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Chemical Composition and Biological Activities of the Essential Oils from Two *Pereskia* Species Grown in Brazil

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The chemical composition of the essential oils of *Pereskia aculeata* Mill. and *P. grandifolia* Haw. (Cactaceae), grown in Brazil, was studied by means of GC and GC-MS. In all, 37 compounds were identified, 30 for *P. aculeata* and 15 for *P. grandifolia*. Oxygenated diterpenes are the main constituents, both in the oil of *P. grandifolia* (55.5%) and in that of *P. aculeata* (29.4%). The essential oils were evaluated for their *in vitro* phytotoxic activity against germination and initial radicle growth of *Raphanus sativus* L., *Sinapis arvensis* L., and *Phalaris canariensis* L. seeds. The essential oil of *P. grandifolia*, at all doses tested, significantly inhibited the radicle elongation of *R. sativus*. Moreover, the antimicrobial activity of the essential oils was assayed against ten bacterial strains. The essential oils showed weak inhibitory activity against the Gram-positive pathogens.

Keywords: *Pereskia aculeata*, *Pereskia grandifolia*, Essential oil, Chemical composition, Biological activities.

The Cactaceae family comprises plants that are desert and semi-desert habitats, which, under environmental stress conditions have developed high defense systems based on phytochemicals, such as alkaloids, flavonoids, terpenes, and tannins, reported to have remarkable biological activities [1]. The genus *Pereskia* is considered the least advanced of the Cactaceae family; it contains 17 species with two sub groups, mainly distributed in the regions between Brazil and Mexico [2]. These plants possess succulent leaves and terminal flowers gathered in cymes [3]. The literature reports that *P. bleo* Kunth, *P. grandifolia* Haw. and *P. aculeata* Mill. have been used as vegetables and in the treatment of various diseases in folk medicine [2]. Moreover, different biological activities have been proved for some *Pereskia* species [4, 5].

P. aculeata Mill. and *P. grandifolia* Haw. are two cacti known in Brazil with the vernacular name "Ora Pro Nobis" and are used in cooking because they have a high nutritional content. The leaves of *P. aculeata* contain high levels of proteins when compared with other plants commonly used as human food [6], and they also contain high levels of carotenoids, minerals, dietary fiber, vitamins A and C, and folic acid [7,8]. Sterols (sitosterol and stigmasterol), flavonoids and phenolic compounds are reported for the leaves of *P. aculeata* [2].

For the first time, Pinto and coworkers [9] reported the cytotoxic and antioxidant activity of *P. aculeata.* The results showed that some fractions inhibited breast cancer cell line MCF-7 and human promyelocytic leukemia cells HL-60 cell proliferation, and that phenolic compounds are the major antioxidant components found in *P. aculeata* leaves.

P. grandifolia is used as a medicinal, edible and ornamental plant [10]. The leaves are traditionally used in Malaysia for the treatment of cancer, hypertension, diabetes and diseases associated with rheumatism and inflammation [11]. Three alkaloids, tyramine, saponins, phenolic compounds, β-sitosterol, vitamin E, and other compounds are reported in this plant [2], as well as sterols and fatty acids [12]. Methanolic and hexane extracts of *P. grandifolia* are reported for their antioxidant and cytotoxic activities on human oropharingeal epidermoid carcinoma KB, MCF7, and human cervical epithelioid carcinoma cells HeLa cell lines. Moreover, different extracts of *P. grandifolia* are reported for their antimicrobial activity [13]. Extracts of this plant showed cytotoxicity to Saos-2 cells in hypoxic versus normoxic conditions [14] and on a panel of tumor cell lines [12]. An infusion and an ethanol extract of the plant were shown to possess hypotensive effects by the involvement of arginine-vasopressine [15].

