparatus for 3 h [39]. The yield of EO was averaged over three experiments and reported as yield of dry weight of the plant material. The extracted oil was dried over anhydrous sodium sulphate and stored at 4 °C until used for the analysis.

Essential oil analysis

The analysis of the *S. africana* EO was performed with a GC–MS system using a BR-5 ms (5% phenyl methyl siloxane) capillary column (30 m length × 0.32 mm column id, 0.25 µm film thickness) coupled with a Thermo DSQ II quadrupole mass spectrometer (EI of 70 eV). The carrier gas was helium at a flow rate of 1.2 mL.min^{-1}; the injection volume was 0.1 µL; injector split mode 1:100. The initial temperature of the column was kept at 70 °C for 1 min and then increased at 10 °C.min^{-1} up to 300 °C. The mass spectrum of each compound was recorded between 40 and 500 Da (m/z^{-1} equivalent unit). Data acquisition and processing were carried out using Xcalibur data system ver. 2.0 [1]. Retention indices (RI) were calculated for all volatile components using the homologous series of n-alkanes (C6–C18).

DPPH radical scavenging assay

The DPPH radical scavenging assay was performed as described by Blois [9] with slight modifications. An aliquot (100 µL) of an extract or ascorbic acid at the different concentration was mixed with 1.9 mL of MeOH DPPH solution (4%). The mixture was left in the dark for 30 min before reading the absorbance at 517 nm with MeOH DPPH solution as blank. The percent DPPH scavenging effect was calculated using the following equation:

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

where A_0 is the absorbance of the control, A_1 is the absorbance of the sample. The IC_{50} values were calculated using the equation obtained from the linear regression analysis.

Antibacterial activity assessment

Antibacterial studies have been evaluated by the method of disc-diffusion. Four bacterial strains were tested in this study, two Gram-positive bacteria namely, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 21332, and two Gram-negative bacteria namely, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853.

The bacterial strains were first grown on Mueller Hinton medium (MHI) at 37 °C for 24 h prior to seeding on to the nutrient agar. The inoculums of the respective bacteria were streaked onto MHI agar plates using a sterile swab. While a serial dilution of EO (pure, 1/2, 1/4, 1/8 and 1/16) was performed to obtain the smallest inhibition zone, the selected EO concentrations for each strain were used in combination with conventional antibiotics (ABs).

Under aseptic conditions, 10 μL of diluted EOs were applied to sterilized Whatman filter paper discs N° 3 (6 mm diameter) and placed on the agar surface. Before incubating at 37 °C, plates were refrigerated at 4 °C for 2–3 h to allow diffusion of the oil. After incubation period, the diameters of the growth inhibition zones were measured to the nearest mm. All the tests were performed in triplicate and the means were calculated as final results. The sensitivity to the EO was classified by the diameter of the inhibition halos as follows: not sensitive (–) for diameter less than 8 mm; sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm and extremely sensitive (+++) for diameter larger than 20 mm [27].

Combination test of EO and ABs

Combined antibacterial action in vitro of EO and gentamicin (10 μg.disc^{-1}), amoxicillin (25 μg.disc^{-1}) and cefazolin (30 μg.disc^{-1}) was assessed following the method of disc-diffusion as described by Moussaoui and Alaoui [23]. First, we evaluated the antibacterial activity of the three antibiotics, which are in the form of ready to use discs; therefore, the antibacterial activity of antibiotics added by 10 μL of selected dilution of *S. africana* EO was evaluated. The interaction between the EO and ABs can produce a synergistic, additive, or antagonistic effect. If the value of the EO/AB combination is significantly higher ($P < 0.05$) than the sum of the individual values, it is a synergistic effect; but an additive effect if it is equal ($P \geq 0.05$). The antagonistic effect, in contrast, occurs when the value of each of the two treatments separately EO/AB is significantly greater than the value of their mixture [31].

Statistical analysis

All data are expressed as mean ± SD, $n = 3$. Statistical significance was analyzed by the one-way ANOVA and post-hoc tests by using the statistical software package STATISTICA 8.0 [15]. Differences with $P < 0.05$ were considered significant.

Results and discussion

Essential oil composition

On hydrodistillation, the aerial parts of *S. africana* yielded 0.76% (v/w) of yellowish essential oil. Our results are in good agreement with those of Lmachraa et al. [19] and Zaiter et al. [38], which by analyzing the same species they obtained yield of 0.86% and 0.8% (v/w) respectively. GC–MS analysis of the essential oil resulted in the identification of forty-six compounds constituting about 91.98% of the total oil (Table 1).

