Artigo

VOLATILE CONSTITUENTS OF *Aristolochia trilobata* L. (*Aristolochiaceae*): A RICH SOURCE OF SULCATYL ACETATE

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Analysis of the volatile fraction of *Aristolochia trilobata* stem led to the identification of 6-methyl-5-hepten-2-yl acetate (23.31 ± 0.28%), limonene (15.43 ± 0.030%), linalool (8.70 ± 0.29%), p-cymene (7.81 ± 0.12%), bicyclogermacrene (4.21 ± 0.11%), and spathulenol (4.17 ± 0.14%) as the major constituents of the essential oil. Linalool (29.51 ± 0.49%), 6-methyl-5-hepten-2-ol (19.54 ± 0.82%), 6-methyl-5-hepten-2-yl acetate (8.92 ± 0.16%), and α-terpineol (4.62 ± 0.05%) were identified as major constituents of the hydrolate. The compound 6-methyl-5-hepten-2-yl acetate was isolated for the first time from this plant and was identified as the major component of the volatile fraction.

Keywords: *Aristolochia trilobata*; essential oil; 6-methyl-5-hepten-2-yl acetate.

INTRODUCTION

The genus *Aristolochia* consists of ~500 species distributed mainly in Asia, Africa, South America, and North America.[1] In recent studies, many of these plants have shown diuretic, analgesic, anti-inflammatory, and anti-cancer activity.[2]

Several species of *Aristolochia*, which present similarities both in terms of their botanical characteristics and their properties, can be found in Brazil. The species most commonly used in folk medicine are *A. triangularis, A. esperanzae, A. ridicula, A. brasiliensis, A. arcuate,* and *A. gigantea*. Considering the above-mentioned similarities, these species share the same common names in Brazil, which include *jarrinha*, *cipó-mil-homens*, *mil-homens*, *milone*, *papo-de-peru*, *erva-de-urubu*, and *jiboinha*.[3]

Extensive research has been carried out on the plants of this genus, mainly on extracts of the leaves, stems, and roots. A variety of activities have been attributed to them, these include bactericidal, anti-inflammatory, anti-trypanosomal, and anti-tumoral.[4-7]

Chemical compounds that have been identified in these plants, both in the essential oils and in organic solvent extracts, include aporphines, amides, quinolines, lignanes, diphenyl ethers, flavonoids, benzenoids, steroids, and terpenoids.[1]

The essential oils of the species belonging to the genus *Aristolochia* are comprised mainly of monoterpenes and sesquiterpenes, the commonly occurring terpenes being germacrene and caryophyllene, which are the major compounds in most cases.

Aristolochia trilobata L. is a species of *Aristolochia* found in Central and South America, and has several applications in traditional medicine in these regions.[8]

One such medicinal use in treating injured dogs has been reported by Lans *et al.*,[9] who carried out a study on hunters in Trinidad and Tobago. According to this study, species of the genus *Aristolochia*, in particular *A. trilobata* and *A. rugosa*, are widely used in treatment for dogs that have been bitten by snakes or scorpions.[9] In the same country, *A. trilobata* is used for treating stomach ache, colic, poisoning, and diabetes in human patients, as well as to facilitate the removal of the placenta and abortion.[10-12]

The use of this plant for treating snake bites is not restricted to Trinidad and Tobago. Studies have revealed that *A. trilobata* has also been used for this purpose in Brazil and Nicaragua.[13,14] In Brazil, it has been reported that *A. trilobata* is also used as a fungicide.[15]

Studies reveal that *A. trilobata* is used as an antimalarial agent in French Guiana.[16] In Dominica, this plant is used for treating intestinal problems.[17]

An infusion (tea) or a plant extract of *A. trilobata* is used in folk medicine. One study determined the chemical composition of the methanolic extracts of the root and stem; four aristolochic acids and one aristolactam were identified.[18]

The stem is found in markets and fairs in Aracaju, Sergipe State, Brazil, and is commonly used by the population in *cachaça* (sugar cane spirit) infusions and ingested in this form.

In this paper, the first study on the chemical composition of the essential oil of the stem of *A. trilobata* is reported. The analysis of the hydrolate is also reported.

RESULTS AND DISCUSSION

The average yield of the oil (oil mass/plant mass) was 0.22%, with a standard deviation of 0.05%. The chemical constituents of the essential oil are shown in Table 1. The main constituents of the essential oil were 6-methyl-5-hepten-2-yl acetate (sulcatyl acetate) (23.31 ± 0.28%), limonene (15.43 ± 0.03%), linalool (8.70 ± 0.29%), p-cymene (7.81 ± 0.12%), bicyclogermacrene (4.21 ± 0.11%), and spathulenol (4.17 ± 0.14%).

