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Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
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Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Table of Contents: Volume 8 Number 8 25 February, 2014

ARTICLES

Research Articles

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Full Length Research Paper

Phytochemical investigation and antioxidant activity of extracts of *Lecythis pisonis* Camb.

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Phytochemical investigation of the ethanol extract of leaves, twigs and fruit shell of *Lecythis pisonis* Camb. revealed the presence of squalene, α- e β-amyrin, lupeol, 3β-friedelinol, ursolic and oleanolic acids, (*E*)-phytol, sitosterol, stigmasterol, campesterol, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rustinoside. Structural elucidation was achieved using ultra violet (UV), nuclear magnetic resonance (NMR), and mass spectrometry. This is the first report of the occurrence of flavonoids, together with squalene, lupeol and campesterol in *L. pisonis*. The EtOH extract of the leaves showed high antioxidant activity, which can be associated in part with the high level of phenols and flavonoids.

Key words: Lecythidaceae, *Lecythis pisonis*, triterpenes, flavonoids, antioxidant activity, total phenolic content, total flavonoid content.

INTRODUCTION

Plants of the Lecythidaceae family are representatives of many neotropical forests in the Americas, Africa and Asia. This family has about 25 genera and 400 species in three subfamilies: Foetidioideae, Planchonioideae and Lecythidoideae (Mori, 2001). The genus *Lecythis* is little studied from the chemical point of view. There are reports in the literature on the chemical speciation of selenium (Se) in nuts of *Lecythis minor* (Dernovics et al., 2007), chemical analysis of the essential oils from flowers of *Lecythis usitata* (Andrade et al., 2000) and leaves of *Lecythis persistens* and *Lecythis poiteau* (Courtois et al., 2009). The species *Lecythis pisonis* Camb popularly known as sapucaia, is distributed in Brazil, within the state of Piaui and from Pernambuco to São Paulo as well as in the Amazon region (Corrêa, 1978). Traditionally, the infusion prepared from the bark of the tree is astringent and used in the treatment of diarrhea, while the leaves are used as diuretic and tonicardiac in tea or infusion or in baths to relieve itching (pruritus) and the fruits are used in the treatment of diarrhea and syphilis (Braga et al.

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2007; Denadai, 2006). The phytochemical study of the leaves of this species led to the isolation of pentacyclic triterpenoids, phytol, sitosterol and stigmasterol (Oliveira et al., 2012). The ethanol extract and the mixture of ursolic and oleanolic acids obtained from the leaves demonstrated antipruritic and cytotoxic activities (Silva et al., 2012; Oliveira et al., 2012). Other studies also showed that ethanol extract, eterea fraction and mixture of ursolic and oleanolic acids from leaves of *L. pisonis* exhibited antinociceptive activity in models of acute pain in mice (Brandão et al., 2013).

As part of our studies aimed at exploring the chemical constituents and pharmacological potential of plant species of the cerrado and transition area in the state of Piauí, this present work describes the isolation and identification of chemical constituents from the ethanol extract of leaves, twigs and fruit shell of *L. pisonis* as well as the evaluation of the antioxidant potential and determination of content phenols and flavonoids total.

MATERIALS AND METHODS

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA and Bruker Avance III spectrometer at 500/400 and 125/100 MHz, respectively, using $CDCl_3$ or DMSO-d_6 as solvents. The samples were analysed by gas chromatography–mass spectrometry (GC-MS) on an Agilent Technologies 7890A GC system coupled to a 5975 VLMSD mass spectrometer equipped with a 7683B series injector device and DB-5 column (J&W, 30 m × 250 mm × 0.25 mm), with injector temperature at 250°C and temperature at the interface of 310°C. The injected volume was 1.0 ml (5 mg ml^{-1}) in the split mode (10:1). Helium was used as carrier gas, at a velocity of 1 ml min^{-1}. Column temperature was initially maintained at 200°C for 4 min, followed by a heating gradient of 6°C min^{-1} until reaching 290°C. This temperature was maintained for 15 min after which a new heating gradient of 2°C min^{-1} was applied until reaching 305°C. This temperature was maintained for 5 min. The obtained mass spectra were compared to library data Nist 0.5. The absorption measurements were determined by an UV-Vis spectrophotometer PerkinElmer, Lambda 25. The chromatographic plates were prepared using Fluka silica gel G and the revealed were made by spraying the plates with a solution of $Ce(SO_4)_2$. The atmospheric pressure chromatographic columns were prepared with silica gel 60 (70 to 230 mesh) from Acros Organics or Sephadex LH-20 (Aldrich). For low-pressure chromatography column Büchi Switzerland with silica gel (40-60 µm; 12 × 150 mm) coupled to a Büchi B-688 pump was used. The semipreparative high performance liquid chromatography (HPLC) was performed on a Shimadizu LC-6AD system equipped with a Phenomenex column (Luna C18, 5 µm, 150 × 21.2 mm).

Plant

The leaves and twigs *L. pisonis* Camb. were collected in July, 2008 and fruit shell in July, 2010, on the Campus Ministro Petronio Portela of the Universidade Federal do Piauí - UFPI, in the city of Teresina, Piauí State, (South latitude = 05° 02' 53.2", West longitude = 42° 47' 16.8", at the level of 68 m). A voucher specimen was identified and deposited in the Herbarium Graziela Barroso of the UFPI, under accession number TEPB 26488.

Preparation of extracts and partition

The plant material consisting of leaves (2.0 kg), twigs (1.2 kg) and fruit shell (3.0 kg) of *L. pisonis* was air-dried, crushed and extracted for six consecutive times with ethanol at room temperature. The ethanol was removed on a rotary evaporator and the residual water by lyophilization, giving 272 g (13.5%), 72 g (5.9%) and 26 g (0.9%) of EtOH extracts, respectively. The EtOH extracts of leaves (200 g) and fruit shell (24 g) were suspended in H_2O:MeOH (3:2) and subjected to partition with *n*-hexane, ethyl ether and EtOAc, successively, resulting in the following fractions: hexane (60 g, 30% and 5.9 g, 24.5%), ether (24 g, 12% and 2.8 g, 11.6%), EtOAc (21 g, 10.5% and 1.8 g , 7.7%), aqueous (70 g, 37.5% and 10.8 g, 44.9%) and a precipitate separated from the EtOAc phase of the leaves extract (ppt-EtOAc; 10 g, 5%). The EtOH extract of the twigs (60 g) was suspended in H_2O:MeOH (3:2) and partitioned with hexane and EtOAc, giving the following fractions: hexane (5.3 g, 8.8%), EtOAc (16.3 g, 27.1%) and aqueous (30 g, 50%).

Isolation and fractionation of the constituents

The hexane fractions of leaves (8.0 g), twigs (4.1 g) and fruit shell (5.2 g) were submitted to column chromatography (5.0 × 50 cm; 3.0 × 50 cm and 3.0 × 50 cm) of silica gel, eluted with *n*-hexane:EtOAc: (100:0), (98:2), (95:5), (9:1), (8:2) and (7:3) providing 102, 100 and 60 fractions, respectively. After removal of the solvent on a rotary evaporator, the fractions were regrouped according to thin layered chromatography (TLC) analysis. Fractionation of the *n*-hexane fraction of leaves provided 11 groups (A_1 to K_1). Group B_1 (fractions 6 to 7, 89 mg) was purified on Sephadex LH-20 eluted with *n*-hexane:CH_2Cl_2 (1:4) giving 32 mg of compound 1. Group D_1 (fraction 25, 175 mg) was suspended in *n*-hexane providing, after separation of the supernatant, an amorphous solid corresponding to the compound 2 (112 mg). Groups F_1 (fractions 34 to 38, 839 mg) and H_1 (fractions 43 to 59, 714 mg) were purified on Sephadex LH-20 eluted with *n*-hexane:CH_2Cl_2 (1:4) providing 124 mg of the mixture of compounds 3 to 5 and 11 and 31 mg of a mixture of compounds 8 and 9, respectively. Group J_1 (fractions 98 to 100, 350 mg) was purified on Sephadex LH-20 column eluted with *n*-hexane:CH_2Cl_2 (1:4) and CH_2Cl_2-acetone (3:2), giving 42 mg of a mixture of compounds 6 and 7.

For GC-MS analysis, an aliquot of 5 mg of these substances was treated with a solution of diazomethane in ethyl ether providing the methylated derivatives. Fractionation of the hexanic fraction of the twigs provided 7 groups (A_2 to G_2). Groups B_2 (fraction 30, 107 mg) and D_2 (fractions 46 to 54, 138 mg) were purified on Sephadex LH-20 column eluted with n-hexane:CH_2Cl_2 (1:4) giving 32 mg of the mixture of compounds 3 to 5 and 11 and 25 mg of the mixture of compounds 8 to 10, respectively. Group F_2 (fractions 96 to 97, 48.6 mg) was purified on Sephadex LH-20 column eluted with *n*-hexane:CH_2Cl_2 (1:4) and CH_2Cl_2:acetone (3:2), giving 14.5 mg of the mixture of compound 6 and 7. Fractionation of the hexanic fraction of fruit shell provided 5 groups (A_3 to E_3). Groups B_3 (fractions 37 to 38, 267 mg) and D_3 (fractions 46 to 51, 300 mg) were purified on Sephadex LH-20 eluted with n-hexane: CH_2Cl_2 (1:4) giving 48 mg of a mixture of compounds 3 to 5 and 85 mg of the mixture of 8 and 9, respectively.

The EtOAc fraction leaves (10 g) was submitted to column chromatography (5.0 × 50 cm) of silica gel, eluted with $CHCl_3$:

MeOH: (100:0), (95:5), (9:1), (8:2) and (1:1), giving 60 fractions.

After removal of solvent on a rotary evaporator and analysis by TLC, the fractions were pooled into 5 groups (A to E). Group B (fractions 8 to 15, 375 mg) was fractionated on columns of Sephadex LH-20, eluted with CH_2Cl_2:acetone (3:2) and silica gel low pressure eluted with *n*-hexane:$CHCl_3$:MeOH (50:46.5:3.5) giving 15 mg of a mixture of compounds 6 and 7. Group D (fractions 42 to 47, 768 mg) was applied on a column of Sephadex LH-20 eluted with MeOH giving 40 fractions which were pooled into 5 groups (D_1 to D_5). Group D_2 (fractions 20 to 25, 417 mg) was purified by semi-preparative HPLC eluted with MeOH:H_2O (42:58) to provide compounds 12 (85 mg) and 13 (9.5 mg).

Squalene (1): ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.8 (C-1), 131.4 (C-2), 124.5 (C-3), 26.9 (C-4), 39.9 (C-5), 135.3 (C-6), 124.5 (C-7), 26.8 (C-8), 39.9 (C-9), 135.1 (C-10), 124.5 (C-11), 28.4 (C-12), 28.4 (C-13), 124.5 (C-14), 135.1 (C-15), 39.9 (C-16), 26.8 (C-17), 124.5 (C-18), 135.3 (C-19), 39.9 (C-20), 26.9 (C-21), 124.6 (C-22), 131.4 (C-23), 25.8 (C-24), 17.8 (C-25), 16.1 (C-26), 16.1 (C-27), 16.1 (C-28), 16.1 (C-29), 17.8 (C-30).

3β-Friedelinol (2): ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.7 (C-1), 36.9 (C-2), 72.4 (C-3), 49.1 (C-4), 38.3 (C-5), 41.6 (C-6), 17.5 (C-7), 53.1 (C-8), 37.7 (C-9), 61.2 (C-10), 35.5 (C-11), 30.5 (C-12), 38.7 (C-13), 39.6 (C-14), 32.2 (C-15), 35.9 (C-16), 29.9 (C-17), 42.7 (C-18), 35.2 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 11.4 (C-23), 16.1 (C-24), 18.1 (C-25), 19.9 (C-26), 18.5 (C-27), 31.9 (C-28), 34.8 (C-29), 31.6 (C-30).

Lupeol (3): EIMS: *m/z* (rel. int.) 426 (9, [$M^{+\cdot}$]), 411 (3), 207 (54), 189 (100), 175 (24), 161 (17), 135 (59), 121 (80), 95 (82), 55 (52).

α-Amyrin (4): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 27.1 (C-2), 79.2 (C-3), 38.7 (C-4), 55.3 (C-5), 18.5 (C-6), 32.9 (C-7), 40.0 (C-8), 47.8 (C-9), 36.8 (C-10), 23.4 (C-11), 124.5 (C-12), 139.7 (C-13), 42.2 (C-14), 28.9 (C-15), 26.7 (C-16), 33.9 (C-17), 59.2 (C-18), 39.5 (C-19), 39.5 (C-20), 31.2 (C-21), 41.6 (C-22), 28.1 (C-23), 15.6 (C-24), 15.6 (C-25), 16.9 (C-26), 23.4 (C-27), 28.1 (C-28), 17.6 (C-29), 21.5 (C-30).

β-Amyrin (5): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 27.4 (C-2), 79.2 (C-3), 38.9 (C-4), 55.3 (C-5), 18.5 (C-6), 32.8 (C-7), 38.9 (C-8), 47.8 (C-9), 37.6 (C-10), 23.7 (C-11), 121.8 (C-12), 145.3 (C-13), 41.8 (C-14), 26.1 (C-15), 27.1 (C-16), 32.6 (C-17), 47.3 (C-18), 46.9 (C-19), 31.2 (C-20), 34.8 (C-21), 37.3 (C-22), 28.2 (C-23), 15.6 (C-24), 15.6 (C-25), 16.9 (C-26), 26.1 (C-27), 28.5 (C-28), 33.5 (C-29), 23.8 (C-30).

Ursolic acid (6): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.7 (C-1), 23.0 (C-2), 78.8 (C-3), 36.9 (C-4), 55.2 (C-5), 18.3 (C-6), 33.9 (C-7), 39.4 (C-8), 47.5 (C-9), 36.9 (C-10), 23.3 (C-11), 125.5 (C-12), 138.1 (C-13), 42.0 (C-14), 27.7 (C-15), 23.0 (C-16), 47.8 (C-17), 52.8 (C-18), 39.1 (C-19), 41.2 (C-20), 30.7 (C-21), 36.8 (C-22), 27.9 (C-23), 16.8 (C-24), 15.5 (C-25), 16.8 (C-26), 23.5 (C-27), 181.0 (C-28), 16.9 (C-29), 21.1 (C-30).

Oleanolic acid (7): ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.5 (C-1), 27.7 (C-2), 78.8 (C-3), 38.6 (C-4), 55.2 (C-5), 18.3 (C-6), 32.5 (C-7), 39.4 (C-8), 47.5 (C-9), 36.9 (C-10), 23.0 (C-11), 122.3 (C-12), 143.5 (C-13), 41.7 (C-14), 27.7 (C-15), 23.3 (C-16), 46.4 (C-17), 41.2 (C-18), 45.9 (C-19), 30.6 (C-20), 33.9 (C-21), 32.5 (C-22), 27.9 (C-23), 15.5 (C-24), 15.4 (C-25), 16.9 (C-26), 25.8 (C-27), 181.2 (C-28), 33.0 (C-29), 23.5 (C-30).

Sitosterol (8): ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.0 (C-7), 32.0 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.5 (C-19), 36.3 (C-20), 18.9 (C-21), 34.0 (C-22), 26.2 (C-23), 45.9 (C-24), 29.3 (C-25), 19.9 (C-26), 19.2 (C-27), 22.8 (C-28), 12.0 (C-29).

Stigmasterol (9): ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.4 (C-1), 31.8 (C-2), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.9 (C-6), 32.0 (C-7), 32.0 (C-8), 50.3 (C-9), 36.6 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.5 (C-19), 36.9 (C-20), 21.2 (C-21), 138.5 (C-22), 129.4 (C-23), 50.3 (C-24), 31.8 (C-25), 21.2 (C-26), 18.9 (C-27), 25.5 (C-28), 12.2 (C-29).

Campesterol (10): EIMS: *m/z* (rel. int.) 400 (25, [$M^{+\cdot}$]), 382 (70), 315 (25), 289 (15), 273 (13), 255 (38), 55 (100).

(*E*)-phytol (11): ^{13}C NMR (100 MHz, $CDCl_3$): δ 59.6 (C-1), 123.2 (C-2), 140.5 (C-3), 39.5 (C-4), 25.3 (C-5), 36.8 (C-6), 32.7 (C-7), 37.4 (C-8), 24.6 (C-9), 37.4 (C-10), 32.9 (C-11), 37.4 (C-12), 24.9 (C-13), 39.5 (C-14), 28.1 (C-15), 22.8 (C-16), 22.8 (C-17), 19.9 (C-18), 19.9 (C-19), 22.8 (C-20).

