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Research Article

Antidiabetic Potential of Aerial Parts of *Mollugo pentaphylla* L. *and* Characterization of a β-Amyrin Type of Triterpenoid

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ABSTRACT

The aim of this work is to evaluate antidiabetic potential of aqueous extract and its various fractions prepared from the aerial parts of *Mollugo pentaphylla* L. Additionally, isolation and characterization of a β-amyrin type of triterpenoid from aqueous extract of this plant was also studied. The aqueous extract and its fractions obtained from column chromatography (excluding the n-hexane fraction) have significant (p<0.01) effet on glucose reduction. The n-butanol, ethyl acetate and ethanol fractions of the aqueous extract of aerial parts of *Mollugo pentaphylla* at levels 200 mg/kg body weight showed significant antidiabetic properties in alloxan induced albino Wistar male rats. The isolated compound obtained from aqueous extract was characterized using different spectral analytical methods. Data of fourier transform infrared (FTIR) spectroscopy, proton-nuclear magnetic resonance (^{1}H-NMR) spectroscopy, ^{13}C-NMR spectroscopy and liquid chromatography–mass spectrometry (LC-MS) of an isolated compound are in support of the presence of a β-amyrin triterpenoid nucleus, substituted with a sugar moiety resulting in the triterpenoid saponin. On the basis of the chemical and spectral evidences, the nomenclature of the triterpenoid saponin is, 2, 2, 6a, 6b, 9, 9, 12a-heptamethyl-10-(4′, 5′, 6′-trihydroxy-3′-(3″, 4″, 5″, 6″-tetrahydroxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy)-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2H-picene-4a-carboxylic acid. The structural novelty is the absence of the carboxyl group of the oleanolic acid in the present compound at the 'C-4a position', configurating it a different β-amyrin triterpenoid.

Keywords: *Mollugo pentaphylla*, spectral analytical methods, triterpenoid, β-amyrin.

INTRODUCTION

Mollugo pentaphylla L. (Aizoaceae, carpet weed in English and Pitta saga, locally) (Fig. 1) is a perennial herb found throughout India, Ceylon, Malacca, China, Japan and Fiji. It is a procumbent herb with adventitious roots and trifoliate leaves with small, obvate/clubshaped leaflets of size around 1 cm, maximally; flowers are white, pentamerous and bisexual. Further, the plant is eaten as a bitter vegetable occasionally; and it is medicinally, stomachic, aperient, antiseptic and antiulcertive[1]. Ethnopharmacologically, leaf-paste is taken orally and applied externally for the treatment of skin allergy[2]. The plant is an emmenagogue on females in India and Indonesia[3]. Hot aqueous extract of dried entire plant in India is used for whooping cough. It has antimicrobial/ antibacterial ability, as it is used for oral infections[4]. An antifungal triterpenoid from the plant had been too recorded[5]. Decoction of the entire plant is used against hepatitis in Taiwan[6]. *Mollugo pentaphylla*, with the folklore name, *Peh-Hue-Juwa-Chi-Cao* in Taiwan, is used as an antineoplastic, antitoxic and diuretic agent[7,8]. Anti-inflammatory, analgesic and antipyretic effects[9,10], and antipyretic activity of the plant in albino mice were too recorded[11]. Protective effect of the whole plant against carbon tetrachloride induced hepatotoxicity in rats had been reported[12]. *In vitro* antihelminthic activity of *Mollugo pentaphylla* is recorded[13]. Spermicidal activity of an antifungal saponin was obtained from this herb[14]. The plant is reported to contain flavones (apigenin and mollupentin), mollugogenol A, triterpenoid, mollugogenol B, mollugogenol D, oleanolic acid, and a steroid the β-sitosterol[15]. Usually, an aqueous extract contains comparatively more polar phytochemicals than other solvent extracts of a plant[16]. Herein, we report the characterization of a novel isolated compound of the aqueous extract of the aerial parts of the plant, after due spectral analysis, fourier transform infrared (FTIR) spectroscopy, proton-nuclear magnetic resonance (^{1}H-NMR) spectroscopy, ^{13}C-NMR spectroscopy and liquid chromatography–mass spectrometry (LC-MS) of the isolated compound that support the presence of β-amyrin type triterpenoid nucleus, substituted with a sugar moiety

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Figure 1: *Mollugo pentaphylla* L.

resulting in a novel triterpenoid saponins, whose nomenclature is given. Further, the present study aims to evaluate antidiabetic potential of the crude plant-extracts and its bioactive fractions, derived using column chromatography with solvents, n-hexane, n-butanol, ethyl acetate and ethanol along with the standard antidiabetic drug, glibenclamide. This plant with a bitter taste is edible as a vegetable locally, and it has been known to have the antidiabetic property in the local cultural memory as the ethnic information. But this plant has not been vividly studied for a scientific evaluation previously. The bio-potentiality of the eluted ethanolic fraction is demonstrated here as a potent source of antidiabetic drug with the iconic animal model of male albino Wistar rats, a study never done before for this plant.