Notable compounds in the hydrolate, due to their high content, were linalool (29.51 ± 0.49%), 6-methyl-5-hepten-2-ol (19.54 ± 0.82%), 6-methyl-5-hepten-2-yl acetate (sulcatyl acetate; 8.92 ± 0.16%), and α-terpineol (4.62 ± 0.05%). These compounds were also identified in the essential oil, but in different percentages. It should

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be noted that oxygenated compounds constituted 88.11% of the total hydrolate composition (Table 1).

In this study, sulcatyl acetate (Figure 1) was identified by nuclear magnetic resonance (^{1}H and ^{13}C NMR; both 1D and 2D), gas chromatography coupled with mass spectrometry (GC-MS), and infrared (IR) analyses (spectra are included in supplementary material). In addition, there are only a few reports of this compound in the literature. The absolute configuration of the chiral center was determined by polarimetry and by comparison with the data in literature. The compound was determined to be (*R*)-(–)-sulcatyl acetate.[19]

There is only one report of sulcatyl acetate from a plant source, by Makholela and Manning,[20] who studied the volatile compounds associated with the aroma of the flowers of the species *Struthiola ciliate*. The percentage of sulcatyl acetate was only 0.04%.[20] Two other papers have reported on the presence of sulcatyl acetate in nature. In the first of these studies, the authors analyzed the volatile compounds present in the venom of five species of wasp of the genus *Polistes*, a genus found in the Mediterranean region of Europe. The percentages of sulcatyl acetate present in the analyzed wasp venom samples were 4.14% (*P. dominulus*), 0.34% (*P. gallicus*), 4.30% (*P. nimphus*), 0.86% (*P. sulcifer*), and 1.78% (*P. olivaceus*).[21] In the second study, the volatile compounds present in the venom of only one wasp species, *P. dominulus*, was investigated, and the percentage of sulcatyl acetate was 1.78%.[22] In other publications, sulcatyl acetate is cited as a participant in the enzymatic resolution of 6-methyl-5-hepten-2-ol (sulcatol), an enantiomerically pure pheromone of ambrosia beetles.[23,24] Thus, the identification of this compound as a major component of the volatile compounds originating from a plant source, in this case *A. trilobata*, is of great relevance. Other compounds with significant presence in essential oils of other species of the same genus have been reported.

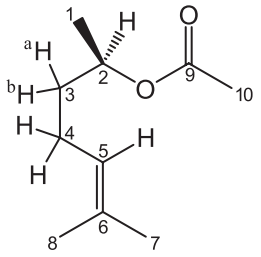

Figure 1. *(R)-6-methyl-5-hepten-2-yl (sulcatyl acetate), essential oil isolated from Aristolochia trilobata*