Quercetin-3-O-rutinoside (12): ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.6 (s, 5-OH), 6.19 (d, *J* 2.0 Hz, H-6), 6.38 (d, *J* 2.0 Hz, H-8), 7.55 (d, *J* = 2.0 Hz, H-2'), 6.84 (d, *J* = 8.5 Hz, H-5'), 7.65 (dd; *J* 2.5 and 8.5 Hz, H-6'), 5.34 (d, *J* 7.5 Hz, H-1''), 4.39 (d, *J* 1.5 Hz, H-1'''), 1.18 (d, *J* 6.0 Hz, H-6'''), 2.75-3.70 (H-2" to H-6", H-2"' to H-5"'). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.4 (C-2); 133.1 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.3 (C-7), 93.7 (C-8), 156.4 (C-9), 104.0 (C-10), 121.6 (C-1'), 116.3 (C-2'), 144.7 (C-3'), 148.5 (C-4'), 115.2 (C-5'), 121.9 (C-6'), 101.4 (C-1''), 74.2 (C-2''), 76.5 (C-3''), 70.4 (C-4''), 76.0 (C-5''), 68.3 (C-6''), 100.8 (C-1'''), 70.6 (C-2'''), 70.6 (C-3'''), 71.9 (C-4'''), 68.3 (C-5'''), 17.9 (C-6''').

Kaempferol-3-O-rutenosideo (13): ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.8 (s, 5-OH), 6.18 (d, *J* 2.0 Hz, H-6), 6.41 (d, *J* 2.0 Hz, H-8); 7.98 (d, *J* 9.0 Hz, H-2'/H-6'), 6.88 (d, *J* 9.0 Hz; H-3'/H-5'), 5.30 (d, *J* 7.5 Hz, H-1''), 4.38 (d, *J* 1.0 Hz, H-1'''), 0.98 (d, *J* 6.0 Hz; H-6'''), 3.00-3.90 (H-2" to H-6", H-2"' to H-5"'). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.2 (C-2), 133.3 (C-3), 177.9 (C-4), 161.5 (C-5), 99.3 (C-6), 164.7 (C-7), 94.1 (C-8), 156.7 (C-9), 104.1 (C-10), 121.3 (C-1'), 131.4 (C-2'/6'), 115.7 (C-3'/5'), 160.3 (C-4'), 101.6 (C-1''), 74.5 (C-2''), 76.8 (C-3''), 70.7 (C-4''), 76.2 (C-5''), 68.5 (C-6''), 100.9 (C-1'''), 70.8 (C-2'''), 70.9 (C-3'''), 71.9 (C-4'''), 68.5 (C-5'''), 18.0 (C-6''').

Determination of antioxidant activity and dosage of total phenolics and flavonoids

The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical consumption, and the quantification of phenolic compounds determined by Folin-Ciocalteu method and expressed in gallic acid equivalent (GAE) according to Sousa et al. (2007) and Costa et al. (2010). The total flavonoid content was determined by molecular absorption spectrometry, following the methodology described by Sobrinho et al. (2010). Stock solutions of EtOH extracts and fractions (1000 µg ml^{-1}) were prepared and then an aliquot of 300 µl of these solutions was transferred to 10 ml flasks to which 0.24 ml of acetic acid, 4 ml of a methanolic solution of pyridine at 20% and 1 ml of a methanolic solution of aluminum chloride (50 mg ml^{-1}) were added, completing the volume with distilled water. Control was prepared in parallel.

Table 1. Chemical constituents of hexane fractions of *Lecythis pisonis.*

Compounds	Hexane fractions		
	Leaves	Twigs	Fruit shell
Squalene (1)	X	-	-
3β-Friedelinol (2)	X	-	-
Lupeol (3)	X[a]	X	X
α-Amyrin (4)	X	X	X
β-Amyrin (5)	X	X	X
Ursolic acid (6)	X	X	-
Oleanolic acid (7)	X	X	-
Sitosterol (8)	X	X	X
Stigmasterol (9)	X	X	X
Campesterol (10)	-	X[a]	-
(*E*)-phytol (11)	X	X	-

[a]Only identified by GC-MS.

Table 2. Antioxidant activity (EC_{50}), total phenolics (TP) and flavonoids content (TFC) of *Lecythis pisonis* extracts.

Samples	EC_{50} (μg ml^{-1}±SD)	TP (mg GAE g^{-1} DPM±SD)	TFC (mg RE g^{-1} DPM±SD)
EtOH leaves	49.04±1.65	56.78±0.13	30.04±0.02
EtOH twigs	71.52±3.11	29.52±0.30	3.77±0.19
EtOH fruit shell	ND	0.88±0.01	0.73±0.00
Rutin	47.08±4.65	-	-

EC_{50} = effective concentration; GAE = gallic acid equivalent; RE = rutin equivalent; DPM = dried plant material; SD = standard deviation; ND = not determined.

After 30 min, the absorbance of samples was measured at 420 nm using glass cuvets. The total flavonoid content (TFC) was determined by interpolating the absorbance of the samples against a calibration curve constructed with standard rutin at concentrations of 3, 6.5, 10, 13.5, 17 and 21 mg L^{-1}, obtained from a stock solution of 1000 mg L^{-1} in MeOH:H_2O (7:3). To each flask containing 10 ml of these solutions, 0.24 ml of acetic acid, 4 ml of a methanolic solution of pyridine at 20% and 1 ml of methanol solution of aluminum chloride (50 mg ml^{-1}) were added completing with distilled water. After 30 min at room temperature, lecture was performed using a spectrophotometer at 420 nm. Values are expressed as milligrams of equivalent rutin per gram of dried plant material (mg of ER g^{-1} of DPM). The straight line equation is: $A = 0.0262C - 0.0072$, where A is the absorbance, C is concentration and linear correlation coefficient of $r = 0.999$. All analyzes were performed in triplicate ($n = 3$).

RESULTS

The phytochemical study of hexane fractions obtained after partition of the EtOH extract of the leaves, twigs and fruit shell of *L. pisonis* resulted in the isolation and identification by GC-MS, ^{1}H and ^{13}C NMR of seven triterpenoids (1 to 7), three steroids (8 to 10) and a diterpenoid (11) listed in Table 1 and Figure 1. The triterpenoid lupeol (3) α- and β-amyrin (4 and 5) and steroids sitosterol (8) and stigmasterol (9) were identified in all samples. Squalene (1) and 3β-friedelinol (2) were identified only in leaves, while campesterol (10) was identified only in the twigs. The ursolic (6) and oleanolic (7) acids were identified in the leaves and twigs, but were not obtained from fruit shells. Fractionation of the EtOAc fraction, resulting from the partition of EtOH extract of the leaves of *L. pisonis* resulted in the isolation of the flavonoids quercetin-3-*O*-rutinoside (12) and kaempferol-3-*O*-rutinoside (13) (Figure 1). The structural identification of these compounds was based on analysis of UV spectra, NMR (1D and 2D).

The antioxidant activity (AA%) of the EtOH extracts of leaves, twigs and fruit shells of *L. pisonis* and positive control (rutin) in concentrations ranging from 25 to 250 μg ml^{-1}, are shown in Figure 2. The extracts and control showed concentration dependent antioxidant activity. Table 2 presents the results of the evaluation of EtOH extracts of leaves, twigs and fruit shells on the content of flavonoids and phenolics and total antioxidant activity expressed by EC_{50}. The EtOH extract of the fruit shell

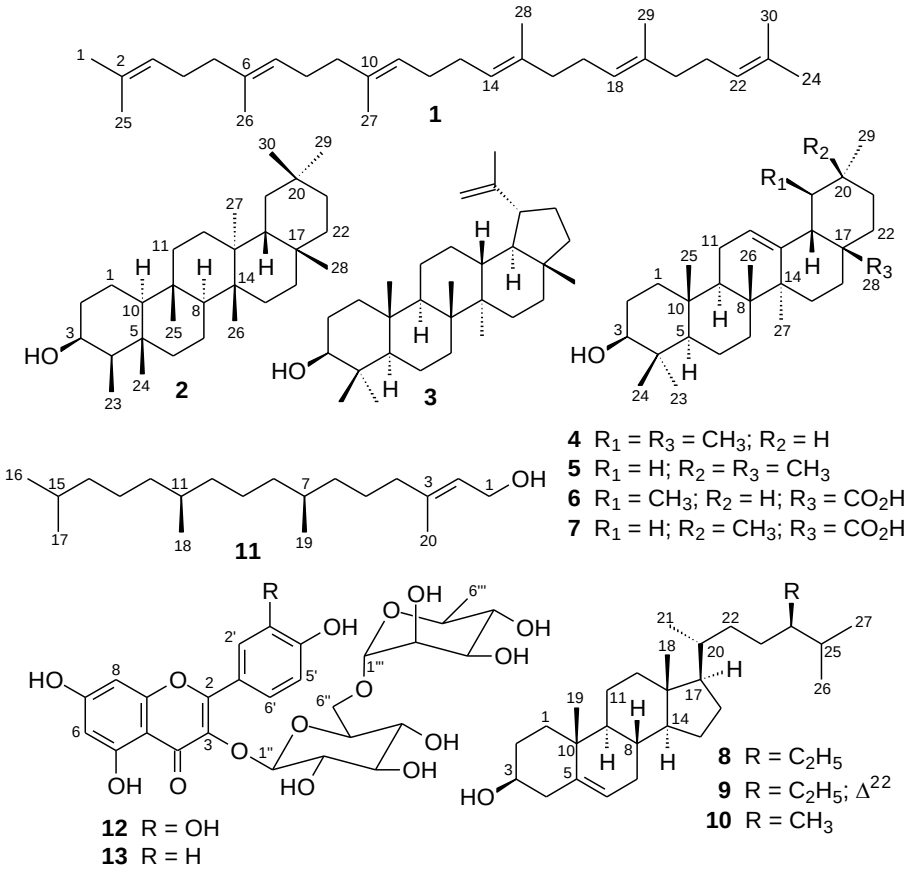

Figure 1. Substances isolated from extracts *Lecythis pisonis.*

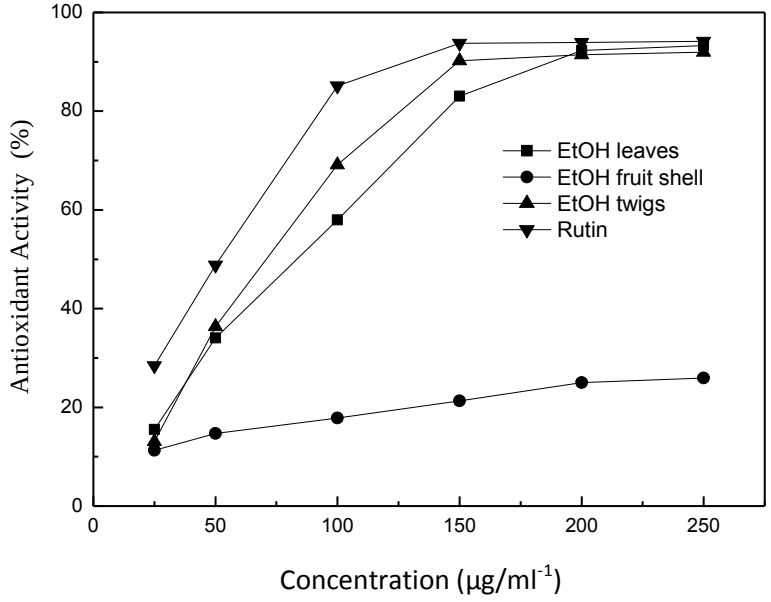

Figure 2. Percentage of antioxidant activity (AA%) of EtOH extracts of leaves, twigs and fruit shell of *L. pisonis*. Positive control: rutin.

Table 3. Total phenols (TP) and total flavonoid content (TFC) in fractions of a partition of ethanol extracts of *L. pisonis.*

Fraction	TP (mg GAE g^{-1} DPM±SD)	TFC (mg RE g^{-1} DPM±SD)
Hexane leaves	ND	ND
Ether leaves	5.53±0.12	4.39±0.00
EtOAc leaves	6.40±0.04	5.21±0.00
Aqueous leaves	42.81±1.02	10.59±0.01
Hexane twigs	0.35±0.00	ND
EtOAc twigs	7.56±0.13	2.37±0.40
Aqueous twigs	10.81±0.22	6.51±0.22

GAE = gallic acid equivalent; RE = rutin equivalent; DPM = dried plant material; SD = standard deviation; ND = not determined.

showed no significant antioxidant activity. The total phenols and flavonoid contents, determined in relation to the dry plant material, lying in the range 0.88 to 56.78 mg of GAE g^{-1} and of 0.73 to 30.04 mg of ER g^{-1}, respectively, with the highest concentrations of these constituents being obtained from the EtOH extract of the leaves (Table 2). Table 3 shows the levels of total phenols and flavonoids of partition fractions of EtOH extracts of leaves and twigs, which proved to be richer in these constituents. Significant differences between fractions were observed ($p < 0.05$). The highest total phenols and flavonoids contents were recorded for the aqueous fraction of the leaves; however it was not possible to determine the flavonoid in hexane fractions of leaves and twigs due to the absence or low content of these constituents.

DISCUSSION

The structural identification of substances 1 to 11 was performed by GC-MS analysis, ^{1}H and ^{13}C NMR and comparison with literature data (Olea and Roque, 1990; Rahman and Ahmad, 1992; Mahato and Kundu, 1994; Junges et al., 2000; Salazar et al., 2000; De-Ekankul et al., 2003). The lupeol (3, leaves) and campesterol (10, twigs) were identified solely on analysis by GC-MS due to the low concentration of these substances in samples. Squalene, lupeol and campesterol are reported for the first time in *L. pisonis*.

The UV spectra of compounds 12 and 13 showed two absorptions maxima at 255/265 and 355/346 nm characteristic of flavonol. ^{1}H NMR spectra (DMSO-d_6) showed typical signals of glycosylated flavonols at δ 5.34/5.30 (d, *J* = 7.5 Hz, H-1'') related to β-D-glucose, δ 4.39/4.38 (d, *J* = 1.5/1.0 Hz, H-1''') assigned to α-L-rhamnose, signals at δ 12.6/12.8 regarding the hydroxyl hydrogens at C-5 chelated to carbonyl and two doublets at δ 6.19/6.18 (*J* = 2.0 Hz, H-6) and δ 6.38/6.41 (*J* = 2.0 Hz, H-8), for the aromatic ring A. The main difference observed in the ^{1}H NMR spectra of these compounds concerned the B ring, as flavonoid 12 showed signals corresponding to three hydrogens, a double doublet at δ 7.65 (1H; *J* = 2.5 and 8.5 Hz, H-6') and two doublets at δ 7.55 (1H; *J* = 2.0 Hz, H-2') and δ 6.84 (1H; *J* = 8.5 Hz, H-5'), characteristic of the 3',4'-dihydroxyflavonol (Fathiazad *et al*. 2006). However, flavonoid 13 showed signals for four hydrogen at δ 7.98 (2H; d, *J* = 9.0 Hz, H-2'/H-6') and 6.88 (2H; d, *J* = 9.0 Hz, H-3'/H-5') characteristic of 4'-hydroxyflavonol (Song et al., 2007). The interglycosidic connection [rhamnopyranosyl(α1'''→6'')glucopyranose] was defined in the heteronuclear multiple bond correlation (HMBC) contour map by the correlation of anomeric hydrogen signal at δ 4.39/4.38 (H-1''') with δ 68.3/68.8 (C-6''), while the location of the diglucoside rutinose moiety [rhamnopyranosyl(α1'''→6'')glucopyranose] at C-3 of the aglycone was determined by the correlation of the signal of the anomeric hydrogen at δ 5.30/5.34 (H-1'') with δ 133.1/133.3 (C-3). A comparison of the NMR data obtained with those reported in literature (Fathiazad et al., 2006; Song et al., 2007) allowed the identification of compounds 12 and 13 as quercetin-3-*O*-rutinoside (rutin) and kaempferol-3-*O*-rutinoside (nicotiflorin), respectively. This is the first report of the identification of flavonoids 12 and 13 in *L. pisonis* and it is also the first described occurrence of the flavonoid kaempferol-3-*O*-rutinoside in Lecythidaceae.

