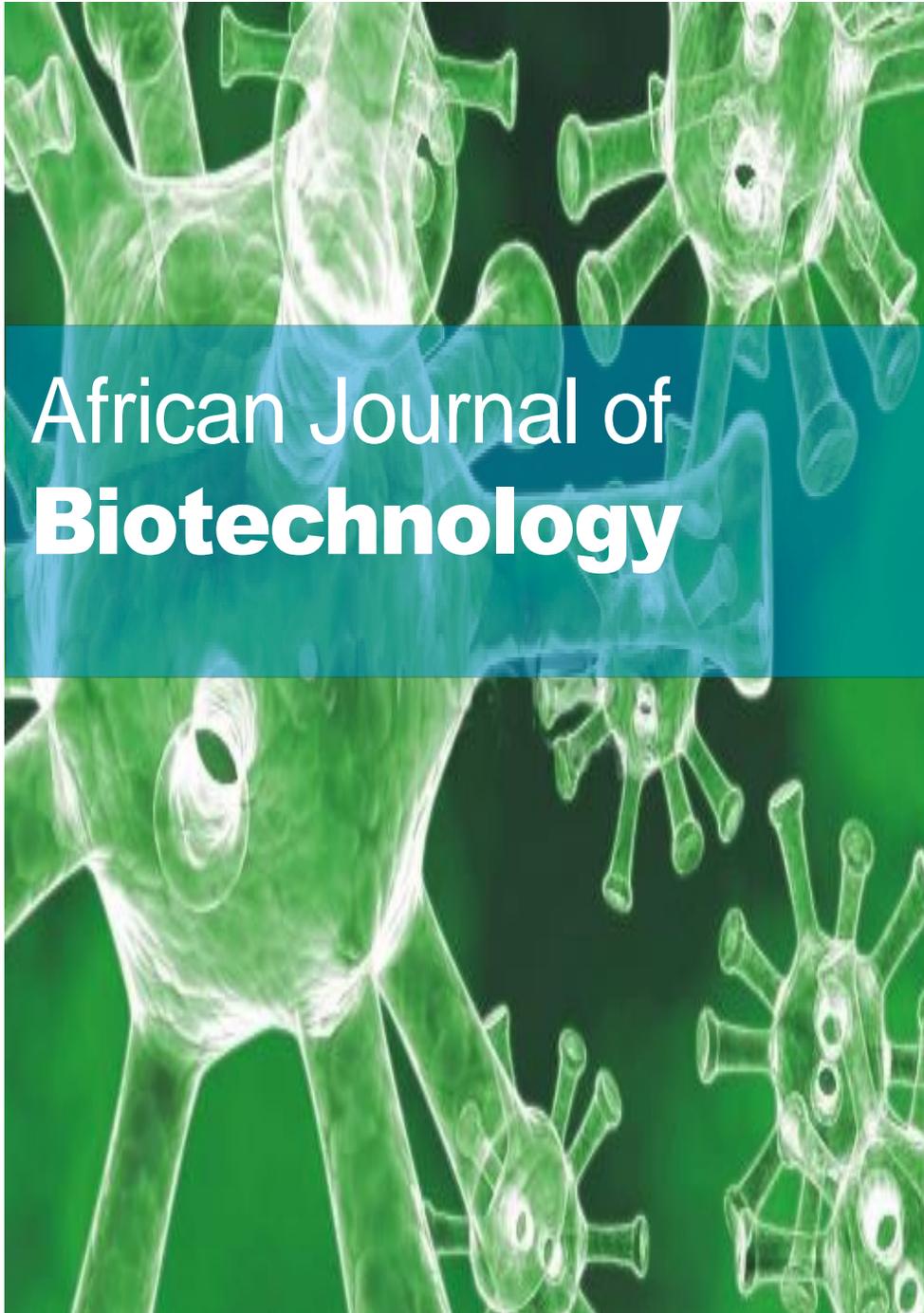


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Full Length Research Paper

Antibacterial saponins from the leaves of *Polyscias fulva* (Araliaceae)

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Saponins are a major family of secondary metabolites that occur in a wide range of plant species. Bioassay - guided fractionation of extract of the leaves of *Polyscias fulva* led to the isolation of three known saponins named, 3-O- α -L-arabinopyranosyl-hederagenin (1), 3-O-[α -L-rhamnopyranosyl(1-2)- α -L-arabinopyranosyl]-hederagenin (2) and 3-O-[rhamnopyranosyl-(1 \rightarrow 2)-xylopyranosyl]-Olean-12-en-28-O-[rhamnopyranosyl-(1 \rightarrow 4)-glucopyranosyl-(1 \rightarrow 6-glucopyranosyl)] ester (3). Leaves of the plant were collected from Kakamega rain forest in Kenya, dried under shade and ground into fine powder and extraction was done using methanol. The methanol extract was subjected to column chromatography and the fractions purified using preparative high performance liquid chromatography (HPLC). The bioactivity of the pure compounds was done using disc diffusion method. The three compounds exhibited moderate activities against Gram positive bacterium (*Staphylococcus aureus* ATCC25922) and Gram negative bacterium (*Klebsiella pneumoniae* ATCC13883). Compound 1 was found to be the most active against *K. pneumoniae* (8.00 \pm 1.00 mm) and *S. aureus* (10.00 \pm 1.73 mm) followed by compound 2 with inhibition zones of 7.66 \pm 0.57 and 7.33 \pm 0.57 mm against *K. pneumoniae* and *S. aureus*, respectively. Compound 3 was the least active against both *K. pneumoniae* (7.33 \pm 0.57 mm) and *S. aureus* (7.00 \pm 1.00 mm). The results obtained indicate that compounds 1, 2 and 3 exhibit potential as possible sources of antibacterial agents.

Key words: Antibiotics, bacterial infections, antibiotic resistance, *Polyscias fulva*, saponins.

INTRODUCTION

Saponins are a major family of secondary metabolites that occur in a wide range of plant species (Hostettman et al., 1995). They are naturally occurring glycosides characterized by their strong foam forming property in aqueous solution (Gl-stndag and Mazza, 2007; Man et al., 2010; Negi et al., 2013). Various members of this important family of plant secondary metabolites are

exploited commercially for a variety of purposes including drugs and medicines, precursors for hormone synthesis, adjuvants, foaming agents, sweeteners, taste modifiers and cosmetics. Since many saponins have potent antimicrobial activity, the natural role of these molecules in plants is likely to be in conferring protection against attack by potential pathogens (Morrissey and Osbourn,

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1999; Negi et al., 2013).

Bacterial infections constitute a major public health problem in developing countries where the high cost of antibiotics makes them unaffordable to the majority of the population (Adwan et al., 2010). Population increase, traditional inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for various diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments (Sharma and Manu, 2013).

Among these plants is *P. fulva* which belongs to the family Araliaceae, a medium size and fast growing deciduous tree of the tropical forests of sub-Saharan Africa (Bedir et al., 2001). In Kenya, the plant grows around Elburgon, North of Mt. Elgon, West of Mt. Kenya, North of the Nandi forests and wetter highlands areas like Kakamega forest (Orwa et al., 2009). The plant has been used traditionally to cure many diseases, for instance, in west and central Africa it is traditionally used to treat malaria, fever and mental illness (Ndaya et al., 2002). It has been reported that the Araliaceae family and precisely the genus *Polyscias* are considered as a rich source of triterpene glycosides (Gopalsamy et al., 1990).

This study reports the isolation and characterization of three compounds of triterpene saponins from the leaves of *P. fulva* collected from Kakamega forest in the western part of Kenya.

MATERIALS AND METHODS

Plant material

Leaves of *P. fulva* were collected from Kakamega rain forest which is at an altitude of 0°10' to 0°21'N 34°58' E. The plant materials were then taken to the Centre for Herbal Research at Egerton University, Kenya where they were dried under shade for three weeks.

Extraction of phytochemicals

Leaves of *P. fulva* were dried under shade and at room temperature to prevent the loss of labile compounds and to retain their natural active compounds. The plant materials were turned over periodically during drying to avoid growth of moulds. The materials were ground separately to fine powder using a Thomas-wiley mill model 4. Six kilograms of the *P. fulva* ground materials were soaked in methanol at room temperature for 24 h with periodical shaking. The contents were then filtered through Whatman no. 1 filter paper and the filtrate was concentrated in vacuum at 50°C using Buchi Rotavapor R-205 rotary evaporator. The methanol crude extracts were placed in the fume hood to total dryness.

Preparative high performance liquid chromatography

The methanol extracts of *P. fulva* were purified using preparative high performance liquid chromatography equipped with uv-vis detector. The stationary phase used was C-18 column (250 mm by 20 mm, 10 µm). Anisocratic mobile phase consist of acetonitrile: Water (5:95 v/v) was delivered at a flow rate of 15.000 ul/min and the elution profiles were read at different wavelengths. The

methanol extract of *P. fulva* yielded three major fractions namely 1, 2 and 3. The compounds 1, 2 and 3 were divided into two portions each; one portion of each compound was used for 1 and 2D high field NMR spectroscopy and mass spectroscopy while the other portions were subjected to assays against selected bacteria.

Disc diffusion assay of pure compounds

The disc diffusion method for antibacterial susceptibility testing was carried out according to the standard method by Zaidan et al. (2003). The pure extracts were screened for antibacterial activity against *S. aureus* and *K. pneumoniae*. Nutrient agar mixed with bacteria at a concentration of 1×10^5 cfu/ml were poured in Petri dishes and allowed to cool. The plant extracts equivalent to 1 mg/ml, dissolved in methanol were applied to sterile paper discs (6 mm diameter). The solvent were then allowed to evaporate and the discs deposited on the surface of the inoculated agar plates and incubated for 24 h at 37°C. Zones of inhibition were measured in millimeter after 24 h of growth. The negative control used in this experiment was 1% dimethyl sulfoxide (DMSO) whereas 30 µg/disc chloramphenicol discs were used as the positive control. All tests were performed in triplicates.

Data analysis

Mean inhibition zones were calculated and equality of means was analyzed using one-way analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD), a Post-Hoc Analysis, was used to determine if there was any significant difference between the means of the isolates. Data analysis was performed using R statistical software version 3.3.1.

Nuclear magnetic resonance (NMR) spectroscopy

The ^1H , ^{13}C , DEPT, HSQC, COSY and HMBC NMR spectra were recorded on the Bruker Advance 500 MHz NMR spectrometer at the Technical University of Berlin, Germany. The readings were done in DMSO and chemical shifts assigned by comparison with the residue proton and carbon resonance of the solvent. Tetramethylsilane (TMS) were used as an internal standard and chemical shifts were given as δ (ppm). The structures were then simulated using ACD NMR manager program to obtain the chemical shifts of proton. The off-diagonal elements were used to identify the spin-spin coupling interactions in the ^1H - ^1H COSY (Correlation spectroscopy). The proton-carbon connectivity, up to three bonds away, was identified using ^1H - ^{13}C Heteronuclear Multiple Bond Correlation (HMBC) spectrum. The ^1H - ^{13}C Heteronuclear Single Quantum Coherence (HSQC) spectrum were used to determine the connectivity of hydrogen to their respective carbon atoms.

Mass spectrometry

Mass spectra of the compounds were recorded on Finnigan Tripple Stage Quadrupol Spectrometer (TSQ-70) with electron spray ionization (ESI) method in negative and positive ion mode. Thermo XcaliburQual computer software was used in analysis of the mass chromatograms.

RESULTS AND DISCUSSION

The methanol extract was subjected to extensive

Table 1. NMR spectroscopic data for the aglycone moieties of compounds 1-3.

