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ARTICLES

Full Length Research Paper

Terpenoids from *Phaulopsis imbricata* (Acanthaceae)

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The whole plant of *Phaulopsis imbricata* (Forssk.) Sweet (Acanthaceae) was collected at Bansoa, Cameroon, shade dried and extracted by maceration in methanol. This study was carried out to isolate secondary metabolites from this plant species that has not been investigated so far. Two lupane-type triterpenoids, one β-type carotenoid, one eudesmane-type sesquiterpenoid, and one sterol glycoside were isolated from the dried methanol extract using solvent partitioning, column chromatography and re-crystallization. They were identified as lupeol, betulin, (*all-E*)-lutein, cryptomeridiol, and sitosterol 3-*O*-β-D-glucopyranoside, respectively. The structures of the isolated compounds were elucidated on the basis of spectroscopic methods including 1D- and 2D- nuclear magnetic resonance (NMR), infrared (IR) and mass spectrometry (MS). This is the first report of these compounds from the genus *Phaulopsis*. To the best of our knowledge, *P. imbricata* is also the first species of the genus to be phytochemically studied.

Key words: *Phaulopsis imbricata*, chromatography, spectroscopy, terpenoids, lupeol, betulin, (*all-E*)-lutein, cryptomeridiol.

INTRODUCTION

The genus *Phaulopsis* (Acanthaceae) comprises about 22 species encountered in tropical Africa and Asia (Ke et al., 2011). *Phaulopsis imbricata* (Forssk.) Sweet (syn. *Aetheilema anisophyllum* R.Brown) is an erect herb of about 1 m high mainly distributed in West Africa (Kayode and Omotoyinbo, 2008; Brummet, 2005). It has been used as medicinal plant for the treatment of pain, arthritis, rheumatism, skin diseases, diarrhoea, dysentery,

stomachache, nausea and sores (Burkill, 1985). It is also used as chewing stick for oral and dental healthcare (Kayode and Omotoyinbo, 2008). Another species, *P. fascicepala*, has been reported to possess antioxidant activity (Adesegun et al., 2009).

Many workers have isolated terpenoids from some species of Acanthaceae (Berrondo et al., 2003; Tamokou et al., 2011; Sudhanshu et al., 2000). But, according to

our knowledge, no phytochemical study has been reported from the genus *Phaulopsis*. This study approach has been to investigate the chemical constituents of *P. imbricata*. We now report, herein, the isolation and structural identification of two triterpenoids (lupeol and betulin), one carotenoid [(*all-E*)-lutein], one sesquiterpenoid (cryptomeridiol), and one sterol glycoside (sitosterol 3-*O*-β-D-glucopyranoside) from the methanol extract of the whole plant of *P. imbricata*.

MATERIALS AND METHODS

General

Melting points (m.p.) were recorded with a Reichert microscope and uncorrected. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) with APT program were recorded at room temperature in CDCl₃, using a Bruker DMX 500 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) with the solvent signals as reference relative to TMS ($\delta = 0$) as internal standard, while the coupling constants (*J* values) are given in Hertz (Hz). COSY, NOESY, HSQC and HMBC experiments were recorded with gradient enhancements using sine shape gradient pulses. The Infra red (IR) spectra were recorded with a Shimadzu FTIR-8400S Infrared spectrophotometer and Gas chromatography-Mass spectrometry (GC-MS) data were obtained with an Agilent 6890N Network GC system/5975 Inert XL Mass Selective Detector at 70 eV and 20°C. The GC column (VARIAN, USA) was a CP- Sil 8 CB LB/MS, chromapack capillary column (0.25 mm × 30 m, film thickness 0.25 μm). The initial temperature was 50°C for 1 min, and then heated at 10°C/min to 300°C. For the carrier gas, helium was used with a flow rate of 1.20 ml/min. Kovat's retention index (KI) was determined using a calibration curve of *n*-alkanes. Column chromatography was run on Merck silica gel 60 (70 to 230 mesh) (MERCK, Germany) and gel permeation on Sephadex LH-20 (SIGMA-ALDRICH, St. Louis, MO, USA) while thin layer chromatography (TLC) was carried out on silica gel GF₂₅₄ pre-coated plates (MERCK, Germany) with detection accomplished by spraying with 50% H₂SO₄ followed by heating at 100°C, or by visualizing with a UV lamp at 254 and 365 nm.

Plant

The whole plant of *P. imbricata* was collected from Bansoa, West Region, Cameroon, in January, 2010. Authentication was done by Mr Paul Mezili, a retired Botanist of the Cameroon National Herbarium, Yaoundé, where the voucher specimen (No. 19290 / SRF / Cam) is deposited.

Extraction and isolation of compounds

The fresh plant of *P. imbricata* was dried under shadow for two weeks. The powdered material (2 kg) was extracted three times by maceration with MeOH (10 L) at room temperature (three days for each time). Evaporation of solvent under vacuum afforded 109 g of crude extract. A portion of this extract (105 g) was successively extracted with *n*-hexane, EtOAc and *n*-butanol. TLC analysis showed that the *n*-hexane and EtOAc extracts (21 and 3 g, respectively) were qualitatively the same. They were thus combined and a portion (23 g) subjected to silica gel (70 to 230 mesh) column chromatography eluted with gradient of *n*-hexane-EtOAc (100:0, 9:1, 4:1, 7:3, 1:1 and 0:100) followed by gradient of EtOAc-MeOH

(100:0, 19:1, 9:1 and 0:100). Sixty-six fractions of 300 ml each were collected and combined on the basis of TLC profile to give six major fractions A to F (A: 1 to 9; B: 10 to 20; C: 21 to 27; D: 28 to 45; E: 46 to 53; F: 54 to 66). Fraction A (3.2 g) was purified on a silica gel column eluted with gradient of *n*-hexane-EtOAc (10:0, 9:1, 4:1 and 7:3). 25 fractions of 50 ml each were collected to afford compound **1** (31 mg) after re-crystallization of sub-fractions 11 to 19 (A2). Fraction B (5 g) was chromatographed on Sephadex LH-20 column eluted with the isocratic *n*-hexane-CH₂Cl₂-MeOH (7:4:0.5). 21 fractions of 10 ml each were collected to afford compound **2** (14 mg) from sub-fraction B2 (fractions 8 to 17) and mixture of chlorophylls. Fraction C (2.5 g) was also passed through Sephadex LH-20 column chromatography eluted with the isocratic *n*-hexane-CH₂Cl₂-MeOH (7:4:0.5). 14 fractions of 10 ml each were collected to give compound **3** (11 mg) after re-crystallization of sub-fraction C1 (fractions 1 to 9) in *n*-hexane. Fraction D was also chromatographed on Sephadex LH-20 column eluted with the isocratic *n*-hexane-CH₂Cl₂-MeOH (7:4:0.5). 20 fractions of 10 ml each were collected yielding three sub-fractions. Sub-fraction D2 (9 to14) was purified through silica gel column chromatography eluted with the gradient CH₂Cl₂-MeOH (100:0, 49:1, 19:1, 9:1, 7:3 and 1:1) giving 10 mg of compound **4**.

The *n*-butanol extract (20 g) was also subjected to silica gel (70 to 230 mesh) column chromatography eluted with the gradient EtOAc-MeOH (100:0, 19:1, 9:1, 4:1 and 0:100). Seventeen fractions of 300 ml each were collected and combined on the basis of TLC profile to give three major fractions G to I (G: 1 to 8; H: 9 to 11; I: 12 to 17). Fraction G (3.7 g) crystallized to afford compound **5** (101 mg). An attempt to purify fractions E, F, H, I and filtrate of G failed, affording only complex mixtures in each case.