MATERIALS AND METHODS

Extraction and isolation

A lot of 750 g powdered aerial parts of *Mollugo pentaphylla* L. (voucher specimen registration number, SPS/SOAU/2008/005) was defatted with petroleum ether at 60-80 ^{0}C, exhaustively. The concentrated plant residue was refluxed with 2 L of distilled water for 48 h, and then the mass was filtered. The filtrate was concentrated in a rotary evaporator under the reduced pressure, a dark brownish viscous mass of 32 g was obtained. Slurry of the concentrated aqueous extract of 25 g, dissolved in an aliquot of 40 mL methanol was prepared with silica gel of size 60–120 mesh, for column chromatography. The slurry was air dried and chromatographed over the silica gel column packed with 100 % n-hexane and the column was eluted successively with n-hexane with a volume of 1L, as well as with different mixtures of n-hexane: ethyl acetate, 9:1, 8:2, 7.5:2.5, 6:4, and 1:1, with volumes of 2L each. Various fractions were collected separately and were matched by TLC to check homogeneity of a particular compound. Elutions with n-hexane-ethyl acetate (1:1) were done repeatedly and eluted masses with similar *retardation factor* (*Rf*) values were pulled together, and crystallization of the pure compound was done. The isolated pure compound was subsequently subjected for the characterization through FTIR, ^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz and LC-MS procedures to ascertain its chemical structure.

Preparation of the test samples and maintenance of animals

Extract and its solvent fraction from column chromatography of *Mollugo pentaphylla* and the standard drug glibenclamide (2.5 mg/kg) were suspended separately in 25 % Tween 20 in distilled water and used as test drug for oral administration. Healthy male albino Wistar rats, weighing 150–200 g body weight were collected. The selected animals were housed in acrylic cages at temperature 20–25 °C and relative humidity 45-55 % under 12 h light/dark cycle, fed with standard rodent diet for one week in order to adapt to the laboratory conditions and water *ad libitum.* The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols.

Evaluation of the extract and its fractions in oral glucose tolerance test

Oral glucose tolerance test (OGTT) was followed by a method followed often[17]. Healthy rats were Balkanized into seven groups of six animals each: Group I served as solvent control and received only the vehicle, Tween+water at 2 ml/kg body weight (bw). Group II received glibenclamide at the dose 5 mg/kg bw. Group III received aqueous extract of *M. pentaphylla* leaves at a dose of 200 mg/kg bw. Groups IV, V, VI and VII received n-hexane, n-butanol, ethyl acetate and ethanol fractions of the aqueous extract of aerial parts of the plant at levels 200 mg/kg bw, respectively. All animals were ingested with glucose (2 g/kg) in distilled water 30 min after the administration of the test drug treatments. The blood glucose levels were measured at 0, 0.5, 1, 2, and 4 h interval, respectively.

Evaluation of the extract and major fractions in alloxan induced diabetic rats

The acclimatized animals were kept fasting for 24 h with water. Experimental diabetes was induced by a single i.p. injection at the dose of 150 mg/kg alloxan monohydrate in normal saline solution. After 1 h, the animals were provided feed *ad libitum*. After 5 days of alloxan injection, the animals with fasting blood glucose between 200-300 mg/dl were considered as diabetic and were segregated into seven groups of six rats each. Blood glucose levels of each rat were estimated at 1, 2, 4, 6, 8 and 10 h respectively[18.]

Spectral analysis

Spectral analyses were done with the following instruments: infra-red (IR) study by Bruker-FTIR-8400S spectrophotometer using KBr powder, ^{1}H NMR and ^{13}C NMR spectra with Bruker DRX-500 NMR spectrometer using trimethylsilane as the internal standard, LC-MS by Shimadzu-Mass spectrophotometer, TLC with silica gel GF_{254}, column chromatography by silica gel with the mesh size 60-120 (Merck), and elemental analysis by Perkin Elmer-2400 Auto system. Molecular weight of the compound was determined by Rast's method, which was close to the theoretical value.

