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Research Article

Secondary metabolites of *Santolina africana*: chemical profiles and assessment of biological activities

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Abstract: Chemicals and antibiotics are serious problems that cause the resistance of bacteria and the persistence of chemical residues in food. These chemical products affect human health and promote diseases. Therefore, the use of natural resources, especially plants, appears as an alternative to avoid the harmful impacts of such products. Plant active substances such as essential oils, alkaloids and phenols are of great interest to scientists and have been studied for their biological activities. Essential oils (Eos) from the stems of *Santolina africana* were extracted by hydrodistillation and analyzed by Gaz Chromatography/ Mass Spectrometry (GC-MS). The antioxidant activity of crude extracts and Eos was evaluated by the DPPH assay and the antibacterial activity was evaluated by the disc diffusion method and the broth microdilution method against Gram-positive strains (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative strains (*Escherichia coli*, *Salmonella paratyphi,* and *Pseudomonas aeruginosa*). *S.africana* Eos from Morocco and Tunisia were found to be rich in artemisia ketone (35.4% and 44.3%, respectively), santolina alcohol (16.2%and 3.2%, respectively) and isoborneol (6.1% and 26.6%, respectively). Methanol extracts were rich in phenolic and flavonoids contents and showed the highest DPPH radical scavenging activity. Results exhibited the sensitivity of the strains to essential oils from *S. africana* especially against Gram-positive bacteria. This current research will provide new information about this plant that can be used as a natural antioxidant and antibacterial for industrial purposes.

1. INTRODUCTION

Since ancient times, plants have been used in food and traditional medicine. The use of herbal remedies for the prevention and treatment of diseases was often explained by their usage, since their biological activities were not yet proven. In recent years, the interest of scientists in plants has led to a systematic examination of bioactive molecules extracted from medicinal and aromatic herbs and plants. Scientific research demonstrated their pharmaceutical, medicinal and biological properties and exhibited their beneficial effects on health. Medicinal species have

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been shown to possess anti-inflammatory (Darwish *et al.*, 2020), antiviral (Javed *et al.*, 2021), anticancer (Dai *et al.*, 2010; Andrade *et al.*, 2018), antidiabetic (Béjaoui *et al.*, 2017), antimicrobial and antioxidant properties (Khammassi *et al.*, 2022). These properties are attributed to the bioactive compounds in essential oils (Eos) and phenolic compounds that are of wide interest in the pharmaceutical and food industries. Eos were complex mixtures of volatile secondary metabolites, including monoterpenes, sesquiterpenes and phenylpropanoids (Ismail *et al.*, 2014). They have been reported for their antioxidant, antifungal and antibacterial activities (Khammassi *et al.*, 2023). A special relevance was also gained to phenolic compounds that exhibited anti-inflammatory, antimicrobial, antioxidant and herbicidal properties (Khammassi *et al*., 2022; Khammassi *et al.*, 2023).

S. africana (synonym: *Ormenis africana*) belongs to the Asteraceae family, is one of endemic species of the North of Africa (Tunisia, Algeria and Morocco). It is growing wild on the mountains and on the rocky slopes (Pottier Alapetite,1981). This plant is a green sub-shrubspecies with a strong odor, woody stems and yellow flowers. It is used in traditional medicine for its therapeutic effects. In Tunisia, it is used as an antidiabetic and for the treatment of colic and abdominal pain. A mixture of this plant and honey is used for the treatment of cardialgia ulcers (Ben Mansour, 2011; Bel Hadj Salah-Fatnassi, 2017). In Morocco, *S.. africana* is utilized as a stomachic, anthelmintic, abortifacientand vermifuge. It is also reported for its hypoglycemic effects (Lmachraa *et al.*, 2014). Essential oils of *S. africana* have been reported to have antioxidant, antimicrobial (Boudjedjou, 2019), antidiabetic (Béjaoui *et al.*, 2013) and antinflammatory activities (Malti *et al*., 2019). These volatile extracts have also been studied for their applicationas natural substances for agricultural control and plant protection. In fact, Eos from *S. africana* revealed fungicidal activity against phytopathogenic fungi (Khammassi *et al.*, 2018). The chemical composition of the Santolina genus essential oils has been studied. Monoterpenes such as 1,8-cineole, myrcene, artemisia ketone and camphor were the main components of the volatile fractions of some Santolina species from different regions of the world (Tundis *et al.*, 2018).

To our knowledge, few studies have been conducted on the chemical composition of *S. africana* essential oil from Tunisia and Morocco. Thus, the aims of this stuy were to investigate the variations in chemical composition of essential oils *S. africana* stems from the two countries and to evaluate the antioxydant and antibacterial activities of their volatile fractions and their crude extracts. Such study may provide natural alternative to chemical products and give solutions for the problems caused by antibiotics and synthetic antioxidants that affect human health.