RESULTS

Compound **1** was obtained as white pellets from MeOH. It had a melting point (m.p.) of 216°C and showed the following spectral characteristics:

IR (KBr) V$_{max}$ cm⁻¹: 3300, 3080, 2920, 1640, 1470, 1420, 1390, 1045, 885. EIMS: m/z (rel. int.) = 426[M]⁺ (22), 207 (51), 189 (84), 161 (48), 135 (91), 121 (98), 95 (100), 81 (86), 55 (65), 41 (38).

Compound **2** was obtained as white crystals from MeOH. m.p. 237-238 °C. It showed the following spectral characteristics:

IR (NaCl) V$_{max}$ cm⁻¹: 3456, 3080, 2925, 1639, 1480, 1460, 1345, 1060, 885. EIMS: m/z (rel. int.) = 442[M]⁺ (4), 207 (52), 189 (81), 135 (82), 121 (72), 95 (100), 81 (93), 55 (80). HR-EI-MS: m/z = 442.3786 [M]⁺ (calcd. 442.3811 for C₃₀H₅₀O₂). ^{1}H- and ^{13}C-NMR spectral data (Table 1).

Compound **3** was obtained as reddish crystals from *n*-hexane. m.p. 190-191°C. It showed the following spectral data:

IR (NaCl) V$_{max}$ cm⁻¹: 3500, 2962, 2921, 1662, 1390. EIMS: m/z (rel. int.) = 568 [M]⁺ (1), 550 (12), 479 (3), 442 (5), 313 (3), 135 (80), 121 (75), 95 (100), 81 (90), 55 (83). HR-EI-MS: m/z = 568.4276 [M]⁺ (calcd. 568.4280 for C₄₀H₅₆O₂). ^{1}H- and ^{13}C-NMR spectral data (Table 2).

Table 1. ^{1}H- and ^{13}C/APT- NMR spectral data of betulin (**2**) (CDCl$_3$, δ in ppm), and, selected HMBC data for **2**.

No	δ$_C$	APT	δ$_H$, mult.(J in Hz)	HMBC (H→C)
1	38.7	CH$_2$	0.72, m; 1.62, m	2, 10, 25
2	27.4	CH$_2$	1.42, m; 1.58, m	1, 3, 4, 10
3	79.0	CH	3.18, dd (15.0, 5.0)	1, 4, 5, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.67, m	3, 4, 6, 10, 23, 25
6	18.3	CH$_2$	1.38, m; 1.52, m	4, 5, 7, 8, 10
7	34.0	CH$_2$	1.39, m; 1.39, m	5, 6, 8, 9, 14, 26
8	40.5	C	-	-
9	50.4	CH	1.27, m	7, 8, 10, 11, 12
10	37.2	C	-	-
11	20.8	CH$_2$	1.21, m; 1.42, m	8, 9, 10, 12, 13
12	25.0	CH$_2$	1.02, m; 1.63, m	9, 11, 13, 14, 18
13	37.5	CH	1.60, m	8, 12, 14, 17, 18, 19, 27
14	42.5	C	-	-
15	27.0	CH$_2$	1.05, m; 1.67, m	13, 14, 16, 17, 27
16	29.7	CH$_2$	1.21, m; 1.90, m	14, 15, 17, 18, 22, 28
17	47.8	C	-	-
18	48.9	CH	1.57, m	12, 13, 14, 16, 17, 19, 20
19	47.8	CH	2.38, m	13, 17, 18, 20, 21, 29, 30
20	150.5	C	-	-
21	29.7	CH$_2$	1.42, m; 1.95, m	17, 18, 19, 20, 22
22	34.6	CH$_2$	1.02, m; 1.83, m	16, 17, 18, 21, 28
23	27.4	CH$_3$	0.93, s	3, 4, 5, 24
24	15.9	CH$_3$	0.76, s	3, 4, 5, 23
25	16.3	CH$_3$	0.82, s	1, 5, 9, 10
26	16.1	CH$_3$	0.98,s	7, 8, 9, 14
27	15.4	CH$_3$	0.90, s	8, 13, 14, 15
28	60.6	CH$_2$	3.33, d (10.0); 3.80, d (10.0)	16, 17, 18, 22
29	109.7	CH$_2$	4.58, *br* s; 4.66, *br* s	19, 20, 30
30	19.1	CH$_3$	1.68, s	19, 20, 29

Compound **4** was obtained as white crystals from MeOH. m.p. 141 to 143°C. It showed the following spectral characteristics:

IR (NaCl) V$_{max}$ cm^{-1}: 3600-3200, 2920, 2848, 1456, 1377, 1238, 1166, 1049. EIMS: m/z (rel. int.) = 240 [M]$^+$ (1), 222 (1), 204 (17), 189 (33), 149 (100), 123 (39), 109 (52), 95 (45), 43 (65). HR-EI-MS: m/z = 240.2094[M]$^+$ (calcd. 240.2089 for C$_{15}$H$_{28}$O$_2$). ^{1}H- and ^{13}C-NMR spectral data (Table 3).

Compound **5** was obtained as white powder. m.p. 252-254 ^{o}C. IR (KBr) V$_{max}$ cm^{-1}: 3500-3300, 2953, 2866, 1108.

DISCUSSION

Compound **1** was considered to be a triterpenoid due to a positive (violet coloration) Liebermann Burchard test. Its

electron impact mass spectrum (EIMS) showed molecular ion peak at m/z 426 (GC-MS). Analysis of the IR spectrum suggested that it contained a hydroxyl group (3300 cm^{-1}) and a terminal double bond (3080, 1640, 885 cm^{-1}). On the basis of TLC, m.p. and IR analyses, compound **1** was suggested to be lupeol, a lupane-type triterpenoid previously isolated in our laboratory (Tene et al., 2009). The identity of compound **1** was confirmed by comparison of its mass spectrum with that of lupeol in the NIST GC-MS library (NIST, USA).

Compound **2** showed a positive (violet coloration) Liebermann Burchard test and was suggested to be a triterpenoid. Its EIMS indicated a molecular ion peak at m/z 442 and a molecular formula C$_{30}$H$_{50}$O$_2$ on the basis of its HR-EIMS (442.3793 [M]$^+$). The IR spectrum showed vibration bands corresponding to hydroxyl group (3456 cm^{-1}) and a terminal double bond (3080, 1639, 885 cm^{-1}). Its ^{1}H NMR spectrum showed five methyl singlets at δ$_H$

Table 2. ^{1}H- and ^{13}C/APT- NMR spectral data of (all-*E*)-lutein (**3**) (CDCl$_3$, δ in ppm), and, selected HMBC data for **3**.

