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Chemical Composition and Antioxidant Activity of *Solenostemma oleifolium* Essential Oil From Southern Algeria

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Abstract

Introduction: *Solenostemma oleifolium* is a species that grows in extremely dry conditions. It is widespread at the foot of cliffs and in rocky areas. It is a medicinal plant used for the treatment of diabetes, respiratory disorders, rheumatism, stomach pain, urinary tract infections and febrifuge. As a part of this research program on natural compounds with antioxidant properties, the main objective of this study was to determine the chemical composition and the antioxidant activity of essential oil of *S. oleifolium*.

Material and Methods: In this study, the aerial parts of the plant were hydrodistilled in a Clevenger-type apparatus. The isolated essential oil was analyzed using gas chromatography mass spectrometry (GC-MS). The antioxidant activity of the essential oil was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing power (FRAP).

Results: The essential oil of *S. oleifolium* was principally characterized by oxygenated monoterpenes (94.3%) represented by linalool (59.0%), α-terpineol (14.5%) and geraniol (12.4%), followed by small amounts of nerol (3.7%) and piperitone (3.6%). The results of the antioxidant activity of essential oil showed an interesting propriety in the quenching of DPPH radical, with an IC50 of 3.3 g/L. On the other hand, essential oil showed the presence of the reductive effect, which increased with an increase in concentration.

Conclusions: The results of this research showed that the *S. oleifolium* essential oil presented an interesting antioxidant property. Actually, it could be proposed as a new potential source of natural additives for the food or pharmaceutical industries.

Keywords: Essential Oil, Antioxidant Activity, *Solenostemma Oleifolium*, DPPH, FRAP

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Introduction

Antioxidants are in the form of food supplements or in the diet, they are essential to prevent many diseases. They are substances that act against oxidation, a process that leads to the formation of free radicals in the body. These free radicals are harmful and can, for example, contribute to the development of different types of cancers. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are 2 food additives that protect fatty foods from oxidation.[1] They are also found in cosmetics for the same reason. Studies have shown that both these synthetic antioxidants affect the nervous system and increase the risk of allergies and certain cancers.[2] Many studies are increasingly interested in the therapeutic effects of naturally occurring antioxidants which are supposed to protect living organisms from oxidative damages.[3]

The use of essential oils is expanding in several sectors and in many industrial fields. Several essential oils have been attributed to good antioxidant properties, which can be exploited to protect other materials, such as food, from rancidity or to prevent the oxidative stress that contributes to the appearance of degenerative diseases.[4] Essential oils and their components are known to possess antioxidant activities.[4] The essential oil of basil, cinnamon, clove, nutmeg, oregano, and thyme possesses antioxidant properties due to their major terpenes such as thymol and carvacrol.[5] In addition, the essential oil of *Melissa officinalis*, whose main components were terpenoids such as neral, geranial, citronellal, isomenthone, and menthone showed free radical scavenging activity.[6] *Solenostemma oleifolium* belongs to the family of Asclepiadaceae and grows in the desert areas of Mali, Libya and Egypt. It is also widely distributed in Algerian Sahara. This species grows in dry condition areas and is widespread at the foot of the cliffs and in rocky areas. It is frequently used in traditional medicine against rheumatism, stomach pain,

urinary tract infections, diabetes and respiratory disorders. *S. oleifolium* is a shrub that can grow up to 60 cm tall. The leaves are opposite, decussate, simple and entire. The petiole is 1 to 2 mm long; blade lanceolate. The flowers are bisexual, regular, 5-merous, white, fragrant; pedicel 2–5 mm long; calyx lobes oblong, corolla 3 mm long, apex acute. This plant is known as vernacular names: Ardjel.[7,8] As part of the program of this research on natural compounds with antioxidant property, the main objective of this study was to determine the chemical composition and the antioxidant activity of the essential oil of *S. oleifolium* growing in south Algeria.

