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Chemical Composition and Antifungal Activity of Volatiles from Three *Opuntia* Species Growing in Tunisia

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Abstract: The chemical composition of the volatiles isolated by steam distillation from leaves, flowers and fruits of *Opuntia lindheimeri var. linguiformis* L. Benson, leaves and flowers of *Opuntia macrorhiza* Engelm and leaves of *Opuntia microdasys* (Lehmann) gathered in the sea cliff of Monastir town (Tunisia), has been studied by GC and GC-MS. Remarkable differences were noted between the composition and the constituent percentage of the different studied organs. The most important compounds found in leaves, flowers and fruits of *Opuntia lindheimeri var. linguiformis* were tetradecanoic acid (3.15-13.57%), hexadecanoic acid (8.5-17.33%), butyl tetradecanoate (8.05-21.47%) and (E)-3-Butyldiene phthalide (6.92-15.77%). In the flowers volatile extract of *Opuntia macrorhiza*, the main compound found was Butyl tetradecanoate (21.14%). The volatile extract from *Opuntia microdasys* leaves was mainly rich in hexadecanoic acid (13.13%), (*E*)-3-Butyldiene phthalide (21.4%) and butyl tetradecanoate (5.91%). Volatile components extracts were tested against the fungi *Alternaria solani*, *Botrytis cinerea*, *Fusarium solani* f. sp *cucurbitae*, *Fusarium oxysporum* f. sp *niveum*, *Pythium ultimum* and *Rhizoctonia solani*. The strongest inhibitory effect of the all volatile extracts was observed against *Alternaria solani*.

Key words: *Opuntia*, volatile extracts, chemical composition, antifungal activity

INTRODUCTION

Opuntias Mill., Prickly pears (*Cactaceae*, *Opuntioideae)*, is a very large genus of cacti, about 360 species varying in size from 5 cm tall miniature plants to 10-30 m tall trees. They are native from Canada, to Chile and Argentina, but widely naturalised throughout the world, found in dry places and rocky slopes (Anderson, 1999). Prickly pears have paddle-like stems, which vary in size and shape even on the same plant, interrupted by constricted joints and minute leaves that soon fall. Many species bear spines. In *Opuntia species* the stem segments are up to 40 cm long. Evenly spaced across the joints are areoles out of which spines and glochids (prickles) grow. Flowers, up to 10 cm across, grow from the areoles on the top edge of the joint, but some species also flower from side areoles. Though most are yellow, orange, or red, some species have white or pink also (Hewitt, 1994). *Opuntias* produce egg-shaped and generally edible fruit with flat tops that ripens to red or purple. The use of *Opuntia* as a source of food for humans, a source of green forage and a much appreciated source of water for livestock has been very important in the arid and semi-arid regions of northern Mexico and in many arid countries (Haustein, 2004).

In Tunisia we found many species belonging to *Cactaceae* family, all have been introduced about 200 years ago. *Opuntia species* either spiny or spineless varieties are abundant and widely distributed in large thickets across the country, used as forage as soil stabilizer, or defensive barrier and for its delicious fruits. Some *Opuntia* species have been cultivated specially as ornamental plant in parks and botanical gardens, such as *O. microdasys* (Lehmann) Pfeiffer var. *pallida*, *Opuntia macrorhiza* Engelm., *Opuntia lindheimeri* var. *linguiformis*.

As far as our literature survey could ascertain, volatile extracts analysis and antifungal activities of the

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three *Opuntia* species studied, have not previously been published, although some reports concerning the constituents of *Opuntia ficus indica* were found (Bernhard *et al*., 2001).

In this research, we report composition and antifungal activity of the volatile compounds extracted from different aerial three *Opuntia* species organs. As a target organism were used 6 fungal species frequently isolated in Tunisian crops, fruits or soils.