2. MATERIAL and METHODS

2.1. Plant Material

Stems of *S. africana* were collected from the El Krib Sud region in the gouvernorate of Siliana (North of Tunisia) and from Taounate Province, located in the Fes region (North of Morocco). Ten samples were harvested from each country and identification was conducted by Dr Amri Ismail. A voucher specimen was deposed in the herbarium of INRGREF.

2.2. Essential Oils Extraction and Analysis

The essential oils (Eos) have been extracted from 100g of air-dried plant material by a Clevenger apparatus (European Pharmacopeia Method, 2008). Hydrodistillation has been conducted for 3hours. Volatile oils were stored at 4°C in darkglass bottles for analysis and the oil yields were calculated.

The composition of essential oils were determined by Gaz chromatography–Mass Spectrometry (GC-MS) analyses. GC/MS was performed on a Hewlett Packard 5972 MSD System. Eos separation was performed usinga HP-5 MS capillary column (30 m x 0.25 mm i.d., 0.25 mm film thickness) and helium as carrier gas (1.2 mL/min).

The GC-MS oven temperature was set at 50°C for 1 minute and then rose to 240°C at 5°C/min) and it remained at 204°C for 4 minutes.The injector and the detector were adjusted at temperatures of 250°C and 280°C, respectively. 0.1 mL of diluted samples (1%) were injected in splitless mode. ChemStation was the software used to process the mass spectra and chromatograms. Composition was identified based on mass spectra with comparison with Wiley spectra and Retention Index from alkanes (C9-C28 on the HP-5) helped in the identification of the chemical compounds.

2.3. Extract Preparation

50g of air-dried *S. africana* stems were powdered and extracted by maceration with distilled water or methanol. The extracts were filtered by filter paper (Whatman No. 4), evaporated with a rotary evaporator and then stored at 4°C for further analysis (Mau *et al.*, 2001).

2.4. Preliminary Phytochemical Screening

Extracts were dissolved in specific reagents through standard procedures and tested for phytochemical screening using standard methods (N'Guessan *et al.*, 2009; Karumi *et al.*, 2004; Dahoun, 2003).

2.5. Total Phenolic Content

The total phenolic content (TPC) was determined by the Folin-Ciocalteau assay using gallic acid as a standard phenolic compound according to the method described by Slinkard and Singleton (1977) and slightly modified by Dewanto *et al.* (2002). Briefly, 1 mL of each diluted extract was added to 1 mL of the Folin-Ciocalteu's phenol reagent and shaken. 3 minutes later, 1 mL of Na_2CO_3 (2 %) solution was added to the mixture. After incubation at room temperature for 90 minutes, absorbance against a reagent blank was read at 760 nm. The experiment was carried out in triplicates. TPCs were expressed as milligrams of gallic acid equivalents (GAE) per gram of weight (mg GAE/g DW).

2.6. Total Flavonoid Content

The total flavonoid content (TFC) was determined according to the method of chang *et al.*, (2002) by mixing 75 µL of $NaNO_2$ (7%) with 250µL of extract. 150µL of $AlCl_3$ (10%) and 500µL of NaOH (1 M) were then added to the mixture. After an incubation of 15 minutes at room temperature, the absorbance was read at 510 nm. Total flavonoids were determind using a calibration curve (50-600µg/mL) and presented in milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

2.7. Determination of Radical-Scavenging Activity

The free radical-scavenging activity of each sample (essential oils/extracts) was evaluated with a DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay (Wu *et al.*, 2006) with sligh modification. DPPH solution (10^{-4} M) was prepared in methanol. 1mL of each sample at different concentrations is added to 3 mL of the DPPH mixture, shaken and then allowed to sit for 30 minutes in the dark. The absorbance was then measured at 517 nm. Synthetic antioxidant butylatedhyroxytoluene (BHT) served as standard and assays were conducted in triplicate.

The percentage inhibition of DPPH radical was calculated according to the following formula:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \text{ X } 100$$

The concentration providing 50% of the radical scavenging activity (IC_{50}) was then determined.

2.8. Antibacterial Activity of Essential Oils and Crude Extracts

The antibacterial activity was evaluated using the agar diffusion method (National Committee for Clinical Laboratory Standards NCCLS, 2003), against five bacteria strains; Gram-positive: *Bacillus subtilis, Staphylococcus aureus*; Gram-negative: *Salmonella paratyphi, Pseudomonas*

aeruginosa, *Escherichia coli*. The bacterial strains were grown on Muller Hinton medium (MHI) at 37°C for 24 hours.

100 µL of microorganism suspension adjusted to 10^6 CFU/mL was spread on petri dishes containing nutrient agar medium. Discs (8 mm of diameter) of sterile filter paper were then put in the plates and impregnated with 10 µL of essential oils or 100 µL of extracts. The petri dishes were incubated for 24hours at 37°C and inhibition zones (mm) were determined. The bacteria with a clear zone of inhibition of more than 12 mm were considered to be sensitive. For each test, the experiment was performed in triplicate. The antibacterial activity of Eos or extracts was compared with two antibiotics ;ampicillin (10 µg/disc) and spiramycin (10µg/disc).