No	δ$_C$	APT	δ$_H$, mult.(J in Hz)	HMBC (H→C)
1	37.2	C	-	-
2	48.4	CH$_2$	1.48, t (10.7)	-
			1.84, dd (6.1, 13.5)	-
3	65.1	CH	4.02, m	-
4	42.6	CH$_2$	2.03, dd (10.7, 15.0)	3, 5, 6
			2.37, m	2, 3, 5, 6
5	126.2	C	-	
6	137.7	C	-	
7	125.6	CH	6.12, m	
8	138.5	CH	6.11, m	
9	135.7	C	-	
10	131.3	CH	6.15, m	
11	125.0	CH	6.63, m	
12	137.6	CH	6.36, d (15.6)	
13	136.5	C	-	
14	132.6	CH	6.26, m	
15	130.1	CH	6.63, m	
16	29.5	CH$_3$	1.09, s	1, 2, 6
17	30.3	CH$_3$	1.09, s	1, 2, 6
18	21.7	CH$_3$	1.74, s	4, 5, 6
19	12.9	CH$_3$	1.99, s	8, 9, 10
20	12.9	CH$_3$	1.99, s	12, 13, 14
1'	34.1	C	-	-
2'	44.7	CH$_2$	1.37, dd (7.1, 13.5)	1', 3', 16', 17'
			1.84, dd (6.1, 13.5)	1', 3', 16', 17'
3'	65.9	CH	4.27, br s	-
4'	124.5	CH	5.55, br s	2', 3', 5', 6', 18'
5'	138.0	C	-	-
6'	55.0	CH	2.39, m	1', 2', 4', 5', 7', 16', 18'
7'	128.8	CH	5.43, dd (10.9, 15.6)	1', 5', 6', 8', 9'
8'	137.7	CH	6.15, m	6', 7', 9'
9'	135.1	C	-	-
10'	130.8	CH	6.15, m	-
11'	124.8	CH	6.63, m	-
12'	137.6	CH	6.36, d (15.6)	-
13'	136.4	C	-	-
14'	132.6	CH	6.26, m	-
15'	130.1	CH	6.63, m	-
16'	24.3	CH$_3$	1.02, s	1', 2', 6', 17'
17'	28.8	CH$_3$	0.87, s	1', 2', 6', 16'
18'	22.9	CH$_3$	1.63, s	4', 5', 6'
19'	13.2	CH$_3$	1.93, s	8', 9', 10'
20'	12.9	CH$_3$	1.99, s	12', 13', 14'

Table 3. ^{1}H- and ^{13}C/APT- NMR spectral data of cryptomeridiol (**4**) (CDCl$_3$, δ in ppm), and, selected HMBC data for **4**.

No	δ$_C$	APT	δ$_H$, mult.(J in Hz)	HMBC (H→C)
1	41.0	CH$_2$	1.09, m; 1.42, m	9, 14
2	20.2	CH$_2$	1.57, m	3
3	43.5	CH$_2$	1.38, m; 1.80, d (12.5)	1, 2, 4, 5, 15
4	72.4	C	-	-
5	54.8	CH	1.22, m	6, 14, 15
6	21.5	CH$_2$	1.05, m; 1.92, d (12.2)	4, 5, 7, 8, 10, 11
7	49.9	CH	1.38, m	8, 11
8	22.5	CH$_2$	1.30, m; 1.59, m	7, 9, 11
9	44.6	CH$_2$	1.15, m; 1.47, m	5, 7
10	34.5	C	-	-
11	73.0	C	-	-
12	27.1	CH$_3$	1.23, s	7, 11, 13
13	27.3	CH$_3$	1.23, s	7, 11, 12
14	18.7	CH$_3$	0.88, s	1, 5, 9, 10
15	22.6	CH$_3$	1.13, s	3, 4, 5

0.76, 0.82, 0.90, 0.93 and 0.98, a vinyl methyl group at δ$_H$ 1.68 (3H-30, s), a double doublet of one oxygenated methine proton at δ$_H$ 3.18 (H-3, dd, 5.0, 15.0), two doublets of one oxygenated methylene protons at δ$_H$ 3.33 (H-28a, d, 10.0) and 3.80 (H-28b, d, 10.0) and two broad singlets of a terminal double bond, one proton each at δ$_H$ 4.58 (H-29a) and 4.66 (H-29b) (Table 1). The ^{13}C NMR/APT revealed 30 carbon signals including 6 methyl groups, 12 methylene groups, 6 methine carbons and 6 quaternary carbons (Table 1). The oxygenated carbon signals appeared at δ$_C$ 60.6 (C-28) and 79.0 (C-3) and the double bond at δ$_C$ 109.7 (C-29) and 150.5 (C-20), characteristics of triterpenes of the lup-20(29)-ene type (Mahato and Kundu, 1994). In the HSQC spectrum correlations observed between H-3 (δ$_H$ 3.18) and C-3 (δ$_C$ 79.0), H-28a (δ$_H$ 3.33) and C-28 (δ$_C$ 60.6), H-28b (δ$_H$ 3.80) and C-28 (δ$_C$ 60.6), H-29a (δ$_H$ 4.58) and C-29 (δ$_C$ 109.7), H-29b (δ$_H$ 4.66) and C-29 (δ$_C$ 109.7), suggested compound **2** to be a 3, 28-dihydroxylup-20(29)-ene. The positions of the two hydroxyl (3-OH and 28-OH) and vinylidene (C=CH$_2$) groups were confirmed in the HMBC spectrum by correlations between H-3 (δ$_H$ 3.18) and C-1 (δ$_C$ 38.7), C-4 (δ$_C$ 38.9), C-5 (δ$_C$ 55.3), C-23 (δ$_C$ 27.4), and C-24 (δ$_H$ 15.9), H-28a (δ$_H$ 3.33)/H-28b (δ$_H$ 3.80) and C-16 (δ$_C$ 29.7), C-17 (δ$_C$ 47.8), C-18 (δ$_C$ 48.9) and C-22 (δ$_C$ 34.6), H-29a (δ$_H$ 4.58)/H-29b (δ$_H$ 4.66) and C-19 (δ$_C$47.8), C-20 (δ$_C$ 150.5) and C-30 (δ$_C$ 19.1). Furthermore, correlations observed in the NOESY spectrum between H-3 (δ$_H$ 3.18, dd, J = 5.0, 15.0 Hz) and H-5 (δ$_H$ 0.67, m), and H-3 (δ$_H$ 3.18) and H-23 (δ$_H$ 0.93, s) suggested the 3-OH group to be β-oriented.

Compound **2** was identified to 3β,28-dihydroxylup-20 (29)-ene (betulin), a lupane-type triterpenoid previously reported from other sources (Tinto et al., 1992; Joshi et

al., 2013).

