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Chemical Composition and Biological Activities of the Essential Oil from *Anredera cordifolia* Grown in Brazil

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The chemical composition of the essential oil of *Anredera cordifolia* (Ten.) Steenis (Basellaceae), grown in Brazil, was studied by means of GC and GC-MS analysis. In all, 19 compounds were identified, accounting for 91.6% of the total oil; hydrocarbons were the main constituents (67.7%). The essential oil was evaluated for its *in vitro* potential phytotoxic activity against germination and initial radicle growth of *Raphanus sativus* L., *Sinapis arvensis* L., and *Phalaris canariensis* L. seeds. At 1.25 µg/mL and 0.625 µg/mL, the oil significantly promoted the germination of *S. arvensis.* Moreover, the antimicrobial activity of the essential oil was assayed against ten bacterial strains. The essential oil showed a weak inhibitory activity against the Gram-positive pathogens.

Keywords: *Anredera cordifolia*, Essential oil, Chemical composition, Biological activities.

The genus *Anredera*, family Basellaceae, includes up to 12 species of perennial, twining or climbing, succulent or mucilaginous, often tuberous vines.

A. cordifolia (Ten.) Steenis is a plant native to South America, from Paraguay to Southern Brazil, Uruguay and Northern Argentina [1]. The specific name, *cordifolia*, refers to the heart-shaped leaves of the species, which has a climbing slender stem about 30 cm long, and dark green leaves with small and numerous white flowers. This plant presents small, irregular, green or light brown air tubers [1,2]. It is known by common names such as “Madeira vine” (South America), “Binahong” (Indonesia) and “Dhen San Chi” in China [3]. It is used traditionally to treat skin disease, hypertension, inflammation and gout [3].

Martinevski and coworkers [2] reported the nutritional value of *A. cordifolia*, known as *bertalha* in Brazil, where it is considered an unusual vegetable, with high nutritional value and known as spinach gaucho, leaf-fat and leaf-santa [4]; it can be used as a leafy vegetable because it has shown neither toxicity nor mutagenic effects [5].

A. cordifolia leaves are reported to contain triterpenoid and steroid saponins, alkaloids, flavonoids, polyphenols, quinones, monoterpenoids, sesquiterpenoids, coumarins and polysaccharides [3,6,7]. Zeid and coworkers [8] also reported phytol, α-pinene and 6,10,14-trimethyl-2-pentadecanone.

Some studies have reported the antibacterial and wound healing activity of *A. cordifolia* leaf extract [9,10], and Kumalasari and Sulistyani [11] recorded the antifungal activity of the ethanol extract of this plant against *Candida albicans.* Sukandar and coworkers [3] reported that the combination of *Zea mays* L. and *A. cordifolia* leaf extracts improved kidney function in a rat model of kidney failure through reduction of oxidative stress, probably due to the presence of flavonoids in the plants. Djamil and coworkers [7] reported the antioxidant activity of the flavonoid fraction of *A. cordifolia* leaves.

The aim of this paper was to study the chemical composition of the essential oil obtained from the leaves of *A. cordifolia*, grown in Brazil, to evaluate its 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 0.06% of a pale yellow oil (on a dry mass basis). Table 1 shows the chemical composition of the essential oil of *A. cordifolia*; compounds are listed according to their elution order on a HP-5MS column. In all, 19 compounds were identified, accounting for 91.6% of the total oil. Hydrocarbons were the main constituents of the oil (67.7%): the main compounds were *n*-hexadecane (11.6%), 2-hexyl-1-decanol (10.1%) and *n*-octadecane (6.3%). Moreover, the oil contains a high percentage of 6-methyl-α-ionone (23.9%), a carotenoid-derived volatile compound.

Only one literature reference reports the chemical composition of the essential oil obtained from aerial parts of *A. cordifolia*. The major compounds of the oil were phytol (15.3%), α-pinene (9.0%) and 6,10,14-trimethyl-2-pentadecanone (6.1%); the total oxygenated compounds constituted 57.4% [8]. The composition of our sample is quite different from the above reported composition, suggesting that different factors can affect the oil composition.

The essential oil was evaluated for its 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. At 1.25 µg/mL and 0.625 µg/mL, the oil significantly promoted the germination of *S. arvensis* (Table 2). The radicle elongation of radish and wild mustard did not appear significantly sensitive to the essential oil (Table 3).