2.9. Minimum Inhibitory (MIC) and Minimum Bactericide (MBC) Concentrations

The Minimum Inhibitory Concentration (MIC) was determined by the broth micro dilution method according to National Committee for Clinical Laboratory Standard-NCCLS (1999). Experiments were carried out in nutrient broth. Broth tubes containing 10^6 CFU/mL were filled with differentconcentrations of essential oils or extracts (1.5-7 mg/mL).Then, the samples were incubated using an incubator shaker to distribute the volatiles oils and subsequently examined for evidence of the growth. Each test was repeated in triplicate. DMSO in the broth tube served as a negative control.

When no visible growth was observed after incubation, the minimum inhibitory concentration (MIC) of the volatile oil or extract was determined. From tubes presenting no visible growth, 20 mL were spread on suitable nutrient agar petri dishes. The plates were then incubated for 24 hours. After subculturing, the lowest concentration of the essential oil or extract at which no visible growth was observed was considered as minimum bactericidal concentration (MBC).

2.10. Statistical Analysis

Analysis were conducted in triplicate and presented as average values. The data gained were subjected to a variance analysis (ANOVA) using SPSS software (Version 21.0) and analysed by means of the multiple comparison Student-Newman-Keuls test. Values of $p \leq 0.05$ were considered significantly different.

3. RESULTS and DISCUSSION

3.1. Phytochemical Analysis

Preliminary phytochemical screening of *S. africana* methanol (Me) and aqueous (Aq) extracts is reported to contain phenols, flavonoids, sterols, polyterpenes, tannins, and quinones. Saponins were found only in water extracts (Table 1) whereas results showed the absence of alkaloids in all the extracts. This result was not in accordance with those reported for plant extracts of the Asteraceae family. In fact, the aqueous and methanol extracts of *Chromolaena odorata* leaves showed the presence of alkaloids (N'Guessan *et al*., 2009). Plants contain different groups of phenolic compounds. Plant phenolics have gained considerable interestdue to their biological functions (Bouazi*et al*., 2009).

Table 1. Phytochemical screening of *S.africana* crude methanol and aqueous extracts.

		Metabolites							
Extracts		Phenols	Flavonoids	Sterols and polyterpenes	Catechic tannins	Gallic tannins	Quinones	Saponins	Alkaloids
MeOH	Morocco	++	++	++	+	++	++	-	-
	Tunisia	++	++	++	+	++	+	-	-
Aqueous	Morocco	+	+	+	+	+	+	+	-
	Tunisia	+	+	+	+	+	+	+	-

++: strong; +: medium; - : poor (according to the color intensity). The measuring was repeated intriplicate.

3.2. Yields and Chemical Analysis

As shown in Figure 1, *S. africana* methanol extract yields were 9.12 % and 9.43 %, for Morocco and Tunisia, respectively. Whereas, aqueous extract yields did not exceed 1.71 %. Volatile oil yields of stems of *S. africana* from Tunisia and Morocco were 0.79 % and 0.9%, respectively. These yields are relatively in agreement with the data reported by Lmachraa *et al.*, (2014). However, they are lower when compared with the yield of *Santolina chamaecyparissus* var. *insularis* aerial parts (Poli*et al.*, 1997). The EO yield of stems from Algerian *S. africana* distilled in a Kaiser-Lang apparatus was 0.95 % (Zaiter *et al.*, 2015). Another study conducted on *S. africana* aerial parts from different locations in Algeria showed Eos yields ranging from 0.03 to 0.17 %. The variations in essential oil yields can be due to different factors such as plant parts, the date of harvest and environmental conditions. Moreover, Eos yiels can be affected by the physiological characteristics of the plants, genetic factors, soil type and methods of extraction (Saoud *et al.*, 2013; Mohammad *et al.*, 2022).

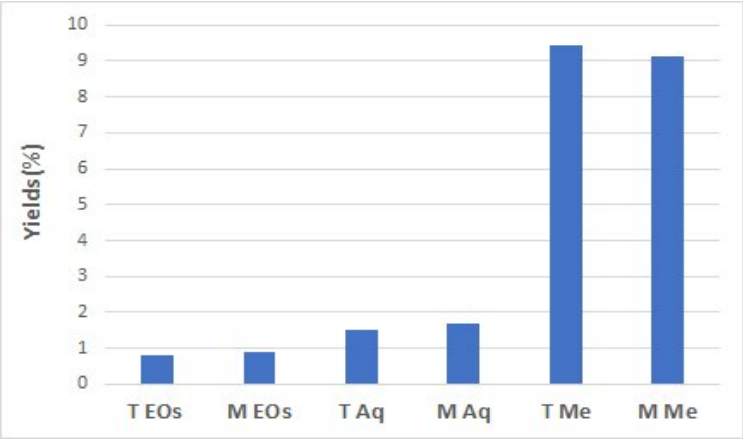


Figure 1. Yields of *S. africana* stems from Morocco and Tunisia.