MATERIALS AND METHODS

Plant material: Three plants were tested in our study: *Opuntia lindheimeri var linguiformis*, *Opuntia macrorhiza*, *Opuntia microdasys*. The plants were collected in the sea cliff bordering Monastir town (Tunisia) in April 2005 (latitude 35°46'0"N, longitude 10°59'0"E, coastal region, east of Tunisia, with a sub-humid climate). Identification was performed in laboratoire de Biologie Végétale et Botanique, Ecole Supérieure d'Horticulture et d'Elevage de Chott-Mariem, Université de Sousse, Sousse, Tunisia.

Extraction of volatile compounds: Each plant was divided in different parts: *Opuntia lindheimeri var linguiformis* (leaves, flowers and fruits), *Opuntia macrorhiza* (leaves and flowers), *Opuntia microdasys* (leaves). An appropriate amount of each plant sample were weighed (Table 1) then extracted by a steam distillation for 3-5 h. Five hundred milliliters (500 mL) of each aqueous distillate was subjected to two successive extractions with chloroform ($CHCl_3$) (2x100 mL). After decantation and separation, the recuperated organic layer, containing volatiles was dried over anhydrous sodium sulphate, then filtrated and concentrated under reduced pressure.

Analyses of volatile extracts

Gas chromatograph: HP 5890-series II equipped with; Flame Ionisation Detectors (FID), HP5MS 30 m ×0.25 mm ID, 0.25 μm film thickness fused capillary column. The carrier gas was nitrogen (1.2 mL min^{-1}). The oven temperature program was 1 min isothermal at 50°C, then 50-280°C at rate of 5°C min^{-1} and held isothermal for 1 min. The injection port temperature was 250°C, detector 280°C. Volume injected 0.1 μL of 1% solution (diluted in hexane). Percentages of constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

GC/MS: The analysis of the volatile constituents were run on a Hewlett-Packared GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP5MS capillary column (30 m x 0.25 mm ID, film thickness of 0.25 μm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 mL min^{-1}. Oven temperature was programmed (50°C for 1 min, then 50-280°C at 5°C min^{-1}) and subsequently, held isothermal for 20 min. Injector port: 250°C, detector: 280°C, split ration 1:50. Volume injected: 0.1 μL of 1% solution (diluted in hexane).

Mass spectrometer: HP 5972 recording at 70 eV; scan time 1.5 sec; mass Range 40-300 amu. Software adopted to handle mass spectra and chromatograms was a Chem.Station.

Identification of the compounds: The components of the volatile extracts were identified by comparison of their mass spectra with those of a computer library (Wiley 275 library). Further confirmation was done by referring to Retention Index data generated from a series of alkanes (C_9-C_{28}) (Shibamato, 1987; Adams, 1995).

Table 1: Plant parts, dry weights and yield of the volatile extracts obtained from different organs of *Opuntia* species

Plant parts	Dry weight (g)	Weight of volatile fraction (mg)	Yield (%)
Opuntia lindheimeri var. *linguiformis*			
Leaves	2710	20	7.38×10^{-4}
Fruits	631	8	1.26×10^{-4}
Flowers	231	5	2.16×10^{-4}
Opuntia macrorhiza			
Leaves	3823	8	2.09×10^{-4}
Flowers	427	3	7.02×10^{-4}
Opuntria microdasys var. *pallida*			
Leaves	2507	10	3.9×10^{-4}

Table 2: Fungal isolated used to test the antifungal activity of plant extracts

Fungus	Source	Plant part sampled	Location	Collection date
Botrytis cinerea	Vine	Fruit	Bou argoub	11/05/2002
F. Oxysporum f. sp. *niveum*	Watermelon	Roots	Skhira	23/05/2001
F. Solani f. sp. *cucurbitae*	Watermelon	Roots	Skhira	23/05/2001
Pythium ultimum	Watermelon	Roots and stems	Regueb	23/05/2001
Alternaria solani	Tomato	Leaves	Chott mariem	12/11/2002
Rhizoctonia solani	Watermelon	Roots and stems	Jebeniena	05/07/2001

Antifungal activity

Test organisms: Six phytopatogenic fungal species were used for the antifungal testing, namely: *Botrytis cinerea*, *F. Oxysporum f.* sp. *Niveum*, *F. Solani f.* sp. *Cucurbitae*, *Pythium ultimum*, *Alternaria solani*, *Rhizoctonia solani*. These isolates were identified and samples of each fungus were deposited in the collection bank at the Plant Pathology Laboratory (Ecole Supérieure d'Horticulture et d'Elevage de Chott-Meriem, Université de Sousse, Tunisia) (Table 2).