Table 1. Constituents of essential oil and hydrolate of *A. trilobata*

Compound	[a]RRI$_{cal}$	[b]% oil ± Std. Dev.	% hydrolate ± Std. Dev.
α-pinene	926	1.26 ± 0.021	-
camphene	938	0.70 ± 0.015	-
β-pinene	971	0.57 ± 0.092	-
1-octen-3-ol	973	0.073 ± 0.13	1.93 ± 0.01
6-methyl-5-hepten-2-one	979	-	0.53 ± 0.09
myrcene	985	0.74 ± 0.010	-
6-methyl-5-hepten-2-ol	988	0.91 ± 0.064	19.54 ± 0.82
α-phellandrene	1005	0.18 ± 0.012	-
δ-3-carene	1006	0.077 ± 0.067	-
p-cymene	1022	7.81 ± 0.12	0.40 ± 0.01
limonene	1027	15.43 ± 0.030	0.40 ± 0.01
1,8-cineole	1030	0.24 ± 0.12	1.08 ± 0.02
benzene acetaldehyde	1041	-	0.08 ± 0.05
(*E*)-β-ocimene	1044	3.40 ± 0.020	0.08 ± 0.01
cis-linalool oxide (furanoid)	1069	-	0.82 ± 0.04
trans-linalool oxide (furanoid)	1085	-	0.70 ± 0.02
linalool	1098	8.70 ± 0.29	29.51 ± 0.49
1-octen-3-yl acetate	1105	0.043 ± 0.075	-
phenylethyl alcohol	1110	-	0.17 ± 0.02
6-methyl-5-hepten-2-yl acetate	1124	23.31 ± 0.28	8.92 ± 0.16
camphor	1146	0.48 ± 0.0058	2.73 ± 0.06
cis-linalool oxide (pyranoid)	1168	-	0.30 ± 0.06
borneol	1170	0.20 ± 0.0058	2.00 ± 0.12
terpinen-4-ol	1178	0.057 ± 0.098	2.89 ± 0.14
p-cymen-8-ol	1185	-	0.49 ± 0.07
α-terpineol	1193	0.45 ± 0.021	4.62 ± 0.05
cis-piperitol	1198	-	0.23 ± 0.07
verbenone	1206	-	3.22 ± 0.12
trans-carveol	1217	-	0.33 ± 0.04
citronellol	1224	0.10 ± 0.10	0.60 ± 0.08
thymol methyl ether	1236	0.46 ± 0.015	-
carvone	1242	-	1.10 ± 0.01
geraniol	1248	-	0.13 ± 0.14
isobornyl acetate	1282	0.52 ± 0.010	0.09 ± 0.08
carvacrol	1295	-	0.19 ± 0.01
p-vinyl-guaiacol	1306	-	0.17 ± 0.04
citronellyl acetate	1350	0.60 ± 0.0	0.12 ± 0.01
α-copaene	1374	0.76 ± 0.015	-
β-elemene	1387	0.62 ± 0.021	-
(*E*)-caryophyllene	1418	3.65 ± 0.085	0.41 ± 0.01
β-copaene	1428	0.54 ± 0.044	-
aromadendrene	1436	0.60 ± 0.0058	-
(*E*)-β-farnesene	1448	1.39 ± 0.11	0.33 ± 0.03
α-humulene	1453	0.48 ± 0.0058	-
allo-aromadendrene	1457	0.69 ± 0.015	-
γ-muurolene	1472	0.48 ± 0.026	0.29 ± 0.03
germacrene D	1479	2.20 ± 0.071	-
viridiflorene	1488	0.86 ± 0.26	-
bicyclogermacrene	1493	4.21 ± 0.11	0.53 ± 0.08
γ-amorphene	1512	0.37 ± 0.045	-
γ-cadinene	1510	1.81 ± 0.13	-
selina-3,7(11)-diene	1539	0.90 ± 0.035	-
spathulenol	1574	4.17 ± 0.14	3.26 ± 0.18
caryophyllene oxide	1581	-	0.31 ± 0.01
globulol	1585	-	1.22 ± 0.13
viridiflorol	1593	-	0.16 ± 0.02
1-*epi*-cubenol	1622	0.36 ± 0.030	-
α-cadinol	1653	0.46 ± 0.038	0.27 ± 0.03
eudesm-7(11)-en-4-ol	1696	0.48 ± 0.060	0.40 ± 0.04
Monoterpene hydrocarbons		30.167	0.88
Oxygenated monoterpenes		36.143	82.49
Sesquiterpene hydrocarbons		19.56	1.56
Oxygenated sesquiterpenes		5.47	5.62
Total compounds identified		91.34	90.55

[a]RRI$_{cal}$ Relative retention index calculated using a homologous series of n-alkanes (C9-C18) in an apolar capillary column DB-5MS. [b] Analysis carried out in triplicate.

For examples, limonene, a monoterpene frequently found in several plants of a wide variety of genera, is present in high concentrations in the following species of this genus: *A. gibertii* (38.5%), *A. arcuata* (8.7%), *A. galeata* (10.5%), *A. malmeana* (10.3%), *A. melastoma* (34.5%), *A. debilis* (7.3%), and *A. indica* (6.9%).[25-30]

Another compound previously identified in *Aristolochia* is linalool. This compound is also found in the volatile fraction of plants of other genera, but has a significant presence (16.6%) in *A. gigantea* in this genus.[29]

Bicyclogermacrene and spathulenol are also frequently found in species of *Aristolochia*. In particular, bicyclogermacrene is one of the major compounds in several species, including *A. arcuata* (10.0%), *A. chamissonis* (24.0%), *A. cynanchifolia* (38.8%), *A. esperanzae* (22.7%), *A. paulistana* (40.3%), *A. gigantea* (18.9%), *A. cymbifera* (8.5%), *A. elegans* (15.2%), *A. galeata* (11.9%), *A. macroura* (15.3%), *A. melastoma* (9.2%), and *A. triangularis* (10.7%).[31]

EXPERIMENTAL

Plant material

The plant material was collected in October 2011 from the municipality of Estância, Sergipe State, Brazil (geographical coordinates: S = 11º 14′ 22.4″ and W = 37º 25′ 00.5″). The plant was identified by Diogo Araújo (MSc) and a voucher specimen was deposited at the herbarium of the Federal University of Sergipe (ASE) under voucher number ASE 23.161.