T EO: Tunisian essential oil; M EO: Moroccan essential oil; T Me: Tunisian methanolic extract; M Me: Moroccan methanolic extract ; T Aq: Tunisian aqueous extract; M Aq: Moroccan aqueous extract

3.3. GC-MS Analysis

GC-MS analysis revealed a total of forty eight compounds, accounting 99.1 % and 98.9 % of the total Moroccan and Tunisian oils, respectively (Table 2 and Figure 2). Oxygenated monoterpenes (OM) were the main groups in *S. africana* Eos, followed by monoterpene hydrocarbons (MH). Sesquiterpene hydrocarbons (SH) ranged from 3.6% to 8.6%. However, previous investigations have demonstrated the dominance of hydrocarbon components in comparison with the oxygenated components in volatile fractions from other species of the Santolina genus (Zaiter *et al.*, 2015; Liu *et al.*, 2007).

Our results indicated that artemisia ketone was the most abundant in both essential oils, with variation in percentage between the two countries. Essential oil from Morocco was found to contain 35.4% artemisia ketone, 16.2 % santolina alcohol, 6.1 % isoborneol and 5.1 % β-oplopenone. Likewise, in Tunisian essential oil, artemisia ketone (44.3%), isoborneol (26.6%) and santolina alcohol (3.2 %) were identified as major compounds. In this study, the percentage and components of *S.africana* essential oil exhibited variation that can be caused by geographical origins (Díaz-Maroto *et al.*, 2006). Our findings were compared with other research involving other countries. It was reported that Eos extracted from aerial parts of *S.africana* in the flowering stage in three locations in the Eastern of Algeria (Batna province) were composed of germacrene D, spathulenol, myrcene, α-bisabolol, β-pinene, cys-chrysanhenol, 1,8-cineole, capillene, camphor santolina alcohol, lyratol and terpinen-4-ol (0.1-6.7 %) (Malti *et al.*, 2019).

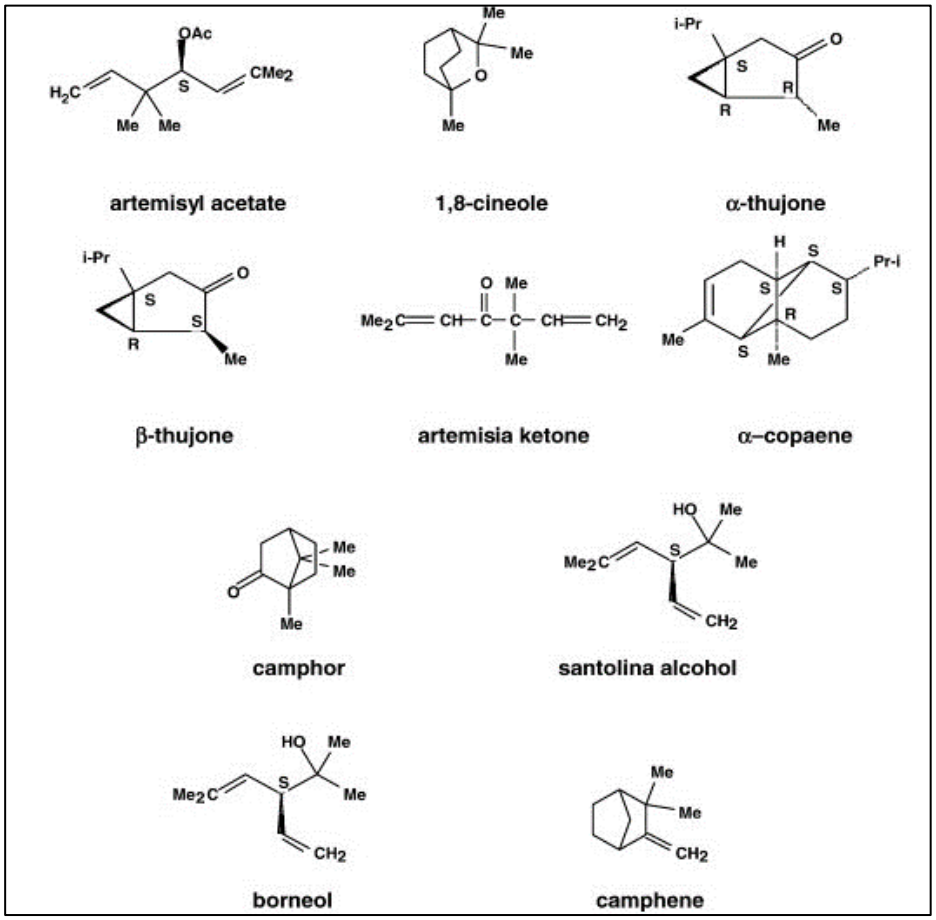
Table 2. Chemical profile of *S. africana* Eos from Tunisia and Morocco.