Evaluation of antifungal activity of volatile extracts: The disc diffusion method was used for antifungal screening (Al-Mughrabi *et al*., 2001). Fungal broth culture aliquots were added to Potato Dextrose Agar medium and distributed uniformly in 9 cm petri dishes. Once the substrate solidified, four discs of Wattman N°5 paper were placed in petri dishes. Each small disc was moistened with 20 μL of volatile extracts. In the case of the control, the small disc was moistened with the same volume of SDW+Tween-80 (10%). The diameter of the zone of inhibition (mm) around the discs was measured after cultivation at 25°C for 8 days and compared with control. The test was performed in triplicate.

RESULTS AND DISCUSSION

Botanical description of the three *Opuntia* species: *Opuntia microdasys* (Lehmann) Pfeiffer var. *pallida*, is a short branching shrub, with flattened oblong to rounded pads from 10 to 15 cm of long, with dense areoles, without true spines, loading short gold yellow (var. *pallida*) numerous glochids which are blown by wind. Flowers are lemon yellow slightly tinged with red, 3-5 cm in diameter, produce lobular dark red fruit.

O. macrorhiza Engelm., Mexicanrose Prickly Pear is a nearly prostrate *Opuntia* easily distinguished by its yellow flowers, 5-6 cm long, joints which are noticeably glaucous (bluish) and very slender and long spines (about 8 cm long).

O. lindheimeri var. *linguiformis*, large sprawling to semi-erect bush, about 2-4 m tall and spreads out 10 m or more, distinctive blue-green large pads, rounded at base, cow's tongue shape (var. *linguiformis)*, with a sharp pointed apex, about 15 cm wide and 20 cm long. Flat areoles, short, golden-brown glochids and most are on the joint's edges, very short on the face areoles. Nearly spineless except on or near the edge, 1-4 round spines per areole, cream to yellow occasionally brown to black 3-8 cm long. Flowers yellow, turning orange-red with age, bloom in May-June. Oval, plum-colored edible fruit and turns red later.

Chemical analyses of volatile extracts compounds: The composition of the volatile extracts obtained from the different studied organs is reported in Table 3 together with the Retention Indices (RI) calculated and percentage for each compound. The constituents are arranged by function and were found mainly to be carboxylic acids, ketones, esters, aldehydes, alcohols, hydrocarbons. Those compounds commonly found in volatile fractions of plants (Mailhebiau, 1994; Brun *et al*., 2001; Harzallah-Skhiri *et al*., 2005) and were dispersed differently within organs (Macchioni *et al*., 2003). We reported difference in the volatile compound composition between different organs from the three plants. Investigation did on volatile fractions of some plants have shown differences between species as well as varieties from genera as *Ficus*, *Morus* and *Opuntia* (Nacef *et al*., 2003). Bouazizi *et al*. (2002) found also difference between the composition in volatile compounds of *Amaranthus augustifolia* and *A. lividis*. Volatile extracts from all organs of *Opuntia* were characterized by the predominance of carboxylic acids and esters in comparison with the rest of the constituents (Table 3).

The volatile extract from leaves of *Opuntia lindheimeri var linguiformis* showed as main fractions carboxylic acids (30.73%) and esters (22.74%). This organ contained more of tetradecanoic acid (13.57%), whereas, the fruits and the flowers are richer in hexadecanoic acid (17.33 and 16.7%, respectively). Nonanoic acid was identified only in the leaves (7.16%) and the fruits (3.96%) and pentadecanoic acid was detected only in the leaves (1.5%) of the same plant (Table 3). The main ester found in volatile extract obtained from the leaves of the above plant was (E)-5-Butyldiene phthalide (13.17%). Only one alcohol nonadecan-1-ol (3.19%) and no aldehyde were found in this organ. Five hydrocarbons represent 9.44% of the totality of the volatile compounds (Table 3). The major fraction present in the volatile extracts of the flowers and the fruits from the same plant was esters (34.78 and 24.89%, respectively) (Table 3). Only fruits containing significant amount of alcohols (12.7%) from which eicosan-1-ol was present at appreciable content (8.55%).