HR-EIMS of compound **3** showed the molecular ion at m/z 568.4276 [M]$^+$ in agreement with the molecular formula C$_{40}$H$_{56}$O$_2$ (13° of unsaturation). The IR spectrum showed bands for hydroxyl group (3500 cm^{-1}) and double bond (1662 cm^{-1}). ^{13}C-NMR/APT indicated signals for forty carbon atoms including ten methyls, three methylenes, eighteen methines and nine quaternary carbons (see Table 2). Its ^{1}H NMR spectrum showed signals for four angular methyl groups at δ$_H$ 0.87 (3H-17', s), 0.99 (3H-16 and 3H-17, s) and 1.02 (3H-16', s), six vinyl methyl groups at δ$_H$ 1.63 (3H-18', s), 1.74 (3H-18, s), 1.93 (3H-19', s), 1.99 (3H-19, 3H-20 and 3H-20', s) and three methylene groups at δ$_H$ 1.37 (H-2'α, dd, J = 7.1, 13.5 Hz), 1.48 (H-2α, t, J = 10.7 Hz), 1.84 (H-2α, H-2'α, each dd, J = 6.1, 13.5 Hz), 2.03 (H-4α, dd, J = 10.7, 15.0 Hz) and 2.37 (H-4α, m). Also on this spectrum, three aliphatic methine protons resonated respectively at δ$_H$ 2.39 (H-6', m), 4.02 (H-3, m) and 4.27 (H-3', br s) (Table 2). The proton sequence of the compound was deduced from the ^{1}H-^{1}H COSY and the gradient HMQC spectra while the HMBC spectrum permitted the construction of the skeleton of **3**. ^{1}H- and ^{13}C-NMR spectral data suggested the presence in the molecule of two different and six-membered aliphatic rings, each bearing one hydroxyl (C-3 / C-3'), one double bond (C-5, C-6 / C-4', C-5') and three methyl (C-16, C-17, C-18 / C-16', C-17', C-18') groups. Each cyclohexenyl moiety is attached in one end of a symmetrical polyene system. But due to the structure difference of the two rings the molecule is not symmetric (Figure. 1 and Table 2). Signals of the polyene region of the ^{1}H NMR spectrum were identical to that of the (*all-E*)-lutein, as previously described (Aman et al., 2005). The structure was determined as (3*R*, 3'*R*, 6'*R*)-
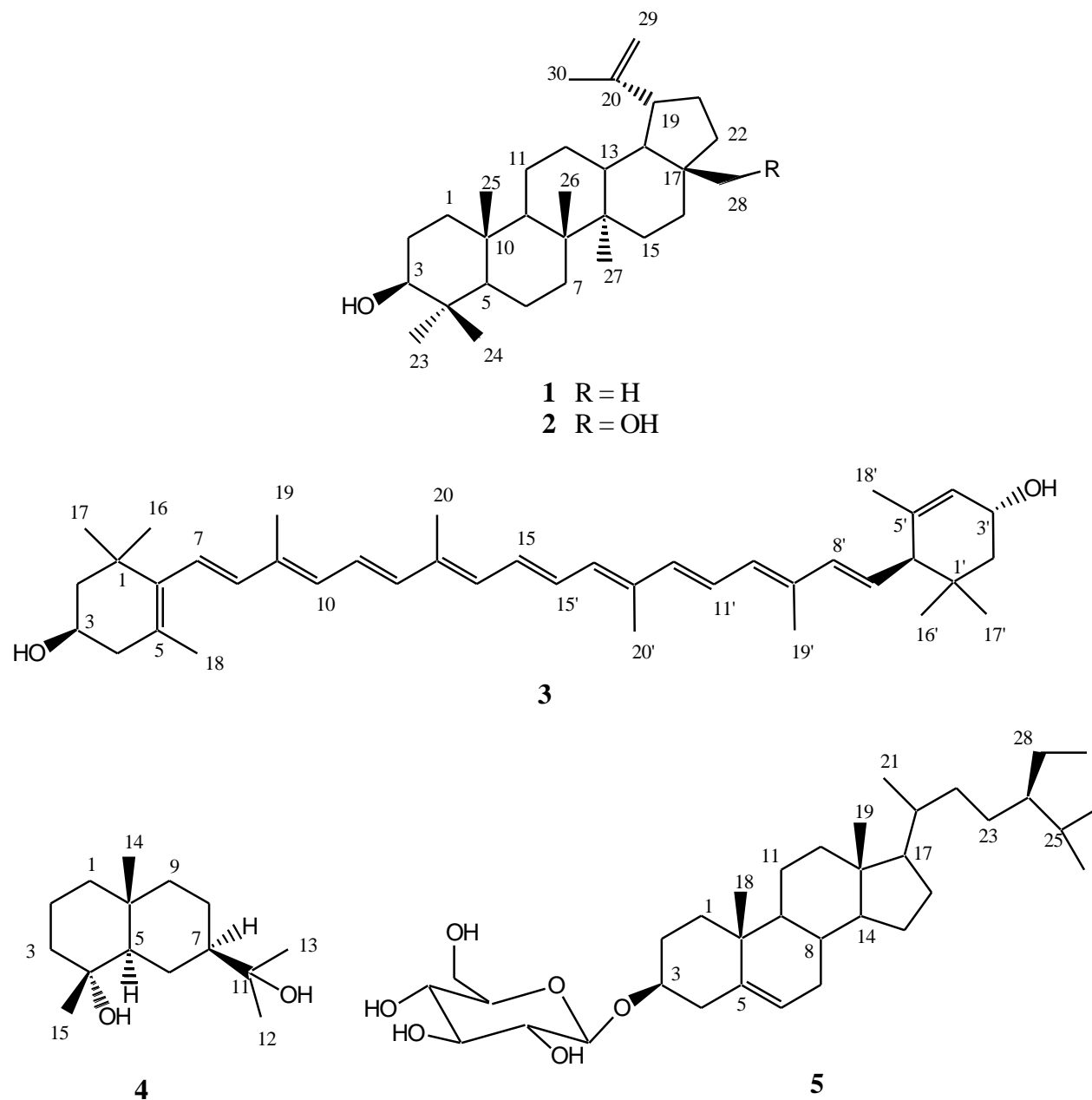
1 R = H
2 R = OH

3

4

5

Figure 1. Structures of the isolated compounds.

β,β-carotene-3,3'-diol using ^{1}H, ^{13}C/APT, HMBC, HSQC, ^{1}H-^{1}H COSY and NOESY spectra. Its NMR data (see Table 2) agree with those reported in the literature (Srividya and Vishnuvarthan, 2014). (*all-E*)-Lutein (**3**) was previously isolated from *Rhus leptodictya* (Songca et al., 2012).

The EIMS of compound **4** showed a molecular ion peak at m/z 240 and a molecular formula $C_{15}H_{28}O_2$ on the basis of its HR-EIMS (240.2094 [M]$^+$). The IR spectrum showed a vibration band at 3400 cm^{-1} corresponding to hydroxyl group. Its ^{13}C NMR/APT spectrum showed fifteen carbon signals including four methyls (δ$_C$ 27.3, 27.1, 22.6, 18.7),

six methylenes (δ$_C$ 44.6, 43.5, 42.0, 22.5, 21.5, 20.2), two methines (δ$_C$ 54.8, 49.7), and three quaternary carbons (δ$_C$ 73.0, 72.4, 34.5) two of which are oxygenated (Figure 1 and Table 3). Its ^{1}H NMR spectrum showed signals for four tertiary methyl groups three of which are attached to carbons bearing oxygen atom, at δ$_H$ 0.88 (3H-14, s), 1.13 (3H-15, s) and 1.23 (3H-12 and 3H-13, s). The two methine protons appeared respectively at δ$_H$ 1.22 (H-5, m) and 1.38 (H-7, m). Other signals were attributed to the methylene protons of the molecule (Table 3). The proton sequence of the compound was deduced from the ^{1}H-^{1}H COSY and the gradient HMQC spectra while the HMBC
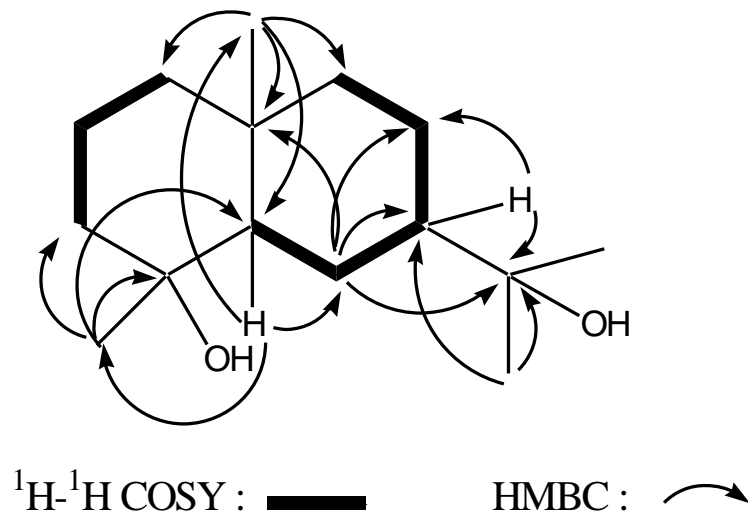
^1H-^1H COSY : ▬▬▬   HMBC : ⌒➔

Figure 2. Selected correlations for compound **4**

spectrum permitted the construction of the skeleton of **4** (Figure 2). In the NOESY spectrum, correlations observed between H-5 ($δ_H$ 1.22) and H-7 ($δ_H$ 1.38), and between 3H-14 ($δ_H$ 0.88) and 3H-15 ($δ_H$ 1.13) permitted the attribution of configurations at the chiral centers of compound **4**. The ^1H and ^{13}C NMR data (Table 3) were in good agreement with those reported in the literature for cryptomeridiol, an eudesmane-type sesquiterpenoid previously isolated from *Artemisia pygmaea* (Irwin and Geissman, 1973) and *Blumea basalmifera* (Ruangrunsi et al., 1985).