Materials and Methods

Plant Material and Extraction of the Oils

Aerial parts of *S. oleifolium* were collected from Tamanrasset area (Algeria) 25°48'32.87"N 8°08'25.53"E, at 1276 m altitude during the month of April 2018. The collected plants were identified by Pr. Noury Benabadji, Laboratory of Ecology and Ecosystem Management, University of Tlemcen, (Algeria). Voucher specimens were deposited in the herbarium of the University of Tlemcen (CI.SO1.04.18). Essential oil was obtained from dry aerial parts (400 g) by hydrodistillation (in 4000 mL distilled water) for 4 hours using a Clevenger-type apparatus. The obtained essential oil was pale yellow in color with a yield (w/w) of 0.5%.

Gas Chromatography

Gas Chromatography (GC) analyses were carried out using an HP Agilent 6890 gas chromatograph (FID) equipped with an HP-5MS (HP Agilent) fused silica capillary column, 30 m × 0.25 mm, 0.25 µm film thickness, which was perfused with helium at a flow-rate of 1 mL/min and operated with a split ratio of 10, essential oil injection volume was 0.2 µL without dilution whereas the injection temperature was set at 250°C. The column temperature was programmed isothermally at 80°C for 2 minutes, after which the temperature was increased to 240°C at a rate of 15°C/min.

Retention indices (RI) of the compounds were determined relative to the retention times of the series of n-alkanes (C_5-C_{30}) with linear interpolation, using the Van den Dool and Kratz equation[8] and software from HP Agilent 6890. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) spectra were obtained using the following conditions: carrier gas helium; flow rate 0.3 mL/min; split-less mode. Same as GC/FID, essential oil injection volume was 0.2 µL without dilution whereas the injection temperature was set at 250°C. The oven temperature program was set at 60°C for 8 minutes increased at 2°C/min to 250°C and held at 250°C for 15 minutes; and the electron impact mass spectral analysis was carried out at ionization energy of 70 eV. Constituents were tentatively identified by comparison of their GC RI, determined with reference to a homologous series of C_5–C_{30} n-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries).

DPPH Free Radical Scavenging Assay

The free radical-scavenging activity of essential oil was measured using DPPH as described earlier.[10] Different concentrations of essential oil (0.01–30 g/L) were mixed with 1 mL of 90 µM DPPH solution and filled up with 95% MeOH to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 hour at room temperature, against ascorbic acid as a positive control. For each sample, three replicates were recorded. The absorbance was read against a blank at 515 nm. The RSC in percent was calculated by the following equation:

$$I\% = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] x100\%$$

Accordingly, A_{blank} is the absorbance of the control reaction (without oils), and A_{sample} is the absorbance in the presence of essential oils. From the obtained RSC values, the IC_{50} values, which represented the concentrations of the essential oil caused 50% neutralization, were determined by linear regression analysis. The essential oil samples were analyzed in three replications.

Reducing Power

The reducing power of aerial parts essential oil was determined as described earlier.[11] Accordingly, 1 mL of different concentrations of essential oil (0.5, 0.75, 1.0, 1.5 and 2.0 g/L) in methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue–green colour was measured at 700 nm. The EC_{50} value (g/L) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control. Tests were carried out in triplicate.

Statistical Analysis

All data are presented as the mean ± standard deviation of three replicates. Correlation analyses of radical scavenging activities and FRAP were carried out using Microsoft Office Excel 2016.

Results

Chemical Composition of Essential Oil

The total number of chemical components identified in essential oil was twenty-three, accounting for 97.8% of the total oil composition. Among them, 17 monoterpenes, 5 sesquiterpenes and 1 non-terpenic compound were identified (Table 1). The chemical composition of *S.*

oleifolium essential oil was represented practically only by oxygenated monoterpenes (95.3%). The other compounds such as monoterpene hydrocarbons (1.7%), sesquiterpene hydrocarbons (0.2%), oxygenated sesquiterpenes (0.5%) and no-terpenic compounds (0.1) were present in very small amounts (Table 1). The main components of this oil were linalool (59.0%), α-terpineol (14.5%) and geraniol (12.4%), followed by appreciable amounts of nerol (3.7%) and piperitone (3.6%) (Table 1).