S/N	1(CDCl ₃)		2(CDCl ₃)		3(CDCl ₃)	
	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H
1	38.4, CH ₂	0.85/1.49	38.6, CH ₂	1.49	38.7, CH ₂	0.85/1.53
2	27.6, CH ₂	0.99/1.65	27.6, CH ₂	0.98/1.66	27.7, CH ₂	0.87
3	80.2, CH	3.49	79.7, CH	3.50	88.2, CH	3.01
4	40.9, Cq	-	40.9, Cq	-	40.9, Cq	-
5	47.5, CH	1.51	47.5, CH	1.52	55.5, CH	0.70/0.73
6	17.6, CH ₂	1.17	17.5, CH ₂	1.38	18.1, CH ₂	1.47
7	32.5, CH ₂	1.19/1.43	32.5, CH ₂	1.15	32.6, CH ₂	1.15
8	39.3, Cq	-	39.3Cq	-	39.3, Cq	-
9	46.5, CH	1.18	46.6, CH	1.19	47.5, CH	1.47
10	36.2, Cq	-	36.0, Cq	-	36.2, Cq	-
11	23.0, CH ₂	1.48	23.0, CH ₂	1.91`	22.9, CH ₂	1.59
12	122.0, CH	5.16	122.0, CH	5.16	122.1, CH	5.17
13	147.7, Cq	-	144.0, Cq	-	143.8, Cq	-
14	41.0, Cq	-	41.0, Cq	-	41.0, Cq	-
15	23.4, CH ₂	1.81	23.3, CH ₂	1.81	23.3, CH ₂	1.79
16	25.5, CH ₂	1.72	25.7, CH ₂	1.72	26.1, CH ₂	0.86
17	45.6, Cq	-	45.6, Cq	-	45.6, Cq	-
18	41.2, CH	2.74	41.2, CH	2.74	41.1, CH	2.74
19	46.1, CH ₂	1.05/1.61	46.1, CH ₂	1.03	46.0, CH ₂	1.07/1.61
20	30.8, Cq	-	30.6, Cq	-	30.6, Cq	-
21	33.7, CH ₂	2.28	33.7, CH ₂	1.11/2.28	33.6, CH ₂	1.32
22	32.4, CH ₂	1.61	32.3, CH ₂	1.01	32.1, CH ₂	1.48
23	63.1, CH ₂	3.08/3.41	62.9, CH ₂	3.09/3.31	27.8, CH ₃	0.93
24	13.4, CH ₃	0.58	13.4, CH ₃	0.57	16.7, CH ₃	0.74
25	16.0, CH ₃	0.88	16.0, CH ₃	0.87	15.7, CH ₃	0.85
26	17.3, CH ₃	0.71	17.3, CH ₃	0.70	17.1, CH ₃	0.68
27	26.0, CH ₃	1.10	26.0, CH ₃	1.10	25.9, CH ₃	1.07
28	173.0, Cq	-	178.6, Cq	-	175.6, Cq	-
29	33.2, CH ₃	0.86	33.2, CH ₃	0.88	33.2, CH ₃	0.87
30	23.8, CH ₃	0.87	23.8, CH ₃	0.87	23.8, CH ₃	0.86

Chemical shifts in ppm from TMS is the internal standard .

spectroscopic studies such as ¹H NMR, DEPT NMR and mass spectrometry as described in the methodology resulting in the elucidation of three triterpene glycosides (saponins; **1-3**).

Compound **1** (10.27 mg) was obtained as a dark brown oily substance (Figure 1). The 1D and 2D NMR spectral data of compound **1** are summarized in Table 1. The mass spectral data of compound **1** gave a molecular ion peak at m/z 605.40 corresponding to its (M+H)⁺ ion suggesting the molecular formula as C₃₅H₅₆O₁₁. The ¹H-NMR spectra of compound **1** showed signals of six methyl groups at δ_H 0.58 (H-24), 0.88 (H-25), 0.71 (H-26), 1.10 (H-27), 0.86 (H-29), 0.87 (H-30), an olefinic group at δ_H 5.16 (H-12), which were characteristics signals for the oleanane skeleton with a hydroxyl group at C-23. These signals indicate a pentacyclic structure hence this compound was identified as an olean-12-ene type

pentacyclic triterpene and this was confirmed by comparison of its NMR data with those of known olean-12-ene type derivatives (Maillard et al., 1992; Mahato and Kundu, 1994; Beaudelaire et al., 2016). Additionally, ¹H NMR spectra of compound **1** also showed the presence of anomeric protons at δ 4.19 (H-1') indicating the presence of a sugar in its structure.

DEPT NMR spectra displayed a total of 27 carbons which consisted of two carbons of a trisubstituted double bond, one anomeric carbon, six methyl groups, 12 methylene groups, nine methine groups and the other missing seven signals were found to be quaternary carbons. The presence of a signal of a carbonyl ester group at δ 173.0 (C-28) suggested the compound as oleanolic acid (Onoja and Ndukwe, 2013).

The HSQC spectrum was used to assign protons directly attached to carbon atoms. This spectrum showed

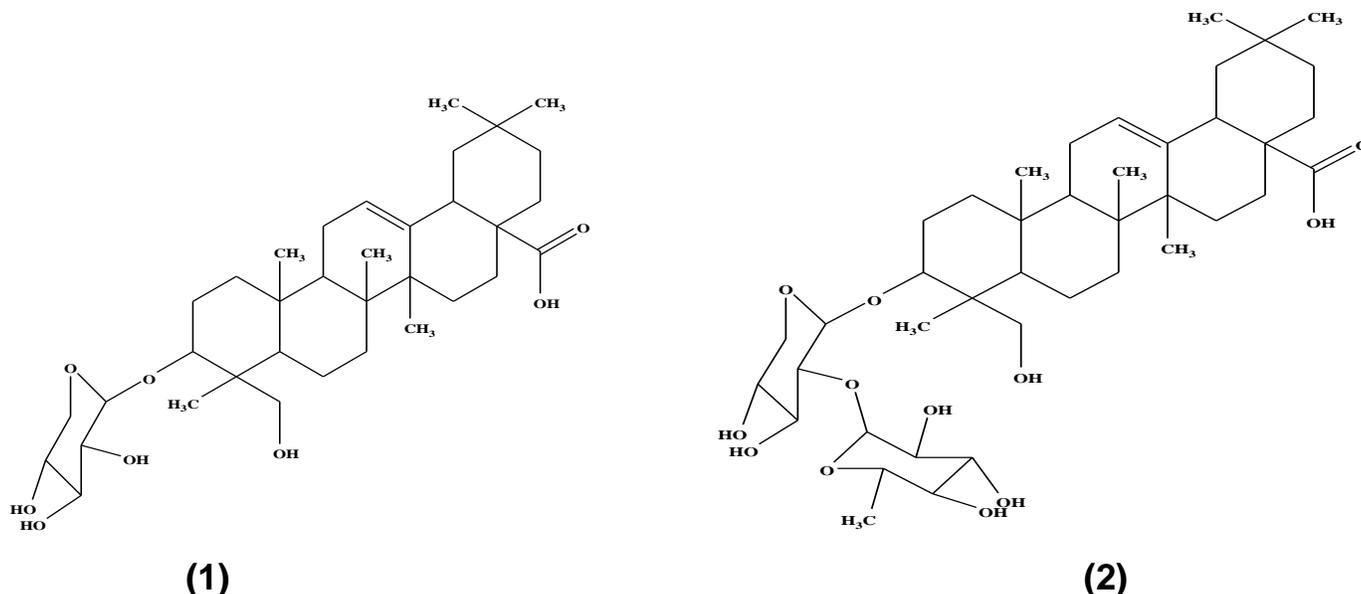


Figure 1. Isolated compounds 3-O- α -L-arabinopyranosyl-hederagenin (1) and 3-O-[α -L-rhamnopyranosyl (1-2)- α -L-arabinopyranosyl]-hederagenin (2).

correlation between proton δ_{H} - 0.85/1.49 (C-1), 0.99/1.65 (C-2), 3.49 (C-3), 1.51 (C-5), 1.17 (C-6), 1.19/1.43 (C-7), 1.18 (C-9), 1.48 (C-11), 5.16 (C-12), 1.81 (C-15), 1.72 (C-16), 2.74 (C-18), 1.05/1.61 (C-19), 2.28 (C-21), 1.61 (C-22), 3.08/3.41 (C-23), 0.58 (C-24), 0.88 (C-25), 0.71 (C-26), 1.10 (C-27), 0.86 (C-29) and 0.87 (C-30). Additionally, one anomeric signal at δ 4.19 (H-1') was observed giving HSQC correlations with one anomeric carbon at δ 105.2.

The proton - proton COSY correlations for compound 1 were also determined. COSY spectrum gave information on the correlation between protons attached to adjacent carbon atoms. The protons H-11 (δ_{H} - 1.48) correlated with proton H-12 (δ_{H} - 5.16) and proton H-18 (δ_{H} - 2.74) correlated with proton H-19 (δ_{H} - 1.05/1.61).

The NMR values for all the protons and carbons were assigned on the basis of ^1H - ^1H COSY, HSQC and HMBC experiments. The attachment of the sugar molecule at position C-3 of the aglycone was established by the HMBC correlation between the δ_{H} 4.19 (H-1') and δ_{C} 80.25 (C-3). The ^1H NMR spectrum of the glycone portion showed the presence of three oxymethine protons at δ_{H} - 3.30, 3.60, 3.31 together with a methylene proton at 3.32/3.65 for H-5' and anomeric proton at δ_{H} - 4.19. The sugar molecule was therefore identified as Arabinose (Joshi et al., 1992; Njateng et al., 2015). Thus, with all the correlations considered, the compound was identified as a monosaccharide triterpenoid saponin of olean-12-en-28-oic acid aglycone with the molecular formula $\text{C}_{35}\text{H}_{56}\text{O}_8$. This compound has been isolated before from the stem bark of the same plant (*P. fulva*) and was established to be 3-O- α -L-arabinopyranosyl-hederagenin (Joshi et al., 1992; Njateng et al., 2015), however, it is the first time to be isolated from the leaves of the same plant.

Compound 2 was also obtained as a dark brown oily substance with a mass of 30.81 mg (Figure 1). Its molecular formula was established as $\text{C}_{41}\text{H}_{66}\text{O}_{12}$. The aglycone region in the DEPT NMR spectra showed great similarity to that of compound 1. The six sp^3 hybrid carbon signals at δ_{C} 13.4, 16.0, 17.3, 23.8, 26.0 and 33.2, and the two sp^2 hybrid carbon signals at δ_{C} 122.0 and 144.0 (Onoja and Ndukwe, 2013) together with the information from ^1H NMR analysis (six methyl proton singlets at δ_{H} 0.57, 0.70, 0.87, 0.87, 0.88 and 1.10 and a vinyl proton at δ_{H} 5.16) indicated that the aglycone possesses an olean-12-ene skeleton.

The HSQC correlations showed correlation between protons absorption at δ_{H} - 1.49 (C-1), 0.98/1.66 (C-2), 3.50 (C-3), 1.52 (C-5), 1.38 (C-6), 1.15 (C-7), 1.19 (C-9), 1.91 (C-11), 5.16 (C-12), 1.81 (C-15), 1.72 (C-16), 2.74 (C-18), 1.03 (C-19), 1.11/2.28 (C-21), 1.01 (C-22), 3.09/3.31 (C-23), 0.57 (C-24), 0.87 (C-25), 0.70 (C-26), 1.10 (C-27), 0.88 (C-29) and 0.87 (C-30). The presence of six methyl signals were characteristics signals for the oleanane skeleton with a hydroxyl group at C-23. Additionally, anomeric protons signals in NMR spectrum at δ_{H} 4.34 and δ_{H} 5.06 together with carbon signals at δ_{C} 100.38 and 103.40 in the DEPT NMR data suggested that compound 2 was a glycoside with two sugar units.

The DEPT NMR spectrum showed a total of 14 methine (CH) signals at δ_{C} - 41.2, 46.6, 47.5, 66.2, 68.5, 70.8, 70.9, 72.5, 73.3, 74.6, 79.7, 100.3, 103.4 and 122.0 and a total of 12 methylene carbons (CH_2) signals absorption at δ_{C} - 17.5, 23.0, 23.3, 25.7, 27.6, 32.3, 32.5, 33.7, 38.6, 46.1, 62.9 and 64.8. It also showed a total of seven methyl (CH_3) signals at δ_{C} - 13.4, 16.0, 17.3, 18.2, 23.8, 26.0 and 33.2. The other missing seven signals

were found to be quaternary carbons. The HMBC spectrum of Compound **2** was almost similar to the one for compound **1** with the only difference occurring with two sugar moieties substituent at position C-3. Similar to compound **1** the aglycone of compound **2** was confirmed to have a pentacyclic olean-12-ene type triterpene skeleton.

The signals of the aglycone's C-12 and C-13 at δ 122.0 and δ 144.0, respectively show the presence of two olefinic carbons (Mehta et al., 2004; Xu et al., 2010) and a signal at δ 178.6 (C-28) which shows the presence of a carboxylic acid at that position, thus, confirming that the aglycone is of olean-12-ene skeleton and therefore olean-12-en-28-oic acid aglycone.

The presence of two sugar moieties is evidenced in the signals at δ_c 103.4 and 100.3 confirming the presence of two anomeric carbons. The ^1H NMR spectrum showed anomeric proton signals at δ_H 4.34 and δ_H 5.06 and one methyl group signal at δ_H 1.07 suggesting the occurrence of rhamnopyranosyl unit.

The sugar moieties were assigned mainly from ^1H - ^1H COSY, HSQC and HMBC experiments which allowed the identification of one rhamnopyranose unit with anomeric protons resonating at δ_H 5.06 and one arabinopyranose unit with the anomeric protons at δ 4.34. The position of the sugar chain at C-3 was determined by the HMBC correlations. A correlation between proton H-1' signal (δ_H 4.34) and C-3 signal (δ_c 79.7) of the aglycone indicated that this pentose is directly attached to the aglycone. Further correlations were observed between signals of ara C-2' (74.6) and Rha H-1" (δ 5.06). ^1H - ^1H COSY correlations was also observed between proton H-1' (δ_H 4.34) and H-2' (δ_H 3.52), proton H-1" (δ_H 5.06) and H-2" (δ_H 3.67) and between proton H-5" (δ_H 3.71) and H-6" (δ_H 1.07) (Table 2).