The results indicated that the *S. africana* EO was a complex mixture of sesquiterpene hydrocarbons (25.2%), monoterpene hydrocarbons (24.89%), oxygenated sesquiterpenes (22.01%) and oxygenated monoterpenes (19.8%). In agreement, previous studies have confirmed the prevalence of hydrocarbon fraction compared to the oxygenated components in EOs isolated from many species of the genus *Santolina* [12,18,38].

The major constituents identified in our sample were β-pinene (11.65%), germacrene D (10.33%), 1,8-cineole (9.46%), sabinene (7.18%), hedycaryol (5.79%), α-bisabolol (5.63%) and α-curcumene (4.5%). Compared to previous reports, there are fewer similarities with the oil of the same species collected in Algeria, which characterized by prevalence of β-pinene (12.78%), 1,8-cineol (10.02%), curcumene (7.96%), and myrcene (6.94%) [38], and to the oil of *S. rosmarinifolia* from Algeria, in which the main components are: germacrene-D (30.2%), β-myrcene (12%), tricosane (10.6%), β-pinene (10.1%), sabinene (7.0%) and pentacosane (6.7%) [11]. However, our results are different from those obtained for oils extracted from the same species collected from different areas of North Africa [4,12,19]. For instance, the main compounds identified in Tunisian *S. africana* were terpinen-4-ol (54.96%), α- terpineol (14.06%), borneol (8.37%), *rans*-chrysanthenol (3.65%), camphor (3.61%), and 1,8-cineole (3.59%) [4]. While the essential oil of Moroccan sample was dominated by camphor (54.30%), borneol (17.24%), bornyl acetate (8.61%) and 1,8-Cineole (5.27%) [19]; the main constituents of the essential oil of Algerian *S. africana* flowers were acenaphtane (25.23%), calarene (21.54%), ocimene (17.44%) [12]. These findings support the suggestion that studying the EOs composition of a larger number of populations of this species (as others species of the genus *Santolina*) can be helpful in chemiotaxonomy [30].

Antioxidant activity

As displayed in Table 2, the antioxidant activity values for the investigated EO concentrations (from 0.28 to 18 mg.mL^{-1}) varied between 23.3% to 92.9% with IC_{50} inferior to 1.13 mg.mL^{-1}, but it has performed a lower potent to scavenging the

Table 1
Chemical composition of essential oil from Algerian *S. africana*.

Peak no.	Compound name	Retention index (RI)	Percentage (%)
1	(Z)-Hex-3-en-1-ol	857	0.08
2	Santolinatriene	908	0.11
3	α-thujene	924	0.11
4	α-Pinene	932	1.23
5	Camphene	946	0.42
6	Verbinene	948	2.23
7	**Sabinene**	968	**7.18**
8	**β-pinene**	978	**11.65**
9	α-Terpinene	1021	0.2
10	*o*-Cymene	1022	0.28
11	**1,8-Cineole**	1031	**9.46**
12	γ-Terpinene	1062	0.32
13	Terpinolene	1086	1.16
14	(z)-p-menth-2-en-1-ol	1092	0.76
15	Chrysanthenone	1124	0.48
16	Camphor	1140	2.17
17	cis-β-terpineol	1140	0.49
18	cis-Verbenol	1140	1.97
19	Borneol	1147	0.9
20	Terpinene-4-ol	1151	1.35
21	α-terpineol	1161	1.33
22	Myrtenol	1198	0.27
23	Myrtenyl acetate	1324	0.1
24	p-Mentha-1,4-dien-7-ol	1325	0.13
25	δ-Elemene	1335	3.68
26	Eugenol	1360	0.39
27	α-Copaene	1374	0.5
28	(Z) Caryophyllene	1408	0.64
29	cis-α-Bergamotene	1411	0.56
30	α-Caryophyllene	1415	0.2
31	α-Guaiene	1437	0.25
32	α-Curcumene	1470	4.5
33	**Germacrene D**	1480	**10.33**
34	γ-Muurolene	1478	0.18
35	δ-cadinene	1523	0.49
36	cis-α-Bisabolene	1527	3.87
37	**Hedycaryol**	1540	**5.79**
38	Spathulenol	1578	3.65
39	Caryophyllene oxide	1582	0.78
40	Isoaromadendrene epoxide	1579	0.31
41	Globulol	1590	0.32
42	α–epi-Cadinol	1640	0.32
43	Cubenol	1645	0.28
44	β-Eudesmol	1665	1.3
45	Eudesm-7(11)-en-4-ol	1672	3.63
46	**α-Bisabolol**	1685	**5.63**
Total identified			**91.98**
Yield (g/100 g dry weight)			**0.76**
Monoterpene hydrocarbons			**24.89**
Oxygenated monoterpenes			**19.8**
Sesquiterpene hydrocarbons			**25.2**
Oxygenated sesquiterpenes			**22.01**

The chemical groups of identified compounds with corresponding portion in analyzed essential oil are presented in bold.