Compound **5** was identified to sitosterol 3-*O-β*-D-glucopyranoside after TLC, m.p. and IR analyses, compared to a referenced sample previously isolated in our laboratory (Tene et al., 2008). This compound is widely distributed in plant kingdom.

Conclusion

The phytochemical study of the whole plant of *Phaulopsis imbricata* (Acanthaceae) afforded five known compounds including lupeol, betulin, (*all-E*)-lutein, cryptomeridiol and sitosterol 3-*O-β*-D-glucopyranoside. To the best of our knowledge, this plant is phytochemically studied here for the first time. In addition, pure compounds are here reported for the first time from the genus *Phaulopsis*. The antimicrobial activity of the extracts and pure compounds from *P. imbricata* will be evaluated, with a view of verifying the hypothesis that this plant species is used by traditional healers for specific medicinal ends.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antimicrobial and phytotoxicity activities of aqueous crude extract from the Amazonian ethnomedicinal plant *Bellucia grossularioides* (L.) Triana

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The use of plants or their parts as alternative therapies for disease is very common in many countries worldwide. Secondary metabolites present in the extracts of certain plants can inhibit or halt the development of certain pathogen species. The present study assessed the possible antimicrobial activities of a popular crude aqueous extract. The aerial parts of the Amazon tree *Bellucia grossularioides* (L) Triana (popularly known as Muúba or Angry-Jambo) were prepared according to folk recommendations and tested against four microorganisms related to health concerns in three concentrations (20, 10 and 5 mg/ml). The results showed no antimicrobial potential against *Staphylococcus aureus*, *Candida albicans* and *Candida krusei*, which cause furunculosis and leukorrhea, respectively. Additionally, growth inhibition of the toxigenic fungus *Aspergillus parasiticus* was assayed *in vitro* and the results showed no inhibitory activity for any of the tested concentrations. These findings contradict the traditional knowledge and may assist the targeting of future therapeutics practices. However, an inhibitory effect was observed for all forms of the preparations and concentrations tested on the roots of *Allium cepa*, indicating phytotoxic effects.

Key words: Medicinal plants, antifungal, antibacterial, *Allium cepa*.

INTRODUCTION

Historically, nature has been considered a source of numerous drugs for use in clinical practice. According to Onofre et al. (2015), the use of plants to cure diseases is as ancient as humanity and folk knowledge largely contributes to these practices. Kokanova-Nedialkova et al. (2009) reported that approximately 75% of the world's population uses medicinal plants to treat diseases. There is an archive concerning the use, with large frequencies

of medicinal plants found in Europe, Asia, and North and South America (Song et al., 2014).

Barreiro and Bolzani (2009) reported that the search for natural bioactive substances is increasing around the world. Cragg and Newman (2007) showed that many of these substances formed a part of the evaluable prescriptions used in modern medicine. Pinto (2011) affirmed that the use of medicinal plants by the population was very common. Lizcano et al. (2010) related that plants with therapeutic potential were frequently used in different phytogeographical regions, such as the Amazonian region in South America.

Battisti et al. (2013) suggested that Brazil is a mega diverse country and thus had a great deal of popular knowledge concerning the cultivation and use of medicinal plants. The majority of people who use medicinal plants are influenced by friends and family, and the cultivation of these specimens is performed in gardens or other private areas (Storey and Salem, 1997). This confirms the presence of an oral transmission of traditional knowledge from one generation to the next. According to Onofre et al. (2015), the utilization of natural products represents an important tool to promote public health in specific ethnic groups. Some communities simply do not have another way to treat diseases. Moreover, sometimes the decision to use medicinal plants is related to cultural aspects.

Despite this, Veiga Júnior et al. (2005) affirmed that there were always some risks associated with this practice. Indeed, few studies are available concerning the pharmacological activities of these plants. In tropical countries, medicinal plants are an invaluable health source and provide folk medicine with great possibilities to assist conventional treatments and combat diverse types of diseases (Souza et al., 2004). Brazil possesses almost 19% of the world's flora, of which the Amazon Forest is one of the most rich and diversified area on the planet; however, roughly 99% of the medicinal plants do not have its efficacy and pharmacological safety proven, making necessary a phytochemical and pharmacological approach, which may represent a great economic potential to be explored by the pharmaceutical industry (Meneguetti et al., 2015). As a consequence, this region possesses huge biotechnological potential (Souza et al., 2004; Possimoser et al., 2012).

The use of medicinal plants can be a sustainable activity and aid in the development of tropical regions by the cultivation, growth, processing, industrialization and commercialization of renewable forest products (Possimoser et al., 2012). Often, ethnomedicinal plants show a satisfactory effect. However, the described effects of these plants do not evade the need to study the efficacy and safety of their use (Lourenço et al., 2009). Many studies have demonstrated that the bioactivity of medicinal plants provides important information for the development of new drugs (Motter et al., 2004). Toledo et al. (2001) reported that there was a trend for the use

plants for therapeutics. Rates (2001) reported that these preparations were normally used on a home scale and were prepared in crude extract form or as enriched fractions.

To a great many people, the terms "medicinal plant, ethnomedicinal plant or natural product" indicate the absence of chemical products or are synonymous with safety. This association is risky because many of these plants contain toxic compounds. Therefore, the incorrect use of phytotherapies can produce severe damage to health (Lourenço et al., 2009). Mei et al. (2006) observed that despite the frequent use of *Symphytum officinale* L. (confrei) as an anti-inflammatory agent, this plant contained carcinogenic substances. Similarly, Costa et al. (2008) verified the presence of genotoxicity in *Bidens pilosa* L., which is another plant with medicinal uses.

Bellucia grossularioides (L) Triana was described in 1867 as a species from the Mellastomataceae family. This species occur in Amazonian and savannah regions and is generally named "jambodamata, jamboselvagem, ormuúba". *B. grossularioides* (L) Triana is an arboreal woody plant that can reach approximately 15 m high; blossom and fructification occur between March and September (Costa and Mitja, 2010). Rodrigues et al. (2007) classified the fruit as a berry with a juicy pulp and citrus flavor. These studies affirmed that this fruit had a good nutritional composition for humans and others animals (Figure 1).

From the *Bellucia* genus, only *B. grossularioides* and *Bellucia pentâmera* have been subjected to phytochemical studies. Both of these plants are used in traditional medicine as vermifugal, anti-leukorrhea, antiofidic and abscess treatments (Cruz and Kaplan, 2004; Lima et al., 2011a; Moura, 2014, 2015).

Despite these reports, there is no evidence for their toxicity or the efficacy of their use to cure these diseases. Beyond the medicinal practices related to the *Bellucia* genus, their wood is very useful for construction and furniture building and their fruits are used in the alimentation of humans and other animals.

Knowledge about medicinal plants can offer good opportunities to discover new bioactive substances with therapeutic properties and to assist in the battle against resistant microorganisms. The latter factor rises especially important because resistance to traditional drugs has become a serious problem to public health worldwide (Oliveira et al., 2013b; Silva et al., 2013c).

However, despite the ethnobotanical studies of some *Bellucia* species, no evaluation of the production of anti-microbial substances by these plants has been performed. Furthermore, the action of substances from these plants against *Staphylococcus* species or *Candida* species, which are the tiological agents of the diseases against which these plants are ethnobotanically recommended, has not been investigated (Onofre et al., 2015; Vasquéz et al., 2014; Mukku et al., 2013).