According to electrospray ionization - mass spectrometry (ESI-MS), the positive electron mass spectrometry showed peaks at m/z 751 $[\text{M}+\text{H}]^+$, 773 $[\text{M}+\text{Na}]^+$, 789 $[\text{M}+\text{K}]^+$ and 1523 $[2\text{M}+\text{Na}]^+$ upon positive ionization mode and at 749 $[\text{M}-\text{H}]^-$, 785 $[\text{M}+\text{Cl}]^-$ and 1499 $[2\text{M}-\text{H}]^-$ upon negative ionization mode. Thus, on the basis of the above evidence and analysis, compound **2** has been identified as a disaccharide triterpenoidsaponin of olean-12-en-28-oic acid aglycone with the molecular formula of $\text{C}_{41}\text{H}_{66}\text{O}_{12}$. As compound **1**, compound **2** was isolated before from the stem bark of *P. fulva* and given the name 3-O- $[\alpha\text{-L}$ -rhamnopyranosyl (1-2)- $\alpha\text{-L}$ -arabinopyranosyl]-hederagenin (Joshi et al., 1992; Njateng et al., 2015) however, it is isolated for the first time from the leaves from the this plant.

Compound **3** (Figure 3) was also isolated as a brownish amorphous powder and its molecular formula was determined as $\text{C}_{59}\text{H}_{96}\text{O}_{25}$ based on molecular ion peak at 1227 $[\text{M}+\text{Na}-\text{H}]^+$ and 1242 $[\text{M}+\text{K}+\text{H}]^+$ upon positive ion mode and at 1203 $[\text{M}-2\text{H}]^-$ upon negative ionization mode.

The ^1H NMR spectrum of compound **3** displayed seven

methyl signals at δ 0.68, 0.74, 0.85, 0.86, 0.87, 0.93 and 1.07 which is a characteristic of a typical oleanane type triterpene acid, an oxymethine at δ 3.01 and olefinic protons at δ 5.17 indicating that the aglycone is of olean-12-ene skeleton and therefore olean-12-en-28-oic acid aglycone (Mehta et al., 2004; Xu et al., 2010). It also displayed five anomeric proton signals at δ 4.31, 4.27, 5.21/5.33, 4.69 and 5.04 signifying the presence of five sugar units.

The DEPT NMR spectrum showed a total of 51 signals and the other missing eight signals were quaternary signals. Out of the total of 59 signals, 30 signals were assigned to the oleanolic acid moiety and the remaining 29 signals to the saccharide portion. The DEPT spectra of the aglycone displayed seven methyl signals at δ 15.7, 16.7, 17.1, 23.8, 25.9, 27.8 and 33.2; ten methylene (CH_2) signals at δ 18.1, 22.9, 23.3, 26.1, 27.7, 32.1, 32.6, 33.6, 38.7 and 46.0. It also showed a total of five methine (CH) signals at δ 41.1, 47.5, 55.5, 88.2 and 122.1. The other missing eight signals were quaternary signals. The first carbon was found to resonate at δ 38.7 (C-1), 27.7 (C-2) and 88.2 (C-3). Quaternary carbons at C-4, C-8, C-10, C-13, C-14, C-17, C-20 and C-28 were found to be absorbed at δ 40.9, 39.3, 36.2, 143.8, 41.0, 45.6, 30.6 and 175.6, respectively. Carbon 5 were found to be absorbed at δ 55.5 while C-6, C-7, C-9, C-11, C-12, C-15, C-16, C-18, C-19, C-21 and C-22 were found to resonate at δ 18.1, 32.6, 47.5, 22.9, 122.1, 26.1, 23.3, 41.1, 46.0, 33.6 and 32.1 respectively. Carbons C-23, C-24, C-25, C-26 and C-27 resonated at δ 27.8, 16.7, 15.7, 17.1 and 25.9 while carbon C-29 and C-30 were found to be absorbed at δ 33.2 and 23.8, respectively.

The HMBC spectrum of compound **3** was almost similar to the one of compounds **1** and **2** with difference occurring with two sugar moieties substituent at position C-3 and in addition to an ester linkage between a trisaccharide chain and the aglycone at position C-28. Further confirmation with ^1H - ^1H COSY correlations showed that there was a strong correlation between protons H-9 (δ 1.47) and H-11 (δ 1.59) and proton H-11 (δ 1.59) and H-12 (δ 5.17) and proton H-18 (δ 2.74) and H-19 (δ 1.07/1.61).

The sugar portion of compound **3** contained in the ^1H NMR spectrum five anomeric proton signals at δ 5.04, 4.27, 5.21, 4.69, 5.04 and two methyl signals at δ 1.08 and 1.09 suggesting the occurrence of two rhamnopyranose units. The sugar moieties were assigned mainly from ^1H - ^1H COSY, HSQC and HMBC experiments which allowed the identification of one Arabinopyranose unit with the anomeric proton signal at δ 5.04, two glucopyranose units with anomeric protons resonating at δ 4.69 and 5.21/5.33 and two rhamnopyranose units with anomeric protons resonating at δ 4.27 and 5.04. Considering the δ values of the signals due to C-3 (δ 88.2) and C-28 (δ 175.6), saponin compound **3** was a 3, 28 bisdesmoside, thus, it has been identified as a bisdesmosidic pentasaccharide triterpenoid saponin of olean-12-en-28-

Table 2. NMR spectroscopic data for the sugar moieties of compounds 1-3.

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1'	105.2, CH	4.19	103.4, CH	4.34	104.2, CH	4.31
2'	71.5, CH	3.30	74.6, CH	3.52	76.9, CH	3.23
3'	73.1, CH	3.31	72.5, CH	3.18	72.3, CH	3.18
4'	68.0, CH	3.60	66.2, CH	3.58	69.7, CH	3.17
5'	65.6, CH ₂	3.32/3.65	64.8, CH ₂	3.32/3.67	64.2, CH	3.67
1''			100.3, CH	5.06	103.0, CH	4.27
2''			70.8, CH	3.67	74.2CH	2.99
3''			73.3, CH	3.48	72.8CH	3.57
4''			70.9, CH	3.49	75.6, CH	3.07
5''			68.5, CH	3.71	67.9, CH	3.59
6''			18.2, CH ₃	1.07	18.1, CH ₃	1.08
1'''					94.4, CH	5.21
2'''					72.8, CH	3.11
3'''					76.9, CH	3.40
4'''					70.8, CH	3.47
5'''					69.0, CH	3.84
6'''					68.0, CH ₂	3.91
1''''					100.9, CH	4.69
2''''					75.9, CH	3.21
3''''					77.3, CH	3.36
4''''					70.8, CH	3.67
5''''					68.7, CH	3.87
6''''					60.4, CH ₂	3.42
1'''''					100.4, CH	5.04
2'''''					68.4, CH	3.66
3'''''					74.9, CH	3.57
4'''''					71.1, CH	3.60
5'''''					72.6, CH	3.53
6'''''					18.2, CH ₃	1.09

oic acid aglycone. The structure of the sugar chain at C-3 was unambiguously defined by the HMBC correlations and compared with those from previous study (Mehta et al., 2004). A correlation between C-3 signal (δ 88.2) of the aglycone and Ara H-1 signal (δ 4.30) indicated that this pentose was directly attached to the aglycone. Further correlations were observed between signals of Xyl C-2 (δ 76.9) and Rha I H-1 (δ 4.27). ^1H - ^1H COSY correlations was also observed between proton H-1 (δ 4.27) and H-2 (δ 3.57). The structure of the oligosaccharide chain at C-28 was identified from the HMBC correlations between signals of Glc I C-4 (δ 69.0), Glc II H-1 (δ 4.69), Glc II C-4 (70.8) and Rha II H-1 (δ 5.04). Correlating signals due to Glc I H-1 (δ 5.21/5.33) and aglycone C-28 (δ 175.6) provided a definitive evidence of an ester linkage between a trisaccharide

chain and the aglycone. Thus, on the basis of the aforementioned evidence and analysis, compound 3 was found to be 3-O-[rhamnopyranosyl-(1 \rightarrow 2)-xylopyranosyl]-olean-12-en-28-O-[rhamnopyranosyl-(1 \rightarrow 4)-glucopyranosyl-(1 \rightarrow 6) glucopyranosyl] ester (Figure 2). This compound is isolated for the first time from this plant, however, it has the same skeleton with Hederasaponin B isolated from the leaves of *Hedera helix* L. (Nanyoung et al., 2017), the only difference occurring at position C-23.

Biological activities of the compounds

Secondary metabolites isolated from *P. fulva* leaf extracts were subjected to antibacterial activity against Gram

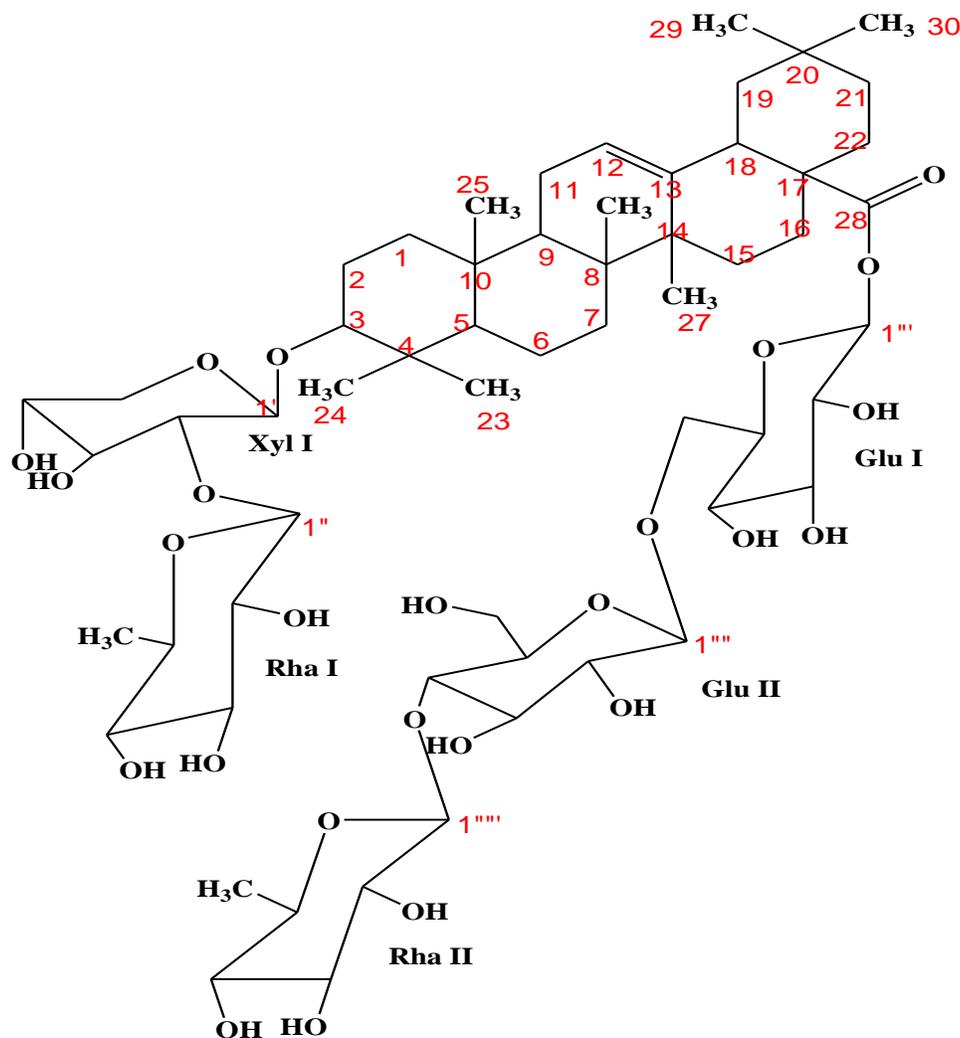


Figure 2. Isolated compound 3-O-[rhamnopyranosyl-(1-2)-xylopyranosyl]-Olean-12-en-28-O-[rhamnopyranosyl-(1-4)-glucopyranosyl-(1-6)-glucopyranosyl] ester.