N°	Compounds	RI	Class	Formula	Area %		Identification
					Morocco	Tunisia	
1	Santolina triene	908	MH	$C_{10}H_{16}$	1.2	1.5	MS, RI
2	α-Tricyclene	926	MH	$C_{10}H_{16}$	0.2	1.1	MS, RI
3	α-Pinene	939	MH	$C_{10}H_{16}$	3.1	2.8	MS, RI, Co-In
4	Camphene	954	MH	$C_{10}H_{16}$	2.6	0.8	MS, RI
5	α-Sabinene	975	MH	$C_{10}H_{16}$	2.8	2.4	MS, RI
6	β-Pinene	979	MH	$C_{10}H_{16}$	4.7	2.9	MS, RI, Co-In
7	β-Myrcene	990	MH	$C_{10}H_{16}$	0.6	1.9	MS, RI, Co-In
8	α-Terpinene	1017	MH	$C_{10}H_{16}$	-	0.6	MS, RI, Co-In
9	*p*-Cymene	1024	MH	$C_{10}H_{14}$	0.5	0.8	MS, RI, Co-In
10	Santolina alcohol	1040	OM	$C_{10}H_{18}O$	16.2	3.2	MS, RI
11	Artemisia ketone	1062	OM	$C_{10}H_{16}O$	35.4	44.3	MS, RI
12	(*Z*)-Sabinene hydrate	1070	OM	$C_{10}H_{18}O$	1.1	0.3	MS, RI
13	α-Terpinolene	1088	MH	$C_{10}H_{16}$	0.3	0.9	MS, RI, Co-In
14	Linalool	1096	OM	$C_{10}H_{18}O$	3.6	0.2	MS, RI, Co-In
15	α-Thujone	1102	OM	$C_{10}H_{16}O$	0.8	-	MS, RI
16	β-Thujone	1114	OM	$C_{10}H_{16}O$	-	0.8	MS, RI, Co-In
17	*Trans*-Pinocarveol	1139	OM	$C_{10}H_{16}O$	0.6	-	MS, RI
18	*iso*-Menthone	1162	OM	$C_{10}H_{18}O$	-	0.6	MS, RI, Co-In
19	Isoborneol	1160	OM	$C_{10}H_{18}O$	6.1	26.6	MS, RI, Co-In
20	Terpinen-4-ol	1177	OM	$C_{10}H_{18}O$	1.2	-	MS, RI, Co-In
21	*p*-Cymen-8-ol	1182	OM	$C_{10}H_{14}O$	-	0.2	MS, RI
22	α-Terpineol	1188	OM	$C_{10}H_{18}O$	1.2	0.3	MS, RI, Co-In
23	Myrtenal	1195	OM	$C_{10}H_{14}O$	0.6	0.2	MS, RI, Co-In
24	(*E*)- Piperitol	1196	OM	$C_{10}H_{18}O$	0.5	0.2	MS, RI
25	(*Z*)-Carveol	1216	OM	$C_{10}H_{16}O$	0.2	0.3	MS, RI
26	Carvacrol methyl ether	1244	PP	$C_{11}H_{16}O$	0.8	0.4	MS, RI
27	α-Santalene	1417	SH	$C_{15}H_{24}$	1.3	-	MS, RI
28	(*Z*)-β-Farnesene	1442	SH	$C_{15}H_{24}$	0.6	-	MS, RI
29	α-Humulene	1454	SH	$C_{15}H_{24}$	0.5	0.6	MS, RI, Co-In
30	Allo-aromadendrene	1460	SH	$C_{15}H_{24}$	0.9	0.6	MS, RI
31	Germacrene D	1485	SH	$C_{15}H_{24}$	0.4	-	MS, RI, Co-In
32	Bicyclogermacrene	1500	SH	$C_{15}H_{24}$	0.8	-	MS, RI
33	α -Muurolene	1500	SH	$C_{15}H_{24}$	0.3	0.4	MS, RI
34	β-Bisabolene	1505	SH	$C_{15}H_{24}$	0.2	0.4	MS, RI
35	γ-Cadinene	1513	SH	$C_{15}H_{24}$	0.8	0.3	MS, RI
36	δ-Cadinene	1523	SH	$C_{15}H_{24}$	0.2	-	MS, RI
37	α-Cadinene	1538	SH	$C_{15}H_{24}$	0.2	-	MS, RI
38	β-Calacorene	1565	SH	$C_{15}H_{24}$	0.1	-	MS, RI
39	Germacrene B	1561	SH	$C_{15}H_{24}$	0.4	0.1	MS, RI
40	Germacrene D-4-ol	1575	OS	$C_{15}H_{26}O$	0.3	-	MS, RI
41	Spathulenol	1578	OS	$C_{15}H_{24}O$	0.9	1.2	MS, RI
42	Caryophyllene oxide	1583	OS	$C_{15}H_{24}O$	0.2	-	MS, RI
43	Guaiol	1600	OS	$C_{15}H_{26}O$	-	0.8	MS, RI
44	β-Oplopenone	1607	OS	$C_{15}H_{24}O$	5.1	-	MS, RI
45	1-epi-Cubenol	1628	OS	$C_{15}H_{26}O$	0.8	0.8	MS, RI
46	(*Z*,*E*)-Farnesol	1722	OS	$C_{15}H_{26}O$	0.2	0.4	MS, RI
47	(*Z*,*Z*)-Farnesol	1698	OS	$C_{15}H_{26}O$	0.2	-	MS, RI
48	(*E*,*E*)-Farnesol	1742	OS	$C_{15}H_{26}O$	0.4	-	MS, RI
Total identification					99.1	98.9	
Monoterpene hydrocarbons (MH)					16	15.7	
Oxygenated monoterpenes (OM)					67.5	77.2	
Phenylpropanoid derivates (PP)					0.8	0.4	
Sesquiterpenes hydrocarbons (SH)					8.6	3.6	
Oxygenated sesquiterpenes (OS)					6.7	2.4	

