ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:3 (2016) 341-348

records of natural products

Chemical Composition, Larvicidal and Cytotoxic Activities of the Essential Oils from two *Bauhinia* Species

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(Received October 09, 2014; Revised May 19, 2015; Accepted August 05, 2015)

Abstract: The essential oils obtained by hydrodistilation from leaves of *Bauhinia pulchella* Benth. and *Bauhinia ungulata* L. were analysed by GC-FID and GC-MS. The major components of *B. pulchell*a essential oil were identified as α-pinene (23.9%), caryophyllene oxide (22.4%) and β-pinene (12.2%), while in the *B. ungulata* essential oil were caryophyllene oxide (23.0%), (*E*)-caryophyllene (14.5%) and α-copaene (7.2%). The essential oils were subsequently evaluated for their larvicidal and cytotoxic activities. Larval bioassay against *Aedes aegypti* of *B. pulchella* and *B. ungulata* essential oils showed LC_{50} values of 105.9 ± 1.5 and 75.1 ± 2.8 μg/mL, respectively. The essential oils were evaluated against four human cancer cells lines**:** HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma), NCI-H292 (lung carcinoma) and HEP-2 (cervical adenocarcinoma), showing IC_{50} values in the range of 9.9 to 53.1 μg/mL. This is the first report on chemical composition of essential from leaves of *B. pulchella* and on larvicidal and cytotoxic activities of the essential oils.

Keywords: *Bauhinia pulchella*; *Bauhinia ungulata*; *Aedes aegypti*; cytotoxic activity.

1. Introduction

Bauhinia (family: Fabaceae, subfamily: Caesalpinioidae) is one of the largest genera of the subfamily, comprising about 500 species of shrubs and small trees distributed in tropical regions [1]. Species of *Bauhinia* genus are popularly known as "cow's paw" or "cow's hoof" due to their leaf format [2] and have been frequently used in folk medicine to treat diabetes [3]. Other activities include

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antimalarial [4], anti-inflammatory [4,5], antioxidant [5-9], antimicrobial [4,5,8], cytotoxic [4,8,10,11], inhibition of acetylcholinesterase [12] and proteinase inhibition [13]. This genus is known as a prolific source of structurally diverse secondary metabolites such as alkaloids [2], cyanoglucosides [6], steroids [10,14,15], triterpenoids [15], oxepin derivatives [15-17], bibenzyls [4,18], and especially flavonoids [19]. In the recent years, essential oils have received considerable attention due to their broad range of biological activities including larvicidal against *Aedes aegypti* [20-23] and cytotoxic properties [24].

A literature survey revealed that the essential oil of *Bauhinia pulchella* Benth. has not been reported, but there is one reporting about the chemical composition of the air-dried leaves from *Bauhinia ungulata* L. [25].

As part of a search for bioactive essential oils from Brazilian northeast flora, this study reports the chemical composition of the essential oils from leaves of *Bauhinia pulchell*a and *Bauhinia ungulata*, as well as the evaluation of their biological activity as larvicidal and cytotoxic agents.

2. Materials and Methods

2.1. Plant Material

The leaves of *Bauhinia pulchella* Benth. and *Bauhinia ungulata* L. were collected in May 2013 and April 2013, in São Benedito and Caucaia Counties, State of Ceará, Brazil, respectively. The plants were identified by Edson Pereira Nunes, and voucher specimens (#54266 and #54609) were deposited at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Brazil.

2.2. Extraction of the Essential Oils

Samples of fresh leaves of both species were subjected to hydrodistillation in a Cleavenger-type apparatus for 2 hours. The isolated oils, after drying over anhydrous sodium sulfate and filtration, were stored in sealed glass vials and maintained under refrigeration until further analysis. The yields (w/w) were calculated based on the fresh weight of the leaves. The oil yields for *B. pulchella* and *B. ungulata* were 0.01% and 0.02%, respectively.