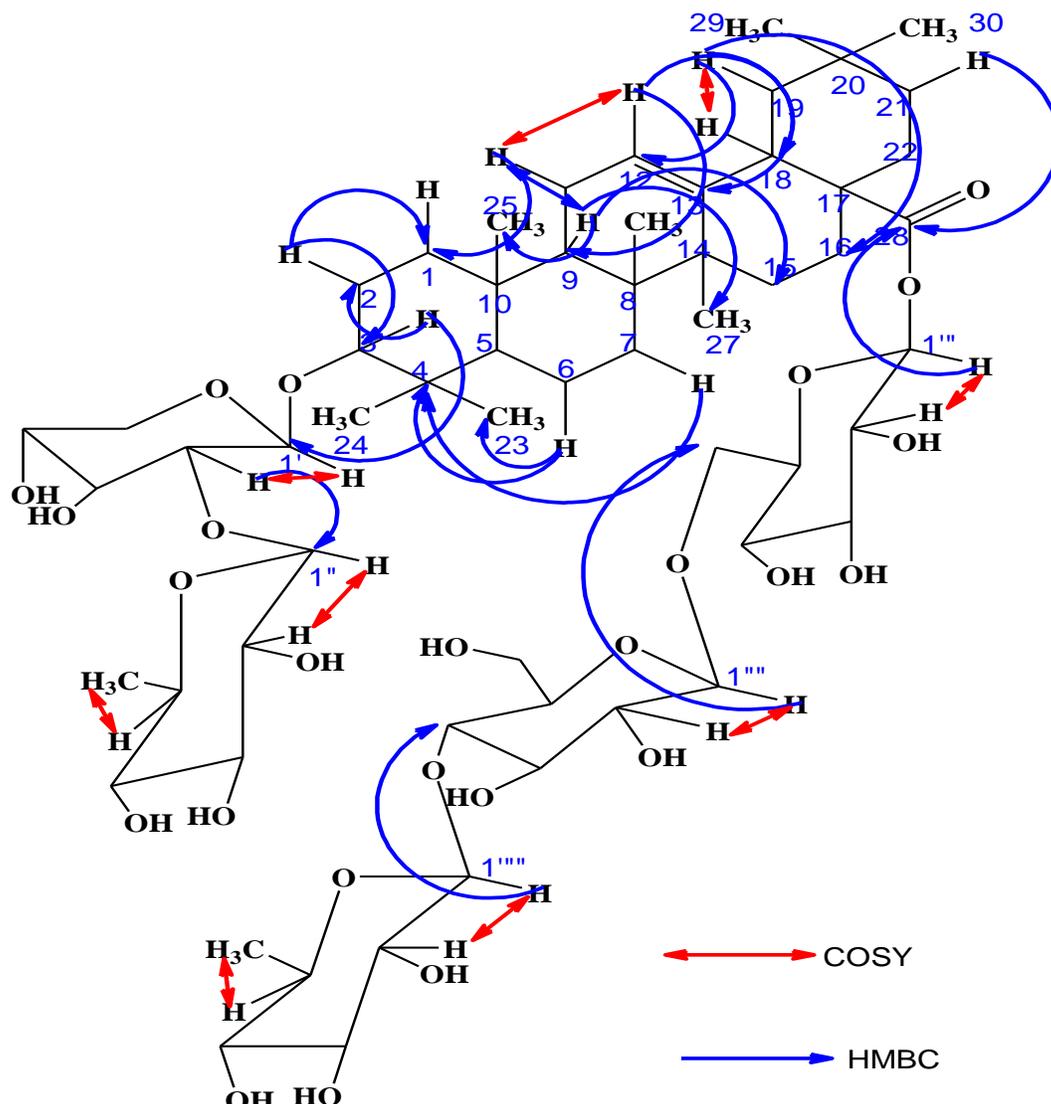
negative bacteria (*K. pneumoniae*) and Gram positive bacteria (*S. aureus*) using disc diffusion assay test and inhibition zones were measured and recorded (Table 3). The crude extract and the isolated compounds generally demonstrated antibacterial activities. The most sensitive bacterium was *S. aureus*. Compound **1** was the most active against both *K. pneumoniae* and *S. aureus* with inhibition zones of 8.00 ± 1.00 and 10.00 ± 1.73 mm, respectively. Compound **3** on the other hand, was the least active against both *K. pneumoniae* and *S. aureus* with inhibition zones of 7.33 ± 0.57 and 7.00 ± 1.00 mm, respectively.

The antibacterial properties for the crude extract and compounds can be explained by the presence of potentially active secondary metabolites detected in them. Among the compounds isolated from the crude methanol extract, compound **1** was more active on the

two tested microorganisms. This difference in activity may be attributed by the presence of hydroxyl group in position 23 and presence of 3-O-[xylopyranosyl] group in the compound (Njateng et al., 2017). Compound **2** that result from the addition of 3-O-[rhamnopyranosyl] group to compound **1** was less active. This modification may have slightly reduced the antibacterial activity of compound **2**. The substitution of position C-23 with a methyl group and the addition of O-[rhamnopyranosyl-(1→4)-glucopyranosyl-(1→6)-glucopyranosyl] group in position 28 to compound **3** could have attributed to being less active against both bacteria compared to compound **1** and **2** (Njateng et al., 2017). According to Pavithra et al. (2010) saponins possess antimicrobial activities. The mean inhibition zones of methanol extract and pure compounds in comparison with chloramphenicol showed that they were significantly less active than

Table 3. Inhibition diameters (mm) for the *Polyscias fulva* against test organisms.

Extracts/treatment	Test organisms (diameter in mm, n=3)	
	<i>K. pneumoniae</i>	<i>S. aureus</i>
Methanol crude	9.33±0.57 ^{bc}	10.33±1.52 ^{bc}
Compound 1	8.00±1.00 ^c	10.00±1.73 ^{bc}
Compound 2	7.66±0.57 ^c	7.33±0.57 ^{bc}
Compound 3	7.33±0.57 ^c	7.00±1.00 ^c
Chloramphenicol	16.00±0.00 ^a	17.00±0.00 ^a
1% DMSO	0.00±0.00 ^d	0.00±0.00 ^d

**Figure 3.** Structure of compound 3 showing selected COSY and HMBC correlations.

chloramphenicol. The means of compounds 1, 2 and 3 against *K. pneumoniae* are not significantly different while compounds 1 and 3 and the methanol extract are not

significantly different against *S. aureus*.

Within a column, extracts sharing the same letter(s) are not significantly different while those with different letter

(s) are significantly different ($\alpha = 0.05$, Turkey's test).

Conclusion

The results obtained show that compounds **1**, **2** and **3** isolated from the leaves of *P. fulva* are promising antibacterial agents.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Uterotonic effect of aqueous extract of *Launaea taraxacifolia* Willd on rat isolated uterine horns

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Launaea taraxacifolia is used by the traditional health practitioners in Sokoto to ease labour pains and augment labour thereby facilitating child birth. This study examined the effect of the aqueous extract of the whole plant on isolated uterus from non-pregnant rats pre-treated with stilbestrol and from pregnant rats in late gestation. This was compared to the effects of uterine contraction agonists, namely; oxytocin, histamine and acetylcholine. The possible mechanism of uterotonic activity was investigated using antagonists such as piroxicam, mepyramine and atropine. The aqueous extract of *L. taraxacifolia* produced a dose-dependent uterotonic activity. In the stilbestrol treated non-pregnant uterus, the force generated at 400, 800 and 1600 mg/ml was 1.469, 1.624 and 1.793 times greater than the control, respectively. In the pregnant rat uterus, a dose of 400, 800 and 1600 mg/ml generated a force of contraction that was 1.36, 1.51 and 1.66 times greater than the control, respectively. When the relative potency was compared to oxytocin, the gold standard uterotonic, *L. taraxacifolia* at 1600 mg/ml was found to be 0.08 times more potent than 0.4 µg/ml oxytocin in the stilbestrol treated non-pregnant rat uterus. In the pregnant rat uterine strip however, oxytocin was 0.17 times more potent than 1600 mg/ml *L. taraxacifolia*. Pre-treating the tissue with either atropine or mepyramine before administering the extract showed an inhibitory effect while piroxicam completely abolished its uterotonic effect, showing a probable moderate stimulation of muscarinic and histamine receptors but majorly the oxytocin receptors by *L. taraxacifolia*. A preliminary phytochemical screening of the extract shows the presence of saponins, tannins, flavonoids and steroids. A dose of 2000 mg/kg/oral of *L. taraxacifolia* was found to be well tolerated in rats with no sign of toxicity. Thus, *L. taraxacifolia* contains phytochemicals with uterotonic properties thereby justifying its ethnobotanical use in easing labour.

Key words: *Launaea taraxacifolia*, uterotonic, labour, isolated uterus.

INTRODUCTION

Since time immemorial, plants have been used for their effects upon sex hormones particularly for suppressing fertility, regularizing menstrual cycle, relieving dysmenorrheal, treating enlarged prostate, menopausal symptoms, breast pain and during and after childhood

(Williamson et al., 1996). This is of importance especially in developing countries where modern medicine and health care is both inaccessible and unaffordable hence more than 80% of the populace continues to rely on traditional form of medicine (WHO, 2003). This form of medicine is

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also used in solving female reproductive health issues.

Uterotonic plants are plants that stimulate uterine contraction and are therefore used to assist labour, remove retained placenta, control post partum bleeding and as an abortifacient (Watcho et al., 2010). They are also of importance in facilitating uterine contraction following a miscarriage to reduce hemorrhage (Roqaiya et al., 2015). Various plants have been used to regulate a number of issues relating to pregnancy and delivery as well as post-partum complications. Examples of such plants are *Monechma ciliatum* (Uguru et al., 1998), *Musanga cecropioides* (Ayinde et al., 2006), *Harpagophyllum procumbens* (Mahomed and Oyewole, 2006), *Ficus asperifolia* (Watcho et al., 2011), *Ananas comosus* (Monji et al., 2016), unripe fruits of *Carica papaya* (Praveena et al., 2017) and *Steganotaenia Araliacea* (Goma et al., 2017); all of which are used to ease the birthing process due to their uterotonic and oxytocic properties; plants such as *Ricinus communis* (Raji et al., 2006), *Strychnos potatorum* (Shah et al., 2009) and *Macrotyloma axillare* (Odhiambo et al., 2017) have been reported to have contraceptive property, while *Lawsonia inermis* (Mudi et al., 2011), *Bamusa vulgaris* (Yakubu et al., 2009) and *Millettia aboensis* (Onyegeme-Okerenta et al., 2016) possess abortifacient properties. In a review of herbs with uterotonic property, Roqaiya et al., (2015) reported 16 plants species whose uterotonic property have been validated in both *in vivo* and *in vitro* models.

Launaea taraxacifolia common names wild lettuce or African lettuce and locally known as Noomen barewa in Hausa or Yanrin in Yoruba is one of the plants that are claimed by the locals to ease birthing process and alleviate labour pains and it is therefore used for that purpose. It grows as a weed along road sides and in bushes in many African countries. It is a perennial herb up to 150 cm tall, with creeping root system. Its stem is erect, often woody at the base (Adebisi, 2004). In Nigeria, the plant is found in the far north (Sokoto, Kebbi and Zamfara States) as well as among the Yoruba speaking states in the south. In Nigeria, the plant is fed to nursing cattle to increase milk production and also given to livestock to induce multiple births. In Benin, it is used as a febrifuge while in Ghana; the leaves are rubbed on the limbs of backward children to induce them to walk (Burkill, 1997). It is sometime burnt for its ash which is used as vegetable salt (Adebisi, 2004). It has also been reported as antimicrobial as it is established to have activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Gbadamosi et al., 2012). *L. taraxacifolia* provides protection against cisplatin-induced hepatorenal damage through its antioxidant activities (Adejuwon et al., 2014). A review of ethnopharmacological and nutraceutical relevance of *L. taraxacifolia* documented effects such as antidiabetic, antihypertensive, anticancer, antimalarial, antibacterial and antiarthritis properties (Adinortey et al., 2018). Aboderin et al. (2017) however

reported some degree of toxic effects on the liver and kidney of albino rats treated with aqueous of *L. taraxacifolia* and emphasized the need for caution in its use for medicinal purposes. The aim of this study was to investigate the uterotonic property of this plant and the possible mechanism of action in order to validate its ethnobotanical use. This study could provide a useful guide to the discovery of lead compounds with oxytocic properties which can be of great benefit in the management of aforementioned gynecological conditions.

MATERIALS AND METHODS

Plant material

The whole plant of *L. taraxacifolia* was collected fresh from the wild in Talata Mafara area of Zamfara State. The plant was identified and authenticated at the herbarium of Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where an herbarium specimen with voucher number UDUH/ANS/0010 was deposited.

Experimental animals

Healthy female rats weighing between 130-150 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. The animals were kept under standard environmental conditions with access to feed and water *ad libitum*. The animals were divided into two categories.

Drugs and chemicals

The following drugs were purchased from Sigma Chemical (St Louis, MO, USA): oxytocin, histamine, mepyramine, acetylcholine hydrochloride, stilbestrol; while piroxicam and atropine were purchased from a local pharmacy. All other chemicals used were of analytical grades.

Preparation of extract

The whole plant was dried under shade to constant weight and ground manually using mortar and pestle. Forty gram (40 g) of the powdered plant material was extracted by maceration using distilled water. It was evaporated to dryness over water bath at 50°C and the percentage yield was calculated.

Oestrogen-dominated non-pregnant rats

All the animals in this category were pretreated with stilbestrol (0.1 mg/kg subcutaneous) for 24 h prior to use in order to induce oestrus phase. Vaginal smears were taken immediately before the animals were sacrificed in order to ascertain that the animals were in oestrus phase. Female rats in oestrus were used for the study.