However, no articles are reported on the chemical composition of the essential oils of *P. aculeata* and *P. grandifolia*, and their phytotoxic activity. Therefore, the aim of this paper was to study the chemical composition of the essential oils obtained from leaves of *P. aculeata* and *P. grandifolia,* grown in Brazil, to evaluate their possible *in vitro* effects against germination and initial radicle elongation of *Raphanus sativus* L. (radish), *Sinapis arvensis* L. (wild mustard) and *Phalaris canariensis* L. (canary grass), and the potential antimicrobial activity against ten selected microorganisms.

Hydrodistillation yielded 0.03% and 0.09% of essential oil (on a dry mass basis) for *P. aculeata* and *P. grandifolia*, respectively. Table 1 shows the chemical composition of the two *Pereskia* oils; compounds are listed according to their elution order on a HP-5MS column. In all, 37 compounds were identified, 30 for *P. aculeata*, accounting for 92.1% of the total oil, and 15 for *P. grandifolia*, accounting for 92.6% of the total oil. Oxygenated diterpenes are the main constituents, both in the oil of *P. grandifolia* (55.5%) and in that of *P. aculeata* (29.4%). In the oil from *P. aculeata*, the main compounds are phytol (29.4%), hexadecanoic acid (17.4%) and linoleic acid (12.7%). Other compounds, in a lesser amount are heptadecane (1.9%) and 14-hydroxy-4,5-dihydro-caryophyllene (1.6%). In the oil from *P. grandifolia*, manool oxide (30.1%),

Table 1: Essential oil compositions (%) of *Pereskia grandifolia* (PG) and *P. aculeata* (PA).

N.	Compound	Ki[a]	Ki[b]	PG	PA	Identification[c]
1	1-Tetradecene	1390	1433	-[d]	0.2	1,2
2	9-Decenyl acetate	1397		0.9	-	1,2
3	(*E*)-*β*-Ionone	1485	1958	t[e]	0.1	1,2
4	*n*-Pentadecane	1485	1500	1.7	0.3	1,2,3
5	10-*epi*-Italicene ether	1511	1856	0.8		1,2
6	6-Methyl-*α*-ionone	1512		-	7.2	1,2
7	Methyl isovalerate	1520		-	0.4	1,2
8	Citronellyl butyrate	1522		-	0.3	1,2
9	*cis*-Dihydro-mayurone	1587		-	t	1,2
10	Caryophyllene oxide	1584	2008	-	0.3	1,2,3
11	1-Hexadecene	1590	1654	-	0.3	1,2
12	*n*-Hexadecane	1598	1200	4.2	1.3	1,2,3
13	*α*-Muurolol	1642		-	0.5	1,2
14	*α*-Cadinol	1656	2255	-	0.9	1,2
15	14-Hydroxy-(*Z*)-caryophyllene	1661	2357	-	0.6	1,2
16	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1661		-	0.6	1,2
17	Eudesma-4(15),7-dien-1β-ol	1690		-	0.3	1,2
18	Heptadecane	1698	1700	4.2	1.9	1,2,3
19	14-hydroxy-4,5-dihydro-Caryophyllene	1706		-	1.6	1,2
20	*cis*-Thujopsenal	1709		6.7	0.9	1,2
21	*n*-Octadecane	1796	1800	9.2	1.0	1,2,3
22	Cyclopentadecanolide	1806		-	t	1,2
23	Alkane not identified	1808		-	1.2	1,2
24	Demethyl isotorquatone	1831		0.8	-	1,2
25	Nonadecane	1899	1900	2.5	2.9	1,2,3
26	Methyl hexadecanoate	1925	2208	-	2.6	1,2
27	Tetrahydro rimuene	1962		1.7	-	1,2
28	Hexadecanoic acid	1966	2931	-	17.4	1,2
29	Manool oxide	1966		30.1	-	1,2
30	Ethyl hexadecanoate	1989		-	0,6	1,2
31	*n*-Eicosane	1998	2000	-	2.9	1,2,3
32	13-*epi*-Manool oxide	2011		0.3	-	1,2
33	Isopropyl hexadecanoate	2026		-	0.7	1,2
34	Methyl linoleate	2091		-	3.0	1,2
35	Linoleic acid	2099		-	12.7	1,2
36	*n*-Heneicosane	2096	2100	4.4	-	1,2,3
37	Phytol	2109	2622	25.1	29.4	1,2
	Total			**92.6**	**92.1**	
	Oxygenated Monoterpenes			**-**	**0.3**	
	Oxygenated Sesquiterpenes			**8.3**	**6.1**	
	Diterpene Hydrocarbons			**1.7**	**-**	
	Oxygenated Diterpenes			**55.5**	**29.4**	
	Hydrocarbons			**27.1**	**12.0**	
	Others			**-**	**44.3**	

