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Chemical composition and antimicrobial activities of Tunisian *Salsola vermiculate* L.

Sana Gannoun[a], Adel Mahfoudhi[b,c], Guido Flamini[d], Ahmed Noureddine Helal[a] and Zine Mighri[b*]

[a] Laboratory: Bioressources, Integrative Biology and Valorization BIOLIVAL(LR14ES06), Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia
[b] Research Unit: Applied Chemistry and Environment (UR13ES63), Faculty of Sciences of Monastir, University of Monastir, Tunisia
[c] Faculty of Sciences of Bizerte, University of Carthage, Tunisia
[d] Department of Pharmacy, University of Pisa, Via Bonanno, 6, 56126 Pisa, Italy

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ABSTRACT

The chemical composition of Salsola vermiculate L. leaves, roots and stems volatile fractions was determined by gas chromatography. The major compounds of leaves volatile fraction were carvone (52.2%) and β-caryophyllene (5.8%). The major constituents of roots volatile fraction were carvone (49.9%) and cumin aldehyde (4.4%). The stems volatile fraction was dominated by carvone (53%), limonene (17.4%) and linalool (11.3%). The antimicrobial activity of S. vermiculate leaves, roots and stems extracts was evaluated toward Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumanii. The ethanolic roots extract was the most active against S. aureus with a MIC value of 0.28 mg/mL. However, E. coli and P. aeruginosa were the most resistant bacteria. The antifungal activity was tested against Candida krusei, Candida parapsilosis and Candida glabrata. These activities were weak with inhibition zone diameters ranging from 6 to 9.5 mm.

Key words: *Salsola vermiculate* L.; Extracts; Volatile fractions; Chemical composition; Antimicrobial activities.

INTRODUCTION

The World Health Organization estimates that plant extracts or their active ingredients are used in traditional medicine by more than 80% of the world's population [1].Over 50% of all modern clinical drugs are products of natural origin and natural products play an important role in drug development programs in the pharmaceutical industry[2]. Many researchers around the world have studied the effects of herbal extracts in microorganisms [3-12].

Salsola vermiculata L. (Mediterranean saltwort), a member of the Chenopodiaceae family, is one of the dominant perennial species in the Mediterranean arid zone. This species is distributed throughout the Middle East and North Africa, including Algeria, Egypt, Iran, Israel, Jordan, Lebanon, Libya, Morocco, the Mediterranean islands of Sardinia and Sicily, Spain, Syria, and Tunisia [13].

In the present study, we report the chemical analysis of volatile fractions obtained by hydrodistillation of *S. Vermiculate* leaves, stems, and roots. Furthermore, the antimicrobial activities of *S. vermiculate* extracts were evaluated against the following pathogenic bacteria and fungi: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*.

EXPERIMENTAL SECTION

2.1. Plant material
Salsola vermiculate L. was collected in the region of Monastir, Tunisia (latitude: 35°75', longitude: 10°82') in February 2012. The fresh plants, previously separated into leaves, stems and roots, were air-dried under shade at room temperature and grounded. Then the powder was stored at room temperature, protected from light and humidity, for subsequent analysis.

2.2. Microorganisms
The tested pathogenic bacteria were *Staphylococcus aureus* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25923) and a clinical isolate *Acinetobacter baumanii.* The pathogenic fungi were *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019).

2.3. Preparation of *S. vermiculate* L. extracts
Leaves, stems and roots powders (60g each one) were separately extracted by Soxhlet until total exhaustion of the plant material using three solvents of increasing polarity: chloroform, butanol and ethanol. At the end of extraction, each extract was paper filtered and concentrated to dryness under vacuum at 40 °C.

2.4. Preparation of *S. vermiculate* L. volatile fractions
Leaves, stems and roots powders (20g each one) were separately submitted to hydrodistillation in a Clevenger-type apparatus for 4 h. The obtained volatile fractions were collected, dried over sodium sulfate, and stored in a refrigerator at 4 °C until use.

2.5. Analytical Gas chromatography
The GC-FID analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (both 30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250°C; carrier gas helium (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl). The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (l.r.i.) relative to the series of n-hydrocarbons [14].

The GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from60°C to 240°C at 3°C/min; carrier gas helium at 1 ml/min; injection of 0.2 μl (10% hexane solution); split ratio 1:30.Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial and home-made library mass spectra built up from pure substances and components of known oils and MS literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

2.6. Antimicrobial assay
2.6.1. Antibacterial activity of *S. vermiculate* L. extracts
a. Determination of the minimum inhibitory concentration (MIC)
The MIC is defined as the lowest concentration of antibiotic that inhibits any visible bacterial growth after twenty-four hours [15, 16].

To determine the MICs of the extracts studied, we used the microdilution technique using 96-well ELISA plates round bottom [17, 18]. The initial extract concentration, prepared by dissolving in 10% DMSO, was 10 mg/mL. The antibacterial test for each sample is repeated three times.

The wells of column one and two were used as negative control: the wells of column one, 150 μL of the bacterial inoculum and those in column two is added, is placed 100 μL of 10% DMSO and 50 μL of the bacterial inoculum.

The last column of the wells were used as positive controls where each well contains 100 μL of 10% DMSO and 50 μL of broth. Is deposited in the wells of the third to the ninth column 100 μL of 10 % DMSO and is brought with the data for column three, 200 μL of the sample to be tested (10 mg / mL), then serially diluted in half each time realized by taking 100 μL of the column three, the diluent in the column four and so on until in column nine. Series of dilutions being made, is placed in the treated wells 50 μL of the bacterial suspension tested.

Then all plates incubated in an incubator at 37°C for ten-eight hours then proceeds to their observation with the naked eye: the lowest concentration that gave an inhibition of bacterial growth in the minimum inhibitory concentration (MIC) of the sample with respect to the test strain.

b. Determination of the minimum bactericidal concentration (MBC)

MBC corresponds to the lowest concentration, which can kill 99.9% or more of the initial inoculums during twenty-four hours of incubation at 37°C [19, 20]. The MBC is determined by streaking, of 10 µL of the contents of each well whose concentration is equal or superior to the MIC, on nutrient agar.

2.6.2. Antifungal activity of *S. vermiculate* L. extracts

We used the diffusion method on solid medium (Sabouraud Chloamphenicol). Therefore, we have prepared the fungal inoculums, in sterile physiological water NaCl 9‰, from a culture of twenty-four hours. The inoculum was adjusted to a value of 1 McFarland using a densitometer (Bio Merieux). In a second step, we flooded the surface of Sabouraud medium with 2-3 ml of fungal inoculum. Then, we removed the excess using a sterile Pasteur pipette. The dishes were dried by incubation in the oven for 15 minutes at 37°C.

Disks of n°3-sterile Whatman paper and 6 mm in diameter are used. We impregnate each of them with 20 µL of test samples, which corresponds to 0.4 mg/disc of tested volatiles (either 20 mg/mL).

Subsequently, the discs impregnated with the volatile fractions were placed on the surface of Sabouraud medium in the presence of control disks impregnated only by solubilizing solvent extract and a positive control disc (Fluconazole). After incubating Petri dishes at 37°C for ten-eight hours, we proceed to measure the diameters of the inhibition zones (ϕ-IZ) surrounding the discs [21].

RESULTS AND DISCUSSION

3.1. Chemical analysis of *S. vermiculate* L. volatile fractions

The chromatographic analysis of *S. vermiculate* volatile fractions permitted us to identify forty-four compounds belonging to several chemical classes as shown in Table 1.

The volatile fraction of *S. vermiculate* leaves contains twenty-eight compounds representing 95.9% of total constituents. The major compounds of this fraction are carvone (52.2%), cumin aldehyde (6%), β-caryophyllene (5.8%) and linalool (7.1%). However, sixteen compounds were identified in the volatile fraction of *S. vermiculate* stems representing 98.5% of total constituents. This volatile fraction is dominated by carvone (53%), limonene (17.4%), linalool (11.3%) and β-caryophyllene (7.5%).