Pregnant rats

Female animals were mated with male overnight. The morning after mating occurred, each female was examined for the presence of a vaginal plug or a vaginal swab was taken to detect any sperm under light microscopy. The presence of a vaginal plug or sperm

positivity was designated as day 0 of gestation. Pregnant animals were housed two per cage with access to water and feed *ad libitum*. They were kept till late pregnancy (Day 16 to 20) before use.

Preliminary phytochemical analyses

The quantitative phytochemical analysis was done using standard protocols. These include Van Buren and Robinson (1981) method for tannins; Obadoni and Chuko (2001) method for saponins; Borham and Kocipai-Abyzan (1994) method for flavonoids; Molischi's test for carbohydrate (Trease and Evans, 1999); and Okeke and Elekwa (2003) method for steroids.

Acute oral toxicity

The acute oral toxicity study was done using the "Up and Down method" in healthy adult female albino rats according to OECD guidelines No. 425 (OECD, 2008). A limit dose of 2000 mg/kg was used for the study. Five female rats were labeled for identification. An animal was picked at a time, weighed and dosed with equivalent volume of extract containing 2000 mg/kg body weight dissolved in distilled water as a vehicle after overnight fasting. Oral administration of drug was done using gastric feeding tube. Each animal was observed after dosing for the first 5 min for signs of regurgitation and then kept in a metallic cage. Each was then observed every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for the short-term outcome according to the specifications of the OECD. The animals were monitored for a total of 14 days for the long term possible lethal outcome.

Experimental design

Each of the pregnant and stilbestrol- pretreated non-pregnant female rats were anaesthetized using chloroform. The uteri were promptly removed, cleaned of the connective tissues and cut into strips of about 1 cm in length. Each uterine strip was mounted in an organ bath of 25-ml capacity containing De Jalon solution of the following composition (mM) NaCl 153.85, KCl 5.64, CaCl₂ 0.55, MgSO₄ 0.08, NaOH 12.5 and glucose 2.78. The physiological salt solution was maintained at 37°C and continuously aerated with carbogen (that is, 5% carbon dioxide + 95% oxygen gas mixture). Each preparation was subjected to a resting tension of 1.0 g and allowed to equilibrate for 30 min before it was challenged with *L. taraxacifolia* / other drugs used.

Drug challenges

After an equilibration period of 30 min, normal myometrial contractions were recorded at baseline. Uterine contractile responses were elicited by adding oxytocin (0.4 µg/ml), acetylcholine (0.4 µg/ml), histamine (0.4 µg/ml) and aqueous extract of *L. taraxacifolia* (400, 800 and 1800 mg/ml) to the De Jalon's solution. Each dose of the drug was allowed to act for 10 min and the amplitude of the contraction recorded by means of an isotonic transducer connected to a single channel recorder which was calibrated to record change in the tension generated on g versus displacement basis. Piroxicam (0.4 µg/ml), atropine (0.4 µg/ml) and mepyramine (0.4 µg/ml) were then used to antagonize the responses of the isolated uterus to oxytocin, acetylcholine or histamine and to the aqueous extract of *L. taraxacifolia*.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM).

Data was analysed using student t-test. P<0.05 was considered to be statistically significant (Figure 1).

RESULTS

Percentage yield

The percentage yield obtained for the aqueous extract of the whole plant of *L. taraxacifolia* was 17.38%.

Preliminary phytochemical analysis

The result of the preliminary qualitative phytochemistry showed the presence of carbohydrate, saponins, tannins, flavonoids and steroids.

Acute oral toxicity

At a limit dose of 2000 mg/ml, all the rats in the short and long term observation survived and no mortality or obvious signs of toxicity was recorded after the 14 day observation period. The LD₅₀ is therefore more than 2000 mg/ml.

Dose dependent effect of *L. taraxacifolia* on uterine contraction on non-pregnant rats

Aqueous extract of *L. taraxacifolia* evoked a dose dependent contraction of the uterine smooth muscle. In the control, the force of contraction recorded was 0.58 g, which was the baseline contraction in oestrogenised rat's uteri. At 400 mg/ml, the force generated was 1.469 times greater than the control. Meanwhile, the force increased by 1.624 and by 1.793 times, following administration of 800 and 1600 mg/ml *L. taraxacifolia*, respectively, with 1600 mg/ml *L. taraxacifolia* producing the maximum tension (Emax). The agonists used also elicited contractions of varying degree with oxytocin having the highest amplitude, with a force of contraction 1.577 times greater than the control while the force increased by 1.272 and 0.567 for acetylcholine and histamine, respectively. The dose of *L. taraxacifolia* that produced the Emax (1600 mg/ml) showed a force of contraction greater than that of 0.4 µg/ml oxytocin. This is shown in Figure 2.

Effect of atropine, mepyramine and piroxicam on the Emax induced by 1600 mg/ml *L. taraxacifolia* in non-pregnant uterine strip

In Figure 3, administration of muscarinic receptor antagonist, atropine into the bathing solution containing isolated uterine tissue pre-exposed to 1600 mg/ml *L.*



Figure 1. Flow chart of methodology.

taraxacifolia resulted in the E_{max} decreasing by 0.17 times. Meanwhile, administration of a histamine H1 antagonist mepyramine resulted in the E_{max} decreasing by 0.16 while a prostaglandin (PG) inhibitor, piroxicam an antagonist of oxytocin completely abolished its effect.

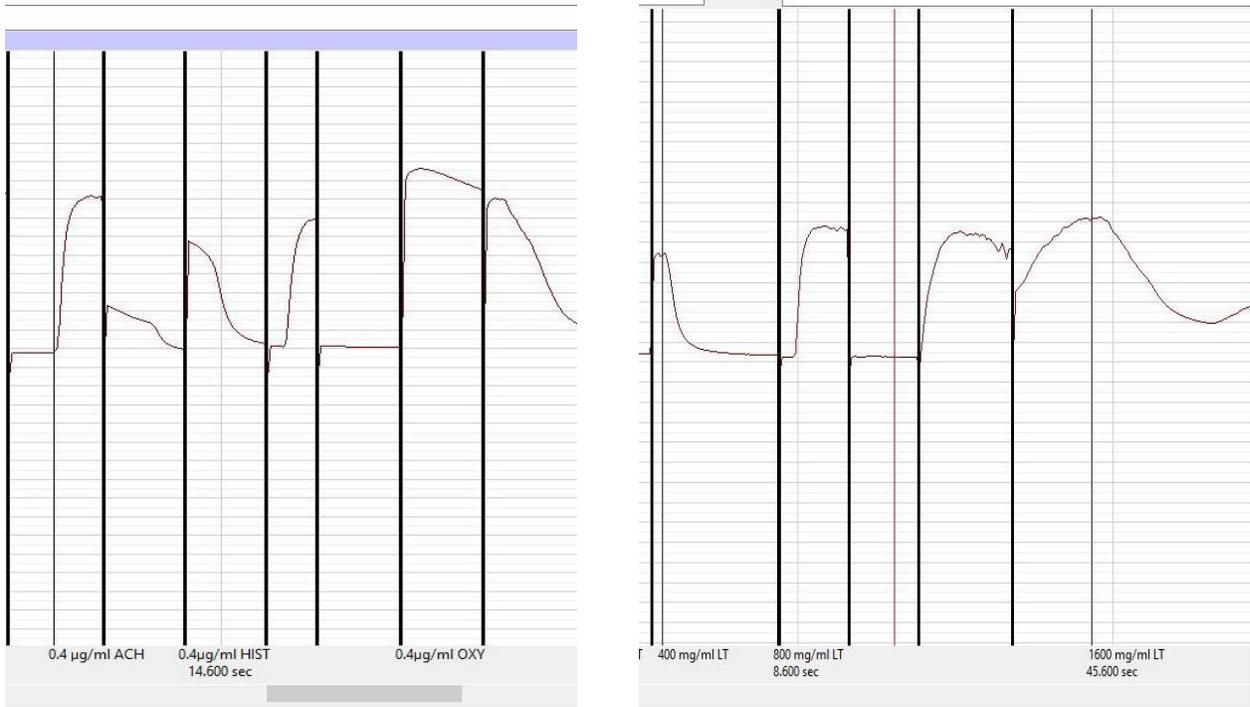
Dose dependent effect of *L. taraxacifolia* on uterine contraction on pregnant rats

The aqueous extract of *L. taraxacifolia* also evoked a dose dependent contraction of the uterine smooth muscle obtained from pregnant uterus in the third trimester (day 18 gestation). In the control, the force of contraction recorded was 0.60 g, which was the baseline contraction in pregnant rat's uteri. At 400 mg/ml, force generated was 1.36 times greater than the control. Meanwhile, the force increased by 1.51 and by 1.66 times, following administration of 800 and 1600 mg/ml *L. taraxacifolia*, respectively. The agonists used also elicited contractions of varying degree with oxytocin having the highest

amplitude, with a force of contraction 2.19 times greater than the control while the force increased by 0.755 and 0.69 for acetylcholine and histamine, respectively. The amplitude of contraction obtained with *L. taraxacifolia* and acetylcholine in the pregnant rats uterine strips were lower than that obtained in the non-pregnant rats. However, for oxytocin and histamine the forces of contraction in the pregnant rat uterine strip were 0.28 times and 0.11 higher than that obtained in the non-pregnant rats, respectively. This is presented in Figure 4.

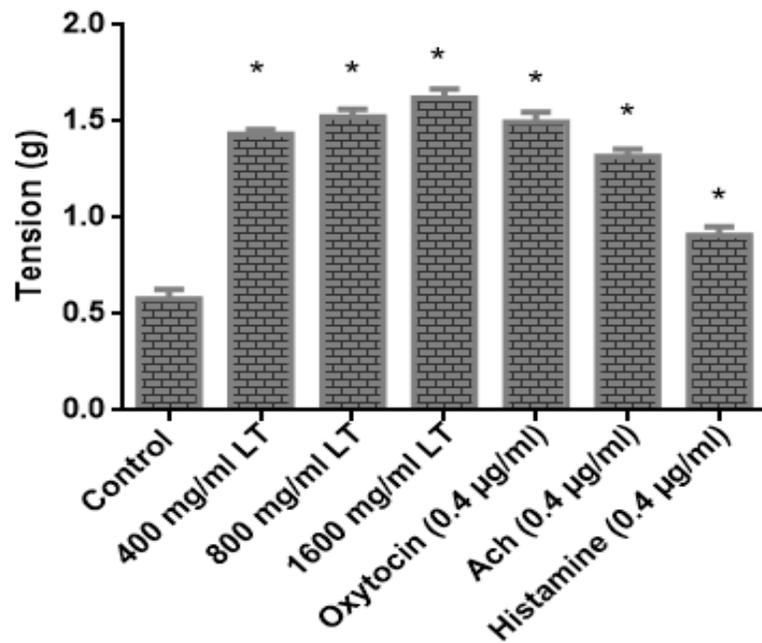
Effect of atropine, mepyramine and piroxicam on the E_{max} induced by 1600 mg/ml *L. taraxacifolia* in pregnant rat uterine strip

In Figure 5, administration of muscarinic receptor antagonist, atropine into the bathing solution containing isolated uterine tissue pre-exposed to 1600 mg/ml *L. taraxacifolia* resulted in the E_{max} decrease by 0.12 times. Meanwhile, administration of a histamine H1



A

B



C

Figure 2. The effect of *L. taraxacifolia* on uterine contraction. **(A)** Tracing of isometric uterine contraction following administration of various agonists. **(B)** Tracing of isometric uterine contraction following administration of various concentration of *L. taraxacifolia*. **(C)** Mean tension generated from isolated uterine horns obtained from different oestrogenized rats, which were exposed to various doses of *L. taraxacifolia* at concentrations ranging between 400 to 1600 mg/ml and different agonists. There was a dose-dependent increase in the tension with increasing doses of *L. taraxacifolia*. n=5 (* p<0.05 as compared to control).