Table 1. Chemical Composition of *Solenostemma oleifolium* Essential Oil

N°[a]	Components	Ria[b]	Ria[c]	EO %[d]	Identification[e]
1	Hexanal	805	804	0.1	RI, MS
2	α-Pinene	932	930	0.1	RI, MS
3	β-Myrcene	991	992	0.3	RI, MS
4	Limonene	1027	1027	0.4	RI, MS
5	(Z)-β-ocimène	1037	1040	0.2	RI, MS
6	(E)-β-Ocimene	1050	1050	0.4	RI, MS
7	(E)-Linalool oxide	1074	1073	0.2	RI, MS
8	α-Terpinolene	1088	1087	0.3	RI, MS
9	Linalool	1113	1115	59.0	RI, MS
10	Camphor	1145	1147	0.1	RI, MS
11	Nerol oxide	1153	1156	0.2	RI, MS
12	Terpinen-4-ol	1179	1180	0.2	RI, MS
13	α-Terpineol	1200	1202	14.5	RI, MS
14	Nerol	1228	1230	3.7	RI, MS
15	Piperitone	1260	1259	3.6	RI, MS
16	Geraniol	1265	1268	12.4	RI, MS
17	α-Citral	1277	1280	0.1	RI, MS
18	Thymol	1303	1301	1.3	RI, MS
19	(E)-β-Damascenone	1385	1383	0.2	RI, MS
20	(E)-β-Caryophylene	1418	1417	0.2	RI, MS
21	(E)-Geranyl acetone	1456	1454	0.1	RI, MS
22	Caryophyllene oxide	1581	1582	0.1	RI, MS
23	β-Eudesmol	1653	1653	0.1	RI, MS
% Identification				**97.8**	
% Monoterpene hydrocarbons				0.1	
% Oxygenated monoterpenes				95.3	
% Sesquiterpene hydrocarbons				0.2	
% Oxygenated sesquiterpenes				0.5	
%No-terpenic compounds				1.4	

[a] Order of elution is given on apolar column (DB-5).
[b] Retention indices of literature on the apolar column (lRIa) reported from Konig et al.; 2001 and National Institute of Standards and Technology, 1999.
[c] Retention indices on the apolar DB-5 column (RIa).
[d] Percentage calculated by GC-FID on non-polar DB-5 capillary column.
Abbreviations: RI, retention indices; MS, Mass spectra in electronic impact mode.

Antioxidant Activity

The antioxidant activity was evaluated using the radical scavenging activity and reducing power methods, using ascorbic acid as positive control. Free radical scavenging capacity of the essential oil measured by DPPH assay is shown in Table 2. The essential oil was able to reduce the stable free radical DPPH to the yellow colored diphenylpicrylhydrazine with an IC_{50} value of 23.3 g/L, which was lower to positive control (ascorbic acid IC_{50} = 0.047 g/L).

The reducing power was used to measure the antioxidant capability of essential oil. Reductive ability was determined by monitoring the Fe^{3+} to Fe^{2+} transformation in the presence of the essential oil. Figure 1 depicts the reducing power of *S. oleifolium* essential oil. According to the results shown in Figure 1, essential oil showed the presence of the reductive effects, with EC_{50} of 36.12 g/L which was slightly lower to positive control (EC_{50} = 45.03 g/L).

Discussion

In this study, *S. oleifolium* essential oil composition and its antioxidant activity were evaluated. According to the literature survey of this research, there is only one report of the chemical composition of the essential oil of *S. oleifolium*. The chemical composition of this specie from Mascara (Algeria) showed that the essential oil was dominated by thujone (43,7%), trans-sabinene hydrate (10.4%), eugenol (8.4%), 1.8-cineole (7.9%), limonene (4.1%) and α-pinene (3,57%).[11] The chemical composition of essential oil studied by Chouitah et al[12] and this study showed significant differences in their chemical compositions. This difference may be explained by the differences in ecological conditions, altitude and climate. However, genetic differences are much higher than those caused by varying environmental conditions, which causes the occurrence of several chemical races or chemotypes within the same species.[13] The results of this study on *S. oleifolium* revealed the existence of many components that are used in the pharmaceutical and cosmetic industries. For example, linalool is a monoterpene compound which exists in many plants. Linalool displays antimicrobial, anti-inflammatory, analgesic, antihyperalgesic and antioxidant activities.[14] Geraniol is widely used in cosmetics; it is used as tonic. Its action is essentially felt on the skin. It can also be used as a masking agent, that is to say it masks the odors of other components of the cosmetic.[15] The antioxidant properties of *S. oleifolium* essential oil are probably related to their active components. Indeed, linalool, is a terpenoid alcohol, with a floral and fresh smell. It is found in a majority of essential oils such as lavender and mint. It has been proven that linalool reduces the levels of nuclear factor-erythroid.[16] In