In the volatile fraction of roots, thirty-three constituents were identified and represent 94.2% of total constituents. The majority compounds are carvone (49.9%), β-caryophyllene (8.5%), linalool (8.2%) and cumin aldehyde (4.4%). Oxygenated monoterpenes (67.8%, 69.4% and 64.6%) are respectively the dominant class of volatile fractions of leaves, stems and roots of *S. vermiculate*. The main major component of this class is carvone. Teuschere E et al. (2005) [22] showed that the essential oils from the fruits of *Anethum graveolens* L. are rich in carvone (50-60%) with stomachic, carminative and diuretic properties.

Table 1. Chemical composition of *S. vermiculate* volatile fractions

Compounds	l.r.i.	*S. vermiculate* L. volatile fractions		
		Leaves	Stems	Roots
α-pinene	941	1.3	-	1.1
5-methyl-3-heptanone	945	-	1.8	-
Camphene	955	-	-	0.4
Sabinene	977	-	0.2	0.3
β-pinene	982	0.6	0.9	0.5
Myrcene	992	-	0.3	-
2-pentyl furan	993	0.7	-	0.6
Limonene	1032	0.7	17.4	0.8
1,8-cineole	1034	1.0	0.7	0.7
Linalool	1101	7.1	11.3	8.2
Nonanal	1103	0.6	-	0.4
Camphor	1145	-	0.7	0.4
Isoborneol	1158	-	1.2	-
4-terpineol	1179	-	0.3	-
α-terpineol	1191	-	1.0	-
Decanal	1206	0.8	-	0.6
Verbenone	1206	-	-	-
Cumin aldehyde	1241	6.0	1.2	4.4
Carvone	1243	52.2	53.0	49.9
Perilla aldehyde	1272	-	-	0.3
α-terpinen-7-al	1284	0.9	-	0.7
(E)-anethole	1285	1.3	-	0.7
Carvacrol	1301	0.6	-	-
p-vinyl guaiacol	1308	0.6	-	-
α-copaene	1377	0.6	0.6	0.9
β-caryophyllene	1419	5.8	7.5	8.5
α-humulene	1455	-	0.4	0.6
Germacrene D	1482	-	-	0.5
β-selinene	1486	0.6	-	0.5
Valencene	1493	0.7	-	0.5
α-selinene	1496	-	-	-
Bicyclogermacrene	1496	-	-	0.4
β-bisabolene	1509	0.7	-	0.6
Trans-γ-cadinene	1514	0.7	-	-
δ-cadinene	1524	1.3	-	1.2
Caryophyllene oxide	1582	1.2	-	1.1
Carotol	1595	0.5	-	-
α-acorenol	1632	-	-	0.4
T-cadinol	1641	1.0	-	0.5
T-muurolol	1642	1.2	-	1.1
α-muurolol	1646	0.9	-	0.5
Ar-turmerone	1666	2.8	-	3.1
n-octadecane	1800	-	-	0.5
hexahydrofarnesylacetone	1845	3.5	-	3.3
Monoterpene hydrocarbons		2.6	18.8	3.1
Oxygenated monoterpenes		67.8	69.4	64.6
Sesquiterpene hydrocarbons		10.4	8.5	13.7
Oxygenated sesquiterpenes		7.6	0.0	6.7
Phenylpropanoids		1.3	0.0	0.7
Apocarotenes		3.5	0.0	3.3
Non-terpene derivatives		2.7	1.8	2.1
Total identified		**95.9**	**98.5**	**94.2**

3.1. Antibacterial activity of *S. vermiculate* L. extracts

The results showed that the antibacterial activities vary with the pathogen and the nature of the extract (Table 2).

Table 2. Antibacterial effects of stems, roots and leaves extracts of *Salsola vermiculate* L. (10 mg/mL) toward five pathogenic bacteria using the micro dilution method

Bacteria	*S. vermiculate* L.								
	Stems			Roots			Leaves		
	EtOH	$CHCl_3$	BuOH	EtOH	$CHCl_3$	BuOH	EtOH	$CHCl_3$	BuOH
	MIC (mg/mL)								
E. coli	>10	>10	>10	>10	>10	>10	>10	>10	>10
A. baumanii	>10	>10	2.5	>10	3.33	5	>10	6.66	5
P. aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10
E. faecalis	1.12	1.12	1.12	2.04	2.5	1.58	2.04	2.5	2.04
S. aureus	1.58	10	2.04	0.28	2.5	2.5	4.16	2.87	2.04

EtOH: Ethanolic extract; $CHCl_3$: Chloroformic extract; BuOH: Butanolic extract.