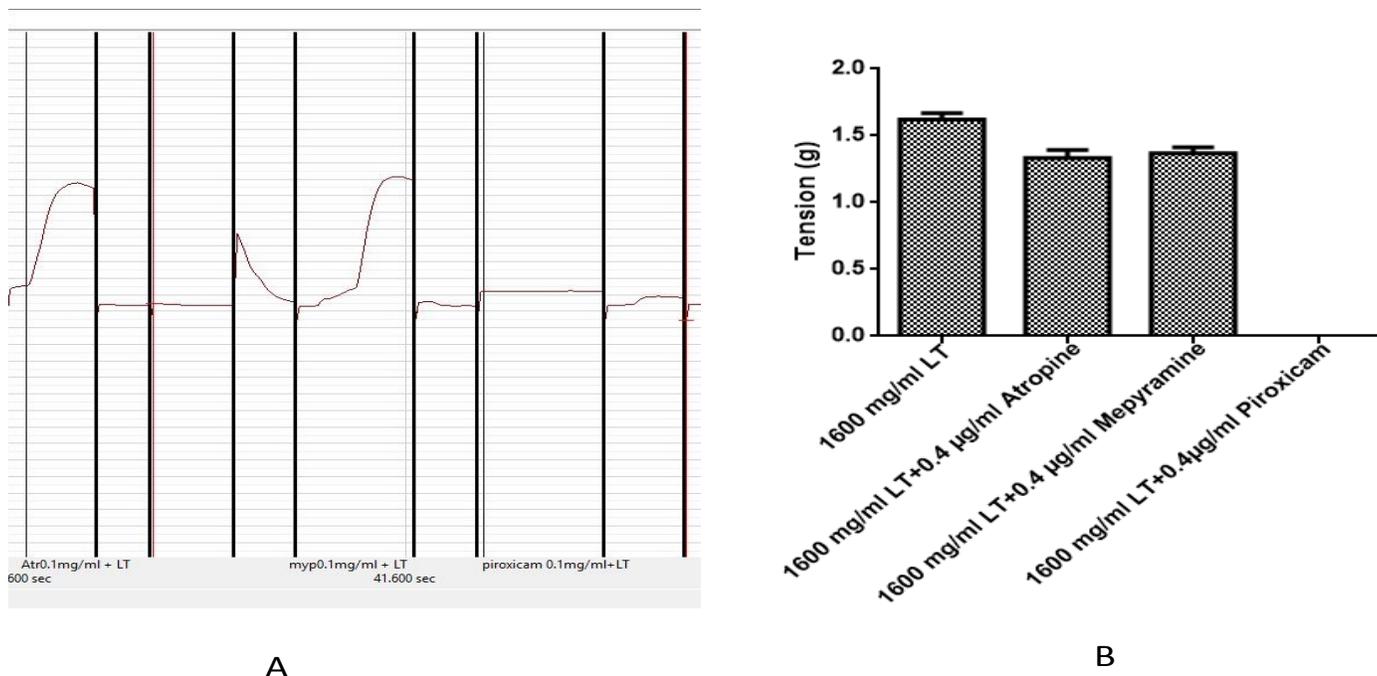


Figure 3. The effect of selected receptor antagonists on *L. taraxacifolia*-induced uterine contraction. **(A)** Representative tracings of isometric uterine contraction following *L. taraxacifolia* administration in the presence of various antagonists. **(B)** Mean Emax following administration of *L. taraxacifolia* at 1600 mg/ml in the presence of atropine, mepyramine and piroxicam. Atropine caused the least inhibition followed by mepyramine. Piroxicam completely abolished the effect of *L. taraxacifolia*.

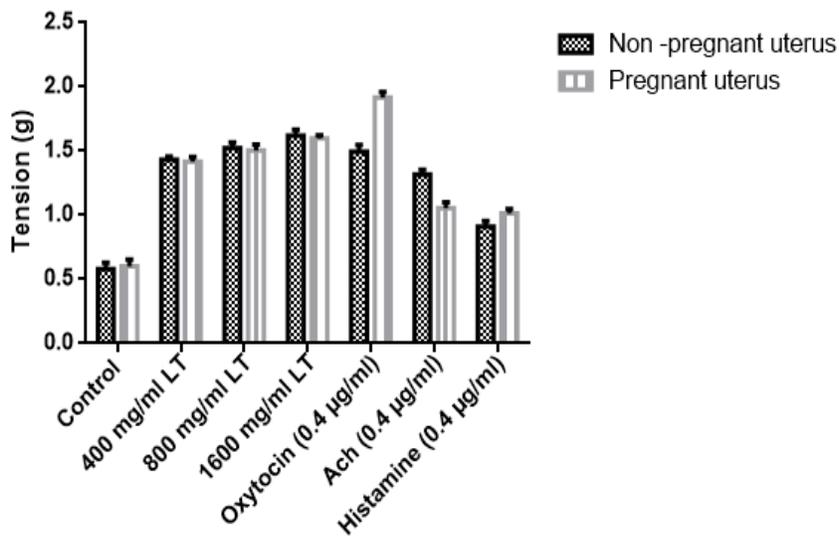


Figure 4. Comparison of mean tension generated from isolated uterine horns obtained from different non-pregnant and pregnant rats, which were exposed to various doses of *L. taraxacifolia* at concentrations ranging between 400 to 1600 mg/ml and different agonists. *L. taraxacifolia* showed a higher uterotonic effect on non-pregnant rats' uterus while effect of oxytocin and histamine were higher on pregnant rats uterus.

antagonist mepyramine resulted in the Emax decreasing by 0.09 while a prostaglandin (PG) inhibitor, piroxicam

completely abolished its effect as obtained in the non-pregnant rats uterine strips.

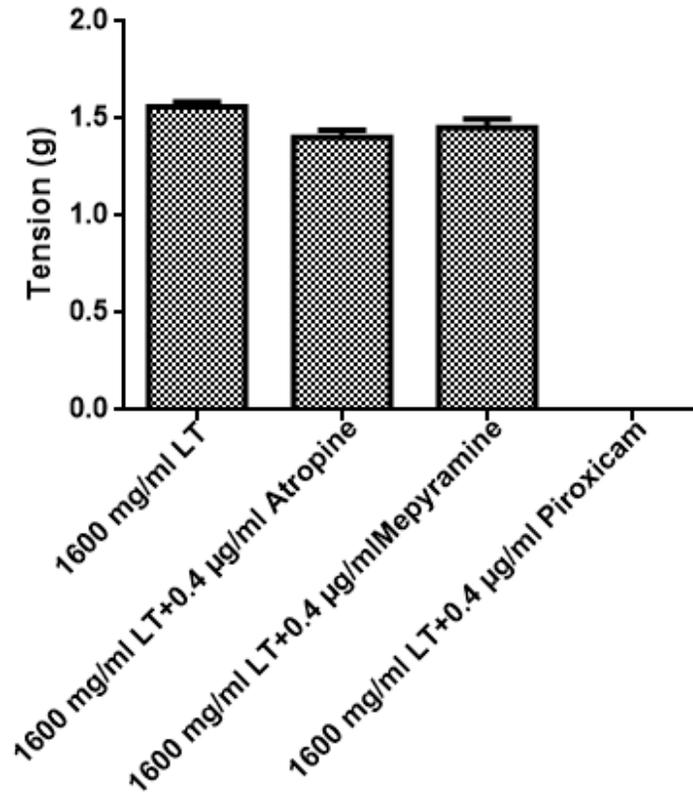


Figure 5. Mean Emax following administration of *L. taraxacifolia* at 1600 mg/ml in pregnant rat uterus in the presence of atropine, mepyramine and piroxicam. Atropine caused the least inhibition followed by mepyramine. Piroxicam completely abolished the effect of *L. taraxacifolia*.

Relative potency of *L. taraxacifolia* as uterotonic

In Table 1, the relative potency of *L. taraxacifolia* was compared to other uterotonic. *L. taraxacifolia* at 1600 mg/ml was 0.08 times more potent than 0.4 µg/ml oxytocin in the non-pregnant rat uterine strips. The Emax produced following administration of 1600 mg/ml *L. taraxacifolia* on non-pregnant rats uterine strip was 1.620 ± 0.048 g. Meanwhile, the Emax produced following administration of 0.4 µg/ml oxytocin, 0.4 µg/ml acetylcholine and 0.4 µg/ml histamine were 1.495 ± 0.053, 1.318 ± 0.032 and 0.909 ± 0.046 g, respectively. In the pregnant rat uterine strip, however, oxytocin was 0.17 times more potent than 1600 mg/ml *L. taraxacifolia*

DISCUSSION

This study has shown the uterotonic effect of aqueous extract of *L. taraxacifolia* and the possible mechanism of action. To the best of our knowledge, this study is the first to display this effect, which justifies the claim that this plant eases birthing process and alleviate labour pain. *L.*

taraxacifolia at 1600 mg/kg was 0.08 times more potent than oxytocin, which is a gold standard uterotonic (Sheldon et al., 2012) in non-pregnant rats. This was however not the case with the pregnant rat uterus. The reason for this is not fully understood but may be due to the presence of different component in the crude extract used, some of which may even have antagonistic effect. Chan et al. (1988) reported that in gravid uterus, just prior to term labour, the parturient uterus usually becomes highly active and responsive to oxytocin. The agonist (oxytocin), been a pure compound will therefore likely result in greater uterine contraction.

The results obtained showed that *L. taraxacifolia* effect is mediated via muscarinic, oxytocin and histamine receptors. The presence of these receptors in the uterus has been previously established (Hay et al., 2010; Abdalla et al., 2004; Kobayashi et al., 1999). These mechanism were confirmed from inhibition of Emax produced by 1600 mg/ml *L. taraxacifolia* following administration of the antagonists to these receptors. Our findings suggest that LH-induced uterine contraction was mediated mainly via the oxytocin receptor. This is because piroxicam, an antagonist of oxytocin completely

Table 1. Relative potency of *L. taraxacifolia* as compared to other uterotonins.

Agent	Tension (g)	
	Non-pregnant	Pregnant
<i>L. taraxacifolia</i> (1600 mg/ml)	1.620±0.048	1.596±0.026
Oxytocin	1.495±0.053	1.916±0.043
Acetylcholine (Ach)	1.318±0.037	1.053±0.048
Histamine	0.909±0.046	1.013±0.034

L. taraxacifolia was 0.08 times more potent than oxytocin in non-pregnant rats while oxytocin was 0.17 more potent than *L. taraxacifolia* in pregnant rat uterus.

abolished the effect of the aqueous extract. Moderate inhibition of the Emax by atropine and mepyramine suggest that LH binding to muscarinic and histamine receptor produced a moderate degree of contraction. There is a possibility that the greatest effect of *L. taraxacifolia* produced following it binding to the oxytocin receptors (as evidenced by the complete inhibition of the Emax by piroxicam) was due to a high number of this receptor expression in the uterus. Previous studies have shown that of all the receptors reported to be present in the uterus, the oxytocin receptor expression in the uterus is the highest (Sanborn, 2001; Grigsby et al., 2006). This is however, not the case with *F. asperifolia* which elicited uterotonic activity via the histamine receptor (Watcho et al., 2011), *M. cecropiodes*, *M. ciliatum* and *Agapanthus africanus* which elicited their uterotonic activity significantly via the muscarinic and oxytocin receptors (Uguru et al., 1998; Ayinde et al., 2006; Veale et al., 1999), and *Ananas comosus* which elicited its uterotonic activity through serotonergic pathway (Monji et al., 2016; Monji et al., 2018).

Pharmacological activities observed in plant extracts are due to the presence of various secondary metabolites they contain (Ayinde et al., 2006). The observed uterine contractility effect in this extract is invariably due to these secondary metabolites. The phytochemical analysis of *L. taraxacifolia* revealed the presence of saponins and steroids both of which have been shown to possess uterine stimulating effect (Watcho et al., 2011; Guo et al., 2008). Similar investigation of different plants with uterotonic properties including *F. asperifolia* (Watcho et al., 2011; *M. ciliatum* (Uguru et al., 1998), *M. cecropiodes* (Ayinde et al., 2006) and *Nymphaea alba* (Bose et al., 2014) all revealed the presence of saponins, tannins, flavonoids and steroids. Tannins, flavonoids and saponins have been shown to be present in uterotonic plants such as *Ficus deltoidea* (Amiera et al., 2014) and *Calotropis procera* (Shamaki et al., 2015). Tannins are thought to elicit their uterotonic effect through affecting calcium availability for uterine tissue and cardiac muscle contraction (Polya et al., 1995; Calixto et al., 1986) while flavonoids, on the other hand, act directly on oestrogen receptors to cause uterine contraction (Revuelta et al., 1997).

The oral acute toxicity studies show that it is safe and well tolerated in rats as no sign of toxicity was observed in all the treated animals. A study which assessed the safety of the ethanol extract of *L. taraxacifolia* in rodents revealed no negative effect on physical, hematological and serum biochemical parameters when doses ranging from 10-5000 mg/kg were administered (Kuatsienu et al., 2012). The findings in this study suggest that the use of *L. taraxacifolia* should be contra-indicated in pregnancy. It however authenticates the folkloric obstetric use of the plant for the induction or acceleration of labour as well as expelling retained placenta among pregnant women in Sokoto, North-west Nigeria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Chemical composition of *Cyperus esculentus* nut and *Phoenix dactylifera* fruit

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This study investigated the chemical composition of *Cyperus esculentus* nut and *Phoenix dactylifera* fruit. The nuts and fruits were purchased as commonly sold in Wukari, Nigeria. They were cleaned, sorted for healthy parts, sun-dried and milled into fine powder. The AOAC, AAS and GC-MS methods were adopted for the proximate, mineral and phytochemical analysis, respectively. The results show that *C. esculentus* nut is higher in percentage moisture, crude protein, crude fiber and lipid compared to *P. dactylifera* fruit, while *P. dactylifera* fruit is higher in percentage dry matter, ash and carbohydrates. *C. esculentus* nut contain higher level (ppm) of magnesium, manganese, copper, zinc and phosphorus than *P. dactylifera* fruit. Potassium, sodium, calcium, chromium and iron are more abundant in *P. dactylifera* fruit. The phytochemical result shows the presence of varieties of chemicals. *C. esculentus* nut and *P. dactylifera* fruit contain certain phytochemicals that are food additives and some that are known to possess anti-inflammatory, hypotensive, antimicrobial, antioxidant, lipid moderating properties and immune booster. Both plant materials contain appreciable amount of macronutrients which are very essential for provision of energy and nourishment of human body system. *C. esculentus* nut and *P. dactylifera* fruit are recommended in general nutrition.