The volatile extract from leaves of *Opuntia macrorhiza* showed a main compound the ester (E)-3-Butyldiene phthalide (14.02%). In the flowers volatile extract this compound represents 7.37% but the major one was butyl tetradecanoate (21.14%) also six hydrocarbons representing 12.42% were found only in this plant organ. On the other hand, carboxylic acids were significant in the flowers (17.77%) but they were minors in the leaves (3.48%).

In the volatile extract of *Opuntia microdasys* leaves, the (E)-3-Butyldiene phthalide continues to be the major

Table 3: Percentage composition of the volatile extracts of the three studied plants

		O. Lindheimeri var. *linguiformis*			*O. macrorhiza O. microdasys* var. *pallida*			
		Percentage						
Component	RI (HP5MS)	L	Fl	Fr	L	Fl	L	Identification
Carboxylic acids								
Tetradecanoic acid	1764	13.57	4.04	2.15	1.18	-	-	MS,RI
Pentadecanoic acid	1864	1.50	-	-	0.47	-	1.73	MS,RI
Hexadecanoic acid	1964	8.50	16.70	17.33	-	7.20	13.13	MS,RI
Nonanoic acid	2150	7.16	-	3.96	1.83	10.57	7.56	MS,RI
Ketones								
(5*E*, 9*E*)-Farnesyl Ketone	1928	1.49	-	-	-	-	-	MS,RI
Undecan-2-one	1276	-	-	-	0.14	-	-	MS,RI
Esters								
Isopentyl hexanoate	1242	-	-	0.49	-	-	-	MS,RI
Linalyl acetate	1244	-	2.23	-	-	-	-	MS,RI
Butyl tetradecanoate	1978	8.05	21.47	8.18	3.70	21.14	5.91	MS,RI
Ethyl octanoate	2171	1.52	4.16	-	-	2.34	-	MS,RI
(*E*)-5-Butyldiene phthalide	2562	13.17	6.92	15.77	14.02	7.37	21.40	MS,RI
Methyl hexadecanoate	1632	-	-	-	-	2.01	-	MS,RI
Benzyl benzoate	1739	-	-	-	-	1.24	-	MS,RI
Aldehydes								
(*E*)-Non-2-enal	1156	-	-	1.35	-	-	-	MS,RI
Benzaldehyde	1499	-	-	1.07	-	-	-	MS,RI
Alcohols								
Hexan-1-ol	1338	-	-	8.55	-	-	-	MS,RI
Eicosan-1-ol	1604	-	-	1.08	-	-	-	MS,RI
α-Bisabolol	1678	-	-	1.91	-	-	-	MS,RI
Nona-1-ol	1772	-	-	3.07	-	-	-	MS,RI
Phytol	1950	-	2.52	-	-	-	-	MS,RI
Nonadecan-1-ol	2166	3.19	-	-	-	-	-	MS,RI
Tridecan-1-ol	1567	-	-	-	0.21	-	-	MS,RI
Hdrocarbons								
Octadec-1-ene	1874	-	-	0.79	-	-	-	MS,RI
Eicosane	2000	3.19	-	0.47	-	-	-	MS,RI
Heneicosane	2100	-	0.56	-	-	-	-	MS,RI
Tricosane	2300	-	0.40	3.10	-	2.55	-	MS,RI
Tetracosane	2400	-	-	-	-	0.72	-	MS,RI
Pentacosane	2500	2.77	0.82	2.01	-	-	0.61	MS,RI
Hexacosane	2600	1.02	-	1.52	-	1.79	-	MS,RI
Heptacosane	2700	-	4.89	1.99	-	4.05	-	MS,RI
Octacosane	2800	1.80	2.08	1.48	-	1.25	1.60	MS,RI
Nonacosane	2900	1.16	-	1.60	-	2.06	0.66	MS,RI
Dotriacontane	3200	-	-	1.47	-	-	1.99	MS,RI
Pentadec-1-ene	1492	-	-	-	-	-	0.29	MS,RI
Docosane	2200	-	-	-	-	-	0.88	MS,RI
Others								
2-Mercaptobenzothiazol	1944	-	-	-	0.87	-	-	MS,RI
Abietatriene	2047	-	-	-	0.35	-	-	MS,RI
Diepi-13-manonyle	2028	-	-	-	-	-	0.64	MS,RI