[a] Kovats retention index determined relative to the t_R of a series of *n*-alkanes (C_{10}-C_{35}) on HP-5 MS column; [b] Kovats retention index determined relative to the t_R of a series of *n*-alkanes (C_{10}-C_{35}) on HP Innowax; [c] 1 = Kovats retention index, 2 = mass spectrum, 3 = co-injection with authentic compound; [d] – = Not detected; [e] t = trace (< 0.1%).

phytol (25.1%), *n*-octadecane (9.2%) and *cis*-thujopsenal (6.7%) are the main constituents.

The literature contains no reference about the chemical composition of the essential oil of *Pereskia* species, and only little information could be found about the essential oils of the Cactaceae family. Bergaoui and coworkers [16] reported the composition of the volatile fraction obtained from leaves, flowers and fruits of *Opuntia lindheimeri* Engelm var. *linguiformis* L. D. Benson, leaves and flowers of *O. macrorhiza* Engelm and leaves of *O. microdasys* (Lehm.) Pfeiff. gathered from the sea cliff of Monastir (Tunisia). The major compounds found in leaves, flowers and fruits of *O. 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 of *O. macrorhiza*, the main compound was butyl tetradecanoate (21.14%), while the volatile fraction obtained from *O. microdasys* leaves was mainly rich in hexadecanoic acid (13.13%), (*E*)-3-butyldiene phthalide (21.4%) and butyl tetradecanoate (5.91%).

The two essential oils were evaluated for their activity against germination (Table 2) and radicle elongation (Table 3) of radish, a species frequently utilized in biological assays, and of wild mustard and canary grass, two weed species. The oils seem to be ineffective against germination (Table 2), but they affected the radicle elongation of *R. sativus* and *S. arvensis* (Table 3). The essential oil of *P. grandifolia*, at all doses tested, significantly inhibited the radicle elongation of *R. sativus*. The same oil significantly inhibited the radicle elongation of *S. arvensis.* At 0.062 µg/mL, the essential oil of *P. aculeata* significantly inhibited the radicle elongation of *R. sativus*.

Table 2: Phytotoxic activity of the essential oils of *Pereskia grandifolia* and *P. aculeata* against germination of *Raphanus sativus*, *Sinapis arvensis* and *Phalaris canariensis*, 120 h after sowing. Results are the mean ± standard deviation (SD).