2.3. GC/MS and GC Analysis of Essential Oils

GC analyses were performed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25μm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL/min. The essential oils were dissolved in ethyl acetate (5 mg/mL) and an injection volume of 0.5 μL was employed, with a split ratio of 1:10. The oven temperature was programmed from 50°C (isothermal for 1.5 min), with an increase of 4°C/min, to 200°C, then 10°C/min to 250°C, ending with a 5 min isothermal at 250°C.

The MS and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m x 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m x 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in the full scan mode (*m/z* 40–350) at a scan rate of 0.3 scan/s using the electron ionization (EI) with an electron energy of 70 eV. The injector temperature was 250°C and the ion-source temperature was 250°C. The FID temperature was set to 250ºC, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL/min, respectively. Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

2.4. Identification of Essential Oils Constituents

Identification of individual components of the essential oils was performed by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons (C_9H_{20}–$C_{19}H_{40}$) was injected under these same conditions and identification of constituents was then performed by comparing the spectra obtained with those of the equipment data bank and by the Kovats index, calculated for each constituent as previously described [26]. Retention indices were obtained with equation proposed by van Den Dool and Kratz [27].

2.5. Larvicidal Biossay

Aliquots of the essential oils tested (50 to 500 μg/mL) were placed in a beaker (50 mL) and dissolved in DMSO/H_2O 1.5% (20 mL). Fifty instar III larvae of *Aedes aegypti* were delivered to each beaker. After 24 hours, at room temperature, the number of dead larvae was counted and the lethal percentage calculated. A control using DMSO/H_2O 1.5% was carried out in parallel. For each sample, 3 independent experiments were run [28]. Larvae of *Aedes aegypti* were collected from mosquito colonies maintained at NUVET – SESA (Núcleo de Controle de Endemias Transmissíveis por Vetor - Secretaria de Saúde do Estado do Ceará).

2.6. Cytotoxicity Assay

The human tumor cell lines used in this work were HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma), NCI-H292 (lung carcinoma) and HEP-2 (cervical adenocarcinoma), and these cells were obtained from Rio de Janeiro Cell Bank (RJ, Brazil). Cancer cells were maintained in RPMI 1640 medium or DMEN supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37°C with 5% CO_2. We assessed the cytotoxicity of the essential oils against four tumor cell lines using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) (Sigma Aldrich Co., St. Louis, MO, USA) reduction assay. For all experiments, tumor cells were plated in 96-well plates (10^5 cells/mL for adherent cells or 3 x 10^5 cells/mL for leukemia). Tested essential oils (3.1-100 μg/mL) dissolved in DMSO 1% were added to each well and incubated for 72 h. Control groups received the same amount of DMSO. After 69 h of treatment, 25 μL of MTT (5mg/mL) was added, three hours later, the MTT formazan product was dissolved in 100 μL of DMSO, and absorbance was measured at 595 nm in plate spectrophotometer (Varioskan Flask, Thermo Scientific). Doxorubicin (0.01–5 μg/mL) was used as positive control. IC_{50} values and their 95% confidence intervals for two different experiments were obtained by non linear regression using Graphpad Prism version 5.0 for Windows (GraphPad Software, San Diego, California, USA).

3. Results and Discussion

3.1. Chemical Composition of the Essential Oils

The chemical composition of the essential oils, including the retention index and the percentage relative to each constituent, is showed in Table 1.

Table 1. Chemical composition of essential oils from leaves of *B. pulchella* and *B. ungulata*