Table 2. DPPH radical-scavenging activity of *Solenostemma oleifolium* essential oil

Sources		Antioxidant activity					IC_{50}
Essential oil	Concentration (g/L)	0.03	0.6	2.0	4.0	6.0	3.3
	DPPH Radical [%]	6.4±1.3	27.3±1.3	42.7±1.6	60.1±2.3	77.7±3.1	
Ascorbic acid	Concentration (g/L)	0.04	0.05	0.06	0.08	0.2	0.047
	DPPH Radical [%]	39.4±3.2	51.0±4.3	68.6±5.1	97.9±6.7	98.4±6.1	

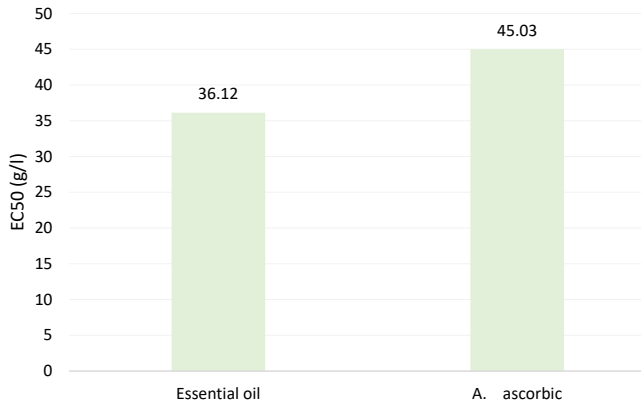

Figure 1. Reducing Power Assay Of Essential Oil and A. ascorbic.

addition, linalool was found to be effective as an antioxidant in guinea pig brains injected with H_2O_2.[14] As it was previously mentioned, α-terpineol proved to possess a potent antioxidant activity against free radicals causing injury.[17] Moreover, α-terpineol exerted cytostatic activities against many human cancer cell lines such as leukemia, ovarian, breast adenocarcinoma and chronic myeloid leukemia.[18] Bicas et al[19] showed that α-terpineol had an antioxidant potential similar to BHA (butylated hydroxyanisole) and possess a potential protective activity in foodstuffs. The antioxidant activity of geraniol has been reported by several authors.[20,21] Geraniol proved to be a good scavenger of DPPH free radical with IC_{50} value of 663 nmol. Geraniol also exerted anti-tumor activity against various cancer cells both *in vitro* and *in vivo* tests.[22,23] On the other hand, the antioxidant activity is probably due to the synergy between components of the oil. It has been proved that the combination of thymol and linalool or limonene and caryophyllene enhances antioxidant activity.[24,25]

Conclusions

In the current study, the chemical composition of the essential oil of *S. oleifolium* from Algeria has been investigated for the first time. The studied chemical composition was mainly composed of oxygenated monoterpenes such as linalool and geraniol, which constitute a range of interesting bioactive compounds. The results of antioxidant activity showed that the essential oil of *S. oleifolium* presented a good antioxidant effect with both tests. This activity may be partly due to major components or to the effect caused by the interaction of all constituents present in the oil. However, further research is necessary to study whether this essential oil can be used in the food or pharmaceutical industries or not.

Authors' Contributions

IC prepared the samples and analyzed the chemical composition; MAD wrote the manuscript; FD performed the antioxidant activities test; DM analyzed the results; and HC supervised the project.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

Ethical Approval

Not applicable.

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