	Pereskia grandifolia	*Pereskia aculeata*
Raphanus sativus	Germinated seeds ± SD	Germinated seeds ± SD
Control	10 ± 0.0	10 ± 0.0
2.5 µg/mL	9.0 ± 0.0	9.7 ± 0.6
1.25 µg/mL	10.0 ± 0.0	9.7 ± 0.6
0.625 µg/mL	9.3 ± 0.6	9.7 ± 0.6
0.250 µg/mL	9.7 ± 0.6	9.3 ± 1.2
0.125 µg/mL	8.7 ± 0.6	9.0 ± 0.6
0.062 µg/mL	8.7 ± 1.5	9.0 ± 1.0
Sinapsis arvensis	Germinated seeds ± SD	Germinated seeds ± SD
Control	8.6 ± 0.6	8.6 ± 0.6
2.5 µg/mL	9.3 ± 0.6	8.7 ± 1.5
1.25 µg/mL	9.3 ± 1.2	9.7 ± 0.6
0.625 µg/mL	9.0 ± 1.7	10.0 ± 0.0
0.250 µg/mL	9.0 ± 1.0	9.3 ± 0.6
0.125 µg/mL	8.7 ± 1.2	9.3 ± 0.6
0.062 µg/mL	8.7 ± 1.2	9.7 ± 0.6
Phalaris canariensis	Germinated seeds ± SD	Germinated seeds ± SD
Control	10.0 ± 0.0	10.0 ± 0.0
2.5 µg/mL	9.0 ± 1.0	9.0 ± 0.0
1.25 µg/mL	10.0 ± 0.0	9.7 ± 0.6
0.625 µg/mL	9.0 ± 1.0	9.7 ± 0.6
0.250 µg/mL	9.7 ± 0.6	9.3 ± 0.6
0.125 µg/mL	9.7 ± 0.6	9.0 ± 1.0
0.062 µg/mL	9.0 ± 1.0	9.7 ± 0.6

Table 3: Phytotoxic activity of the essential oil of *Pereskia grandifolia* and *P. aculeata* against radicle elongation of *Raphanus sativus*, *Sinapis arvensis* and *Phalaris canariensis*, 120 h after sowing. Data are expressed in cm.

	Pereskia grandifolia	*Pereskia aculeata*
Raphanus sativus	Radicle length ± SD (cm)	Radicle length ± SD (cm)
Control	4.0 ± 1.4	4.0 ± 1.4
2.5 µg/mL	2.5 ± 0.8***	2.5 ± 1.3
1.25 µg/mL	2.4 ± 1.1***	3.6 ± 1.2
0.625 µg/mL	2.2 ± 1.1***	3.8 ± 1.2
0.250 µg/mL	2.1 ± 1.4***	3.5 ± 1.6
0.125 µg/mL	3.0 ± 1.2*	2.7 ± 1.5
0.062 µg/mL	2.3 ± 0.9***	1.5 ± 0.7*
Sinapsis arvensis	Radicle length ± SD (cm)	Radicle length ± SD (cm)
Control	1.9 ± 1.0	1.9 ± 1.0
2.5 µg/mL	1.0 ± 0.4**	2.1 ± 1.2
1.25 µg/mL	1.6 ± 1.0	1.6 ± 0.8
0.625 µg/mL	1.2 ± 0.6*	1.5 ± 0.5
0.250 µg/mL	1.0 ± 0.6**	1.4 ± 0.7
0.125 µg/mL	1.0 ± 0.8**	1.8 ± 1.0
0.062 µg/mL	1.0 ± 0.4**	1.8 ± 1.0
Phalaris canariensis	Radicle length ± SD (cm)	Radicle length ± SD (cm)
Control	3.6 ± 0.6	3.6 ± 0.6
2.5 µg/mL	2.9 ± 0.7	3.1 ± 0.8
1.25 µg/mL	3.0 ± 0.6	3.4 ± 1.2
0.625 µg/mL	2.9 ± 0.6	2.8 ± 0.8
0.250 µg/mL	3.0 ± 0.6	3.3 ± 0.8
0.125 µg/mL	2.5 ± 0.9	3.3 ± 0.6
0.062 µg/mL	2.6 ± 0.8	2.7 ± 0.8

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. positive control.