Table 2
Antioxidant activity of *S. africana* essential oil and ascorbic acid using DPPH radical.

Antioxidant activity of EO		Antioxidant activity of AsA	
Conc. of EO (mg.mL^{-1})	P. inhibition (%)	Conc. of AsA (µg.mL^{-1})	P. inhibition (%)
18	92.90 ± 0.60	100	97.48 ± 0.22
9	83.47 ± 3.71	50	97.26 ± 0.11
4.5	71.43 ± 4.96	25	87.38 ± 0.06
2.25	68.77 ± 2.61	12.5	61.45 ± 0.17
1.13	55.30 ± 2.65	6.25	25.71 ± 0.11
0.56	37.70 ± 2.63	3.12	12.55 ± 0.06
0.28	23.30 ± 0.70		

EO, essential oil, P., percentage; Conc., concentration; AsA, ascorbic acid.

Table 3
Antibacterial activity of *S. africana* essential oil. Values were expressed as mean ± SD of growth inhibition zone diameters (mm).

Strains tested	EO concentration pure	1/2	1/4	1/8	1/16
E. coli	29.67 ± 0.58	25 ± 1.0	21.67 ± 0.58	**11.67 ± 1.52**[a]	10 ± 1.0
S. aureus	29 ± 1.0	24.33 ± 0.58	15 ± 1.0	**12.33 ± 0.58**[a]	8.67 ± 0.57
P. aeruginosa	13.33 ± 0.58	**7.67 ± 0.58**[a]	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
B. subtilis	15 ± 1.0	14 ± 1.0	11.67 ± 0.58	**8.0 ± 1.0**[a]	6.0 ± 0.0

[a] The results illustrated in bold correspond to the concentrations selected for the test of the combination of *S. africana* EO with the ABs.

Table 4
Antibacterial activity of conventional antibiotics alone and in combination with *S. africana* essential oil. Values were expressed as mean ± SD of growth inhibition zone diameters (mm).

Test substance	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
GEN	28 ± 1.0	32 ± 1.0	19.67 ± 0.58	22.83 ± 0.29
AMX	26 ± 1.0	29.67 ± 0.58	6.0 ± 0.0	15.33 ± 0.58
CFZ	42 ± 1.0	40.67 ± 0.58	6.0 ± 0.0	28 ± 1.0
GEN + EO	34.67 ± 0.58	49.33 ± 0.58	24.67 ± 0.58	28.33 ± 0.58
AMX + EO	31.67 ± 0.58	36.67 ± 0.58	6.0 ± 0.0	15.33 ± 0.58
CFZ + EO	38.33 ± 0.58	38.67 ± 0.58	6.0 ± 0.0	25.67 ± 0.58
P values				
EO/GEN	0.46 ns	0.027*	0.002**	0.0118*
EO/AMX	1.0 ns	0.39 ns	0.007**	0.013*
EO/CFZ	0.0006***	0.0002***	0.007**	0.025*
Combination effect				
EO/Gent	Additive	Synergistic	Synergistic	Synergistic
EO/AMX	Additive	Additive	Antagonistic	Antagonistic
EO/CFZ	Antagonistic	Antagonistic	Antagonistic	Antagonistic

GEN, gentamicin; AMX, amoxicillin; CFZ, cefazolin; EO, essential oil.
ns, no significant.
* Significant ($P < 0.05$).
** Highly significant ($P < 0.01$).
*** Very highly significant ($P < 0.001$).

DPPH as compared to the AsA ($IC_{50} < 12.5\,\mu g.mL^{-1}$). Similar results were reported in studies of antioxidant effect of EOs from *S. africana* flowers [12], *S. canescens* [34], and *S. chamaecyparissus* aerial parts [25]. Nevertheless, the antioxidant effect of the tested EO is very important when comparing it with the results of the aforementioned reports. Generally, the antioxidant activity of essential oils is related to their major compounds [6], and their high hydrogen-donating capacity [21]. In line with this, the main components of the evaluated EO, such as α-pinene, β-pinene, sabinene, 1,8-cineole, germacrene D, hedycaryol, spathulenol, terpinene-4-ol, α-terpineol, camphor, appear to play an important role as antioxidant agents [2,36].

Antibacterial activity

The antibacterial activity of *S. africana* EO against the tested microorganisms was qualitatively assessed by the inhibition zone diameters. Interestingly, our results indicate that the tested oil exhibited a significant antibacterial activity against all the tested bacteria, but in a concentration dependent-manner (Table 3). Of all the tested concentrations, the pure oil exhibited the strongest effect. The highest inhibition zone diameters were recorded against *E. coli* (29.27 mm) and *S. aureus* (29 mm) respectively, while the lowest inhibition zone diameter was recorded against *P. aeruginosa* (13.33 mm).