Another study conducted on Algerian *S.africana* EO (location Ichemoul, province of Betna) at the flowering stage, exhibited its richness in β-pinene, 1,8-cineole, germacrene D, sabinene, α-bisabolol and hedycaryol (Boudjedjou *et al.*, 2019). Similarly, Zaiter *et al.*, (2015), reported that the essential oil of the aerial parts of *S.africana* from Setif provence (North Eastern Algeria) is composed of β-pinene, myrcene and at less extent, α-pinene. Compared to Tunisian reports, there are notable differences with the oil of *S. africana* leaves collected from Siliana, a province in Tunisia, which is composed of artemisia ketone and isoborneol, presenting 42.96%, 24.30%, respectively (Khammassi, 2018).

In a previous study conducted on *S.africana* aerial part from Morocco (province of Tahanaout), oxygenated monoterpenes were particularly abundant (97.24 %) and camphor (54.3 %), borneol (17.24 %) and 1,8 cineole (5.27 %) were the main coumpounds (Lmachraa *et al.*, 2014).

The variation in the composition of volatile oil can be attributed to the geographical location of the plant and also to the plant parts (seeds, leaves and stems) which explain the aim of this investigation and also explain the exploitation of such bio resources for their biological properties.



RI: retention index; MS = mass spectrometry; Inj = co-injection with authentic compounds; - = absent

Figure 2. Main compounds of *S.africana* essential oil.

3.4. Total phenolic and Flavonoid Content

The results of total phenolic and flavonoids content are presented in Table 3. Statistical analysis revealed that the amounts of total phenolic content were significantly different between Me and aq extracts. In fact, Me extracts were richer in phenolics than aq extracts with amounts of 137.46 mg GAE/g DW and 137.03 mg GAE/g DW for Morocco and Tunisia. Flavonoids contents

differ significantly between extracts and methanol extracts exhibited their richness in flavonoids,with amounts ranging from 31.79 to 38.2 mg QE/g DW.Béjaoui *et al*., (2013)reported that total phenolics and total flavonoid amounts were 50mg GAE/100g DM and 42.56 QE/g of dry mass, respectively, for the methanol extract of *S.africana* leaves collected from the region of ELKEF. Another study conducted by Ben Mansour *et al.*, (2011) exhibited that hydroethanolic extracts from inflorescences contain 312.07 ± 4.81 mg GAE/g dray matter and 73.72 ± 1.98 QE/g of dry mass of the phenolic and flavonoids compounds, respectively. The difference found between our results in comparison to other work may be due to the plant part used, solvent and the method of extraction. Previous research conducted on Asteraceae species showed high amounts of phenols (Wojdyło *et al.*, 2007).

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidative action (Hatano *et al.*,1989).

Table 3. Total phenolic and flavonoid content of methanol and aqueous extracts of *S.africana*.

	Methanol extract		Aqueous extract	
	Morocco	Tunisia	Morocco	Tunisia
Total phenolic (mg GAE/g DW)	137.46± 0.14^{a}	137.03± 0.31^{a}	63.36±0.3^{b}	59.34± 0.2^{c}
Flavonoid content (mg QE/g DW)	38.2 ±0.15^{a}	31.79± 0.22^{b}	9.26±0.58^{c}	7.57±0.21^{d}