The difference in biological activity of the oils could be attributed to their different chemical composition. The oil of *P. grandifolia* was rich in oxygenated diterpenes (55.5%). Macias and coworkers [17] reported the high phytotoxic activity of two diterpenes (2-oxokovalenic acid, 19-hydroxyferruginol) isolated from *Tectona grandis* L. on *Lactuca sativa* L., *Lycopersicum esculentum* Mill., *Lepidium sativum* L., and *Allium cepa* L. Some clerodane diterpenes isolated from the exudate of the fresh aerial parts of *Salvia miniata* Fernald were phytotoxic against *Papaver rhoeas* L. and *Avena sativa* L. final germination [18]. Moreover, Amri and coworkers [19] reported the herbicidal activity of the essential oil of *Pinus nigra* L. subsp. *laricio*, rich in oxygenated diterpenes (38.5%),

particularly manool oxide (38%), on germination and seedling growth of *Phalaris canariensis*, *Trifolium campestre* Schreb. and *Sinapis arvensis*.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the essential oils against ten selected microorganisms are reported in Table 4. The essential oils showed weak inhibitory activity against the Gram-positive pathogens; *Staphylococcus aureus* was the most resistant bacterium. The essential oils did not show any significant activity against Gram-negative bacteria.

Table 4: MIC and MBC* values (μg/mL) of essential oils from the two *Pereskia* species and MIC of the reference antibiotic, chloramphenicol.

	Pereskia grandifolia		*Pereskia aculeata*		C
Bacterial Strain	**MIC[a]**	**MBC[b]**	**MIC[a]**	**MBC[b]**	
Bacillus cereus ATCC 177	n.a.	100	50	100	12.5
Bacillus subtilis ATCC 633	100	>100	n.a	100	12.5
Staphylococcus aureus ATCC 25923	n.a.	>100	n.a.	>100	25
Staphylococcus epidermidis ATCC 12228	50	100	50	100	3.12
Streptococcus faecalis ATCC 29212	n.a.	100	100	n.a.	25
Escherichia coli ATCC 25922	n.a.	>100	n.a.	>100	12.5
Klebsiella pneumoniae ATCC 10031	n.a.	>100	n.a.	>100	50
Proteus vulgaris ATCC 13315	n.a.	>100	n.a.	>100	25
Pseudomonas aeuriginosa ATCC 27853	n.a.	>100	n.a.	>100	100
Salmonella typhi Ty2ATCC 19430	n.a.	>100	n.a.	>1000	6.25

[a] MIC, Minimal Inhibitory Concentration (μg/mL); [b] MBC, Minimal Bactericidal Concentration μg/mL); n.a., not active. C: Chloramphenicol.

In the literature, antimicrobial activities are reported only for crude extracts of some species of *Pereskia.* Philip and coworkers [13] reported the antimicrobial activity of different extracts of *P. bleo* Kunth and *P. grandifolia* against four bacterial pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis.* The ethyl acetate extract of *P. grandifolia* showed some antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Moreover, hexane and methanol extracts of *P. bleo* showed high and moderate, respectively, antibacterial activity towards two Gram-negative bacteria, *P. aeruginosa* 60690 and *Salmonella choleraesuis* [20].

Experimental

Plant material: The leaves of *Pereskia aculeata* Mill. and *P. grandifolia* Haw. were collected from the campus of Universidade Federal do Rio Grande do Sul (Porto-Alegre, Brazil) in October 2013. The plants were identified by Dr Kinupp, and voucher specimens were deposited at Alarich R. Schultz Herbarium of the Universidade Federal do Rio Grande do Sul (Brazil).

Isolation of volatile oil: One hundred g of dried leaves of each *Pereskia* species were ground in a Waring blender and then subjected to hydrodistillation for 3 h according to the standard procedure described in the European Pharmacopoeia [21]. The oils were solubilized in *n*-hexane, filtered over anhydrous sodium sulfate and stored under N_2 at +4°C in the dark, until tested and analyzed.

GC-FID analysis: Analytical gas chromatography was carried out on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a FID and a data handling processor. The separation was achieved using a HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Column temperature: 40°C, with 5 min initial hold, and then to 270°C at 2°C/min, 270°C (20 min); injection mode splitless (1 μL of a 1:1,000 *n*-hexane solution). Injector and detector temperatures were 250°C and 290°C, respectively. Analysis was also made using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm i.d., 0.25 ìm film thickness). In both cases, helium was used as carrier gas (1.0 mL/min).