The EtOH extracts of leaves and twigs, in concentrations of 200 and 250 µg ml^{-1} showed antioxidant activity comparable to percentage of the positive control (rutin), while the EtOH extract of the fruit shell was the least active in all concentrations tested. Antioxidant activity was also evaluated by EC_{50}, thus the EtOH extract of the leaves (EC_{50} = 49.04 ± 1.65 µg ml^{-1}) was comparable to the positive control rutin (EC_{50} = 47.08 ± 4.65 µg ml^{-1}).

According to Rufino et al. (2010), the content of phenolic compounds, expressed as gallic acid equivalents

per gram of dried plant material may be classified into low (< 10 mg GAE g^{-1}), medium (10 to 50 mg GAE g^{-1}) and high (> 50 mg GAE g^{-1}). In this study, the content of phenolic compounds was high for the EtOH extract of leaves, medium for the twigs and low for the fruit shell. A positive correlation between the EC_{50} and the content of phenols and flavonoids of EtOH extracts of leaves, twigs and fruit shells was observed. This behavior is generally expected, considering that the phenolic compounds and in particular flavonoids are free radicals scavengers and consequently exhibit antioxidant properties.

The phenolic contents for the fractional partition EtOH extract of the leaves ranged from 0.06 to 42.81 mg GAE g^{-1} of DPM while flavonoids contents ranged from 2.37 to 10.59 mg ER g^{-1} of DPM. Although the total phenols of EtOAc fraction of leaves was lower than in the EtOAc fraction of the twigs, the content of flavonoids was higher, being explained partly by the presence of flavonoids quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside, that were detected in the EtOAc fraction of leaves but not from twigs, according to TLC analysis.

Conclusions

The phytochemical study of the hexane fractions of the leaves, twigs and fruit shells of *L. pisonis* resulted in the isolation and identification of seven triterpenoids: squalene (1), 3β-friedelinol (2), lupeol (3), α- and β-amyrin, (4 and 5) ursolic and oleanolic acids (6 and 7), three steroids: sitosterol (8), stigmasterol (9) and campesterol (10) and a diterpenoid, the (*E*)-phytol (11). The EtOH extract of the leaves showed the highest antioxidant activity and the highest levels of total phenolics and flavonoids. These results can be partly explained by the presence of flavonoids, quercetin-3-*O*-rutinoside (12) and kaempferol-3-*O*-rutinoside (13), isolated from the EtOAc fraction resulting from the partition of the EtOH extract of leaves. The two flavonoids as well as lupeol, squalene and campesterol are being reported for the first time in *L. pisonis* and the occurrence of kaempferol-3-*O*-rutinoside (13) is also being reported for the first time in the Lecythidaceae family.

Depending on the possible toxic effects of synthetic antioxidants currently used, there is a growing interest in the use of natural products in the pharmaceutical and food industries. Thus, the results suggest that the EtOH extract of the leaves of *L. pisonis* is promising for the development of phytomedicine, cosmeceutical products or adjuvants product where antioxidant activity is desirable.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Altered sex expression by plant growth regulators: An overview in medicinal vegetable bitter gourd (*Momordica charantia* L.)

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Bitter gourd is one of the popular vegetables for its medicinal values. It is monoecious cucurbitaceous plants which have imbalance sex ratio of male-female flowers that causes lower fruit yield. Different research works on cucurbits like bitter gourd and other related crops in respect of plant growth regulators, plant nutrients, and priming practices have been conducted in different parts of the world. Literatures related to the present study have been reviewed and found that bitter gourd genotypes produced larger male-female ratio and the induction of male flower was earlier than that of female ones. Growth regulators have significant positive effect on yield and yield components. Application of gibberellic acid (GA_3) enhanced the length of main vine, but decreased the primary branches while ethylene producing chemicals Canadian Environmental Protection Act (CEPA) increased the number of primary branches per plant. Application of auxin like 1-naphthaleneacetic acid (NAA) at 50 and 100 ppm and CEPA at 150 ppm also proved to be effective in inducing earlier female flowers at lower node. Application of CEPA at 150 ppm and NAA at 50 ppm was found to be the best treatments for reducing sex ratio by increasing the female flowers by suppressing the male ones, and consequently induce higher yield.

Key words: Bitter gourd, sex ratio, medicinal plant, yields.

INTRODUCTION

Value added productivity in the agriculture sector can be further enhanced through increasing the values from existing industries by cultivating new commercial crops such as herbal products. Bitter gourd (*Momordica charantia* L.) a medicinal plant, belonging to cucurbitaceous family is one of the most popular vegetables in Bangladesh and also in other Asian countries namely China, Taiwan, Malaysia, Vietnam, Thailand, India and

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the Philippines. It is adapted to a wide range of environments and can be grown in tropical and subtropical climates (Lim, 1998; Reyes et al., 1994). This vegetable is a different nature's bountiful gifts to mankind, which does not only have fabulous digestional properties, it is a storehouse of remedies for many common ailment. The fruits, leaves and even the roots of *M. charantia* have been used in Ayurveda for a number of diseases such as a bitter stomachic, laxative and anathematic. A compound known as 'charantin' present in the bitter gourd is used in the treatment of diabetes to lower blood sugar level (Anunciado and Masangkay, 2002). The fruit accumulates bitterness with time due to build up of three pentacyclic triterpenes momordicin, momordicinin and momordicilin, and then loses the bitterness during ripening (Begum et al., 1997; Cantwell et al*.,* 1996). The whole extract of the fruit is also advocated in diseases of spleen, liver, rheumatism and gout. The immature fruit of bitter gourd is valued for its bitter flavor, considered to bring out the flavor in other ingredients. It is usually eaten fresh (stuffed and/or sliced) but can also be pickled and has been canned in brine (Vinning, 1995).

The plant also has a rich amount of vitamin C, iron, phosphorus and carbohydrates (Behera, 2004). The small type bitter gourd, 'uchja' fruits contained relatively more protein (2.1%), lipid (1.0%), ash (1.33%) and iron (9.4 mg/100 g) than the large type fruit 'karala', as well as good levels of carbohydrate (7.2%), sugars (0.42%), phosphorous (140 mg/100 g) and ascorbic acid (74 mg/100 g), and was therefore considered to have the best nutritional value (Choudhury, 1990; Kale et al.,1991; Kore et al., 2003). So, 'uchja' is considered as a high priced vegetable throughout the year for its medicinal value and unique taste.

Bitter gourd is a monoecious plant, naturally, inducing greater number of male flowers than the female flowers. This flowering behavior is not advantageous and economical, because it results in lower fruit set and yield, which is a common problem in bitter gourd cultivation. To have the higher yield, the male and female flower ratio needed to be synchronized. Maleness and femaleness can usually be altered by environmental variables such as temperature, photoperiod and nutrition or by the application of plant growth regulators (Krishnamoorthy, 1981).

Attempts have been made to overcome the aforementioned problem, and several investigations have been done in this regard in different parts of the world. Proper and judicious use of plant growth regulators with balanced fertilizer, especially N, P, K is one of the ways to increase the crop yield of bitter gourd. Use of adequate mineral nutrients and balance supply of plants hormone within the plant may play vital role in the control of plant growth and fruit setting capability of bitter gourd. Application of indol acetic acid (IAA) increased the female flowers in cucurbit as suggested by Basu et al*.* (1994). Similarly, higher number of female flower and fruit set in bitter gourd was recorded by enhanced N application (Ali et al., 1995). On the other hand, Samdyan et al. (1994) found that application of N fertilizer in combination of growth regulators produced higher yield as compared to growth regulator alone. Melissa and Nina (2005) reported that exogenous applications of 1-naphthaleneacetic acid (NAA) and gibberellic acid (GA_3) at the 5-leaf stage in bitter gourd induced parthenocarpy. Numerous research works have been done in the use of plant hormones and mineral nutrients separately or in combination, especially in 'uchja'. Keeping these views in mind, the present review works have been initiated with the objectives to investigate the influence of plant growth regulators with mineral nutrients to synchronized male/female flowers by lowering sex ratio and thereby increase fruit setting.

DISCUSSION

Plant growth regulators on growth, sex expression and fruit yield

Exogenous application of growth regulators may shift the sex expression towards femaleness by increasing the production of female flower and suppressing that of male flower in cucurbitaceous plants. Plant growth regulators have positive effect on the production of early flowering and yield. Growth regulators can decrease male and female flower ratio and increasing the number of fruits per plant and individual fruit weight as well as increase the total yield.

The role of plant growth regulators in various physiological and biochemical processes in plant is well known from its identification. Root and flower buds initiation, development of flowers and fruits are controlled by different physiological processes. In many crops like cucurbitaceous ones, these processes can often be altered to man's benefit by proper application of plant growth regulators. The concept that plant growth and development are regulated by a substance produced in minute quantities is one organ that elicits a response in another was first suggested by Julius von Sachs, the father of plant physiology. The term plant growth regulators (PGRs) cover the broad category of organic substances (other than vitamins and nutrients) that in minute amounts, promote, inhibit, or otherwise modify physiological processes (Wareing and Phillips, 1978). The PGRs, where endogenous (phytohormones) or exogenous, elicit essentially the same plant responses. Presently, PGRs are used to control a host of physiological processes in crop production, including flowering and fruiting (fruit set and parthenocarpy), partitioning of

assimilate, germination, growth suppression, and post harvest ripening (Weaver, 1975).

The principle in sex modification in cucurbits lies in altering the sequence of flowering and sex ratio. Besides the environmental factors, endogenous levels of auxins, gibberellins, ethylene and abscisic acid, at the time and the seat of ontogeny determine the sex ratio and sequence of flowering (Leopold and Kriedemann, 1975). Exogenous application of plant regulators can alter the sex ratio and sequence, if applied at 2 or 4 leaf stage, the critical stage at which the suppression or promotion of either sex is possible. Hence, modification of sex to desired direction has to be manipulated by exogenous application of plant regulators once, twice or even thrice, at different intervals (Devies, 1987).

Ravindran (1971) reported that the exogenous application of ethral (2-Chlorothyl phosphonic acid) at concentrations ranging from 200 to 600 ppm induced stunting growth, retardation and male sterility and the production of male flowers significantly reduced in bitter gourd. Similarly, in cucumber (*Cucumis sativus* L.), application of ethral up to 500 ppm (Bhandary et al., 1974) increased the female flowers. They stated that ethrel concentration up to 500 ppm delayed male flowering up to 14 days and advanced female flowering by up to 9 days, while number of male flower were also reduced and female flowers increased by the application of same treatments. Higher ethrel concentrations were detrimental, whereas sex expression of snake gourd could be altered by foliar application of ethephon (ethrel) at 250 ppm and fruit yield could also be increased (Cantliffe, 1976). Mishra et al. (1976) reported that in cucumber maximal suppression of staminate flowers was obtained by the application of 400 ppm of ethrel. In case of fruit yield and yield components like number of pistillate flowers, fruit numbers plants^{-1}, fruit size and fruit weight were increased in *Trichosanthes anguina* plants by the application of ethrel at 50 to 150 ppm. The best result was obtained with ethrel at 150 ppm (Ramaswamy et al., 1976). In *Luffa acutangula* seedlings treated with 500, 1000 or 2000 ppm of ethrel, Patnaik et al. (1974) reported that ethephon treated plants produced pistillate flowers only, but the number of fruits and total yield were inferior to those of untreated plants. Verma et al. (1980) reported that ethrel treatments (50, 100, 150 and 200 ppm) were the most effective in increasing the number of female flowers, producing the largest number of fruits and greatest fruit weight plant^{-1} in cucumber. They further reported that all the treatments reduced the number of male flowers.

Krishnamoorthy (1981) studied the effect of ethrel at 250 to 1000 ppm on growth, flowering and sex expression of *Cucurbita pepo* L. They stated that ethrel increased the number of female flowers and decreased of male flowers. Li (1983) reported that ethrel at 200 or 300 ppm could lower the site of the first female flower, promote the appearance of female flowers, increase the number of fruits and leaf area, reduce the number of male flower, fruit setting and increase yields of three cucumbers. The ethylene-releasing chemical, ethrel enhanced the development of pistillate flowers and delays development of staminate flowers of monoecious cucurbits (Sheshadri, 1986).

Sreeramulu (1987) treated the sponge gourd plants at 3-true leaf stage with ethrel solution (100 mg/L). He observed that ethrel not only increased the number of pistillate flowers, but also hastened the appearance of the first female flower. The effect of ethrel on the staminate flowers was the opposite, that is, it delayed the appearance of the first staminate flower and also decreased the total number of female flowers. The sex ratio (staminate: pistillate) is decreased from 12.1:1 to 6.8:1. Plants were sprayed with different growth regulators at the 2 and 4 true leaf stages. The total yield (2.39 kg plant^{-1}) was the highest in plants treated with ethrel (ethephon) at 100 ppm. The average control yield was 0.69 kg plant (Arora et al., 1985). Singh and Choudhury (1988) stated that ethrel at 50 and 100 ppm induced the first pistillate flowers earlier and at lower nodes in cucumber and bottle gourd, but delayed the appearance of female flowers in water melon. Karim et al. (1990) treated hybrid seedlings of cucumber with water, ethephon (250 and 350 ppm) at 1, 2, 3 and 4 leaf stage. Seedlings treated with ethephon at any stage produced more female flowers than water treated plants. The maximum increase in the number of female flowers occurred with 250 ppm ethephon applied at the 2-leaf stage.

Seed germination was not influenced by ethrel and the first female flower development was marginally earlier with ethrel treatment. Al-Masoum and Al-Masri (1999) reported that cucumber cv. Beit Alpha grown in a greenhouse in 1996 to 1997 was treated with ethephon at 250, 350 and 450 ppm at the seedling stage (2 to 4 true leaves). They obtained positive effect of ethephon on the early and total yield, late number of female flowers, number of male flowers, days to the first female flowers, number of nodes to the first female flower, number of nodes to the first male flower and plant height. Ethephon induced femaleness (pistillate flowers) on the main stem that led to greater fruit production.

Negi et al. (2003) studied the effect of ethephon and row spacing on the growth and yield of bitter gourd. Treatments comprised: two ethephon levels (0 and 250 ppm) and three row spacing (1.0, 1.25 and 1.50 m). Ethephon (250 ppm) reduced the length of main vine and number of branches and delayed the appearance of the first male and female flowers. Increasing row spacing

increased the total number of female flowers per plant and appearance of the first female flower at the lower nodes. The fruit number as well as total fruit yield per plant increased with increased in spacing and decreased with ethephon application.

Choudhury and Singh (1970) reported that NAA 100 ppm, IAA 100 and 200 ppm, Maleic Hydrazide (MH) 50 and 200 ppm were equally effective in suppressing the male flowers and increasing the number of female flowers in cucumber. The effects subsequently increased the percentage of fruit set and ultimately the yield. Bisaria (1974) found that foliar spray of NAA at 100 ppm increased the number of female flower per plant and the sex ratio was reduced in cucurbits. Pandey and Singh (1976) compared the effects of seed soaking for 24 h in solutions of 2, 4- D at 1.5 ppm, MH and NAA, each at 200 pmm and GA_3 at 50 ppm and foliar spraying with 2, 4-D at 0.5 to 1.0 ppm, applied at the 2 true leaf and 4 to 5 true leaf stages. The number of pistillate flowers of *Luffa cylindrica* was increased by seed treatment with MH and NAA at 200 ppm and by spraying with NAA at 100 and 150 ppm, MH at 100 to 200 ppm and GA_3 at 10 ppm, staminate flower numbers were decreased by MH at 200 ppm, NAA at 100 ppm and GA_3 at 10 ppm. The ratio of pistillate: staminate flower numbers was increased by all treatments except 2, 4-D and GA_3 at 25 and 50 ppm. Fruit set was enhanced by all treatments except GA_3 at 50 ppm and 2, 4-D. Yields were increased by seed treatment with NAA at 200 ppm and by spraying with NAA and MH at 150 and 200 ppm, respectively.

Gopalkrishnan and Choudhury (1978) reported that in contrast with Tri-iodobenzoic acid (TIBA), GA_3 in general produced the largest number of female flowers; GA_3 at the lowest concentration of 10 ppm produced more number of female flowers in first year. In the first year MH 100 to 600 ppm as well as NAA and IAA at 50 to 150 ppm induced larger number of female flowers. TIBA from 50 to 200 ppm gave a significant increased in the number of fruits and weight of fruits of water melon.