3.5. Antiradical Scavenging Activity

The Eos and crude extracts were screened for their antioxidant potential by DPPH assays. Statistical results showed that the radical scavenging activity differs significantly between extracts and origins (Figure 3). Methanol extracts were the strongest antioxidants followed by aqueous extracts and revealed high potential as compared with BHT. Eosexhibited the highest IC_{50}= 538.12 and 613.84 µg/mL for Moroccan and Tunisian species, respectively and then they presented the lowest antiradical activities. Research conducted on the antioxidant activity of the methanol extract leaves of *S. africana* showed significant antiradical potential towards DPPH radicals (46 µm Trolox equivalents TE) (Béjaoui *et al.*, 2013). Another study demonstrated the antioxidant activity of EO of *S. africana* aerial parts from Algeria with an IC_{50} value of 1.51 mg/mL (Malti *et al.*, 2019). The antioxidant activity of plant extracts can be attributed to their richness in total phenolics and flavonoids. Many reports showed a correlation between phenolic contents and the antioxidant properties of the plants. The chemical composition of bioactive compounds is one of the major factors that influences the activity of natural antioxidants (Bouaziz *et al.*, 2009; Shahidi & Marian, 2003). In fact, as plant secondary metabolites, the phenolics can react by different mechanisms: by inactivating lipid free radical chains, chelating redox-active metal ions, and avoiding hydroperoxide conversions into reactive oxyradicals. Radical scavenging by polyphenols is the most widely published mechanism for their antioxidant activity. In this radical scavenging mechanism, polyphenols sacrificially reduce reactive oxygen and nitrogen species ROS/RNS, such as •OH, O2•-, NO•, or OONO- after generation, preventing damage to biomolecules or theformation of a more reactive oxygen system (Perron*et al.*, 2009). While polyphenols are primarily recognized for their antioxidant functions, they also have many other biological properties, such as antimicrobial activiy.

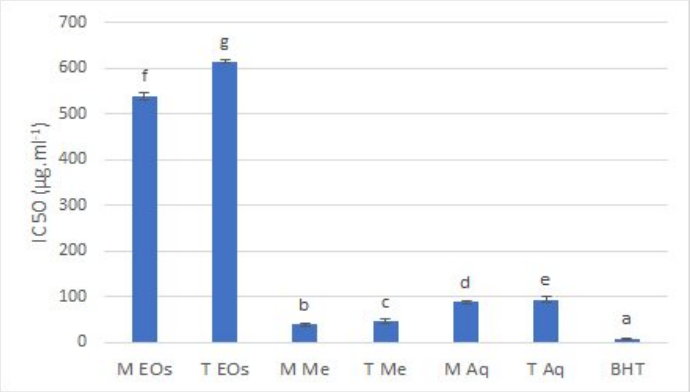


Figure 3. IC_{50} of Eos and extracts of *S.africana*.

M EO: Moroccan essential oil; T EO: Tunisian essential oil; M Me: Moroccan methanolic extract; T Me: Tunisian methanolic extract; M Aq: Moroccan aqueous extract; T Aq: Tunisian aqueous extract.

3.6. Antibacterial Activity

The antibacterial activity of the Eos and crude extracts of *S. africana* aerial parts was evaluated against four bacteria, by the agar disc diffusion method. Results are presented in Table 4 and showed different degrees of bacterial growth inhibition that depended on the strains and the tested sample. Our result showed that essential oils from the two coutries were more effective against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomona aeruginosa*,with growth inhibitionof 13.33 mm to 15.66 mm. The most important effect was observed against *Staphylococcus aureus*. However, Eos revealed moderate activity against *Salmonella paratyphi* and *Escherchia coli* with inhibition zone around 12mm and 12.5mm, respectively. For methanol and aqueous extracts, a very slight inhibition (9-10.5 mm) was observed against all bacteria strains. As compared to the antibiotics, essential oils from *S. africana* were less effective against the tested bacteria strains, except against *S.aueurs*. Indeed, the same sensitivity of the oils of Morocco and Tunisia (15.33 mm and 15.66 mm, respectively) and ampicillin (16 mm) was observed for this bacteria. In accordance with our results, Malti *et al.*, (2019) showed that Eos of Algerian *S.africana* extracted from the aerial parts at full flowering were effective against *S.aureus* (19.7 mm). Boudjedjou *et al.*, (2019)demonstrated also the potent effect of *S.africana* EO at the fowering stage from Algeria against *E. coli* and *S. aureus* and *B. subtilis*,are respectively, of 29.27 mm, 29 mm and 15 mm.

Liu *et al.*(2007) have also reported that *S.corsica* EO revealed remarkable activity against *S. aureus*, but remaind inactive against *P. aeruginosa* and *E. coli*. However, other studies on *S.chamaecyparissus* and *S.rosmarinifolia* Eos recorded a strong inhibition growth of *E. coli* (25 mm and 15 mm, respectively) (Chibani *et al.*, 2013; Salah-Fatnassi*et al.*, 2017).

S.africana essential oils from Morocco and Tunisia displayed activity against *S.aureus*, *B.subtilis* and *E.coli* with MICs of 7 mg/mL. The tested strains were more resistant against crude extracts. *Ormenis* oils displayed a bacterecidal effect against *S.aure*us and *B.subtilis*, with a MFC of 5 mg/mL (Table 5).