GC/MS analysis: Analysis was performed on an Agilent 6,850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2,000 V. Mass spectra were scanned in the range 40–500 amu, scan time 5 scans/s. Gas chromatographic conditions were as reported in the previous paragraph; transfer line temperature, 295°C.

Identification of the essential oil components: Most constituents were identified by gas chromatography by comparison of their Kovats retention indices (Ri) [determined relative to the t_R of *n*-alkanes (C_{10}–C_{35})], with either those of the literature or by comparison of the mass spectra on both columns with those of authentic compounds available in our laboratories by means NIST 02 and Wiley 275 libraries [22]. The component relative concentrations were obtained by peak area normalization. No response factors were calculated.

Biological assay: A bioassay based on germination and subsequent radicle growth was used to study the phytotoxic effects of the two essential oils on seeds of *Raphanus sativus* L. (radish), *Sinapis arvensis* L. (wild mustard), and *Phalaris canariensis* L. (canary grass). The seeds were purchased from Blumen Srl (Piacenza, Italy). The seeds were surface sterilized in 95% ethanol for 15 s and sown in Petri dishes (Ø = 90 mm), containing 5 layers of Whatman filter paper, impregnated with either distilled water (7 mL, control) or test solution of the essential oil (7 mL), at the different assayed doses. The germination conditions were 20 ± 1°C, with natural photoperiod. The essential oil, in water–acetone mixture (99.5:0.5), was assayed at the doses of 2.5, 1.25, 0.625, 0.25, 0.125 and 0.062 μg/mL. Controls performed with water-acetone mixture alone showed no appreciable differences in comparison with controls in water alone. Seed germination was observed directly in Petri dishes, each after 24 h. A seed was considered germinated when the protrusion of the root became evident [23]. After 120 h (on the fifth day), the effects on radicle elongation were measured in cm. Each determination was repeated 3 times, using Petri dishes containing 10 seeds each. Data are expressed as the mean ± SD for both germination and radicle elongation. Data were analyzed using ANOVA followed by the Dunnet's test through GraphPad software (GraphPad Software Inc., San Diego, CA).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth dilution method [24]. Ten bacterial species, selected as representative of the class of Gram-positive and Gram-negative, were tested: *Staphylococcus aureus* (ATTC 25923), *Streptococcus faecalis* (ATTC 29212), *Bacillus cereus* (ATCC 1177), *B. subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella typhi* Ty2 (ATCC 19430) and *Proteus vulgaris* (ATCC 13315). The strains were maintained on Tryptone Soya agar (Oxoid, Milan, Italy); for the antimicrobial tests, Tryptone Soya broth (Oxoid, Milan, Italy) was used. In order to facilitate the dispersion of the oil in the aqueous nutrient medium, it was diluted with Tween 20, at a ratio of 10%. Each strain was tested with a sample that was serially diluted in broth to obtain concentrations ranging

from 100 µg/mL to 0.8 µg/mL. The sample was previously sterilized using a Millipore filter of 0.20 µm. The samples were stirred, inoculated with 50 µL of physiological solution containing 5 × 10^6 microbial cells, and incubated for 24 h at 37°C. The MIC value was determined as the lowest concentration of the sample that did not permit any visible growth of the tested microorganism after incubation. Control, containing only Tween 20, was not toxic to the microorganisms. As positive controls cultures were used containing only sterile physiologic solution Tris buffer. MBC was determined by subculture of the tubes with inhibition in 5 mL of sterile nutrient broth. After incubation at 37°C, the tubes were observed. When the bacteria did not grow, the sample denoted a bactericidal action. Each oil sample was tested in triplicate. Chloramphenicol was used as the standard antibacterial agent.

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