An investigation was conducted to study the influence of various chemicals (ethrel, NAA, Clinical Care Classification (CCC), MH, para-chlorophenoxyacetic acid (PCPA), ascorbic acid and boron) on the growth, flowering and yield of bitter gourd. PCPA at 100 ppm improved plant growth significantly. The treatment of CCC at 250 and 500 ppm produced female flowers about 12 days earlier in comparison to control plant. Maximum fruit yield per plant (3123 g) was produced under CCC 250 ppm followed by ascorbic acid 25 ppm and cycocel 350 ppm (Mangal et al., 1981). Similarly, Ahmad and Gupta (1981) found that the minimum ratio of male to female flower was reached at 1000 ppm of cycocel in case of smooth gourd and at 1500 ppm in bottle gourd and snake gourd. Nodes per female flower as well as days to flower were minimum at 1000 ppm in snake gourd and 1500 ppm in smooth gourd and bottle gourd. Earliest node for first female flower was observed at 1000 ppm in smooth gourd and snake gourd but at 1500 ppm in bottle gourd.

Verma et al. (1984) found that ethrel 100 ppm delayed the appearance of first male and female flowers. Application of MH 200 ppm and boron 3 and 4 ppm produced the earliest female flowers, but at a higher node, while ethrel 100 ppm induced the first staminate and pistillate flower at the lowest nodes at 6.5 and 9.5, respectively. Boron 4 ppm also proved superior to all and MH 100 ppm did not response much. The number of fruits per plant and average weight of fresh fruit was increased significantly in both varieties.

Islam (1995) stated that application of GA_3 was effective in improving the yield components of bitter gourd when applied at low concentration of 10 ppm. The inhibitory effect of GA_3 applied at the rate of 100 ppm was observed on production of fruits with lesser number of filled seeds, dry matter of seeds, weight of 100 seeds, seed yield and percent seed vigor index. Irrespective of concentration, the application of GA_3 reduced the total number of staminate flowers. The ratio between the staminate and pistillate flowers as fruit setting was low. The number, length, diameter and weight of fruits were not influenced by GA_3 application. Wang and Zeng (1996) reported that gibberellic acid at 25 to 100 ppm increased the number of female flowers up to 80 days. Baruah and Das (1997) observed that plants sprayed with NAA at 25 ppm and MH at 50 ppm produced the best yields (5.48 and 4.86 kg $plant^{-1}$, respectively) in *Lagenaria siceraria*. Yield decreased with late sowing dates from 5.49 to 2.62 kg $plant^{-1}$. Tomar and Ramgiry (1997) conducted an experiment and found that plants treated with GA_3 showed significantly greater plant height, number of branches $plant^{-1}$, number of fruit $plant^{-1}$ yield.

Gedam et al. (1998) conducted an experiment in 1992 where bitter gourd plants were sprayed at 40, 55, 70, 80 and 100 days after sowing with GA_3 at 15, 25 and 35 ppm, NAA at 50, 100 and 150 ppm, ethephon at 50, 100, and 150 ppm, MH at 100, 200, and 300 ppm and boron at 2, 4 and 6 ppm with water (control). GA_3 at 35 ppm produced the earliest female flower and NAA at 50 ppm produced the earliest male flower. Fruit maturity was the earliest in plants treated with 50 ppm NAA or 4 ppm boron. Fruit and seed yields were also the highest in these treatments. Melissa and Nina (2005) reported that exogenous applications of NAA and GA_3 at the 5-leaf stage induced parthenocarpy. Fruit length of bitter gourd treated with NAA decreased significantly compared with that of the control. The fruit diameter increased significantly at 100 ppm NAA, possibly due to larger cell size induced by this growth substance. Treatment with GA_3 did

Table 1. Effect of different levels of plant growth regulators and NPK fertilizers on sex expression in bitter gourd.

Treatment	Days to flower		Node of first flowering		Flowers plant^{-1}		Sex ratio
	Male	Female	Male	Female	Male	Female	(M/F)
Plant growth regulators (ppm)							
Control	38	53	10.50	12.20	310	27.42	11.31
GA_3 50	35	49	9.55	11.80	383	29.42	13.02
NAA 50	37	41	8.45	7.00	250	35.14	7.48
NAA 100	36	46	10.75	8.90	285	33.43	8.11
CEPA 150	40	42	8.50	6.50	210	38.33	5.48
CEPA 300	33	43	6.25	9.70	342	31.43	10.88
LSD ($_{0.05}$)	0.95	0.75	0.35	0.85	12.45	1.65	0.55
NPK fertilizers (kg ha^{-1})							
$N_0 P_0 K_0$	45	48	15	9.50	175	24.47	7.15
$N_0 P_{45} K_{60}$	36	46	13	8.83	235	27.26	8.62
$N_{60} P_{45} K_{60}$	38	45	12	8.33	250	32.95	7.59
$N_{90} P_{45} K_{60}$	37	45	12	7.92	350	38.12	9.18
$N_{120} P_{45} K_{60}$	39	46	13	8.83	310	33.63	9.22
$N_{90} P_0 K_{60}$	38	46	14	9.17	288	28.80	10.00
$N_{90} P_{30} K_{60}$	37	45	13	8.42	345	33.69	10.24
$N_{90} P_{60} K_{60}$	35	44	14	8.58	335	34.21	9.79
$N_{90} P_{45} K_0$	37	46	13	8.83	290	33.86	8.56
$N_{90} P_{45} K_{80}$	37	45	13	8.75	350	38.28	9.14
LSD ($_{0.05}$)	1.05	1.14	1.12	1.75	15.85	1.37	0.98

not significantly increase the fruit length while its fresh weight and diameter decreased significantly at 20 ppm compared with the control.

Our results (Table 1) suggested that Canadian Environmental Protection Act (CEPA) and NAA at both concentrations induced first female flower 10 to 12 days earlier than control at lower nodes, whereas GA_3 induced male flowers earlier to all other treatments. CEPA 150 ppm and NAA 50 ppm increased the total number of female flowers by 40 and 29%, respectively. CEPA 150 ppm proved to be the best treatment for reducing the sex ratio.

Combined effect of plant growth regulators and plant mineral nutrients

Pandey and Singh (1973) found that soil application of up to 100 kg N ha^{-1} increased the number of pistillate and staminate flower and the yield where the sex ratio was not affected in bottle gourd. Application of MH approximately doubled the proportion of female flowers and also increased yield. Combined application of N and MH produced more female flowers and the greater yield. Suresh and Pappiah (1991) conducted a trial with bitter gourd where N and P_2O_5 were applied at 0, 40 and 80 and 0, 30 and 60 kg ha^{-1}, respectively and MH was sprayed at 0, 100 and 200 ppm solution. The highest yield was obtained with 80 kg N and 45 kg P_2O_5 ha^{-1} and 200 ppm MH. Samdyan et al. (1994) conducted field trials where bitter gourd plants received N fertilizer at 25, 50 or 75 kg ha^{-1}, with cycocol at 100 or 250 ppm, ethrel at 50 or 100 ppm, GA_3 at 10 or 25 ppm or MH at 25 or 50 ppm. Nitrogen at 75 kg ha^{-1} produced the thickest rind and highest fruit dry matter (DM) content, while 50 kg ha^{-1} gave the highest flesh weight and ascorbic acid and total soluble solid (TSS) contents. Among the growth regulators, MH at 50 ppm gave the thickest and heaviest rind, while cycocel at 250 ppm gave the highest DM, ascorbic acid and TSS contents and flesh thickness and weight. The combination of 75 kg N ha^{-1} + 50 ppm MH gave the thickest and heaviest rind and thickest flesh. Ghosh and Basu (1983) conducted an experiment to study the effect of plant growth regulators on sex expression in *M. charantia* L. by the application of GA_3, IAA and 3-hydroxymethyl oxindole (HMO). All plant growth regulators stimulated female flowering. Both IAA and HMO accelerated ethylene evolution in the seedlings of this plant. While a low concentration of ethrel promoted flowering. The effect was reversed with increased

Table 2. Interaction effect of different levels of NPK fertilizers and plant growth regulators on initiation of first flower (male and female) of 'uchja' (BG-5 genotype) grown in summer, 2007.

NPK fertilizer	*Days to first flowering													
	Control		GA-50		NAA-50		NAA-100		CEPA-150		CEPA-300		Mean	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
$N_0P_0K_0$	49	53	48	49	46	45	38	50	42	44	45	45	45	48
$N_0P_{45}K_{60}$	42	55	32	50	32	42	39	45	38	42	30	44	36	46
$N_{60}P_{45}K_{60}$	45	52	35	48	35	43	35	43	41	41	35	43	38	45
$N_{90}P_{45}K_{60}$	46	53	31	47	36	40	35	46	38	40	33	43	37	45
$N_{120}P_{45}K_{60}$	45	55	35	47	40	40	38	48	40	43	35	45	39	46
$N_{90}P_0K_{60}$	48	53	32	49	39	40	35	44	40	43	32	45	38	46
$N_{90}P_{30}K_{60}$	45	51	36	48	35	41	36	47	37	43	32	41	37	45
$N_{90}P_{60}K_{60}$	43	51	30	49	30	40	38	44	40	42	30	40	35	44
$N_{90}P_{45}K_0$	46	52	35	50	38	42	32	45	42	42	30	43	37	46
$N_{90}P_{45}K_{80}$	45	53	32	48	36	41	36.2	46	40	40	31	42	37	45
Mean	45	53	35	49	37	41	36	46	40	42	33	43	-	-
CV%	4.15		LSD(0.05): F = 4.5,				PGR = 3.25				F × PGR = 6.75			

*No. of days after sowing of seeds. F: Effect of different levels of NPK fertilizers. PGR: Effect of different plant growth regulators. F × PGR = Interaction effect of different levels of NPK fertilizers and plant growth regulators. CV: Coefficient of variance.

concentrations. Surprisingly, GA_3 was the most effective growth regulator in increasing femaleness. In untreated plants, levels of endogenous GA-like substance increased progressively up to the age of 60 days at which the ratio of male to female flowers was lower. Our research findings suggested that combined effect of mineral nutrient NPK at the rates of 90, 45 and 60 kg ha^{-1} with plant growth regulator CEPA 150 ppm was superior to all treatments for increasing the total number of female flowers (Table 1).

Conclusions

Among the many factors which determine the low yield, sex ratio and synchrony of male-female flowers and suitable genotype are more important. Days to first flower initiation, number of total female flower and sex ratio was significantly influence by different genotypes. The days to first male and female flowers varied from 39.4 to 51.17. The results indicated that the days to flowers initiation might be controlled by inherent characters of genotypes.

Application of different doses of plant growth regulators and NPK fertilizer significantly influenced the female flower induction and synchrony of male-female flowers. Application of CEPA at 150 ppm and NAA at 50 ppm produced lower sex ratio by increasing the female flowers by 40 and 28% over control (no spray). Among the plant growth regulators, CEPA is the superior of all for producing maximum number of female flowers $plant^{-1}$. On the other hand, the combined effect of treatment $N_{90}P_{45}K_{60}$ with CEPA 150 ppm was better for producing higher number of female flowers $plant^{-1}$ by enhancing the maximum branches $plant^{-1}$ (Table 2).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Assessment of the medicinal uses of plant species found on termitaria in the Pendjari biosphere reserve in Benin

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Medicinal plants are important in the life of African populations and there is nowadays an increasing need to gathering information related to them. In order to highlight the importance of termitaria to local populations, we investigated within the Pendjari Biosphere reserve the medicinal uses made from plant species found in association with them. We laid out plots in fields and fallows surrounding the Pendjari National Park to assess fifty six termitaria and identify plant species on them. By using specimen and local names of species, group interviews were conducted with the 3 major ethnic groups in the Biosphere reserve. Results show that people perceive termitaria as fertilization materials, and plants in association with them are considered more efficient in traditional medicine than those collected in mounds vicinities. Indigenous people used, for various medicinal purposes, twenty-two (22) plant species consisting of 21 woody and 1 herbaceous belonging to fourteen (14) families. Species used as medicine were relatively different according to the ethnic group. Furthermore, Combretaceae was the most used plant family. A total of thirty (30) diseases and illnesses were treated by plants, and bark was the most used part followed by leaves and roots. We suggest that conservationists and other scientific advisers use our findings to well define conservation programs and increase people's awareness on the sustainable management of termitaria and their ecosystems.

Key words: Medicinal plants, illnesses, termitaria, Pendjari biosphere reserve, Benin.

INTRODUCTION

Since time immemorial, people have gathered plant and animal resources for their needs. Examples include edible nuts, mushrooms, fruits, herbs, spices, gums, game, fodder, fibres used for construction of shelter and housing, clothing or utensils, and plant or animal products for medicinal, cosmetic or cultural uses (Schippmann et al., 2002). In developing countries, even today hundreds of millions of people derive a significant part of their subsistence needs and income from gathered plant and animal products (Walter, 2001). Similarly, Jones et al. (2002) reported that gathering of high value products such as mushrooms (morels, matsutake, truffles), medicinal plants (ginseng, black cohosh, goldenseal) also continues in developed countries for cultural and economic reasons. Medicinal plants play a great role among all the uses and almost 70 to 80% of the world populations use those plants for their primary healthcare (Cunningham, 1993). Moreover, plant species are mostly

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harvested from the wild to satisfy the pharmaceutical factories demands and for the local informal trade (Adjanohoun, 1982; Hamilton, 2004; Grace et al., 2004).

In many parts of the world, the use of medicinal plants has led to close relationships between local populations and their nature. In order to ensure a long term availability of medicinal plants for people, there is a recognition that much attention should be focused not only on the diversity of plants used for that purpose but also on the conservation of the different kinds of habitats including hotspots where these plants are harvested. Among those hotspots, termitaria are recognised as habitats for many plant species (Konate et al., 1999; Loveridge and Moe, 2004; Traoré et al., 2008). Termitaria are also habitats for animal species such as Andros Iguana *Cyclura cyclura cyclura* (Knapp and Owens, 2008) and small vertebrates (Fleming and Loveridge, 2003). Moreover, termite mounds may contribute to sustaining populations of mega herbivores in miombo woodland (Loveridge and Moe, 2004; Frost, 1996; Mobæk et al., 2005).

With regards to the role of termitaria in animal and plant species conservation, it is important to state local perception about them. In order to identify strategies to reduce wind erosion in Burkina-Faso, Leenders et al. (2005) focused their research on the farmer's perception of the role of scattered vegetation in this erosion control. Moreover, Vodouhê et al. (2010) have recently stated that Pendjari's local populations' perceptions of biodiversity conservation were strongly related to locally perceived benefits. These investigations confirm the necessity to take people's perception of termitaria and associated plants into account. So, this paper focused on: (i) The perception of indigenous people about termitaria and plants found in association with them, (ii) the diversity of plants species found on termitaria in fields and fallows surrounding the Pendjari National Park and the medicinal uses that local people make from them.

The aim behind this publication is to highlight the importance of termitaria to Pendjari's local populations through the investigation of the medicinal uses of plant species found in relation with them. Elsewhere, we do hope that conservationists and other scientific advisers from NGOs use our findings to increase people's awareness on the importance to conserve termite mounds and to well define conservation programs towards them. This no doubt constitutes a way to sustain medicinal plants in the Biosphere Reserve of Pendjari.

METHODOLOGY

Study area

The study was carried out in Pendjari Biosphere Reserve located between 10° 30' to 11° 30' N and 0° 50' to 2° 00' E (Figure 1). The reserve covers 471,140 ha of which the Pendjari National Park covers approximately 56.47% and the Pendjari hunting zone only 43.53%. The dry season starts from mid-October to mid-May. The annual mean temperature ranges between 18.6 and 36.8°C in the northern Reserve and in the southern, between 20.5° and 34.2° (Sogbohossou, 2004). Temperatures are highest in March and April and lowest from December to January. Vegetation consists of savannas, dry forest, woodlands and gallery forests. *Terminalia*, *Combretum* and *Acacia* are the predominant genera in shrub savannas (Sinsin et al., 2000) and the most abundant species are *Combretum glutinosum*, *Crossopteryx febrifuga*, *Acacia seyal*, *Acacia senegal*, *Acacia gourmaensis* (MAB UNESCO, 1990). Tree savannas are dominated by *Acacia sieberiana*, *Pseudocedrela kotschyi*, *Terminalia macroptera*, *Detarium microcarpum*, *Burkea africana*, *Afzelia africana* and *Vitellaria paradoxa*. Lastly, gallery forests are mostly characterized by *Diospyros mespiliformis*, *Borassus aethiopium*, *Ficus capensis*, *Khaya senegalensis*, *Parinari congoensis* and *Syzygium guineense*. Almost thirteen ethnic groups surround the Reserve and mainly three groups are predominant: Gourmantché (G), Berba (B) and Wama (W). The main activities generating income in the Biosphere Reserve are crop production, animal raising, trade and ecotourism services.