The inherant effects of Eos can be related to their chemical composition, their proportions and to the interactions between their compounds, which may produce additive, synergistic, or antagonistic effects (Tyagi & Malik, 2011). The antibacterial activity of volatile oils can also be attributed to the sensitivity of the tested strains. In fact, essential oils composed mainly of terpenes, phenolsandaldehydes are known by their hydrophobicity that permet them to accumulate in the membranes of bacteria cells and then cause the permeabilityincrease. Leakage of intracellular constituents and alteration of the microbial enzyme can be produced and then cause the loss of cellular content and cell death. In addition, interaction between the different components can occur to changes in the conformation of the structure, causing a reduction in inhibitory activity (Ceylan & Fung, 2004; Bajpai *et al.*, 2013).

Our study revealed that essential oils inhibit more Gram positive bacteria than Gram negative bacteria. This phenomenon was reported previously by Smith-Palmer *et al.*, (1998). It is not known why Gram negative are more resistant, but it can be attributed to the outer membrane that confer to the bacterial surface strong hydrophilicity and acts as strong permeability barrier (Nikaido *et al.*, 1985). Recent data suggest that essential oils may disrupt the permeability barrier of cell membranes and inhibit respiration (Cox *et al.*, 2000).

Table 4. Antibacterial activity of *S.africana* essential oils and extracts.

		Bacteria				
		Gram positive		Gram negative		
		S.aureus	*B.subtilis*	*E.coli*	*P.aeruginosa*	*S. paratyphi*
Eos (10µL/disc)	Morocco	15.33±0.33^{a}	14±0.66^{c}	12±0.33^{b}	13.33±0.57^{b}	12^{b}
	Tunisia	15.66±0.33^{a}	14.66±0.33^{b}	12.5±0.33^{b}	13.66±0.57^{b}	12.33±0.33^{b}
MeOH extract (10µL/disc)	Morocco	9^{b}	9^{d}	-	-	-
	Tunisia	9^{b}	9.33±0.33^{d}	-	9^{c}	9.66±0.33^{c}
Aqueous extract (10µL/disc)	Morocco	9^{b}	-	-	-	-
	Tunisia	9.33±0.33^{b}	-	10^{c}	9.66±0.33^{c}	-
AMP		16±0.57^{a}	-	16.33±0.66^{a}	18^{a}	15.66±0.33^{a}
SPI		-	18.33±0.66^{a}	-	-	-
DMSO		-	-	-	-	-

AMP: ampicillin; SPI: spiramycin; DMSO: Diméthylsulfoxide

Table 5. MIC and MBC (mg/mL) of essential oils and extracts from *S.africana*.

		S.aureus		*B.subtilis*		*E.coli*		*P.aeruginosa*		*S. paratyphi*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Eos	Morocco	7	5	7	5	7	-	> 7	-	> 7	-
	Tunisia	7	5	> 7	5	7	-	> 7	-	> 7	-
MeOH extract	Morocco	> 7	-	> 7	-	> 7	-	> 7	-	> 7	-
	Tunisia	> 7	-	> 7	-	> 7	-	> 7	-	> 7	-
Aqueous extract	Morocco	> 7	-	> 7	-	> 7	-	> 7	-	> 7	-
	Tunisia	> 7	-	> 7	-	>7	-	> 7	-	> 7	-

4. CONCLUSION

The present study presents a contribution to the analysis of Eos and extracts from *S. africana* traditionally used for several medicinal applications. The chemical composition of *S. africana* essential oils from Morocco and Tunisia showed their richness in oxygenated monoterpenes. Artemisia ketone and isoborneol were the most abundant in both essential oils. Methanol extracts were rich in phenolic and flavonoid contents and exhibited the highest antiradical activity. Regarding antibacterial property, the evaluation of antibacterial activity using the disc diffusion method and the microdilution method for determining MIC and MBC indicated that *S. aureus* and *B.subtilis* were the most sensitive strains against *S.africana* essential oils.Volatile oils and extracts of *S. africana* can be used as natural agents for the interesting activities they have presented. The chemical composition of each extract may have a crucial role in its bioactivity. However, toxicological investigations are required to demonstrate the safety of this plant.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship Contribution Statement

Marwa Khammassi: Investigation, Methodology, Visualization, Formal Analysis, and Writing -original draft, Writing – review & editing. **Sana Khedhri**: Software, Formal Analysis**,** Writing -original draft. **Awatef Slama**: Visualization, Methodology, Formal Analysis**. Meriam Boudkhili:** Data curation, and Formal Analysis, **Ismail Amri:** Investigation, Visualization, Methodology, Supervision, **Lamia Hamrouni**: Supervision, and Validation, **Bassem Jammoussi**: Supervision, and Validation

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