The aim of this study is to evaluate the anti-microbial

Figure 1. (A) *Bellucia grossularioides* tree, (B) leaves, (C) fruits, and (D) flower. Available at: http://www.kew.org/science/tropamerica/imagedatabase.
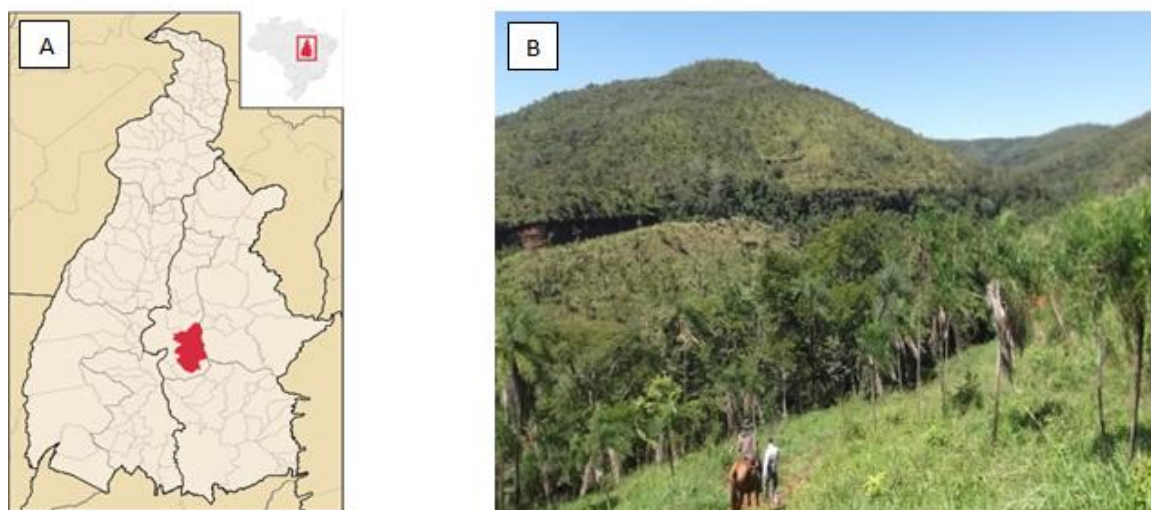
Figure 2. Sample collection area in Propriedadede Deus farm in MontedoCarmo city, Tocantins State, Brazil. (A) Available at: http://www.pt.wikipedia.org; (B) Autor picture.

potential of aqueous crude extract from the aerial parts of the Amazonian and Savannah plant *B. grossularioides,* which is traditionally used as vermifuge, anti-leukorrhea, and abscess treatment.

MATERIALS AND METHODS

Geographic location

The samples were collected in Montedo Carmo city in Tocantins,

Brazil, located at the geographic coordinates S10°42`50"W47°55`67" in the Propriedadede Deus farm close to the Caranã river. This region represents a very preserved area (Figure 2).

Sample collection

The aerial parts (leaves and stems) were collected from 30 specimens of *B. grossularioides* (L.) Triana in March, July and November of 2014 (dry and wet periods). Voucher specimens were created from the branches by Prof. Rodney Viana, herbarium

curator and deposited in the Federal University of Tocantins herbarium with the protocol number HTO10640.

Crude extract preparation

Crude aqueous extracts were prepared using the methodology of Oliveira et al. (2013a) with modifications and the protocols established by the Brazilian sanitary agency (Anvisa, 2011). Only the aerial parts of the plants (leaves and stems) were used; these tissues were dried and prepared by decoction and infusion similar to the method used in the traditional preparation. The samples were splitted into two groups, each with one sample of leaves and one sample of stems (20 g per sample). Then, the groups were split into two sub-groups: one with green leaves and stems and the other with samples dried in incubator at 35°C for 24 h. The dried samples were manually triturated with a mortar and pestle until a powder was obtained.

All groups were subjected to extraction in water (1,000 ml) at 100°C for 15 min. The crude extracts were filtered with Whatman n°5 filters, followed by an additional filtration with Whatman n°1 filters. The final product was kept in dark sterile bottles at 4°C prior to use in the experiments.

Antimicrobial assay

Inhibition of mycelial growth in vitro

These assays followed the methodology proposed by Stangarlin et al. (1999) and Santos et al. (2010). The crude extracts at concentrations of 20, 10, and 5 mg/ml were added to potato dextrose agar (PDA; Difco Lab., Detroit, MI, USA) to obtain filamentous fungi for the *A. parasiticus* tests, Sabouraud dextrose agar (Difco) for the yeast tests and Mueller-Hinton agar (Difco) for the *Staphylococcus* bacteria.

To test the efficiency of the crude extract against *A. parasiticus*, one plug of PDA with a 0.5 cm^2 *A. parasiticus* culture (7 days old) was placed in the center of a PDA dish. Then, the extracts were added and the plates were incubated at 27°C for 48 h. The evaluation of the inhibition of colony growth was performed by measuring the two opposite diameters of the colony. The control was performed by the inoculation of a similar plug in Petri dishes with PDA medium without any antagonistic substances. The results were compared with the control to obtain the percent inhibition. All tests were repeated three times.

To evaluate the efficiency against *Candida albicans* and *Candida krusei*, swabs were submerged in a saline suspension (0.85%) with 1.5×10^6 cells/ml and streaked onto Sabouraud plates containing the different concentrations of the crude extract (NCCLS, 2012). The results were obtained by the observation of the presence or absence of microbial growth over the medium. The negative and positive controls were generated by utilizing sterile distilled water and fluconazole, respectively. To evaluate the effect on *Staphylococcus aureus*, the assays were performed in the same way except that the Sabouraud agar was replaced with Mueller-H into and the antimicrobial gentamicin was replaced with chloramphenicol. The assays were repeated three times for each extract concentration.

Kirby-Bauer test

The Kirby-Bauer tests were performed according to CLSI/NCCLS (2012, 2015) to evaluate the efficiency of the crude extract against *C. albicans, C. krusei*, and *S. aureus*. Filter discs with 10 µl of the extracts at concentrations of 20, 10 and 5 mg/ml were placed onto Petri dishes. The dishes were incubated at 27°C for the yeasts and 37°C for *S. aureus* for 48 h. The controls were generated with gentamicin and chloramphenicol for the bacteria and fluconazole for the yeasts. All tests were repeated three times.

Evaluation of *Allium cepa* roots

The toxicity experiments were conducted as recommended by Fiskesjö (1993) and Meneguetti et al. (2014) based on alterations in meristem germination. The experiments used specimens of *A. cepa* with similar sizes and the same origin (not sprouted and healthy). For each form of the preparation and control, the bulbs were partially submerged in 50 ml of the extracts for germination at 27°C for 72 h. Distilled water was used as a negative control. Aqueous extracts of Muúba (in the forms of infusion or decoction) were used at the following concentrations: 20, 10, and 5 mg/ml. After the beginning of germination, the three major roots of each bulb (for a total of 9 roots per assay) were measured with a digital pachymeter in mm. All tests were repeated three times.

Statistical analysis

The experimental design was completely randomized in a factorial arrangement (9×3) with 9 treatments: three concentrations of the extract and three replicates per treatment. Root growth and elongation data were used to calculate the Relative Growth Index (RGI) and Germination Index (GI) according to the methods of Young et al. (2012). These indices were obtained by the following equations:

$$RGI = RLS/RLC$$

where RLS is the radicle length of the sample and RLC is the radicle length of the control, and

$$GI\% = ((RLS \times GSS)/(RLC \times GLC)) \times 100$$

where RLS is the radicle length RLC×GSC of the sample, RLC is the radicle length of the control, GSS is the number of germinated roots in the sample and GSC is the number of germinated roots in the control. The data were subjected to analysis of variance, and the means were compared using Tukey's test ($P > 0.05$).