Key words: *Cyperus esculentus*, mineral, nutrition, *Phoenix dactylifera*, phytochemical, proximate.

INTRODUCTION

The use of many plant materials which are consumed raw or processed in different forms before consumption as food in human daily food is already on the increase. Most of these plant materials are widely known to be efficient in treatment and management of different forms of diseases. Many are used to improve human health or immune system.

Phoenix dactylifera L, commonly known as Date palm is a member of palm family Arecaceae, and it is one of the major staple crops in Africa and parts of Asia. Date palm grows in many parts of the world. The fruit contains a single seed. It has been reported to grow in South America, parts of the United States, Mexico, Africa and Australia (Al-Harrasi et al., 2014; Hazzouri et al., 2015).

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Figure 1. Photographs of *Cyperus esculentus* nuts (A) and *Phoenix dactylifera* fruits (B).

In Nigeria, it is referred to as “dabino” by the Northerners who often sell it at different markets and strategic locations. The fruit is sweet, oval-cylindrical in shape and serve as energy booster. The name (*dactylifera*) of the species is used to explain the clustered nature (usually referred to as “finger-bearing”) of the fruits produced by the plant. The production of the fruits has increased over the years because of its increasing demand and because it is economical. The fruit is believed to possess lots of medicinal properties. The fruits may be eaten alone or in conjunction with other food materials. Date fruit has been reported to be of high nutritional value and also a good source of vitamin B complex (Eoin, 2016). Therapeutic properties of date fruits, such as anti-proliferative, antibacterial, antioxidant and antifungal have been reported by Al-Alawi et al. (2017). It is believed to be a good aphrodisiac.

Cyperus esculentus L. (Tiger nut) is an edible tuber crop of Cyperaceae family that produces rhizomes which are usually spherical (Devries and Feuke, 1999). Tiger nut is used widely in Africa, America, Arabian Peninsula and parts of Europe for human and other animal consumption (Abaejoh et al., 2006; Sanchez-Zapata et al., 2012). It grows freely and is usually cultivated in Northern Nigeria. In Nigeria, it is called “Aki Hausa” in Igbo, “Aya” in Hausa and “Ofio” in Yoruba. There are two major varieties that are commonly sold in the market: the fresh (yellow/milky colour) and dried (brown colour). These varieties can be processed and eaten in different forms. They can be processed into flavouring agent, milk, yoghurt and other forms of drink (Ezeonu et al., 2016). It can also be used in preparing livestock feed.

Phytochemical analysis of plant materials helps in revealing the medicinal and pharmacological properties

of plant, while proximate composition of plant samples contributes to the overall level of acceptance of plant materials as good sources of food in general nutrition. *C. esculentus* nut and *P. dactylifera* fruit are widely eaten in its raw form as food in many parts of the world and also used in preparation of different drinks. They are the major ingredients in the popular nutritious drink in Nigeria known as “Kunun aya.” Investigation into the phytochemical, mineral and proximate composition of *C. esculentus* nut and *P. dactylifera* fruit will guide in knowing their possible diverse physiological and pharmacological effects as well as the nutritional value and overall acceptance of the plant parts from the users’ stand point. This therefore warrants study into the chemical composition of *C. esculentus* nut and *P. dactylifera* fruit.

MATERIALS AND METHODS

Plant

C. esculentus nut and *P. dactylifera* fruit were bought at New Market, Wukari, Nigeria. They were identified at the Biological Science Department, Federal University Wukari, Nigeria. The seeds in the fruits of *P. dactylifera* were removed and discarded. The plant materials (Figure 1) were sun-dried, milled with manual blender and stored in air-tight containers until required for analysis.

Preparation of plant extracts

Each plant powder was macerated in 70% ethanol for 48 h with occasional shaking, thereafter filtered and the filtrate concentrated to eliminate the ethanol. The crude plant extracts were then used for phytochemical analysis.

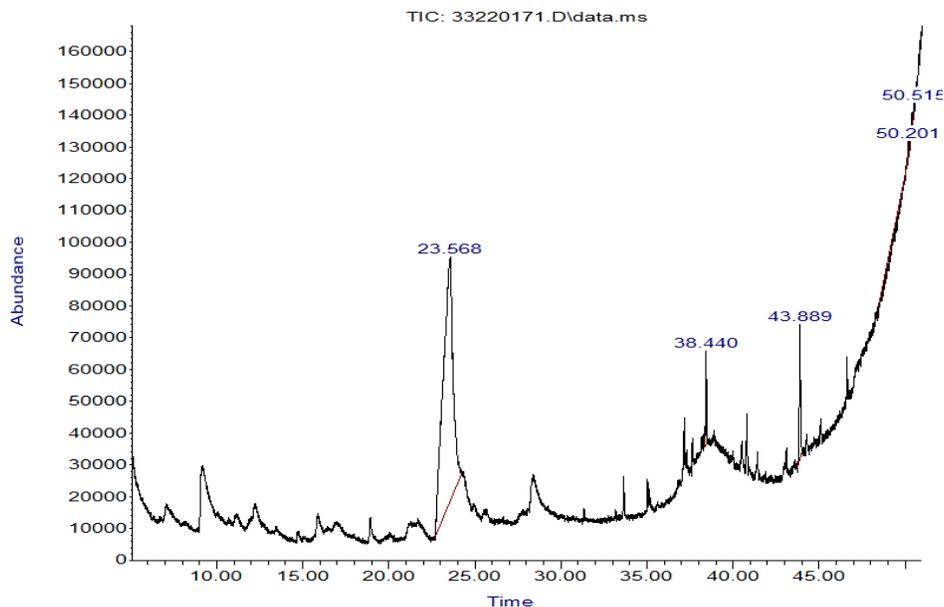


Figure 2. GC-MS chromatogram of ethanolic extract of *Cyperus esculentus* nut (Tiger nut)

Determination of proximate and mineral composition of *C. esculentus* nut and *P. dactylifera* fruit.

The percentage protein, lipid, fiber, ash, moisture, carbohydrates and dry matter of both plant materials were determined using the method of AOAC (2010), while the amount of magnesium, calcium, manganese, chromium, copper, zinc, iron, potassium, sodium and phosphorus in both plant samples were carried out using atomic absorption spectroscopy (model AA280FS), product of Agilent Technologies, U.S.A. The temperature and the inert argon gas flow followed were as recommended by the manufacturer.

Determination of phytochemical composition of *C. esculentus* nut and *P. dactylifera* fruit.

The phytochemical analysis of ethanolic extracts of both plant materials were carried out using gas chromatography-mass spectrometry (GC-MS). The GC (model no 7890B) and MS Detector (model 5977A) were products of Agilent Technologies, U.S.A. It was equipped with column: Agilent HP 5MS ultra Inert (350°C) 30 m × 250 μm × 0.25 μm. The gas used was Helium (He) with flow: 0.7 ml/min, pressure: 4.4867 psi, average velocity: 30.641 cm/s. The injection volume was 1 ml; inlet temperature 250°C; split ratio 20:1, and split flow 14 ml/min. Oven temperature was 60°C with equilibrating time of 1 min; maximum oven temperature 350°C and total run time 35.857 min. The MS tune type is E1; start mass 50; stop mass 550; threshold 150; acquisition type: scan and frequency (scan/s) 2.9. The constituent phytochemicals were identified after matching the spectra with the mass spectra of reference compounds contained in the database of the National Institute of Standards and Technology (NIST 14). The amounts of individual chemical components suggested were expressed as area percent comparable to the total peak area.

Statistical analysis

After the proximate analysis, the results were analyzed statistically

using ANOVA and Paired-Samples T Test using Statistical Package for Social Sciences (SPSS) version 21. Means for each parameter were compared for significance at $p \leq 0.05$ and result presented as mean \pm standard deviation (SD).

RESULTS

The result of proximate analysis shows that *C. esculentus* nut has higher percentage of moisture (4.31 ± 0.11), crude protein (6.06 ± 0.11), crude fiber (10.12 ± 0.28) and lipid (7.46 ± 0.13) compared to *P. dactylifera* fruit, while *P. dactylifera* fruit has higher percentage of dry matter (96.95 ± 0.06), ash (1.97 ± 0.02) and carbohydrates (85.10 ± 0.60) compared to *C. esculentus* nut.

C. esculentus nut contain higher level of magnesium (6.520 ± 0.0006 ppm), manganese (0.084 ± 0.0004 ppm), copper (0.047 ± 0.0002 ppm), zinc (0.763 ± 0.0001 ppm) and phosphorus (2.060 ± 0.0394 ppm) than *P. dactylifera* fruit. Potassium and sodium are more abundant in *P. dactylifera* fruit with 7.067 ± 0.0817 and 4.300 ± 0.1225 ppm, respectively. Calcium, chromium and iron are also more abundant in *P. dactylifera* fruit than in *C. esculentus* nut.

DISCUSSION

This study shows important chemicals present in tiger nut (Table 3 and Figure 2). Some of the phytochemicals are used for discovery of drugs (as starting materials) and also in modern and traditional medicine (Imo and Uhegbu, 2015). 9-Octadecenoic acid is believed to possess hypotensive effect. It is also associated

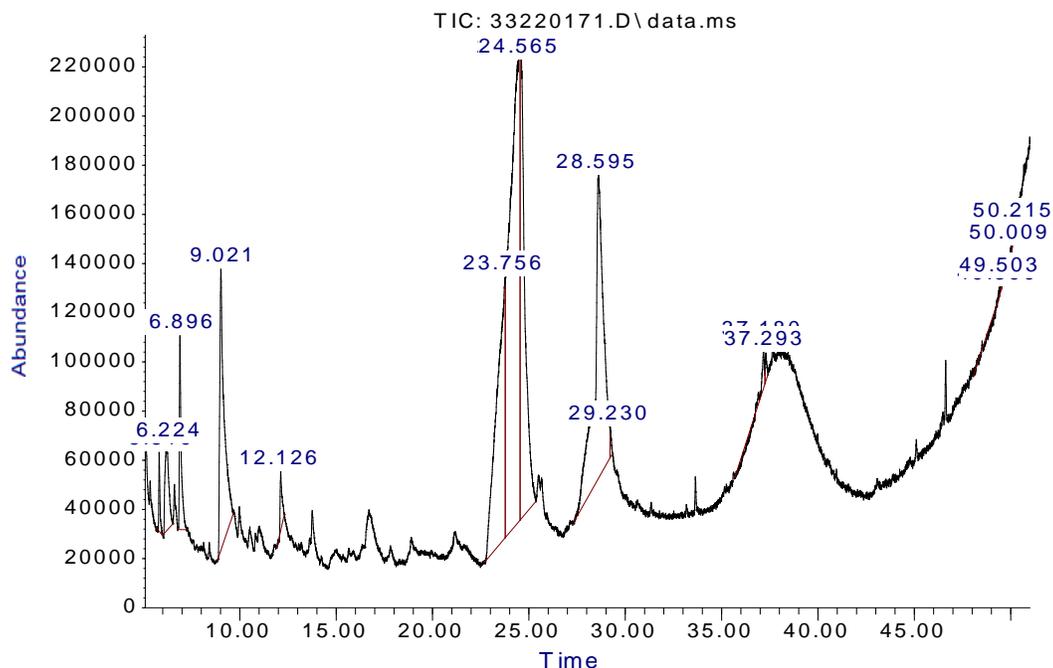


Figure 3. GC-MS chromatogram of ethanolic extract of *Phoenix dactylifera* fruit (Date fruit).

with increased high-density lipoprotein (HDL) cholesterol and possibly reduced low-density lipoprotein (LDL) cholesterol.

Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl is an organic compound that possess antimicrobial activity (Kumaradevan et al., 2015). Hexadecane, 1-(ethenyloxy)- is a component of essential oil. Hexadecane has been reported to exhibit beta-oxidant, thermogenic and anti-inflammatory properties (Callaghan et al., 2009). A variety of hexadecane, 1-(ethenyloxy)- known as Cis-9-Tetradecenoic acid, heptyl ester is a component of adipose tissue triacylglycerol in human (Jiang et al., 1999). It has been reported that it may be used for the treatment of prostate cancer due to its cell death inducer and cytotoxic effect (Iguchi et al., 2001). 9-Octadecenal, (Z)- is a food additive. Oxalic acid, monoamide, n-propyl, dodecyl ester: the conjugate base of oxalic acid (oxalate) is a competitive inhibitor of lactate dehydrogenase, while monoamide is believed to be a neurotransmitter. Its variety known as 2-Ethyl-oxetane is used in drug development, while certain derivatives of it possess antibiotic activity. The properties of these various phytochemical component show that *C. esculentus* nut (tiger nut) may possess anti-microbial, anti-inflammatory, antioxidant, lipid moderating and immune boosting effects. The vitamin E content of tiger nut is reported to collaborate against “the bad cholesterol” because of its antioxidant effect over fats, which is important for coronary heart disease (Chukwuma et al., 2010). Tiger nut has also been documented to aid in activating the circulation of blood, prevention of heart disease, treatment

of bacterial infection and urinary tract infection (Adejuyitan et al., 2009).