The results of the antimicrobial activity showed that *E. faecalis* and *S. aureus* were most sensitive to *S. vermiculate* extracts. MICs values varied from 0.28 to 4.16 mg/mL.

The ethanolic roots extract was the most active extract on *S. aureus* with an MIC of 0.28 mg/mL. *P. aeruginosa* and *E. coli* were the most resistant to the tested extracts. These results are similar to Mohammadi et al. (2004) [23] who showed that *E. coli* were resistant to *Salsola baccata* extracts.

The extracts of *S. vermiculate* were more sensitive to gram-positive bacteria. These results are similar to Samia Rashid et al. (2000) [24] who have shown that extracts of the plant *Salsola fruticosa* have a high antibacterial potentiel on the gram-positive bacteria.

The antibacterial activity of *S. vermiculate* could be attributed to the presence of carvone. This compound was been reported to be one of the most efficient antimicrobial agents of various plants [25]. The mechanism of antibacterial activity of carvone is not completely understood in detail. It has been demonstrated that the mechanism of action of carvone on the growth microorganisms includes the destabilization of the phospholipid bilayer structure, interaction with membrane enzymes and proteins, and its act as a proton exchanger reducing the pH gradient across the membrane [26].

4.2. Antifungal activities of *S. vermiculate* L. extracts

Antifungal activities of *S. vermiculate* stems, leaves and roots extracts were evaluated toward three pathogenic *Candida* species by the method of diffusion in a solid medium. The results are summarized in Table 3.
The results of the antifungal activity showed that these vary according to the pathogen and the plant extract. It also appears that the extracts of stems, roots and leaves of *S. vermiculate* have low antifungal activity shown by an inhibition zone ranging from 6.5 to 9.5 mm.

The butanolic extract of roots was the most active on *Candida parapsilosis* (ϕIZ = 9.5 mm). Mahasneh et al. (1996) [27] studied the antifungal activity of petroleum ether and butanolic extracts from *Salsola vermiculate* and *Salsola villosa*. Both species showed significant antifungal activity against *Candida albicans* and *Fusarium oxysporum*.

The richness of *S. vermiculate* in carvone could explains its antifungal activity. Carvone has both antibacterial and antifungal potential. It showed antimicrobial activity against *Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida albicans*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Enterococcus faecium*, *Escherichia coli*, *Salmonella typhimurium* and *Photobacterium leiognathi* [28].

Table 3. Antifungal activities of *Salsola vermiculate* L. Stems, Leaves and Roots extracts (100 mg/ mL) towards a three species of *Candida*by the method of diffusion in a solid medium.

	***S. vermiculate* L.**									
	Leaves			**Stems**			**Roots**			
	$CHCl_3$	**BuOH**	**EtOH**	**$CHCl_3$**	**BuOH**	**EtOH**	**$CHCl_3$**	**BuOH**	**EtOH**	**Flu**
Candida	**ϕIZ (mm)**									
C. krusei	6.5	7	8	6.5	7.5	8	6.5	8	8.5	30
C. parapsilosis	6.5	7	9	6.5	9	8	6.5	9.5	7	29
C. glabrata	7	7.5	6.5	7	8	7	6.5	9	7	6

EtOH: Ethanolic extract; $CHCl_3$: Chloroformic extract; BuOH: Butanolic extract; Flu: Fluconazole (2 mg/mL)

CONCLUSION

The present study indicated that *Salsola vermiculate* L. leaves, stems and roots volatile fractions are rich in carvone (52.2%, 53.0% and 49.9% respectively). *S. vermiculate* extracts are active in particular on the positive gram bacteria (*E. faecalis* and *S. aureus*). However, they have low antifungal activity against the three tested fungal species (*C. krusei, C. parapsilosis* and *C. glabrata*).

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