RESULTS AND DISCUSSION

Recently, many studies have been performed to verify the antimicrobial activities of plant extracts and many extracts have demonstrated good potential applications against diverse groups of microorganisms (Costa et al., 2008; Silva et al., 2010; Gonçalves et al., 2013). Natural products are available alternative to discover new substances. Currently, approximately 12% of drugs are obtained from plants (Cowan et al., 1999). Mussi-Dias et al. (2012) reported that medicinal plants could be used in different forms and for different purposes.

In this study, the potential for the utilization of aqueous crude extract of *B. grossularioides* was evaluated against four microorganism species with health implications. The extracts were prepared using a method similar to the folk tradition (Pinto-Benitez and Stashenko, 2009; Lima et al., 2011b; Vásquez et al., 2014). The ethnobotanical literature contains various traditional forms of use for the

Table 1. Antimicrobial activities of the aqueous crude extract of *B. grossularioides.*

Microorganisms	Aqueous crude extract*							
	DSI	GSI	DLI	GLI	DSD	GSD	DLD	GLD
Candida krusei	A	A	A	A	A	A	A	A
Candida albicans	A	A	A	A	A	A	A	A
Staphylococcus aureus	A	A	A	A	A	A	A	A

*Results for all tested concentrations (20, 10 and 5 mg/ml). DSI: dried stem infusion; GSI: green stem infusion; DLI: dried leaf infusion; GLI: green leaf infusion; DSD: dried stem decoction; GSD: green stems decoction; DLD: dried leaf decoction; GLD: green leave decoction. A: Absence; P: presence.

Table 2. Average mycelial growth of *A. parasiticus* in Petri dishes supplemented with different concentrations of the aqueous crude extract of *B. grossularioides* after 48 h of incubation at 25°C.

Concentration	Extract							
	DSI	GSI	DLI	GLI	DSD	GSD	DLD	GLD
Control (DW)	22.8^a	36.4^a	22.5^a	27.5^a	22.8^a	37.0^a	23.3^a	41.0^a
20 mg/ml	26.0^a	30.8^a	21.3^a	33.2^a	25.7^a	32.5^a	19.7^a	33.1^a
10 mg/ml	22.9^a	29.6^a	22.2^a	38.6^a	26.7^a	35.1^a	22.9^a	38.0^a
5 mg/ml	23.4^a	33.9^a	24.5^a	37.1^a	25.9^a	35.4^a	25.4^a	27.9^a

Bellucia plant genus, with medical use the most frequent form (Costa and Mitja, 2010; Lizcano et al., 2010; Lima et al., 2011b; Moura et al., 2014; Vásquez et al., 2014; Moura et al., 2015).

Phytochemical studies have been performed on only *B. grossularioides* and *B. pentamera* from the *Bellucia* genus. The species from this genus exhibit considerable chemical variations. One important substance detected in these plants was squalene, which had activities against tumor cells, anti-protozoal (leishmanicidal and trypanomicidal) activities, and was hepatoprotective and an immuno-stimulant (Di Stasi, 1995; Isaza et al., 2007; Satalaya et al., 2009).

This study was designed based on the ethnobotanic use of *B. grossularioides* by the local population in a traditional community from the Amazonian region in Brazil. However, according to Table 1, the *in vitro* experiments did not give any evidence of efficacy for the use of the decoction or the infusion of the aerial parts of this plant as an antimicrobial against *C. albicans, C. krusei*, and *S. aureus,* which are the etiological agents of the diseases for which folk medicine recommends the use of this plant (Mors et al., 2000; Satalaya et al., 2009).

Similar negative results were observed by Kilks (1985), who did not obtain any confirmation for the utilization of the traditional herb *Chenopodium ambrosioides* as an anthelmintic. Guevara et al. (1994) also did not observe antimicrobial activity for *C. ambrosioides*, which had been indicated for use against *Vibrio cholera.* Vieira et al. (2010) tested extracts of *Moringa oleifera* against *S. aureus* and obtained only negative results. Similarly, Gonçalves et al. (2013) reported that medicinal plants frequently used in folk medicine were not effective when tested with the scientific method. According to Mukku et al. (2013) approximately 54% of the seeds of medicinal plants used in ethnobotanic studies did not show effectiveness in laboratory assays. These percentages may be as high as 63% according to Silva et al. (2013b). Aversi-Ferreira et al. (2013) observed only 26.6% of the confirmed activities of medicinal plants in the laboratory. In the assays of this study, the growth of *A. parasiticus* in culture plates was not inhibited by any of the concentrations tested (Table 2).

A. parasiticus is a frequent contaminant of grains, such as peanuts and corn. This pathogen can produce toxic metabolites with carcinogenic proprieties (aflatoxins). Any strategy that can reduce the growth or production of toxic metabolites is very important for public health. These fungi have a great economic importance due to the deterioration of foods (Roze et al., 2011; Prado, 2014; Venegas et al., 2014).

The folk knowledge associated with medicinal plants is an important tool for the research and development of new substances, but the erroneous use of these plants can prejudice health due to side effects or toxicity (Silva et al., 2013c). The inadequate use of phytotherapeutics (that is, self- prescription) can provoke allergies and intoxications (Marinho et al., 2007).

Therefore, it is necessary to scientifically evaluate the efficacy and toxicity of phytotherapeutics because sometimes these prescriptions can be innocuous to disease or induce a worsening in patient health. Kilks (1985) corroborated this idea by suggesting that it was essential that the effectiveness of ethnomedicinal
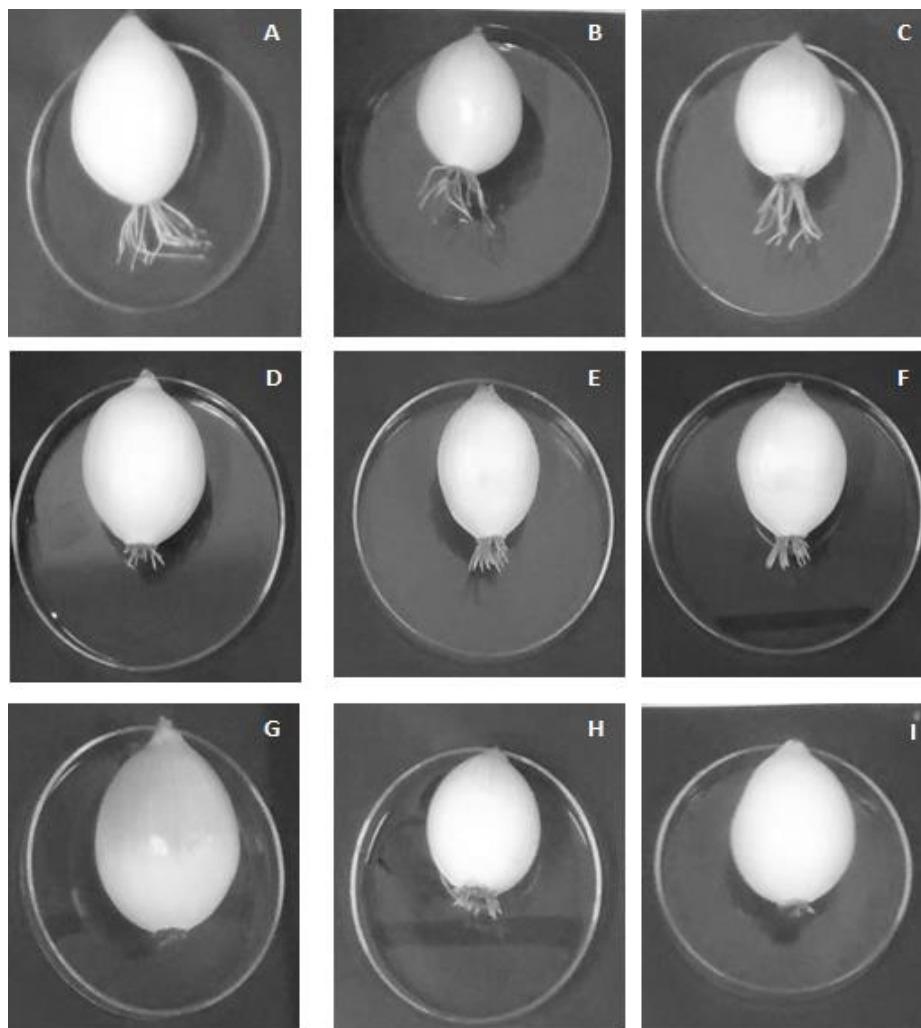
Figure 3. Appearance of *Allium cepa* bulbs after exposure to crude extract of *B. grossularioides* (A) Negative Control; (B) dried stem infusion; (C) green stem infusion; (D) dried leaf infusion; (E) greenleafinfusion; (F) dried stem decoction; (G) green stem decoction; (H) dried leaf decoction; (I) green leaf decoction.

practices be evaluated objectively and safely using appropriate protocols prior to use in health care programs.