Steam distillation of essential oil

Samples of dry *A. trilobata* stem were cut into small pieces and triturated in a four-knife mill (Marconi, model MA680). The essential oil was obtained through the steam distillation process in a Clevenger device. In this method, triturated stem (200 g) and distilled water (1500 mL) were placed in a 2 liter flask, and distillation initiated after coupling with the Clevenger device. Distillation was continued for 180 min after the start of the condensation in the Clevenger device. The yield of essential oil was expressed as a percentage (oil mass/plant mass). The essential oil obtained was stored in a refrigerated amber flask until further analysis.

Liquid-liquid extraction of hydrolate

The hydrolate was obtained by extraction of the distillate collected during steam distillation of volatile components of *A. trilobata*. Initially collected distillate (500 mL) was extracted with diethyl ether. The liquid-liquid extraction was performed by washing 250 mL of the distillate with diethyl ether (3 × 50 mL) at room temperature. The hydrolate extract obtained (100 mg from 1 L of distillate) was evaporated after drying on Na_2SO_4.

General experimental procedures

Gas chromatography-mass spectrometry

The oil sample was analyzed on a Shimadzu QP5050A (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i auto-sampler and the gas chromatograph was interfaced to a mass spectrometer (GC/MS) with a J&W Scientific DB-5MS (Folsom, CA, USA) fused-silica capillary column (30 cm × 0.25 mm i.d., composed of 5% phenylmethylpolysiloxane). Helium (99.999%) was used as carrier gas at a constant flow of 1.2 mL min^{-1} and an injection volume of 0.5 µL was employed (split ratio of 1:83). The injector temperature was 250 °C and the ion-source temperature was 280 °C. The oven temperature was programmed to increase from 50 °C (isothermal for 2 min) to 200 °C with a rate of 4 °C/min, and then at 10 °C/min to 300 °C, where it was maintained for 10 min. Mass spectra (40-550 Da; EI) were acquired at 70 eV with a scan interval of 0.5 s.

Chiral gas chromatography (GC-FID)

Enantioselective GC analysis of (±)-sulcatyl acetate was performed on an Agilent fused-silica capillary column (cyclodex-B; 30 m × 0.25 mm i.d., 0.25 µm film thickness) using a gas chromatograph (Shimadzu model GC 17A), equipped with a flame-ionization detector (FID). The temperature of the oven was programmed to increase from 50 °C (isothermal for initial 1.0 min) to 80 °C at 3 °C/min; after being held at 80 °C for 10 min, the temperature was allowed first to increase at 0.5 °C/min to 95 °C and then at 15 °C/min to 170 °C, where it was maintained for the final 5 min. Helium was used as the carrier gas at a constant flow of 1.2 mL/min, and the injector and detector temperatures were 200 °C and 280 °C, respectively. The injection volume was 0.5 µL (ethyl acetate) with a split ratio of 1:10.

Identification of essential oil constituents

Individual components of the essential oil were identified by computerized matching of the acquired mass spectra with those stored in WILEY8, NIST107, and NIST21 mass spectral libraries of the GC-MS data system. A mixture of hydrocarbons (C9H20–C19H40) was injected under the same conditions and constituents were identified by comparing the spectra obtained with those in the databank and considering the relative retention index (RRI), calculated for each constituent as previously described.[32]

Nuclear magnetic resonance (NMR)

1D and 2D NMR data were acquired at 293 K in $CDCl_3$ on a Bruker AVANCE III 400 NMR spectrometer operating at 9.4 Tesla observing ^1H and ^{13}C at 400.13 and 100.61 MHz, respectively. The spectrometer was equipped with either a 5-mm multinuclear direct detection probe (1D NMR experiments) or a 5-mm multinuclear inverse detection probe (1D NOE and 2D NMR experiments), both with *z*-gradient. One-bond and long-range ^1H-^{13}C correlations from HSQC and HMBC NMR experiments were optimized for average coupling constants of $^1J_{(C,H)}$ and $^{LR}J_{(C,H)}$ of 140 and 8 Hz, respectively. All ^1H and ^{13}C NMR chemical shifts (δ) are reported in ppm relative to the TMS signal at 0.00 ppm, as internal reference, and the coupling constants (*J*) in Hz.