Compound	RI[a]	RI [26]	*B. pulchella* (%)	*B. ungulata* (%)
Tricyclene	919	921	7.3	-
α-Pinene	930	932	23.9	1.4
Camphene	946	946	2.2	-
Sabinene	970	969	1.2	-
β-Pinene	974	974	12.2	-
p-Cymene	1023	1020	0.9	-
Limonene	1027	1024	1.0	-
1,8-Cineole	1030	1026	0.5	-
α-Pinene oxide	1097	1099	1.9	-
Linalool	1100	1095	1.9	-
trans-Pinocarveol	1140	1135	0.9	-
Pinocarvone	1162	1160	0.5	-
Terpinen-4-ol	1180	1174	0.8	-
α-Terpineol	1196	1186	0.6	-
α-Cubebene	1345	1345	-	0.6
Cyclosativene	1366	1369	-	1.2
α-Copaene	1373	1374	1.3	7.2
β-Bourbonene	1380	1387	0.5	1.4
β-Elemene	1387	1389	1.1	4.9
Cyperene	1399	1398	2.2	-
(*E*)-Caryophyllene	1416	1417	2.2	14.5
β-Copaene	1427	1430	1.3	4.6
α-*trans*-Bergamotene	1431	1432	0.2	-
α-Guaiene	1433	1437	-	0.6
6,9-Guaiadiene	1439	1442	-	0.8
$M^{\bullet+}$ 204	1447	-	-	1.0
α-Humulene	1453	1452	-	6.6
allo-Aromadendrene	1457	1458	0.4	3.2
$M^{\bullet+}$ 204	1473	-	0.7	5.0
β-Selinene	1486	1489	1.3	1.4
α-Muurolene	1496	1500	0.7	2.8
γ-Cadinene	1511	1513	0.9	1.6
$M^{\bullet+}$ 204	1517	-	-	2.0
α-Calacorene	1541	1544	-	0.8
$M^{\bullet+}$ 205	1550	-	1.1	1.1
Spathulenol	1576	1577	2.9	1.8
Caryophyllene oxide	1581	1582	22.4	23.0
Humulene epoxide II	1609	1608	1.7	4.6
Junenol	1621	1619	-	0.8
Cubenol	1644	1645	-	1.1
α-Cadinol	1655	1652	0.7	1.0
$M^{\bullet+}$ 204	1659	-	-	1.5
$M^{\bullet+}$ 220	1713	-	-	1.5
Monoterpenes			**55.8**	**1.4**
Sesquiterpenes			**39.8**	**84.5**
Total identified			**95.6**	**85.9**

RI[a]: Relative retention index calculated against *n*-alkanes (C_9H_{20}–$C_{19}H_{40}$) applying the Van den Dool & Kratz (1963) equation. *m/z* (rel. int.): RI[a] = 1447, $M^{\bullet+}$ 204 (17), 189 (15), 161(36), 147 (9), 133 (44), 119 (32), 105 (58), 91 (62), 81 (39), 67 (24), 55 (40), 41 (100);
RI[a] = 1473, $M^{\bullet+}$ 204 (10), 175 (3), 161 (91), 147 (9), 133 (26), 119 (61), 105 (72), 93 (59), 91 (66), 79 (74), 77 (39), 69 (29), 67 (33), 55 (41), 41 (100);

RI^a = 1517, $M^{\bullet+}$ 204 (41), 189 (15), 161 (98), 147 (15), 134 (45), 128 (19), 119 (100), 105 (99), 91 (64), 81 (46), 79 (28), 65 (13), 55 (41), 41 (71);
RI^a = 1550, $M^{\bullet+}$ 205 (1), 177 (2), 157 (2), 147 (5), 133 (9), 121 (10), 106 (33), 93 (37), 91 (43), 79 (49), 69 (29), 67 (26), 55 (27), 41 (100);
RI^a = 1659, $M^{\bullet+}$ 204 (10), 189 (8), 176 (7), 162 (11), 157 (20), 144 (7), 139 (15), 109 (15), 105 (23), 95 (32), 79 (28), 71 (35), 67 (29), 55 (36), 53 (40), 43 (100);
RI^a = 1713, $M^{\bullet+}$ 220 (15), 202 (12), 188 (9), 179 (7), 173 (12), 159 (25), 146 (36), 133 (19), 119 (41), 107 (41), 105 (48), 91 (29), 79 (48), 67 (34), 65 (29), 53 (41), 43 (100).

In the essential oil of *B. pulchella*, twenty-eight constituents were identified representing 95.6% of the total composition. The major components of this essential oil were identified as α-pinene (23.9%), caryophyllene oxide (22.4%), β-pinene (12.2%) and tricyclene (7.3%). The monoterpene fraction amounts 55.8% of the oil, while the sesquiterpene fraction amounts 39.8%.