Muñoz-Solarte and Guerrero-Pepinosa (2013) and Fachinetto et al. (2007) affirmed that *A. cepa* was a secure and trustworthy experimental model to evaluate the toxicity and cytotoxity of different substances and could be used to study the effects of medicinal plant extracts on eukaryotic cells. According to Bagatini et al. (2007), the *Allium* test system is frequently used to evaluate the toxicity of crude extracts based on the morphological alterations of root tissues.

The *A. cepa* test demonstrated good performance compared with other systems, such as eukaryotes and prokaryotes (Bagatini et al., 2007; Fão et al., 2012). *Allium* test is only predictive for the occurrence of toxicity (Fiskesjo, 1993). Muñoz-Solarte and Guerrero-Pepinosa

(2013) agreed with the use of this methodology to evaluate toxicity, mainly due to the ease of performance because only the germination and elongation of the roots were evaluated. Sharma et al. (2012) simplified this method by demonstrating that toxicity was easily measured by the observation of root growth inhibition. Herrero et al. (2012) demonstrated the toxicity of certain compounds present in pharmaceutical products using the macroscopic analysis of *A. cepa* bulbs. In our experiments, the macroscopic analysis of root growth and elongation were performed to determine toxicity (Young et al., 2012; Herrero et al., 2012; Muñoz-Solarte and Guerrero-Pepinosa, 2013; Meneguetti et al., 2014). The modification of the size and number of roots after exposure to some aqueous extracts can be observed in Figure 3.

The crude aqueous extract of *B. grossularioides*

Table 3. Average root elongation of *Allium cepa* exposed to three different concentrations of the aqueous crude extract of *B. grossularioides* for 48 h at 27°C.

Concentration	Treatments							
	GLI	GSI	GLD	GSD	DLI	DSI	DLD	DSD
NC	30.33[a]	30.33[a]	30.33[a]	30.33[a]	30.33[a]	30.33[a]	30.33[a]	30.33[a]
5 mg/ml	22.09[b]	13.78[b]	6.36[b]	1.56[b]	3.11[b]	9.67[b]	0.00[b]	14.14[b]
10 mg/ml	13.41[b]	2.82[b]	0.43[b]	1.65[b]	1.79[b]	7.18[b]	0.00[b]	9.57[b]
20 mg/ml	6.34[b]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.79[b]	0.78[b]	1.80[b]

DSI: dried stem infusion; GSI: green stem infusion; DLI: dried leaf infusion; GLI: green leaf infusion; DSD: dried stem decoction; GSD: green stems decoction; DLD: dried leaf decoction; GLD: green leave decoction. NC: Negative control (distilled water). In the columns, means followed by different letters are significantly different (Tukey's test, $P > 0.05$).

Table 4. Root Growth Index (RGI)/Germination Index (%GI) of *Allium cepa* and toxicity categories of three different concentrations of the aqueous crude extract of *B. grossularioides*.

Concentration	GLI		GSI		GLD		GSD		DLI		DSI		DLD		DSD	
	RGI	GI%	RGI	GI%	RGI	GI%	RGI	GI%	RGI	GI%	RGI	GI%	RGI	GI%	RGI	GI%
NC	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
5 mg/ml	0.71 (I)	37.44	0.45 (I)	24.75	0.20 (I)	0.52	0.05 (I)	0.3	0.10 (I)	1.3	0.31 (I)	4.96	0.00 (I)	0	0.46 (I)	23
10 mg/ml	0.44 (I)	12.32	0.09 (I)	10.17	0.01 (I)	0.013	0.05 (I)	0.25	0.05 (I)	0.4	0.23 (I)	1.61	0.00 (I)	0	0.31(I)	3.41
20 mg/ml	0.20 (I)	10.6	0.00 (I)	0	0.00 (I)	0	0.00 (I)	0	0.00 (I)	0	0.02 (I)	0.02	0.02 (I)	0.14	0.05 (I)	0.05

DSI: dried stem infusion; GSI: green stem infusion; DLI: dried leaf infusion; GLI: green leaf infusion; DSD: dried stem decoction; GSD: green stems decoction; DLD: dried leaf decoction; GLD: green leave decoction. NC: Negative control (distilled water). Toxicity categories: I: inhibition; NSE: no significant effects; S: stimulation.

induced a significant inhibition of root development compared to the control (Table 3). Similarly, Candido et al. (2013) reported the inhibition of germination and root growth in the presence of a *Croton* doctor is extract.

The calculation of the RGI was used as a parameter for the determination of toxicity and was conducted similarly to the method of Young et al. (2012). The results of these analyses are shown in Table 4.

According to Young et al. (2012), the RGI values were different for the three categories according to the observed toxic effects: (a)

inhibition of root elongation (I):0.0<X>0.8; (b) no significant effects (NSE):0.8≤X≥1.2; and (c) stimulation of root elongation (S):X>1.2, where X is the value obtained for RGI. Our data showed inhibition of root growth, which represented a potential toxic effect of the crude aqueous extract of *B. grossularioides*, in all forms and under all tested concentrations. Similar results were reported by Lima et al. (2011b), who showed inhibitory activity of the herbal extract of *Bidens alba* (95.94%) and *B. pilosa* (47.29%) over the tested systems.

Similarly, Trapp et al. (2015) observed that the

aqueous extracts of the leaves of *Prunus myrtifolia* prepared by infusion had an antiproliferative effect on the germination of *A. cepa* roots. Iganci et al. (2006) demonstrated that extracts of different species of "boldo" (ethnomedicinal plants) decreased the germination and development of *A. cepa*, which served as a sensitive bioprobe for these compounds. Rosado et al. (2009) reported a reduction in the root growth of lettuce by the aqueous extracts of "manjericão" (*Ocimum basilicum*). Nobre et al. (2014) demonstrated a reduction in the development of lima bean roots in

the presence of aqueous extracts of three medicinal plants from Brazil. Meneguetti et al. (2014) also reported toxic effects for an aqueous extract of *Maytenus guyanensis* on *A. cepa*, subsequently these results was confirmed in tests in mice, demonstrating the effectiveness of tests *A. cepa*, as compared with other methodological procedures (Meneguetti et al., 2015)*.*

Conclusion

This work showed that the use of aqueous crude extracts of the aerial parts of the medicinal plant *B. grossularioides* prepared by decoction or infusion did not have an anti-microbial effect against *S. aureus*, *C. albicans, C. krusei* and the aflatoxigenic fungus *A. parasiticus in vitro*. However, the crude aqueous extract of *B. grossularioides* had a significant inhibitory effect on the germination and development of *A. cepa* roots in all forms of preparation and concentrations tested, indicating probable toxic effects that need to be confirmed with cytological and mutagenic investigations.

Conflict of interests

The authors have not declared any conflict of interests.

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