Certain chemicals present in date fruit (Table 4 and Figure 3) possess important properties such as anti-microbial and anti-oxidant. 1-Pyrrolidinecarbonitrile has been reported to exhibit potent anti-hyperglycaemic activity (Villhauer et al., 2002). Palmitoleic acid possesses anti-apoptotic activity. 2(1H)-Pyrimidinone which is a variety of 1-Pyrrolidinecarbonitrile has been reported to possess antitumor properties (Driscoll et al., 1991), while (S)-(+)-2-Amino-3-methyl-1-butanol is used for synthesis of benanomicin-pradimicin antibiotics (Tamiya et al., 2007). Some derivatives of 2-Ethyl-oxetane have antibiotic activity. 2R,3S-9-[1,3,4-Trihydroxy-2-butoxy methyl] guanine possess anti-tumour, anti-inflammatory, anti-microbial and anti-oxidant activities (Teoh and Mashitah, 2013). 5-Eicosene (E)-possesses antibacterial property. 2(5H)-Furanone, 5-methyl-; propanoic acid, 2-propenyl ester, 2-ethylhexanal; and butanoic acid, 3-oxo-, propyl ester are used as flavouring agents.

Proximate analysis of *C. esculentus* nut (Tiger nut) showed the percentage abundance of the parameters evaluated to be dry matter (95.70 ± 0.11) > carbohydrates (70.17 ± 0.11) > crude fiber (10.12 ± 0.28) > lipid (7.46 ± 0.13) > crude protein (6.06 ± 0.11) > moisture content (4.31 ± 0.11) > ash content (1.90 ± 0.04). Proximate analysis for *P. dactylifera* fruit (date fruit) showed the percentage abundance of the parameters evaluated to be dry matter (96.95 ± 0.06) > carbohydrates (85.10 ± 0.60) > lipid (3.57 ± 0.16) > crude

Table 1. Proximate composition of *Cypercus esculentus* nut and *Phoenix dactylifera* fruit (%).

Composition	<i>C. esculentus</i> nut (Tiger nut)	<i>P. dactylifera</i> fruit (Date fruit)
Moisture content	4.31 ± 0.11 ^a	3.08 ± 0.03 ^b
Dry matter	95.70 ± 0.11 ^a	96.95 ± 0.06 ^b
Crude protein	6.06 ± 0.11 ^a	3.10 ± 0.13 ^b
Crude fiber	10.12 ± 0.28 ^a	2.99 ± 0.23 ^b
Lipid	7.46 ± 0.13 ^a	3.57 ± 0.16 ^b
Ash content	1.90 ± 0.04 ^a	1.97 ± 0.02 ^a
Carbohydrates	70.17 ± 0.11 ^a	85.10 ± 0.60 ^b

Values are mean ± standard deviation (n=3). Mean in the same row, with different letters of the alphabet as superscript are statistically significant ($p < 0.05$).

protein (3.10 ± 0.13) > moisture content (3.08 ± 0.03) > crude fiber (2.99 ± 0.23) > ash content (1.97 ± 0.02) (Table 1).

Carbohydrate is very essential for energy provision to animals and also for the nourishment of plants and animals (Edeoga et al., 2005). The high carbohydrates and lipid content imply that tiger nut and date fruit are good sources of energy. Therefore, they can play vital roles in the sustenance and nourishment of animal body. This is the reason why there is energy gain after their consumption. Date fruit is highly consumed by those who engage in fasting prior to and at the end of their fasting period because of its high energy supply which is as a result of this high carbohydrates content. The carbohydrates are readily hydrolysed to reducing sugar: a good source of ATP generation. Comparative analysis shows that date fruit contains higher amount of carbohydrates than tiger nut, but lower lipid content than tiger nut. These two plant products are commonly used in preparation of a nutritious drink known as "kunun aya" in Northern Nigeria. Their high carbohydrates and lipid content confirm the reason why the drink made from date fruit and tiger nut could quench hunger after consumption and could supply the needed glucose to produce ATP for work. Date fruit has been reported to possess potential health benefits (Chao and Krueger, 2007; Al-Harrasi et al., 2014).

Tiger nut possesses significant higher fiber content than date fruit. The high fibre content of tiger nut suggests it will aid in the reduction of constipation and can enhance frequent bowel content elimination. However, consumption of high fibre may decrease nutrient utilization, cause intestinal irritation and lower digestibility. This is why excessive consumption of tiger nut may cause stomach discomfort to some consumers. The protein content of tiger nut is almost twice of that in date fruit. Combination of the two plant products in nutrition could complement each other. In biological systems, proteins perform several pharmacological and physiological roles. The protein content of tiger nut suggests it could be a better source of various vital amino acids, but both plant materials can play important role in

growth regulation, immunological protection, enzymatic catalysis and general nutrition. This is because protein is required in these processes.

Both fresh plant materials (as commonly sold in the market) show tiger nut possesses higher moisture content than date fruit. The result shows that date fruit will last longer than tiger nut when stored. The moisture content of the fresh tiger nut which is 4.31% may contribute to the reason why it does not have a long shelf life when stored afresh and also why it usually deteriorate fast (within few days). This is believed to be one of the major reasons why some traders process some of the fresh materials into dried form. This helps to preserve and elongate its shelf life by reducing the condition that may permit increased microbial activities which usually result to its deterioration. There is no statistical difference between the ash content of both plant materials, although date fruit has mildly higher ash than tiger nut. The ash content shows the possible corresponding mineral level in date fruit and tiger nut, which have been elucidated in this study. The proximate results show the important need for the use of date fruit and tiger nut in general nutrition.

The mineral analysis showed appreciable levels of the different mineral elements evaluated (Table 2). Mineral analysis of *C. esculentus* nut (tiger nut) showed the abundance (ppm) of the different minerals evaluated to be in magnesium (6.520 ± 0.0006) > potassium (5.567 ± 0.1206) > sodium (4.000 ± 0.0866) > calcium (2.155 ± 0.0007) > phosphorus (2.060 ± 0.0394) > iron (1.846 ± 0.0015) > zinc (0.763 ± 0.0001) > chromium (0.201 ± 0.0003) > manganese (0.084 ± 0.0004) > copper (0.047 ± 0.0002) order. The mineral analysis for *P. dactylifera* fruit (date fruit) showed the abundance (ppm) of the different minerals evaluated to be in potassium (7.067 ± 0.0817) > magnesium (4.922 ± 0.0004) > sodium (4.300 ± 0.1225) > calcium (4.021 ± 0.0006) > iron (2.238 ± 0.0007) > phosphorus (0.980 ± 0.0430) > zinc (0.229 ± 0.0004) > chromium (0.210 ± 0.0007) > manganese (0.051 ± 0.0003) > copper (0.037 ± 0.0002) order.

Mineral elements are inorganic substances which are very necessary for proper immune function and proper

Table 2. Mineral composition of *C. esculentus* nut and *P. dactylifera* fruit (ppm).

Mineral	<i>C. esculentus</i> nut (Tiger nut)	<i>P. dactylifera</i> fruit (Date fruit)
Magnesium	6.520 ± 0.0006	4.922 ± 0.0004
Calcium	2.155 ± 0.0007	4.021 ± 0.0006
Manganese	0.084 ± 0.0004	0.051 ± 0.0003
Chromium	0.201 ± 0.0003	0.210 ± 0.0007
Copper	0.047 ± 0.0002	0.037 ± 0.0002
Zinc	0.763 ± 0.0001	0.229 ± 0.0004
Iron	1.846 ± 0.0015	2.238 ± 0.0007
Potassium	5.567 ± 0.1206	7.067 ± 0.0817
Sodium	4.000 ± 0.0866	4.300 ± 0.1225
Phosphorus	2.060 ± 0.0394	0.980 ± 0.0430

Values are mean ± standard deviation (n=3).

Table 3. Phytochemical constituents of *C. esculentus* nut (Tiger nut).

Name of compound	RT (min)	Area (%)
Oxalic acid, monoamide, n-propyl, dodecyl ester	23.568	84.500
9-Octadecenoic acid	38.440	3.380
9-Octadecenal, (Z)-	43.889	6.220
Hexadecane, 1-(ethenyloxy)-	50.201	4.370
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- hexadecamethyl	50.515	1.530

Table 4. Phytochemical constituents of *P. dactylifera* fruit (Date fruit).

Name of compound	RT (min)	Area (%)
1-Pyrrolidinecarbonitrile	5.819	0.600
(S)-(+)-2-Amino-3-methyl-1-butanol	6.224	2.320
2(5H)-Furanone, 5-methyl-	6.896	2.540
Propanoic acid, 2-propenyl ester	9.021	8.730
2-Ethylhexanal	12.126	0.730
2-Ethyl-oxetane	23.756	15.290
d-Mannitol, 1,4-anhydro-	24.483	32.840
Acetamide, N-butyl-	24.565	15.910
Butanoic acid, 3-oxo-, propyl ester	28.595	19.620
2R,3S-9-[1,3,4-Trihydroxy-2-butoxy methyl]guanine	29.230	0.230
5-Eicosene, (E)-	37.180	0.210
Palmitoleic acid	37.293	0.300
Cyclotetradecane	49.399	0.330
3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	49.503	0.080
Hexadecane, 1-(ethenyloxy)-	50.009	0.170
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	50.215	0.100

maintenance of some biological processes that are required to sustain the life of human and other vertebrates (Soetan et al., 2010). The minerals do not yield energy but are essential for vital life processes. They are grouped into two major classes: micro and

macro elements. Macro-minerals analysed in tiger nut and date fruit include phosphorus, calcium and sodium, while the micro-minerals include potassium, iron, manganese, copper, magnesium, chromium and zinc. When these essential minerals are deficient in certain

animals such as human, it usually predisposes the animal to certain disease conditions. However, if these minerals are taken in excess, they may cause disruption of homeostatic balance and may also induce some dangerous side effects.

The moderate levels of sodium and potassium show that consumption of date fruit and tiger nut is suitable for maintenance or regulation of osmotic balance between interstitial fluid and bodily cells. The sodium and potassium level of date fruit is slightly higher than tiger nut. Calcium and phosphorus are required by humans and some other vertebrates in appreciable (large) amounts for proper muscle and nerves function, and also for maintenance and construction of bone and teeth. Phosphorus is also essential since it is a component of nucleic acid and adenosine triphosphate (Soetan et al., 2010). Consumption of date fruit and tiger nut could help in supplying calcium and phosphorus to the body system for these important functions. Phosphorus content of tiger nut is higher than that of date fruit.

Iron is required in small amount by humans. Its levels in date fruit and tiger nut are low compared to the macro-minerals evaluated. This shows the use of date fruit and tiger nut in nutrition could help supply iron which is required for the synthesis of haemoglobin (pigment carrying oxygen) needed for proper functioning of red blood cells and for active cellular respiration. The higher level of iron in date fruit shows it will play these important roles more than tiger nut. The cytochromes are known to require iron as a crucial component.

Magnesium, zinc, manganese, chromium and copper are important micro-minerals required by some enzymes by acting as co-factors required for various biochemical processes or pathways. Although, these micro-minerals are low in amounts (except magnesium) in date fruit and tiger nut. Their levels are moderate in regards to the amounts required by humans, since humans require them in small quantities. The result shows that tiger nut and date fruit could aid the proper function of some enzymes required for certain important biochemical processes. However, adequate caution should be taken in the consumption of foods that contains these minerals, since excess or accumulation of some of the minerals could result to toxicity which could be accompanied with many side effects.

Conclusion

Cyperus esculentus nut contains phytochemicals that function as food additives and some that possess anti-inflammatory, hypotensive, antimicrobial, antioxidant, lipid moderating and immune boosting effects. *P. dactylifera* fruit contains phytochemicals that are food additives and possess important properties such as anti-microbial and anti-oxidant, anti-apoptotic, antitumor and anti-hyperglycaemic activities. Both plant materials contain

appreciable amount of carbohydrates, proteins and lipids which are very essential for energy provision to animals, nourishment of human body system and other vertebrates, repair and replacement of dead cells, worn tissues and hormone production. Mineral elements present in date fruit and tiger nut are very essential for proper immune function and proper maintenance of some biological processes that are required for sustenance of life. Consumption of these plant materials will supply some of the minerals which aid maintenance of homeostatic balance, proper nerves and muscle function, maintenance of bone and teeth, and required by some enzymes as co-factors for various biochemical processes. The use of tiger nut and date fruit is recommended in general nutrition and will help improve human health system.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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