Despite the essential oils of many *Bauhinia* species contained mainly sesquiterpenes in their chemical composition [22,25,29,30], the essential oil of *B. pulchella* showed a large percentage of monoterpenes. This result is in accordance with findings about the chemical composition of *B. variegata* essential oil [31].

In the essential oil of *B. ungulata*, twenty-two constituents were identified representing 85.9% of the total composition. Caryophyllene oxide (23.0%), (*E*)-caryophyllene (14.5%), α-copaene (7.2%) and α-humulene (6.6%) were found to be the major constituents. Sesquiterpenes predominated in the oil (84.5%).

It was previously reported that the major components in the essential oil from air-dried leaves of *B. ungulata* were spathulenol (47.7%) and caryophyllene oxide (18.3%) [25]. Despite the compositional differences detected in the two samples of *B. ungulata* from different geographic sites, it is important to emphasize the presence of caryophyllene oxide among the main constituents of both oils.

3.2 Larvicidal and Cytotoxic Activities

The leaf oils from both investigated *Bauhinia* species exhibited activity against instar III larvae of *Aedes aegypti*, showing LC_{50} values of 75.1 ± 2.8 μg/mL for *B. ungulata* and of 105.9 ± 1.5 μg/mL for *B. pulchella*. Temephos® (*O,O'*-(thiodi-4,1-phenylene)bis(*O,O*-dimethyl phosphorothioate) was used as control positive and showed LC_{50} value of 1.4 ± 0.2 μg/mL. The predominance of sesquiterpenes in the essential oil of *B. ungulata* should contribute to the higher larvicidal activity of this oil compared to essential oil of *B. pulchella*, because several studies have shown that essential oils containing high levels of sesquiterpenoid compounds possess significant larvicidal activities [22,32,33]. The literature also reports that caryophyllene oxide exhibit larvicidal properties against *Aedes aegypti* [34,35], and this compound could be responsible for the larvicidal activity of these essential oils.

The essential oils of *B. pulchella* (**A**) and *B. ungulata* (**B**) were submitted to the MTT assay [36] for the evaluation of their cytotoxic effects on HL-60 (human pro-myelocytic leukemia), MCF-7 (human breast adenocarcinoma), NCI-H292 (human lung carcinoma) and HEP-2 (human cervical adenocarcinoma) cell lines (Table 2).

The MTT analysis showed that both essential oils (**A** and **B**) exhibited cytotoxic activity against tested cancer cell lines. The essential oil from *B. ungulata* was more active for MCF-7, NCI-H292 and HEP-2 cell lines, while presented the same degree of cytoxicity as *B. pulchella* oil for HL-60 cell line. The cytotoxic activities of caryophyllene oxide [37], (*E*)-caryophyllene [37,38], α-pinene [39], β-pinene [39] are reported in the literature. Therefore, it is possible that these constituents of the essential oils work synergistically to produce the cytotoxic activity.

The findings of the present study allow to indicate these essential oils as potential natural larvicidal and cytotoxic agents.

Table 2. IC_{50} values of essential oils from leaves of *B. pulchella* (**A**) and *B. ungulata* (**B**)

Sample	IC_{50}, 95% confidence intervals (μg/mL)			
	HL-60	MCF-7	NCI-H292	HEP-2
A	9.94 (8.24-12.00)	53.05 (41.39-67.99)	48.98 (44.22-54.25)	50.42 (42.47-59.87)
B	10.57 (8.81-12.67)	22.25 (17.60-28.11)	23.11 (21.58-25.96)	26.56 (22.34-31.58)
Doxorubicin	0.02 (0.01-0.02)	0.30 (0.20-0.50)	0.20 (0.10-0.50)	0.70 (0.30-1.40)

Acknowledgments

The authors thank the Brazilian agencies CNPq, CAPES, FUNCAP, PRONEX for fellowships and financial support, and Laboratório de Entomologia, Núcleo de Endemias da Secretaria de Saúde do Estado do Ceará, Brazil, where the larvicidal bioassays were performed.

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