Termite mounds sampling

Prior to people's perception and the medicinal uses documentation, we investigated plant species found on termitaria by laying out plots sized 50 m × 50 m in order to take inventory of termite mounds. Then plant species on each mound were noted. A total of 56 mounds were surveyed in fields and fallows. Only the agricultural lands were taken into account as the local populations are not allowed to collect plants from the park. Plant species of which names were not determined in field had been identified at the National Herbarium.

Surveying people's perception about termitaria and mounds-related species

In each of the three ethnic groups (Gourmantché, Waama and Berba), we conducted group interview. The survey groups were made based on age and gender and old people and traditional healers were the most representative (almost 75%) in each group as they are supposed to be guards of traditional knowledge in African societies. Interviewees were asked to list the different categories of termitaria found in their area and the criteria they use to distinguish each one. We also ask participants to list the various beliefs related to termitaria and plants in relation with them. We conducted field trips in the Biosphere Reserve with participants to observe termitaria and identify some plants found on them as well as their local names.

Medicinal plants and uses surveys

In order to gather the diversity of uses as well as similarity, one group of people were interviewed in each of the three major ethnic groups: Gourmantché (G), Berba (B) and Wama (W). Participants were shown specimen and asked to provide the local name. Then they were asked to list the different diseases and illnesses that they treat using the species as well as the parts exploited and modes of preparation applied. There is however some species in our paper of which local names were not provided generally because of the non use of the species concerned by the ethnic group.

Statistical analysis

We performed an X^2 independent test to state whether the plant families used as medicine depend on the ethnic groups that

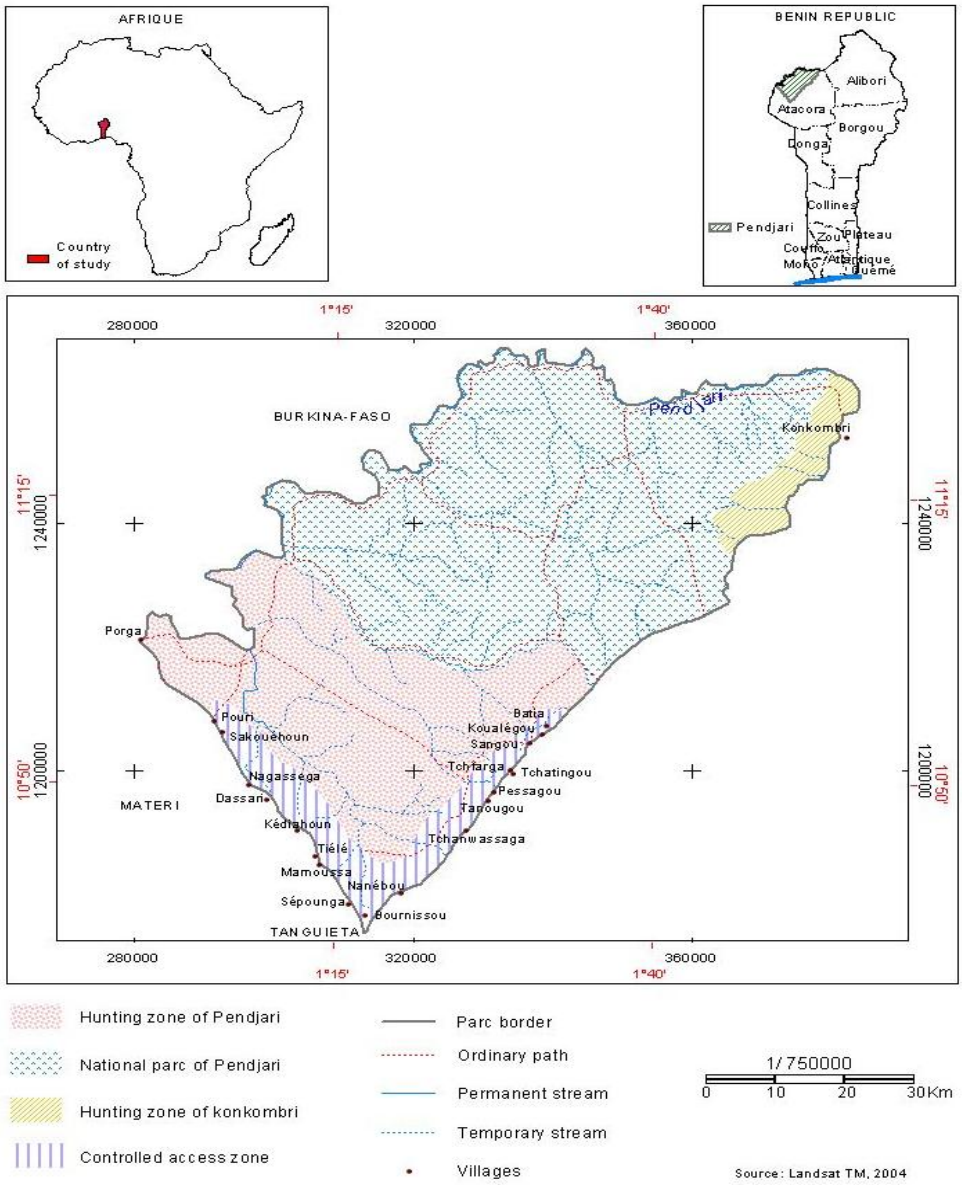

Figure 1. Maps showing the location of the study country Benin in West Africa as well as the study area Biosphere Reserve of Pendjari in Northern Benin. The Atakora chain is the southern border of the reserve whereas it is the Pendjari River which constitutes its north- western border.

Pendjari's people belong to. We considered 0.05 as level of probability. All proportions and graphs presented in this paper were computed using Excel software package.

RESULTS AND DISCUSSION

Indigenous people's perception about termitaria and mounds-related plant species: Ecology and traditional knowledge

Participants in the Pendjari Biopshere Reserve identified termitaria based on four major criteria: The height of the termite mound, its hardness, colour and the size of termite inside the mound. Two categories of termitaria were defined by local populations. The first group is characterised by mounds with height less than 1.2 m, often hard with black colour and small termites inside mounds. The second group is composed of big termitaria (height more than 1.2 m), soft and generally wet. According to indigenous people, big termites are found inside. Termites found in the small mounds (those of the first category) are sometimes used to feed poultry. Local people mentioned the big termitaria as the most common in the Biosphere Reserve of Pendjari. According to the

ethnic groups surveyed, termitaria are places where their ancestors' spirits and other religious spirits dwell. As a consequence, termitaria are respected and for instance, it is forbidden to urinate on termitaria for fear to be punished by ancestors' spirits. Termitaria are also used by hunters to hide from dangerous wild animals that may be possessed by "bad" spirit during hunting party. Elsewhere, according to participants, the presence of large termitaria on crop lands is evidence of fertile soil. Farmers preserved termitaria that occur on their farms and they avoid breaking the termite mounds for fear that termites might devastate their crops.

Plants that occur on termitaria are considered to be medicinally more efficient than plants in the savannah because these plants are connected to the spirit of the anscetors. Participants emphasized that termitaria plant species such as *Capparis sepiaria*, *Combretum micrantum* and *Tamarindus indica* are highly used in traditional medicine. To harvest medicinal plants on termitaria, harvesters take specific precautions. The trunk of the tree is first cut at four sides. In Berba communities prior to plant bark harvest, termitaria were first surrounded with fresh ash the night before harvest. Such harvesting takes place at dawn. This belief was the only one peculiar to Berba communities that we noticed during our research.

The perception of local people that termitaria are housing ancestors' spirit and improve the fertility of their lands was the main reasons why termitaria are preserved. This perception matches with Omari (1990) who found that traditional Africans viewed land and its resources as communal property that belonged not only to the living but to their ancestors and to future generations. Furthermore, it certainly contributes to the conservation of termites diversity.

The taxonomy and the feeding group are the major criteria often used by scientists to classify termite (Eggleton and Tayasu, 2001; Donovan et al., 2008). This study shows that farmers have their own criteria to distinguish termitaria. Linking farmer's knowledge to Western classification, we suggest that termites classification based on their feeding materials be completed by the height of mounds those termites built. This aspect could somehow lead to knowing whether species feeding on the same matter has the same building capacities and then investigate the causes of likely difference in mounds architecture. The indigenous people's perception that plants on termite mounds are more efficient may take off the pressure on these species outside savanna. In addition, the need to observe some ceremonial rites before harvesting plant on the termitaria help limit the amount of material harvested from the limited population of plants on termitaria. So since termitaria materials and plant associated are profitable to Pendjari's populations, all perceptions towards them leads to traditional conservation practices. Similarly, Vodouhe et al. (2010) had recently noticed that Pendjari's local populations' perceptions of biodiversity conservation were strongly related to locally perceived benefits.

Termitaria-related plants species used as medicine

A total of 42 woody plant species and 32 herbaceous were recorded on mounds in fields. Woody species were represented by 35 genera and belonged to 23 botanical families whereas the herbaceous were represented by 24 genera and belonged to 14 families. In fallows, there were 33 woody species represented by 29 genera and belonging to 16 families. In this management area type, 17 herbaceous species represented by 14 genera and belonging to 7 families were recorded. The relative abundance of various families in terms of species recorded on mounds in the various area types is presented in Table 1. Populations in the Biosphere Reserve of Pendjari used as medicine from termitaria, overall twenty-two (22) plant species. These species belong to eighteen (18) genera and fourteen (14) families (Table 2). Among these plants, twenty-one (21) woody and one herbaceous species *Euphorbia convolvuloides* were recorded (Table 2). Among the species used as medicine, *Feretia apodanthera* and *Grewia lasiodiscus* were the most frequent recorded on termitaria in fields. Similarly, *F. apodanthera* and *Flueggea virosa* were recorded as most frequent on mounds in fallows. With regards to the abundance, *F. virosa* was more abundant on mounds both in fields and fallows. In addition, *Diospyros mespiliformis* was also more abundant on termitaria in fields.

Regarding the similarity in plant species used as medicine, we notice (Figure 2) that the most important proportion (55%) of recorded species were mentioned only by one ethnic group (that means by G or B or W). In contrast, a relatively low proportion of species (36%) were mentioned by two of the three ethnic groups while only 9% of the recorded plants were commonly mentioned by all the 3 ethnic groups. Results showed that the plant families used for medicinal purpose were dependent on the ethnic group that people belong to (X^2_{obs} = 15.4 and X^2_{th} = 19.4; df = 26). Combretaceae species [with 4 species (18.18%), 2 genera (11.11%) of which Combretum and Anogeissus] were the most recorded as medicinal plants.

The diversity of termitaria plant species used as medicine in the Pendjari Biosphere Reserve denotes the variety of knowledge that Pendjari's populations have from their nature. Besides, the relation between ethnic groups and plant families used as medicine added to the low proportion of medicinal plant species commonly mentioned by the 3 ethnic groups may reflect the diversity of origins of indigenous people and the variety of ethno botanical knowledge that they inherit from their ancestors. In fact, contrary to Berba and Wama communities that originate from Benin, Gourmantché people are

Table 1. Relative abundance of plant families per type of management area.

Family	Number of genus	Percentage	Number of species	Relative species importance	Species abundance	Relative abundance
Anarcadiaceae	3	8.571428571	4	9.523809524	4	2.631578947
Balanitaceae	1	2.857142857	1	2.380952381	1	0.657894737
Bombacaceae	1	2.857142857	1	2.380952381	1	0.657894737
Boraginiaceae	1	2.857142857	1	2.380952381	3	1.973684211
Capparaceae	2	5.714285714	2	4.761904762	11	7.236842105
Celastraceae	1	2.857142857	1	2.380952381	2	1.315789474
Caesalpiniaceae	3	8.571428571	3	7.142857143	9	5.921052632
Combretaceae	2	5.714285714	4	9.523809524	12	7.894736842
Curcubitaceae	1	2.857142857	1	2.380952381	8	5.263157895
Ebenaceae	1	2.857142857	1	2.380952381	16	10.52631579
Euphorbiaceae	2	5.714285714	2	4.761904762	21	13.81578947
Fabaceae	1	2.857142857	1	2.380952381	1	0.657894737
Liliaceae	1	2.857142857	1	2.380952381	1	0.657894737
Loganiaceae	1	2.857142857	1	2.380952381	2	1.315789474
Malvaceae	1	2.857142857	1	2.380952381	1	0.657894737
Meliaceae	1	2.857142857	1	2.380952381	3	1.973684211
Mimosaceae	2	5.714285714	4	9.523809524	12	7.894736842
Moraceae	1	2.857142857	2	4.761904762	2	1.315789474
Rhamnaceae	1	2.857142857	2	4.761904762	7	4.605263158
Rubiaceae	3	8.571428571	3	7.142857143	15	9.868421053
Sterculiaceae	1	2.857142857	1	2.380952381	1	0.657894737
Tiliaceae	1	2.857142857	2	4.761904762	17	11.18421053
Vitaceae	2	5.714285714	2	4.761904762	2	1.315789474
Total	34	97.14285714	42	100	152	100

Figure 2. Illustration of diversity of knowledge related to termitaria plant species in the Pendjari Biosphere Reserve. A high proportion (55%) of plant species was listed as medicine by one of the ethnic groups while a low proportion (9%) of species were commonly mentioned by the 3 ethnic groups.

from Burkina-Faso. Combreatceae, the most abundant species in sudanian bioclimatic zones (Thiombiano et al., 2006) were the most used as medicine. Whether Combretaceae species are commercialized, this activity added to their medicinal uses can negatively affect the conservation of the botanical group (Painter and Durham, 1995). As a result, we suggest that further researches are needed to state the part of termitaria plant species commercialized in the Pendjari Biosphere Reserve.

To predict termitaria ecosystems viability, we also suggest the documentation of the impacts of the species gathering on their sustainability. The high proportion of medicinal species (55%) mentioned by one of the three ethnic groups reflects the variety of knowledge on plants

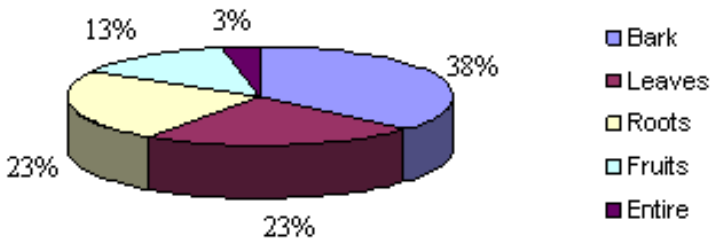

Figure 3. Proportion of diseases treated by plant parts. It reveals that bark is the most used part of plant species found on termitaria in the Biosphere Reserve of Pendjari. Leaves and roots rank respectively second and third in terms of medicinal uses.

among communities. So, the destruction of termitaria will signify not only the loss of cultural knowledge of potential pharmaceutical drugs for the developed world but also the erosion of the sole health care option for many of Pendjari's rural and urban poor. Africa and particularly Benin is a reservoir of knowledge about medicinal plants, however there is within the country no botanical garden installed to conserve medicinal plants. The conservation of termitaria as well as the establishment of gardens for the conservation of medicinal plants for their future and sustainable utilizations is adviced. These gardens and termitaria can also be valued through ecotourism activities that are known to provide employment to people and consequently generate local income.