Table 1: Essential oil composition (%) of *Anredera cordifolia.*

N.	Compound	Ki[a]	Ki[b]	%	Identification[c]
1	1-Tetradecene	1382	1389	0.9	1,2
2	*n*-Tetradecane	1396	1400	1.2	1,2,3
3	2,6,10-Trimethyl-dodecane	1459		1.4	1,2
4	2-Dodecenal, (2E)	1466	1466	1.2	1,2
5	*n*-Pentadecane	1494	1496	5.9	1,2,3
6	*trans*-Cyclohexane,1-(cyclohexylmethyl)-2-methyl	1490		0.1	1,2
7	6-Methyl-α-ionone	1507	1518	23.9	1,2
8	2-Hexyl-1-decanol	1542		10.1	1,2
9	1,10-Decanediol	1551		3.0	1,2
10	*n*-Hexadecane	1596	1600	11.6	1,2,3
11	Tetradecanal	1607	1612	1.6	1,2
12	*n*-Heptadecane	1684	1700	7.3	1,2,3
13	*n*-Pentadecanol	1768	1773	0.4	1,2
14	1-Octadecene	1782	1789	3.1	1,2
15	*n* -Octadecane	1790	1800	6.3	1,2,3
16	*n*–Hexadecanol	1869	1875	3.9	1,2
17	*n*-Nonadecane	1894	1900	4.6	1,2
18	*n*-Eicosene	1986	1988	1.8	1,2
19	*n*-Eicosane	1993	2000	3.3	1,2,3
	Total			**91.6**	
	Hydrocarbons			**67.7**	
	Others			**23.9**	

[a] Kovats retention index determined relative to the t_R of a series of *n*-alkanes (C_{10}-C_{35}) on a 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.

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

Anredera cordifolia	
Raphanus sativus	Germinated seeds ± SD
Control	8.0 ± 2.0
2.5 μg/mL	5.3 ± 1.5
1.25 μg/mL	5.6 ± 0.5
0.625 μg/mL	5.0 ± 1.0
0.250 μg/mL	4.6 ± 0.5
0.125 μg/mL	6.0 ± 1.7
0.062 μg/mL	5.7 ± 1.2
Sinapsis arvensis	Germinated seeds ± SD
Control	8.3 ± 0.6
2.5 μg/mL	9.3 ± 1.2
1.25 μg/mL	10.0 ± 0.0*
0.625 μg/mL	10.0 ± 0.0*
0.250 μg/mL	9.7 ± 0.6
0.125 μg/mL	9.3 ± 0.6
0.062 μg/mL	9.0 ±1.0
Phalaris canariensis	Germinated seeds ± SD
Control	9.3 ± 0.6
2.5 μg/mL	9.0 ± 0.0
1.25 μg/mL	8.7 ± 0.6
0.625 μg/mL	8.7 ± 1.2
0.250 μg/mL	9.6 ± 0.6
0.125 μg/mL	9.7 ± 0.6
0.062 μg/mL	10.0 ± 0.0

Note: * $p < 0.05$ *vs*. control.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of the essential oil against ten selected microorganisms are reported in Table 4. The essential oil showed inhibitory activity against the Gram-positive pathogens, among which were *Bacillus subtilis* and *Staphylococcus epidermidis*. The essential oil did not show any significant activity against Gram-negative bacteria. The antimicrobial activity of this oil is probably due to the high amount of hydrocarbons (67.7%) and the presence of non-regular terpenes, such as 6-methyl-α-ionone.

In the literature, it is reported that various compounds have direct activity against many species of bacteria, such as terpenes and a variety of aliphatic hydrocarbons. Aliphatic alcohols were reported to possess strong to moderate activities against several bacteria and the activity increased with the length of the carbon chain [12]. Agnihotri and coworkers [13] reported the antimicrobial and topical anti-inflammatory activity of essential oil isolated from fruits of *Amomum subulatum* Roxb. This oil was composed mainly of twelve aliphatic hydrocarbons (51.8%), seven aliphatic alcohols (27.4%), three fatty acids (12.4%) and four sesquiterpenoids (8.4%).