L: Leaves; FL: Flowers; Fr: Fruits; -: Compound absent

Table 4: Inhibition (mm)[a] of fungal growth with different parts of *Opuntia lindheimeri var linguiformis*, *Opuntia macrorhiza* and *Opuntia microdasys*

Plant parts/Fungus	*Botrytis cinerea*	*F. oxysporum* f. sp. niveum	*F. solani f.* sp. *cucurbitae*	*Pythium ultimum*	*Alternaria solani*	*Rhizoctonia solani*
Opuntia lindheimeri. var. linguiformis						
Leaves	0	0	0	0	58.83±1.75	
Flowers	0	16.83±0.76[a]	0	0	15±1	
Fruits	0	0	0	0	46.83±7.07	
Opuntia marcrorhiza						
Leaves	0	0	0	0	61.33±0.76	
Flowers	0	0	0	0	61.16±0.76	21±1
Opuntia microdasys var. *pallida*						
Leaves	0	0	0	0	48.33±1.8	

[a]Mean of inhibition is the mean±SE of three repetitions per fungus and per volatile extract

component (21.4%). The latter and the butyl tetradecanoate (5.91%), both representing the ester fraction, constitute one of the most important part (27.31%) of this extract. Pentadecanoic (1.73%), hexadecanoic (13.13%) and nonadecanoic (7.65%) acids were present and represent the second main fraction of this volatile extract.

Eight common components were differently dispersed in the organs of the three studied *Opuntia* species, 3 carboxylic acids (pentadecanoic, hexadecanoic and nonadecanoic acids) with a predominance of the second one. Two esters, butyl tetradecanoate and (E)-3-Butyldiene phthalide, two hydrocarbons, octacosane and nonacosane and only one alcholol, phytol were found essentially in the flowers.

Antifungal activity of different tested volatile extracts: Volatile extracts obtained from different parts of the three tested plants, showed an antifungal effects against some plant pathogenic fungi. In general, extracts from leaves exhibited the stronger antifungal activity (Table 4).

Volatile extract from leaves of *Opuntia lindheimeri var. linguiformis* had the strongest antimicrobial effect (58.83±1.75 mm) then this from fruits (46.83±7.07 mm) and from flowers (15±1 mm) (Table 4). High inhibition of *Alternaria solani* was obtained with *Opuntia macrorhiza* leaves and flowers volatile extracts (61.33±0.76 and 61.16±0.76 mm) (Table 4). A high level of inhibition (58.83±1.75 and 46.83±7.07 mm) (Table 4) against this fungus was obtained with leaves and fruits volatile extract of *Opuntia lindheimeri var linguiformis* and with this of *Opuntia microdasys* leaves (48.33±3.51 mm). Volatile extract obtained from *Opuntia lindheimeri var linguiformis* flowers exhibited a low mean inhibition (15±1 mm) against *Alternaria solani.* All volatile extracts from the three plants studied in this work were with no effect on *Botrytis cinerea*, *Fusarium solani* f. sp. *cucurbitae* and *Pythium ultimum* development.

Fusarium oxysporum f. sp. *niveum* and *Rhizoctonia solani* showed moderately inhibition level by contact with *Opuntia lindheimeri var linguiformis* flowers volatile extract (16.83±0.76 mm) and this of *Opuntia macrorhiza* leaves (21±1 mm) (Table 4) at the concentration of 1 mg mL^{-1}.

Overall the results of this study show that the three *Opuntia species* may be promising source of antimicrobial compounds to be used as alternative to pesticides in the control of plant diseases.

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