According to the literature survey on the antibacterial activity of *S. africana* EOs, only one study has been published [12]. Despite the difference observed in the chemical composition of our EOs, the authors reported that the inhibition zone diameters produced by highest EO ($8000\,\mu g.mL^{-1}$) are 20.15 mm for *B. subtilis*, 19.50 mm *S. aureus*, 7.20 mm for *E. coli* and 6.50 mm for *P. aereginosa*. Similarly, Liu et al. [18] have reported that *S. corsica* EO exhibit a strong activity against *S. aureus,* but remain inactive against *E. coli* and *P. aeruginosa*. However, the EOs of *S. rosmarinifolia* [11] and *S. chamaecyparissus* [30] inhibited the growth of *E. coli* by producing inhibition zone diameters of 25 mm and 15 mm, respectively. This good antibacterial activity is possibly due to the synergistic effect of the main components of *S. africana* EO such as β-myrcene, β-pinene and sabinene [11].

The test of antibiotics susceptibility showed that the activities of conventional antibiotics varied among the strains (Table 4), and most of them were efficient against *E. coli, S. aureus* and *B. subtilis,* while *P. aeruginosa* exhibited a resistance to Amoxicillin and Cefazolin discs.

In agreement, *P. aeruginosa* displays high intrinsic resistance to a wide variety of antibiotics. This is largely due to the reduced penetration of antibiotics across the outer membrane of *P. aeruginosa*, which enables secondary adaptive resistance mechanisms to work more efficiently, including increased efflux and enzymatic antibiotic modifications (e.g. β-lactamase) [10].

Combination effect of EO and ABs

The antibacterial efficacy of *S. africana* EO in combination with the selected ABs was investigated for the first time (Table 4). The combined effects were evaluated in terms of increased or decreased inhibition zone diameters induced by ABs and EO when compared with each alone. Combined addition of EO/GEN against *S. aureus, P. aeruginosa* and *B. subtilis* induced synergistic effects. In contrast, the combined additions of EO/CFZ against all tested strains, and EO/AMX against *P. aeruginosa* and *B. subtilis*, have induced antagonistic effects. According to the results, *E. coli* was not affected by the combined addition of both EO/GEN and EO/AMX, while the latter gave the same result by applying it to *S. aureus,* which means that the tested combinations have induced an additive effect. In other words, the combination of *S. africana* EO with β-lactam antibiotics showed antagonistic and additive effects, while their combination with aminoglycosides antibiotics showed additive and synergistic effect.

Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and bioavailability of one or more substances of an extract. A particular synergistic effect can occur when antibiotics are combined with an agent that antagonizes bacterial resistance mechanisms [35]. Consistent with this, the hydrophobic nature of S. *africana* EO allows it to penetrate microbial cells and presumably cause rupture of the outer membrane and disruption cellular functions [20]. This can increase the permeability of bacterial cell membranes [24], and facilitates the penetration of gentamicin into the cell [29]. Moreover, interaction between different compounds may lead to changes in structural conformation, resulting in the reduction of the inhibitory activity [17], which can explain the additive effect observed with the combination of EO/GEN against *E. coli.* These findings are in agreement with the results of Rosato et al. [29], on synergistic action of certain combinations of gentamicin and essential oils.

The antagonistic and additive effects result from the use of compounds acting on the same target of the microorganism [35], which explains our results regarding the interaction between *S. africana* EO and beta-lactam antibiotics, which seem to act both on the cell membrane.

In the present study, the choice of Gram-negative or Gram-positive bacterial strains seem to be not decisively significant for the research of the combined effect of EOs with antibiotics, which means that the proper EO/AB association act as equally stronger or weaker against all Gram-positive and Gram-negative bacterial strains. This presumed that the outer membrane of the Gram-negative bacteria is not a predominant factor of their resistance [16].

Conclusion

This paper presents an interesting analysis of the chemical composition of aerial part essential oil of *Santolina africana* growing wild in Algeria. Among the forty-six components identified, β-pinene, germacrene D and 1,8-cineole were the main components. *S. africana* EO showed a higher antioxidant activity compared to the oils of species from the same genus. The antibacterial activity of *S. africana* oil displayed significant effect among the different bacterial strains, but remained lower than the activities of the standard antibiotics. The combination of this oil with conventional antibiotics has significant potential to improve the antimicrobial activity and to reduce the antibiotic concentration. The oil acts in synergy with antibiotics probably by binding to targets different from those of the antibiotics.

Conflict of interest

The authors declare no conflicts of interest.

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