Diseases and illnesses

A total of thirteen (30) diseases and illnesses are treated using the twenty-two (22) plant species. Dysentery and headaches were the most mentioned diseases and three species were used in their treatment. Against dysentery, people used *A. leiocarpa, D. mespiliformis* and *Flueggea virosa*. The latter as well as *C. febrifuga* and *G. lasiodiscus* were used against headaches. Stomach aches and diarrhoea rank second as most mentioned and respectively two species *A. leiocarpa* and *G. venusta*, *F. virosa* and *V. paradoxa* were used in their treatment. Among the mentioned plants, *C. febrifuga* has been positively tested as antioxidant species in Mali (Maiga et al., 2005). We remark that *F. virosa* is used to fight against a relatively important number of diseases. So the species may have a great importance to the local population. Among the diseases treated using termitaria plant species, we have malaria against which African nations are fighting in order to reach the Millennium Development Goals. Thus, the sustainable management of termitaria and their ecosystems will somehow contribute to reach these goals. The important number of illnesses (30) treated using termitaria plant species proves that medicinal plants are relevant healthcare alternatives in the Biosphere Reserve of Pendjari. Although indigenous people do not have any Western knowledge, they hold a relevant experience related to illnesses and their treatments since decades. So, there is a need to prioritize the ethno botanical knowledge of medicinal plants in Africa and particularly in Benin for ensuring that this knowledge will be available for future generations.

Plant parts used

The leaves, unripe fruits, bark and roots were the parts used in treatment and the entire plant in the case of the herbaceous *Euphorbia convolvuloides* was used to treat scorpion bites. This latter use represents a threat of overcollection for *E. convolvuloides*. Figure 3 shows that in the treatment of the 30 diseases and illnesses, bark was the most used part (38% of total diseases), followed by leaves and roots in equal proportion of diseases and illnesses (23%). While considering the concept of sustainability according to Prescott-Allen and Prescott-Allen (1996), a society is thought as sustainable when the system consisted of both human conditions and the condition of the ecosystem are satisfactory and improving. This system improves only when both of the conditions improve. So, with regards to the use of bark and roots which can in long term be prejudicial to the species conservation, and in order to ensure an availability of the plant species for future generations, we deeply advise to sensitize local populations about the importance to conserve termitaria and rationally use plants in relation with them.

Conclusion

Apart from the known role of termitaria as game ranching where termites are collected for food purposes, our research reveals that the populations in the Biosphere Reserve of Pendjari have great knowledge of the medicinal uses of plant species in relation with termitaria. Plants harvested from termitaria are used to treat diseases and illnesses and the bark is the most used part. As a result, we suggest future studies to be focused

Table 2. List of medicinal plants and diseases treated. It shows the variety of knowledge that Pendjari's people have of plant species found on termitaria.

Scientific name	Local name	Parts used	Disease/illness treated	Usage	Ethnic group using the treatment
Combretum fragrans (Combretaceae)	Tantamanni (G) Tantam (B) Kurutêdé (W)	Leaves	Abscess	New leaves are passed through fire and laid on the abscess	Gourmantché
Combretum glutinosum (Combretaceae)	Tantapienni (G) Kurudé (W) Tantampui (B)	Leaves	Anaemia	Decoction is prepared and given to kids	Gourmantché
Combretum collinum (Combretaceae)	Fampienni (G) Kurupodé (W) Tantam (B)	Roots	Fever	Decoction prepared	Gourmantché
Anogeissus leiocarpa (Combretaceae)	Bussiébu (G) Séika or Koubu (W)	Bark	Dysentery	Decoction prepared and used to clean kids	Gourmantché
	Qwark (B)		Stomachaches	Porridge prepared with decoction and maize flour	Berba
Diospyros mespilliformis (Ebenaceae)	Bugabu (G) Kabu (W)	Fruits	Dysentery	Fruits are pounded and juice is mixed with cow milk for drinking	Gourmantché
Tamarindus indica (Cesalpiniaceae)	Bupurbou (G)	Fruits	cold and cough	Non-ripe seeds with pulp added to water and sugar for drinking	Gourmantché
	Pussika (W)	Leaves	Rheumatism	Decoction is used for bathing	Gourmantché
			wound	Leaves are triturated and laid on mound.	Gourmantché
Piliostigma thonningii (Cesalpiniaceae)	Nambabu (G)	Leaves	Body impurities	Decoction used to clean kids	Gourmantché
			muscle pain	Decoction used	Berba
	Bakambu (W)		snake bite	Decoction prepared for drinking with leaves of Prosopis africana. Annona senegalensis and Securidaca longepedun-culata	Berba
	Lamangue (B)	Roots	Dysentery	Decoction is used to cook porridge with flour of Sorghum bicolor	Berba
Flueggea virosa (Euphorbiaceae)	Ichilimu (G)	Bark	Headaches	Dried and transformed in powder with 3 corns of Aframomum meleguetta. Head scars are done with the powder	Berba
	Buluyédu(B)	Roots	Diarrhoea	Drinking of decoction	Gourmantché

Table 2. Contd.

	Warambu (G)	Fruits	Convulsion	Unripe fruits are burnt and the smoke is used to treat kids	Gourmantché and Wama
Crossopteryx febrifuga (Rubiaceae)	Samitiré (W)	Leaves	Any persistent illness	Bath with triturated leaves	Berba
	Lapekoe (B)	Bark	Headaches	Use of smoke to treat the sick person	Berba
Feretia apodanthera (Rubiaceae)	Kwalabkanga (G) Yablicataca (W)	Roots	Non drink of milk by babies	Not available	Gourmantché
Grewia lasiodiscus (Tiliaceae)	Yuapienni (G) Arguipodé (W)-	Roots	Hard headaches	Roots are burned and transformed into powder used to make scars	Gourmantché and Wama
	Yuamoahoun (G)	Leaves+Bark	Delay in baby walking	Use of decoction to clean kids	Berba
Grewia venusta (Tiliaceae)	Arguitêdé (W) Sarroui (B)	Roots	Stomach aches	Use of decoction to prepare a porridge with Sorghum bicolour flour	Berba
Ziziphus abyssinica (Rhamnaceae)	Congoanugu G)	Fruits	Non straight look	Crushed and powder used to beautify eyelash	Gourmantché
	Santchiku (B)	Roots	Delay in dentition	Drinking of decoction and its use to clean kids	Berba
Lannea acida (Anacardiaceae)	Ngbantchablidjaga (G) Wassawému (W) Ndoougou (B)	Bark	Swollen body parts	Decoction with cloves of Parkia biglobosa. Application to the swollen part.	Gourmantché et Wama
Lannea microcarpa (Anacardiaceae)	Ngbantchabli (G) Tchiendafa (W) Sebeck (B)	Bark	Premature childbirth	Premature children are laid on bark	Gourmantché et Wama
Annona seneglensis (Annonaceae)	Namussakpê-chibu (G) Nouak (B)	Leaves	Excrements with viscous liquid	Leaves are transformed in powder and drunk with porridge.	Gourmantché et Wama
Azadirachta indica (Asteraceae)	Nimu (G) Neem (W) Titusik (B)	Leaves	Malaria	Triturated leaves are mixed with water and the ill person takes bath with	Gourmantché.. Wama et Berba
Vitellaria paradoxa (Vitaceae)	Bussambu (G) Taambu (W) Tanga (B)	Bark	diarrhoea	Bark is collected from 2 opposite side of the tree and decoction is drunk by the ill person.	Berba

Table 2. Contd.

Balanites aegyptica (Balanitaceae)	Bukpakpakabu (G) Kpakpakabu (W) Koomwack (B)	Bark	Hiccups	Not given	Gourmantché
Ficus sycomorus (Moraceae)	Mukankanbu (G) Kanyjakasire (W) Kank (B)	Bark	Bad luck	The tree is surrounded with fresh ash the night before harvest. The harvest takes place at dawn. The bark is pounded with salts and the water is drunk looking for luck.	Berba
Bombax costatum (Bombacaceae)	Bufuobu (G) Fokubu (W) Sankwaoun (B)	Bark	Pain prior to menstrual cycle	Drinking of decoction	Berba
Euphorbia convolvuloides (Euphorbiaceae)		Entire plant	Bite of scorpion	The entire plant is triturated and strike against the part.	Gourmantché

on the commercialization of termitaria plant species. The added to the local medicinal uses of the concerned species will help know whether there are threats or not towards plant species found on termitaria.

Results from this paper can also serve as argument for scientific advisers and any stake-holders in order to increase people's awareness on the importance to use rationally plant species found on termitaria and to conserve termitaria and their ecosystems.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Hypotensive activity, toxicology and histopathology of different extracts of *Berberis vulgaris*

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A number of plants have been used widely in the traditional system of medicine or Tibb-e-Unani (Unani medicine) in the management of many diseases but these mostly, have not been investigated for their described effects. In this study, the hypotensive, toxicology and histological activities were studied in normotensive albino rats at different doses of ethanolic extract of root pulp (BRE) and aqueous extract of root pulp (BRD). The receptor activity was assessed by using the drugs acetylcholine (Ach) and atropine (Atr) on rat heart. Administration of different extracts (BRE and BRD) showed significant reduction in blood pressure comparable to its respective control. While on pre-treatment with Atr (10^{-4} M) the BRE (10 mg/kg) and BRD (20 mg/kg) did not produce any reduction in blood pressure. These behavior matches exactly to that of acetylcholine (1 µg/kg). The results confirmed that oral and intraperitoneal administration of BRE does not indicate any structural and functional disturbance of liver, heart and kidney up to the dose 100 mg/kg. While, 1000 mg/kg appeared as lethal dose (LD) and all mice died at the interval of 24 h. In conclusion, different extract of BRE used in this study caused hypotensive effect by stimulating non-selective muscarinic receptors. The toxicological, hematological and histopathological results further confirm the safety of BRE up to the dose of 100 mg/kg.

Key words: *Berberis vulgaris*, hypotensive, ethanolic extract of root pulp (BRE), aqueous extract of root pulp (BRD).

INTRODUCTION

The commonest cardiovascular infection affecting the adult population is hypertension (high blood pressure). Hypertension and other “vascular diseases” such as stroke and kidney failure cause 43 percent of all deaths recorded each year (Saleem et al., 2003). World Health Organization (WHO) has identified hypertension as the leading cause of cardiovascular mortality and suggests that more than 50% of the hypertensive populations worldwide are unaware of their condition (Chockalingam, 2007). Control of blood pressure in patient with hypertension is necessary for cardiovascular morbidity and mortality (Walker et al., 2002). Drugs used in the treatment of cardiovascular diseases are very expensive and beyond the reach of a common man. The American

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Heart Association estimated the direct and indirect costs of high blood pressure in 2010 as $76.6 billion (Lloyd-Jones et al., 2010), while on the other hand these drugs produce mild to severe side effects; so side effects associated with these drugs restricted the people to use them.

Barberries have reported as hepatoprotective, hypoglycemic and as a herbal remedy for the treatment of a variety of complaints including diabetes, liver dysfunction (Jaundice), gallbadder pain, gall stone diarrhea, indigestion and urinary tract diseases (Hermenean et al., 2012; Meliani et al., 2011; Jellin et al., 2000; Chevallier, 1996; Gruenwald, 1998). Earlier the aqueous and methanolic extract from *Berberis vulgaris* fruit and root was tested to evaluate its antihypertensive effects on DOCA-induced hypertension in the rats (Fatehi et al., 2005b; Azmat et al., 2009). Present studies led to the determination of hypotensive activity and toxicity of different ethanolic extract of *B. vulgaris* root and root bark.

MATERIALS AND METHODS

Plant

The roots of *B. vulgaris* were yellowish brown, cylindrical, more or less knotty, hard and tough. With the bark intact they are cut into pieces of varying length and a maximum diameter of 45 mm. The bark is internally dark brown and soft. The root in powdered form is bright yellow with a slight odour and a bitter taste.

Extraction

Root (500 gm) of *B. vulgaris* was extracted with ethanol for four times at room temperature. The extracts were combined together and evaporated on rotavapour. At the end yellowish brown colored residue (BRE) was collected and used during the whole study. Another extract (decoction) BRD was prepared by boiling the *Berberis* root in distilled water.

Animals

Adult NMRI mice (20 to 25 g) and Sprague dawley rats (200 to 225 g) of either sex were obtained from Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) and were housed in groups of 6 per cage for seven days prior to experimentation with free access to food and tap water *ad libitum* and kept on a 12 h light/dark cycle. Each experimental group consisted of six animals. All animals were housed in an air-conditioned room at 23 ± 1°C during the quarantine period. The experimental procedures were performed according to Guidelines for Care and Use of Laboratory Animals (National research council, 2011). All experimental procedure was approved by review board of departmental research committee.

Chemicals and drugs

Different chemicals and drugs used in the present study were, acetylcholione and sodium chloride from E. Merck (Germany), atropine sulfate from Boehringer Ingelheim (Germany). Acetylcholine (10^{-6} M) used as positive control while saline (0.9% NaCl) as negative controls, while atropine (10^{-4} M) used for receptor activity. Heparin (Leo Pharmaceutical Denmark) and Pentothal sodium from Abbott Karachi (Pakistan) were used as anaesthetic agent and anticoagulant, respectively.

Instruments for the extraction and recording of blood pressure parameters

Rotavapour (R114 Buchi) was used for the extraction of BRE. The arterial blood pressure was recorded by using research grade blood pressure transducer (Harvard, 60-3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (United kingdom).

Hypotensive activity

Normotensive Sprague-Dawley rats of either sex (200 to 250 g) were anaesthetized with Pentothal sodium (40 mg/kg i.p.) as described by Ulicna et al. (2003). Then the trachea was exposed and cannulated with polyethylene cannula to facilitate spontaneous respiration (Ogochukwu et al., 2009). Drugs were injected (vol. 0.2 to 0.25 ml) through a polyethylene cannula inserted into the external jugular vein followed by a saline flush (0.2 ml). The arterial blood pressure was recorded from the carotid artery via arterial cannula connected to a research grade blood pressure transducer (Harvard, 60 to 3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (UK). The temperature of the animals was maintained at 37°C by using the overhead lamp. Animals were allowed to equilibrate for at least 15 min before administration of any drug.

Measurements

Mean arterial blood pressure was calculated as sum of the diastolic blood pressure plus one-third-pulse width (Adeboye et al., 1999). Changes in blood pressure were expressed as the percent of control values, obtained immediately before the administration of test substance (Saleem et al., 2003). Acetylcholine used as positive control caused 57.61 + 2.31% (mean + SEM, n = 15) fall in mean arterial blood pressure as the dose of 10^{-6} M/kg. The hypotensive studies were carried out on different doses of BRE and BRPD.

Methods for the determination of receptor activity

Normotensive Sprague-Dawley rats of either sex (200 to 250 g) were anaesthetized and their blood pressure was recorded through carotid artery as described earlier. Atropine 10^{-4} M/kg were injected through a polyethylene cannula inserted into the external jugular vein followed by a saline flush (0.2 ml), to block the muscarinic receptors.

The arterial blood pressure was continuously monitored from the carotid artery via arterial cannula connected to a research grade blood pressure transducer (Harvard, 60-3003) coupled with four channels Harvard Universal Oscillograph (Curvilinear, 50-9307) (UK). Animals were allowed to equilibrate for at least 5 min than acetylcholine was administered to check the blockade of receptor than BRD and BRE administered one by one, and change in blood pressure was monitored.

Toxicological/safety evaluation studies in mice

Five groups of NMR-I mice (25 to 30 g) containing twelve animals in each group (six male, six female) were used in this study. All animals were treated orally once daily for 14 consecutive days.

1. Group I was treated with saline served as control.
2. Group II was treated with BRE (100 mg/kg), administered orally (p.o.).
3. Group II was treated with BRE (1000 mg/kg) administered orally (p.o.)
4. Group IV was treated with BRE (100 mg/kg), administered intraperitoneally (i.p.).
5. Group V was treated with BRE (1000 mg/kg) administered intraperitoneally (i.p.).

Animals were weighed daily before the administration of dose. All the animals were kept under observation for nearly two hours after the administration of dose, for any change in behavior or physical activities. Numbers of expired animals were noted at the end of study period. At the end of 7th day all survived mice were anaesthetized with pentothal sodium (40 mg/kg) and autopsied.

Toxicological studies in rats

Two groups of Sprague dawley rats (225 to 250 g) containing twelve animals in each group (six male, six female) were used in this study. All animals were treated intraperitoneally once daily for fourteen consecutive days.

1. Group I was treated with BRE (100 mg/kg).
2. Group II was treated with distilled water.

Autopsy

At the end of 14th day all survived mice and rats were anaesthetized with pentothal sodium 40 mg/ml i.p (Ulicna et al., 2003) and autopsied.