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

Anredera cordifolia	
Raphanus sativus	Radicle length ± SD (cm)
Control	2.8 ± 0.9
2.5 μg/mL	3.1 ± 1.3
1.25 μg/mL	2.0 ± 0.9
0.625 μg/mL	2.2 ± 0.8
0.250 μg/mL	2.0 ± 1.1
0.125 μg/mL	2.3 ± 0.6
0.062 μg/mL	2.4 ± 0.5
Sinapis arvensis	Radicle length ± SD (cm)
Control	1.3 ± 0.5
2.5 μg/mL	3.0 ± 1.2
1.25 μg/mL	1.5 ± 0.7
0.625 μg/mL	3.3 ± 1.1
0.250 μg/mL	2.8 ± 1.3
0.125 μg/mL	1.5 ± 0.9
0.062 μg/mL	2.0 ± 0.9
Phalaris canariensis	Radicle length ± SD (cm)
Control	3.9 ± 0.7
2.5 μg/mL	4.2 ± 0.6
1.25 μg/mL	3.4 ± 0.9
0.625 μg/mL	3.5 ± 1.1
0.250 μg/mL	3.3 ± 0.7*
0.125 μg/mL	3.6 ± 0.6
0.062 μg/mL	3.7 ± 0.6

Note: * $p < 0.05$ *vs*. control.

Table 4: MIC and MBC* values (μg/mL) of essential oil from *Anredera cordifolia* and MIC of the reference antibiotic, chloramphenicol.

	Anredera cordifolia		**C**
Bacterial strain	**MIC[a]**	**MBC[b]**	
Bacillus cereus ATCC 1177	100	n.a.	12.5
Bacillus subtilis ATCC 6633	50	n.a.	12.5
Staphylococcus aureus ATCC 25923	n.a.	n.a.	25
Staphylococcus epidermidis ATCC 12228	25	50	3.12
Streptococcus faecalis ATCC 29212	100	n.a.	25
Escherichia coli ATCC 25922	n.a.	n.a.	12.5
Klebsiella pneumoniae ATCC 10031	n.a.	n.a.	50
Proteus vulgaris ATCC 13315	n.a.	n.a.	25
Pseudomonas aeruginosa ATCC 27853	n.a.	n.a.	100
Salmonella typhi Ty2 ATCC 19430	n.a.	n.a.	6.25

[a] MIC, Minimal inhibitory concentration (μg/mL); [b] MBC, Minimal bactericidal concentration μg/mL); n.a., not active. C: Chloramphenicol.

Kang and coworkers [14] reported that β-ionone, a precursor of carotenoids, possesses a variety of biological properties such as anticancer, antimutagenic and antimicrobial activity, and reduced LPS-induced pro-inflammatory mediators. One reference reports that the crude extracts of *A. cordifolia* leaves did not inhibit growth of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [9].

Previously, Tshikalange and coworkers [15] reported that the chloroform extract of *A. cordifolia* inhibited the growth of bacteria. Gram-positive bacteria appeared to be more susceptible to the inhibitory effect of the extracts (water and chloroform) than Gram-negative ones. The weak activity obtained against Gram-negative bacteria was not surprising as, in general, these bacteria are more resistant than Gram-positive ones.

Experimental

Plant material: The leaves of *Anredera cordifolia* (Ten.) Steenis were collected from the campus of Universidade Federal do Rio Grande do Sul (Porto-Alegre, Brazil) in October 2013. The plant was identified by Dr Kinupp, and voucher specimen was 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 *A. cordifolia* was ground in a Waring blender and then subjected to hydrodistillation for 3 h according to the standard procedure described in the European Pharmacopoeia [16]. The oil was 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 run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm i.d., 0.25 μm 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 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 and also by comparison of mass spectra on both columns with those of authentic compounds available in our laboratories by means NIST 02 and Wiley 275 libraries [17]. 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 essential oil of *A. cordifolia* on seeds of *Raphanus sativus* (radish), *Sinapis arvensis* (wild mustard), and *Phalaris canariensis* (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 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 [18]. 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 [19]. Ten bacteria 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 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 with 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 containing only sterile physiological solution Tris buffer were used. 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 no growth was observed, the sample denoted a bactericidal action. Oil sample was tested in triplicate. Chloramphenicol was used as the standard antibacterial agent.

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