Infrared (IR) spectroscopy

A Perkin Elmer infrared spectrometer with Fourier transform, model BX, was used. The spectra were obtained in the region from 4000 cm^{-1} to 400 cm^{-1}.

Optical rotation

Optical rotation was determined using a Perkin Elmer polarimeter (model P-2000) at the Department of Organic and Inorganic Chemistry of the Federal University of Ceará, Brazil. The measurements were taken using monochromatic sodium light at a wavelength of 589 nm and were expressed using the notation $[\alpha]^T_D$, where T (in ºC) is the temperature at which the measurement was recorded. Chloroform ($CHCl_3$) was used for the dissolution of the samples.

Isolation of sulcatyl acetate

Separations by preparative thin layer chromatography (TLC) were carried out on freshly prepared TLC plates. Silica gel plates (1.0 mm thick) were prepared by evenly spreading ~30 g of Macherey-Nagel

Table 2. NMR data (400 MHz, $CDCl_3$) for 6-methyl-5-hepten-2-yl

Position	^{13}C	^{1}H mult. (*J*)	HMBC[a]	NOE
1	20.0 CH_3	1.21 *d* (6.3)	2 & 3	4.87 (H-2)
2	70.7. CH	4.88 *dqd* (7.7; 6.3; 5.3)	1. 3. 4 & 9	1.21 (H-1)
3a	35.9. CH_2	1.63 *ddt* (13.3; 7.7; 6.3)	1. 2. 4 & 5	
3b		1.49 *dtd* (13.3; 7.4; 5.3)	1. 2. 4 & 5	
4	24.0. CH_2	2.00 *dddqq* (7.4; 7.2; 6.3; 1.0; 0.8)	2. 3. 5 & 6	
5	123.5. CH	5.08 *tqq* (7.2; 1.4; 1.3)	3. 4. 7 & 8	1.68 (H-7)
6	132.1. qC			
7	25.7. CH_3	1.68 *dtq* (1.3; 1.0; 0.4)	5. 6 & 8	
8	17.6. CH_3	1.59 *dtq* (1.4; 0.8; 0.4)	5. 6 & 7	2.00 (H4) & 1.63 (H-3)
9	170.8. qC (C=O)			
10	21.4. CH_3	2.03 *s*	9	

The experiments were carried out at 295 K and the chemical shifts are expressed in ppm in relation to the TMS signal at 0.00 ppm, as the internal reference, and the coupling constants (*J*) in Hz. The exact multiplicities of the signal were determined with the aid of the first-order multiplet simulator/check FOMSC3. [a] The long range ^{1}H-^{13}C correlations (HMBC) were optimized for $J_{H,C}$ 8 Hz.

silica gel 60 in 80 mL of distilled water on glass sheets (20 × 20 cm). After evaporation of the water at ambient temperature, the TLC plates were activated in an oven at 110 °C for 30 min. For visualization, a solution of anisaldehyde in acid and ethanol (ethyl alcohol (90 mL) + sulfuric acid (5 mL) + anisaldehyde (5 mL) + acetic acid (1 mL)) was used, followed by heating at 110 °C. For each preparative TLC, 300 mg of oil was applied and eluted twice using the appropriate solvent system. The average yield of sulcatyl acetate was 15% and had a purity of 85%, as determined by GC-FID.

(2R)-(–)-6-methyl-5-hepten-2-yl acetate

The isolation gave a colorless oil, $[\alpha]_D^{19}$ −3.01 (c 0.2, 4 mL, $CHCl_3$). ^{1}H and ^{13}C NMR data of the same are shown in Table 2.[21]

CONCLUSIONS

Analysis of the volatile fraction of *Aristolochia trilobata* allowed for the isolation and identification of (2*R*)-(–)-6-methyl-5-hepten-2-yl acetate (sulcatyl acetate), limonene, linalool, p-cymene, bicyclogermacrene, and spathulenol as the major constituents of the essential oil. Linalool, 6-methyl-5-hepten-2-ol (sulcatol), 6-methyl-5-hepten-2-yl acetate (sulcatyl acetate), and α-terpineol were the main constituents of the hydrolate. Sulcatyl acetate was identified for the first time in the genus *Aristolochia*, where it is present as a major component of the volatile fraction of the plant.

SUPPLEMENTARY MATERIAL

^{1}H and ^{13}C NMR spectra (including 1D and 2D spectra), IR spectra, MS, and GC-FID chiral chromatogram of sulcatyl acetate, along with the total ion chromatogram (TIC) of the essential oil and the hydrolate of *A. trilobata* are available at http://quimicanova.sbq.org.br in the form of a PDF file, with free access.

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