Estimation of different biochemical parameters

At the end of 14th day all survived rats were anaesthetized with pentothal sodium 40 mg/ml i.p and the blood samples approximately (4 to 8 ml) were withdrawn from cardiac puncture before dissecting the animals with sterile disposable syringe from all treated and control rats, were left at room temperature for 20 min, then incubated at 37°C for 30 min and centrifuged separately in (BHG) Hermle Z230 (Germany) at the speed of 3,000 rpm for 20 min. Supernatants (Serum) were separated out and the residue was discarded. Serum obtained (1 to 3 ml) was subjected for the study of the following parameters: Bilirubin, SGPT, gamma glutammyl transferase (γGT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), CK, aspartate amino transferase (ASAT, SGOT), total protein, albumin, urea uric acid, blood urea nitrogen (BUN), creatinine, high density lipoprotein (HDL), cholesterol, triglycerides (TG), glucose. All tests were performed by using commercial assay kits. Kits used were: Mercko test® for bilirubin, Ecoline® 25 for SGPT. Ecoline® S+ by Szasz method for γGT, Ecoline® 25 for Alkaline Phosphatase, Ecoline® 125 for LDH, Ecoline® 125 for ASAT (GOT), Ecoline® 125 for CK-NAC, Ecoline® S+ by biuret method for Total Protein, Ecoline® 100 by UV test, GIDH method for Urea, Ecoline® 100 by UV test, GIDH method for BUN, Ecoline® S+ 100 by bromocresol green method for Albumin, Ecoline® 100 for Uric Acid (TBHBA), Ecoline® S+ by Jaffé method for Creatinine, Ecoline® 125 for Triglycerides GPO, diagnostica Merck by CHOD-PAP method for HDL-Cholesterol, Ecoline® 125 for Cholesterol, Ecoline® 1000 by GOD-PAP method for glucose. All these kits were purchased from diagnostica Merck (Germany). U-2000 spectrophotometer (Hitachi) was used to measure the absorbance of light.

Histology

Heart, liver, spleen and kidneys were fixed in 10% formalin. After usual processes of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 7 µm slices through Leica RM 2145-rotation microtom. The tissues were stained with hematoxylin and eosin. The slides were studied and photographed through nikon advance trincocular research microscope OPTIPHOT model X2T-21E equipped with Nikon Microphotography system; model UFX-DX-35 and phase contrast N plan.

Statistical analysis

Changes in blood pressure and serum biochemical levels were compared using student's t-test. Values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to be significant.

RESULTS

Effect of various doses of BRE on various blood pressure parameters

The effect of intravenous administration of various doses of ethanolic extract of root (BRE) on various blood pressure parameters has been presented in Table 1.

Effect of 10 mg/kg BRE on various blood pressure parameters

BRE at the dose of 10 mg/kg was found to reduce the systolic, diastolic and mean arterial blood pressure that was 44, 48 and 47% in comparison with their controls, respectively as shown in Figure 1. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$).

Effect of 20 mg/kg BRE on various blood pressure parameters

Intravenous administration of 20 mg/kg dose of BRE showed significant ($p < 0.005$) hypotensive effect. It was found to decrease the systolic, diastolic and mean arterial blood pressure by 43, 47 and 46%, respectively as shown in Figure 1. These reductions in various blood pressure parameters were statistically significant ($p <$

Table 1. Effect of ethanolic extract of root (BRE) obtained at different doses on various BP parameters.

Parameter	10 mg/kg		20 mg/kg	
	Before administration	After administration	Before administration	After administration
Systolic BP	133±4.41(6)	73.67±4.55*(6)	126.5±2.12(6)	71.50±3.54*(6)
Diastolic BP	129.67±1.08(6)	66.33±6.53*(6)	123±2.01(6)	65±5.66*(6)
MABP	130.7±8.53(6)	68.78±5.86*(6)	124.14±0.71(6)	67.17±4.95*(6)

The values have been presented as mean±S.E.M (n). *Represents significant difference after the administration of extracts.

Table 2. Effect of decoction of root (BRD) obtained at different doses on various BP parameters.

Parameter	10 mg/kg		20 mg/kg	
	Before administration	After administration	Before administration	After administration
Systolic BP	139.13±5.9 (10)	107.63±10.16* (10)	130.5±5.86 (10)	71.70±4.88* (10)
Diastolic BP	137.5±5.74 (10)	105.63±10.16* (10)	128.4±5.89 (10)	69.60±4.9* (10)
MABP	138.04±5.79 (10)	106.29±10.06* (10)	129.10±5.88 (10)	70.30±4.89* (10)

The values have been presented as mean±SEM (n). *Represents significant difference after the administration of extracts.

0.005) when compared to its control as shown in (Table 1).

Effect of various doses of BRD on various blood pressure parameters

The effect of intravenous administration of various doses of decoction of root (BRD) on various blood pressure parameters has been presented in Table 2.

Effect of 10 mg/kg BRD on various blood pressure parameters

Intravenous administration of BRD at the dose of 10 mg/kg was found to reduce the systolic pressure, diastolic and mean arterial blood pressure that was 23% in comparison with their controls as shown in Figure 2. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$). At the dose of 10 mg/kg hypotensive effect remained for 91 ± 14.29 s.

Effect of 20 mg/kg BRD on various blood pressure parameters

BRD at the dose of 20 mg/kg was found to reduce the systolic pressure, diastolic and mean arterial blood pressure that was 45% in comparison with their controls as shown in Figure 2. Decreases in various blood pressure parameters were statistically significant ($p < 0.005$) which remained effective for 381 ± 203 s.

Result of receptor activity

The receptor activity was determined in rats results demonstrated that BRE (10 mg/kg) and BRD (20 mg/kg) has been found to decrease the mean arterial blood pressure that was 47 and 45%, respectively when compared with their respective controls (Figures 1 and 2). While, on pre-treatment with Atr (10^{-4} M) the BRE (10 mg/kg) and BRD (20 mg/kg) did not produce any reduction in blood pressure. These behavior matches exactly with that of acetylcholine (1 µg/kg). The use of Ach (10^{-6} M) has resulted in tremendous fall in MABP {124.63 ± 4.93(6) to 67.77 ± 4.11(6)} that was 46% than its respective control. Further, on pre-treatment with Atr (10^{-3} M) the Ach (10^{-3} M) did not show decline in MABP as shown earlier without Atr (10^{-3} M) pre-treatment.

Toxicological studies in mice and rats

Toxicological studies of BRE were carried out in mice. Oral administration of BRE (100 mg/kg) did not show any change in physical behavior of mice while oral administration of higher dose of BRE (1000 mg/kg/d) caused decreased motor activity, corner sitting, hind limb abduction and palpaberal ptosis in early two hours after dosing (Table 3). None of the groups showed any significant change.

Intraperitoneal administration of BRE (100 mg/kg) i.p. showed a lot of symptom like Abdominal cramps, ataxia, decreased motor activity, corner sitting, hind limb abduction and localized paralysis, while 1,000 mg/kg appeared as lethal dose (LD) and all mice died at the

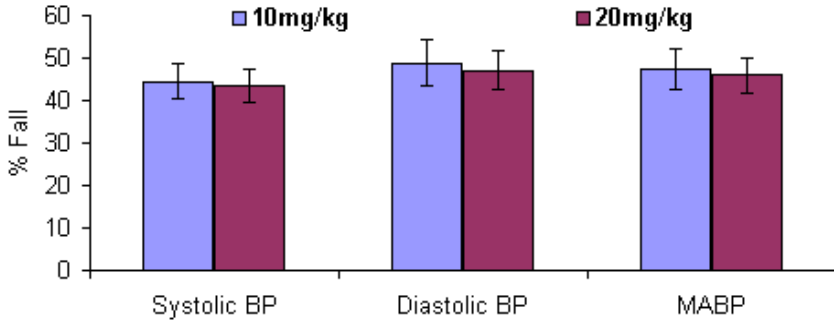

Figure 1. Effect of different doses of BRE on various BP parameters.

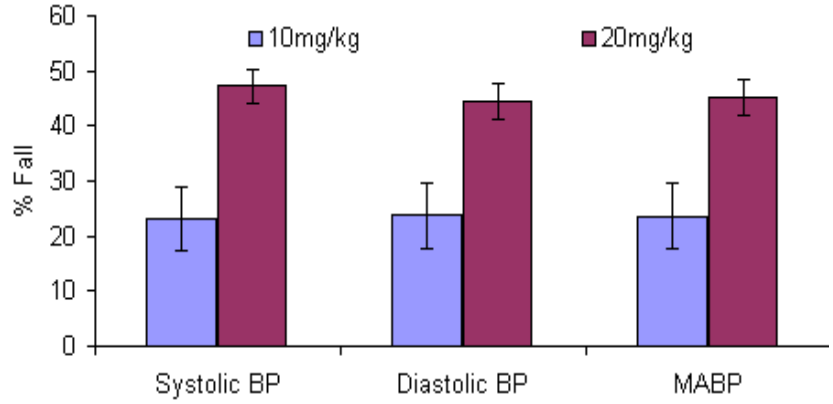

Figure 2. Effect of BRD on various BP parameters.

interval of 24 h (Table 3). Control group did not show any mortality or significant change in their general behavior or physical activities (Table 3). Male and female rats treated with BRE (100 mg/kg-body weight) did not show any mortality as shown in Table 3. None of these animals showed any sign of toxicity but some physical behavioral changes were observed in first 2 h after dosing like decreased motor activity and corner sitting (non quantified observation) as shown in Table 3 and the animal returned to normal within 2 h.

Autopsy

Autopsy revealed after the administration of BRE (100 mg/kg), no gross changes were observed in organs like liver, spleen, heart and kidney. Drug did not cause any internal body hemorrhage.

Effect of BRE on different biochemical parameters

The effects of BRE observed on different biochemical parameters of rat. Oral administration of 100 mg/kg dose of BRE for 14 consecutive days was not found to alter the liver enzyme significantly. It reduces the serum bilirubin, γGT and ALP with respect to its control, although this reduction was statistically non significant ($p > 0.05$). BRE (100 mg/kg) produced slight but non-significant increase in the serum SGPT but this increase was also statistically non significant ($p > 0.05$) as shown in Table 4. Heart profile showed that It reduced the serum CK, LDH and SGOT in comparison to control rats, although these reductions were statistically non significant ($p > 0.05$) as shown in Table 4. It prevented the elevation of lipid profile significantly. It reduces the serum cholesterol and TG with respect to its control, although this reduction was statistically non significant ($p > 0.05$), while an increase was observed in serum HDL level after the administration of BRPM (100 mg/kg) for 14 consecutive days as shown in Table 4. A non-significant decrease in the serum total protein, albumin, urea, uric acid, creatinine and BUN was observed in the BRE (100 mg/kg) treated rats in comparison to control rats. Although these reductions were statistically non significant ($p > 0.05$). A non-significant decrease was also observed in blood glucose level.

Table 3. Toxicological study of different extracts of *Berberis vulgaris* in mice and rats.

S/no	Extract	Animal	Dose	ROA	No of animals	Days	Mortality	Symptoms
	Saline		0.5 ml	I.P	6 Male 6 Female	14	Nil	Nil
			100 mg/kg	Oral	6 Male 6 Female	14	Nil	Nil
1	BRE	Mice	1000 mg/kg	Oral	6 Male 6 Female	14	Nil	Decreased motor activity, corner sitting, hind limb abduction
			100 mg/kg	I.P	6 Male 6 Female	14	Nil	Abdominal cramps, ataxia, decreased motor activity, corner sitting, hind limb abduction and localized paralysis
	Saline		1000 mg/kg	I.P	6 Male 6 Female	14	12	Convulsion and then death
2		Rats	0.5 ml	Oral	6 Male 6 Female	14	Nil	Nil
	BRE		100 mg/kg	Oral	6 Male 6 Female	14	Nil	decreased motor activity and corner sitting

ROA = Route of administration.

Histopathology

A histopathological study was performed on the organs of surviving rats, which were sacrificed at the end of experiment. Histology of hearts, kidneys, liver and spleen of most of the treated animals examined seems unaffected.

DISCUSSION

In the present study, different ethanolic and aqueous extracts of *B. vulgaris* has been tested for its hypotensive effects in rats. The acute intravenous administration of different extract of *B. vulgaris* (BRE, BRD) as well as Ach used as positive reference drug (Lahlou et al., 2002) in this study, all exerted immediate and significant fall in systolic, diastolic and mean arterial blood pressure (MABP) in normotensive anaesthetized rats. Earlier, the methanolic extract of *B. vulgaris* was reported to be hypotensive by Azmat et al. (2009). It is hypothesized that these extracts might be acting like Ach and keeping these assumptions, the receptor activity of extracts has also been tested in the present study by using cholinergic agonist Ach and cholinergic competitive antagonist Atr. The results demonstrate that Ach and different extracts of *B. vulgaris*, when administered alone have reduced the blood pressure. On the other hand, the use of Ach and different extracts of *B. vulgaris*, on Atr pre-treated animal did not show such decline in blood pressure as shown earlier without atropine pre-treatment. These results clearly indicate that effect of *B. vulgaris* is mediated through same receptor and mechanism as established for Ach. Ach and muscarinic receptor agonists can cause vasodilation of most blood vessels, resulting in a decrease in total peripheral resistance (Harvey, 2012). It may therefore be concluded that muscarinic responses may contribute to the cholinergic hypotensive effect of these extracts.

The present study suggests that different extracts of *B. vulgaris* possess significant hypotensive activity. Keeping this view in mind, the toxicological studied were carried out. *B. vulgaris* root extract (BRE) was found to be safe because the results obtained from toxicological studies suggested that after oral administration of BRE at the dose of 100 and 1000 mg/kg and intraperitoneal administration at the dose of 100 mg/kg, no mortality was observed. But intraperitoneal administration at the dose of 1000 mg/kg/day was found as lethal dose (LD_{100}), killing all mice. It is reported that barberry is generally considered safe when consumed orally and appropriately for medicinal purposes, but due to its moderately toxic properties cannot be recommended for consumption in quantities over

Table 4. Effect of BRPM (100 mg/kg) on different biochemical parameters.

Parameter	Control	BRPM treated	P value
Bilirubin	1.36±0.1	1.16±0.19	P>0.05
Alanine aminotransferase (ALAT:SGPT)	14.77±5.98	15.55±1.99	P>0.05
Gamma glutamyl transferase (γGT)	4.24±0.85	3.72±0.67	P>0.05
Alkaline phosphatase (AP)	76.4±16.63	63.26±7.01	P>0.05
Lactate dehydrogenase (LDH)	196.99±16.38	190.23±24.8	P>0.05
Creatine kinase (CK)	32.86±6.41	21±5.56	P>0.05
Aspartate amino transferase (ASAT:SGOT)	77.52±4.75	63.02±6.76	P>0.05
Total protein (TP)	6.4±0.211	6.26±0.13	P>0.05
Albumin	3.595±0.09	3.42±0.1	P>0.05
Urea	30.97±7.32	30.68±5.46	P>0.05
Uric acid	3.66±0.152	3.49±0.24	P>0.05
Blood urea nitrogen (BUN)	18.918±2.88	17.45±2.93	P>0.05
Creatinine	1.02±0.03	0.875±0.07	P>0.05
Cholesterol	87.91±2.76	82.167±3.19	P>0.05
High density lipoproteins (HDL)	46.67±1.68	54.76±2.17	P>0.05
Triglycerides (TG)	119.7±2.32	109.33±2.03	P>0.05
Glucose	113.27±4.16	101.7±4.23	P>0.05

All values are presented as mean+SEM (n=12).

500 mg (Jellin et al., 2000).

Biochemical studies showed that there were non-significant effects on liver, kidney heart and diabetic profile in BRE (100 mg/kg) treated rats. The non-significant data collected after 14 days were interpreted as biological variability normally observed in rats. Different enzyme tested for liver function, cardiovascular functions and kidney function because these levels rise faster in cholestastatic, obstructive disease, hepato-cellular damage, atherosclerosis, muscular dystrophy and acute myocardial infarction. Results suggest that after the administration of BRE showed non-significant changes in serum level of bilirubin, γGT, alkaline phosphatase, SGPT, CK, SGOT, LDH, TG, HDL, total protein, albumin, urea, uric acid BUN, creatinine level. The histopathological study confirmed that BRE at the dose of 100 mg/kg did not produce any change in liver, heart, kidney and spleen.

The results confirmed that oral and intraperitoneal administration of BRE does not indicate any structural and functional disturbance of liver, heart and kidney up to the dose 100 mg/kg.

Conclusion

From the discussion, it is concluded that BRE is a physiologically and pharmacologically active drug because systolic, diastolic and mean arterial blood pressure is influenced by BRE used in the present study. Different extract of BRE used in this study caused hypotensive effect by stimulating muscarinic receptors. The toxicological, hematological and histopathological results further confirm the safety of BRE at the dose of 100 mg/kg.

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ABBREVIATIONS

BRE, Ethanolic extract of root pulp; **BRD**, aqueous extract of root pulp; **DrHMIIPS**, Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences; **p.o**., oral administration; **i.p**., intraparetoneally.

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Antimicrobial activity of the leaf extract and fractions of *Lupinus arboreus*

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The antimicrobial effect of the leaf extract and fractions of *Lupinus arboreus* was investigated. The crude methanol extract (CME) of the dried leaves obtained by 48 h cold maceration was fractionated into n-hexane fraction (HEF), ethyl acetate fraction (EAF), and methanol fraction (MEF); and evaluated using modified agar-well diffusion method. The results showed that the extract and fractions at varying concentrations, exerted strong antimicrobial activity on some of the test organisms. However, a weak activity was observed on the tested fungi-*Aspergillus niger* and *Candida albicans*. Ethyl acetate fraction showed the highest activity on many organisms than extract and other fractions.

Key words: *Lupinus arboreus*, antimicrobial activity, standard drugs, test organisms, extracts and fractions.

INTRODUCTION

The search for novel antimicrobial agents from medicinal plants to combat pathogens has become crucial for avoiding the emergence of untreatable bacterial infections (Bandow et al., 2003; Pfaller et al., 1998). Micro organisms have unfavourable effects on the quality and safety of life. Synthetic chemicals are widely used against these microorganisms. Unfortunately, they develop resistance to many antimicrobial agents. The reason for this high resistance to commonly used antimicrobial agents may not be unconnected with worldwide and indiscriminate use in the environment (Anyim et al., 2010; Mukherjee et al., 2002). In addition, these antimicrobials sometimes cause allergic reaction and immunity suppression. The use of essential oils and plant extracts, therefore, is less damaging in the human health and environment (Isman, 2000; Misra and pavlovstathis, 1997). Plants have provided an arsenal of chemicals to survive attack by a microbial invasion (Martini et al., 2004). Literatures showed that natural products and their derivatives represent more than 50% of the drugs in chemical use with one quarter originating from higher plants (Cragg et al., 1997). *Lupinus arboreus* is easily recognized as a bushy shrub to six feet (1.8 m) tall, with bright yellow sweet-smelling flowers blended with purple and white colours (Pickart and Miller, 1998). Also known as yellow bush, *L. arboreus* occurs as an invasive species in Northern California coastal dunes (Wear, 1998). But in Nigeria, it is planted widely as ornamental plant (Ohadoma et al., 2011). It is highly nutritive and wholesome hence grown for fodder and come close to soybean in protein content (Rachel, 2006). In our previous paper (Ohadoma et al., 2010), i.p LD_{50} of 77.45mg/kg of the methanol leaf extract of *L. arboreus* was reported. This study screened the antimicrobial

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activity of *L. arboreus.*

MATERIALS AND METHODS

Collection and identification of plant

Leaves of *L. arboreus* were collected from Owerri, Imo State, Nigeria and official identification was done by Pharm F. N. Osuala, Head of Pharmacognosy Department, Madonna University where a voucher specimen has been deposited in the herbarium. The leaves were air-dried at room temperature for 28 days and pulverized into fine powder. The powdered leaves (2 kg) was extracted with absolute methanol (Sigma Aldrich, Germany) by cold maceration for 48 h. The mixture was filtered to obtain the methanol extract, which was evaporated using a rotary evaporator (RV 05 Basic IB, IKA Staufen, Germany) and the concentrated methanol extract stored in a refrigerator. Using silica gel column chromatography, dried methanol extract (10 g) was partitioned to yield hexane fraction (HEF), ethyl acetate fraction (EAF) and methanol fraction (MEF). Phytochemical screening of the extract and fractions were carried out (Harbone, 1988).

Test organisms

Pure clinical isolates of *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella paratyphi, Escherichia coli, Staphylococcus aureus, Candida albicans,* and *Aspergillus niger* were obtained from Medical Laboratory Unit of Madonna University Teaching Hospital, Elele, Nigeria.

Determination of sensitivity test and inhibitory zone diameter (IZD)

The modified agar-well diffusion technique was employed (Perez et al, 1990). Each of the test organisms was streaked on the surface of the different sterile sensitivity agar media. Wells were bored on the agar media using sterile cork borer of 6 nm diameter. Exactly 2 drops of the extract prepared as described earlier were accordingly put into the wells and then allowed to stand for 30 min for proper diffusion. Standard drugs (ampicillin 80 µg/ml, tetracycline 40 µg/ml, gentamicin 40 µg/ml, ciprofloxacin 40 µg/ml) were served as control. The plates were then incubated aerobically at 37°C for 24 h.

Determination of minimum inhibitory concentration (MIC)

MIC was determined using the micro broth dilution technique (Irobi et al., 1993). The extract and fractions were incorporated at varying concentrations into nutrient broth respectively containing the test organisms in the test tubes. The control experiment containing the growth medium and each of the test organisms, excluding the extract and fractions were also set. The experiments were incubated at 37°C for 24 h. The lowest concentration of extract and fraction that did not allow microbial growth within the incubation period was taken to be the MIC.

RESULTS

The phytochemical studies showed that methanol extract had the abundance of saponins, glycosides, steroids, terpenes and flavonoids. Resins, protein and reducing sugar occurred in moderate amounts, while alkaloids appeared but in trace amount. Hexane fraction (HEF) contained steroids and terpenes, ethyl acetate fraction (EAF) contained flavonoids and glycosides, while methanol fraction (MEF) contained tannins, saponins and glycosides (Table 1).

Extract and fractions have activity against the test bacteria except *E. coli* and little or no activity on fungi (Table 2). The minimum inhibitory concentration of the extract and fractions on the four organisms that showed sensitivity and IZD are as shown in the Tables 2 to 5.

The results of agar diffusion bioassay of the diluted standard drugs (ampicillin, tetracycline, gentamicin and ciprofloxacin) for MIC determination against the susceptive microorganisms are as shown in the Tables 6, 7, 8, 9 and 10.

DISCUSSION

The solvent extraction of the leaves of *L. arboreus* yielded the crude methanolic extract, while the solvent guided extraction yielded the n-hexane, ethyl acetate and methanolic fractions.

The study revealed the antimicrobial efficacy of *L. arboreus* crude methanolic leaf extract, n-hexane fraction, ethyl acetate fraction and methanolic fraction against clinical isolates of Gram-negative and Gram-positive bacteria responsible for majority of the multidrug resistant infections in Nigeria (Kesah et al., 2003) and *Salmonella* (Akinyemi et al., 2000), urinary tract and asymptomatic genital infections, otitis media and wound infections by *P. aeruginosa* (Oyeka et al., 1995) and *S. aureus* (Akerele et al., 2002), upper respiratory tract infections, periodontal disease and osteomyelitis in children by *Streptococcus* species and *Bacillus* species (Onuba, 1992).

L. arboreus showed appreciable activity against these bacteria using method of agar-well diffusion; *B. subtilis, S. aureus, P. aeruginosa* and *S. paratyphi*, but had weak activity on the test fungi-*A. niger* and *C. albicans*, hence it is broad-spectrum antimicrobial.

Extrapolations from the graph of IZD^2 $(mm)^2$ against log concentration of extract, fractions and standard antimicrobials gave their MIC values. From the result of minimum inhibitory concentration (MIC), it was observed that the greater the IZD produced, the lower the MIC and the more potent the agent. However, the fractions, extract and standard antimicrobials had varying MICs on individual organism. Although, it showed no activity on other organism used in the study, the n-hexane fraction when compared with the other fraction showed the highest activity only on *B. subtilis* (MIC 1.07 mg/ml), followed by ethyl acetate (MIC 1.25 mg/ml), methanolic fraction (MIC 3.98 mg/ml) and crude methanolic extract (MIC 5.62 mg/ml). When the activity of these fractions and extract on *B. subtilis* was compared with those of standard drugs, it was observed that gentamicin (MIC 0.02 µg/ml) showed the highest activity. This was

Table 1. Phytochemical constituents of leaf extract and fractions.

Phytochemical constituent	Extract (12.5 %w/w)	HEF	EAF	MEF
Saponins	+++			+
Glycosides	+++	+++	+++	+
Flavonoids	+++		+++	
Steroids	+++	+++		
Terpenes	+++			
Tannins	++			+
Resins	++			
Protein	++			
Reducing sugar	++			
Alkaloids	+			

Value in parenthesis is the extractive yield. +++ =Abundantly present, ++=moderately present, +=present in trace amount.

Table 2. Sensitivity test and IZD of Isolates.

Parameter	*B. subtilis* IZD	*P. aeruginosa* IZD (Mm)	*S. paratyphi* IZD (Mm)	*E. coli* IZD (mm)	*S. aureus* IZD (mm)	*C. albicans* (mm)	*A. niger* IZD (mm)
n-hexane fraction	14.5	-	-	-	-	-	-
Ethyl acetate fraction	14	11	15	-	15	-	-
Methanol fraction	13	13	16	-	14.5	-	-
Crude methanol extract	16.5	12.5	21	-	11.5	-	-

Where (-) means no inhibition.

Table 3. Result of IZD (mm) and IZD^2 $(mm)^2$ of n-hexane fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0961	-	1.7958	-
B. subtilis	5.0	25.0	4.5	20.2	4.0	16.0	3.5	12.2	3.0	9.00
S. aureus	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-
S. paratyphi	-	-	-	-	-	-	-	-	-	-

Table 4. Result of IZD (mm) and $IZ^2$$(mm)^2$ of ethyl acetate fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0961	-	1.7958	-
B. subtilis	9.0	81	6.0	36.0	4.5	20.2	4.0	16	3.5	12.2
S. aureus	8.0	64	5.0	25.0	4.0	16.0	-	-	-	-
P. aeruginosa	4.5	20.1	4.0	16.0	3.0	9.0	-	-	-	-
S. paratyphi	4.5	20.2	3.5	12.2	3.0	9.0	-	-	-	-

followed by Ciprofloxacin (0.02 µg/ml), then tetracycline (MIC1.33 µg/ml) and finally ampicillin (MIC 5.62 µg/ml) upon the high stock concentration. Ethyl acetate fraction showed the highest activity on the other organisms: *S. aureus* (MIC 6.3 mg/ml), *P. aeruginosa* and *S. paratyphi* (MIC 9.4 mg/ml for both). The methanolic fraction showed least activity on *S. paratyphi* (MIC 21.13 mg/ml), but showed the highest activity on both *B. subtilis* (MIC 8.81

Table 5. Result of IZD (mm) and IZD^2 $(mm)^2$ of methanol fraction.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.00	-	1.6980	-	1.3979	-	1.0989	-	0.7958	-
B. subtilis	4.0	16.0	3.5	12.2	3.0	9.0	3.0	9.0	-	-
S. aureus	6.0	36.0	5.0	25.0	4.0	16.0	-	-	-	-
P. aeruginosa	6.0	36.0	3.0	9.0	-	-	-	-	-	-
S. paratyphi	4.0	16.0	3.0	9.0	-	-	-	-	-	-

Table 6. Result of IZD (mm) and IZD^2 $(mm)^2$ of crude methanol extract.

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (mg/ml)	100	-	50	-	25	-	12.5	-	6.25	-
Log concentration	2.0	-	1.6980	-	1.3979	-	1.0989	-	0.7958	-
B. subtilis	10	100	7.5	56.2	5.0	25.0	3.5	12.2	2.0	4.0
S. aureus	12	144	8.0	64.0	6.0	36.0	4.4	19.3	2.0	4.0
P. aeruginosa	16	256	11.5	132.2	9.0	81.0	6.0	36.0	3.0	9.0
S. paratyphi	14	196	9.0	81.0	8.0	64.0	5.5	30.2	2.5	6.2

Table 7. Result of IZD (mm) and IZD^2 $(mm)^2$ of ampicillin (concentration of stock = 80 μg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (μg/ml)	80.00	-	40.00	-	20.00	-	10.00	-	5.00	-
Log concentration	1.9030	-	1.06020	-	1.3010	-	10.0	-	0.6989	-
B. subtilis	9	81	6	36	5	25	3	9	-	-
S. aureus	20	400	18	324	16	256	15	225	12	144
P. aeruginosa	8	64	6	36	4	16	-	-	-	-
S. paratyphi	9	81	6	36	-	-	-	-	-	-

Table 8. Result of IZD(mm) and IZD^2(mm) of tetracycline (concentration of stock = 40 μg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZ^2
Conc. (μg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	11.00	121.00	10	100	9	81	6	36	5	2
S. aureus	-	-	-	-	-	-	-	-	-	-
P. aeruginosa	16.00	256.00	11	121	10	100	7	49	-	-
S. paratyphi	17.00	289.00	16	256	11	121	10	100	6	36

Table 9. Result of IZD (mm) and IZD^2 $(mm)^2$ of gentamicin (concentration of stock = 40 μg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (μg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	26	676	22	484	21	441	20	400	18	334
S. aureus	26	676	25	625	24	576	21	441	18	334
P. aeruginosa	22	484	16	256	14	196	10	100	6.0	36
S. paratyphi	22	400	18	334	16	256	14	196	12.0	144

Table 10. Result of IZD (mm) and IZD^2 $(mm)^2$ of ciprofloxacin (concentration of stock = 40 μg/ml).

Parameter	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2	IZD	IZD^2
Conc. (μg/ml)	40	-	20	-	10	-	5	-	2.5	-
Log concentration	1.6020	-	1.3010	-	1.00	-	0.6989	-	0.379	-
B. subtilis	22	484	19	381	18	334	16	256	11	121
S. aureus	28	784	26	675	21	441	16	256	15	225
P. aeruginosa	25	625	16	256	9	81	7	49	-	-
S. paratyphi	25	625	21	441	18	334	17	289	16	256

mg/ml) when compared with crude methanolic extract on *B. subtilis* (1.07 mg/ml) and *S. aureus* (MIC 14.12 mg/ml). It also had least activity on *P. aeruginosa* (MIC 39.8 mg/ml) when compared with MIC (22.38 mg/ml) of crude methanolic extract. The ethyl acetate fraction had equal activity on *P. aeruginosa* and *S. paratyphi*. Therefore, the relative low MIC of ethyl acetate fraction on bacteria stems from the fact that it extracts the saponins content of plant leaves which is claimed to possess antibacterial property (Trease and Evans, 2004).

The standard antimicrobials showed very good activity against all the tested organisms with the exception of tetracycline that showed no activity on *S. aureus*. Meanwhile, it suffixes to say that *L. arboreus* leaf extract and fraction (except n-hexane fraction) had broader spectrum of activity than tetracycline on the tested organisms. The greater activity or potency observed with the use of higher dilution (lowest concentration) of the standard antibiotics when compared with the crude extract and fractions is due to their high purity level thus devoid of impurities or contaminant that may antagonized its activities unlike the plant sample extract and fractions.

The Gram-positive bacteria were more susceptible to plant extract, fractions and standard drugs than the Gram-negative bacteria (even the resistance by *E. coli*). This finding agreed with the susceptibility of the microbes to different plant extracts reported by the researchers (Elastal et al., 2003). This could be explained by the fact that the cell wall of Gram-positive bacteria is less complex and lack the natural sieve effect against large molecules (Hawkey, 1998; Geuld and Booker, 2000).

The individual fractions of sample and crude extract showed no activity on *E. coli* and weak activity against the tested fungi (*C. albicans* and *A. niger*). The high content of saponin and tannin (Ohadoma et al., 2010) could be the basis of its antimicrobial action which is in accordance with the claim that plants rich in saponins and tannins have antimicrobial property (Trease and Evans, 2004). Flavonoids which are present could be very useful antioxidant suggesting the plant importance in the prevention and treatment of tumour (Leslie, 1996).

Conclusion

L. arboreus leaf extracts and fractions have exhibited broad spectrum of activity against certain bacteria. In view of this, more study is needed in the areas of isolation, purification and identification of specific constituent with the antimicrobial property as this will help curb the menace of bacterial resistance in chemotherapy and to enhance the exploration of medicinal properties of ethnobotanicals.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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