Table 1: Effect of the aqueous extract and major fractions in oral glucose tolerance test in rats

Group	Treatments mg/kg b.w.	Blood glucose levels (mg/dl) 0 hr	0.5 hr	1 hr	2 hrs	4 hrs	Percent decrease at the end of 4hours
I	Solvent control (Tween + Water)	144.2 ± 5.0	135.2 ± 4.7	129.2 ± 2.9	121.5 ± 1.7	113.5 ± 2.5	21.3
II	Glibencamide, 5	151.83 ± 6.5	134.7 ± 5.3	107.0 ± 4.6	85.16 ± 2.0 ^{c}	72.33 ± 2.3 ^{c}	52.4
III	Aqueous extract, 200	142.8 ± 2.7	128.16 ± 4.2	106.2 ± 5.7 ^{b}	90.66 ± 3.6 ^{c}	81.2 ± 4.4 ^{c}	43.1
IV	n-Hexane fraction, 200	149.7 ± 3.8	139.66 ± 3.1	132.3 ± 5.4	126.5 ± 5.0	118.8 ± 2.2	20.6
V	n-Butanol fraction, 200	139.3 ± 9.7	131.33 ± 6.7	112.7 ± 3.3^{a}	99.5 ± 1.5 ^{b}	99.5 ± 1.5 ^{b}	28.6
VI	Ethyl acetate fraction, 200	152.7 ± 5.7	136.5 ± 5.9	116.3 ± 5.3	105.5 ± 4.3 ^{b}	88.7 ± 3.6 ^{b}	41.9
VII	Ethanol fraction, 200	147.8 ± 2.6	128.2 ± 4.2	106.2 ± 5.7 ^{b}	82.7 ± 3.6 ^{c}	67.5 ± 4.4 ^{c}	54.3
F-value		1.24	0.62	2.89*	13.37**	29.76**	

Values are expressed in Mean ± SEM of six animals. Dunnett's t-test and one-way ANOVA were done. The t-value denotes statistical significance at ^{a}p <0.05, ^{b}p <0.01 and ^{c}p <0.001 respectively, in comparison to Group I. F-value signifies statistical significance at *p <0.05, **p <0.01.

RESULTS AND DISCUSSION

Evaluation of extract and fractions in oral glucose tolerance test

The aqueous extract and its 4 solvent-fractions, when administered 30 min prior to glucose, produced significant reduction (p < 0.01) in the rise in blood glucose levels by OGTT. The test extract and fractions at dose level of 200 mg/kg produced 54.34 %, 41.92 %, 28.58 %, 20.6 %, 46.43 % reduction in blood glucose with respect to ethanol, ethyl acetate, n-butanol and n-hexane fractions and the crude extract, respectively. The animals of Group I had a 21.3 % decrease of blood glucose level in 4 h. However, the rest other groups with the aqueous extract or its fractions had significant to non-significant decrease in glucose levels. The n-hexane fraction, 200 mg/kg bw on treatment had no glucose reduction, in comparison to the Group I level in OGTT. Glucose levels of all treatments at 1 h had a significant reduction at p < 0.05, level, while at 2 and 4 h after drug-administration or the crude aqueous extract or its three fractions (excluding the n-hexane fraction) had the highly significant (p <0.01) dégrée of glucose reduction (Table 1).

Evaluation of extract and fractions in alloxan induced diabetic rats

With alloxan induced rats, the ameliorating effects of the aqueous and ethanolic fraction were nearer to that of the standard drug used for reducing the hyperglycemic condition, Glibenclamide at 5 mg/kg bw with percent values of decrease of 72.1 and 68.7 %, respectively. Glucose level testing was done for the total 10 h, at 1 and 2 h intervals. The aqueous extract and its fractions when administered at 200 mg/kg dose level to alloxan induced hyperglycemic rats, the percentage of fall of hyperglycemia were in the decreasing order, 64.9, 68.7, 55.7, 20.6 and 6.2 % due to the aqueous extract, ethanol, ethyl acetate, n-butanol and n-hexane fractions, respectively, at the end of the 10 h. The crude plant extract and its three fractions ethanol, ethyl acetate caused a highly significant (p < 0.001) degree of reduction of blood glucose level after 1 h of drug-feed, when compared with Group-1, as discernible from conclusions from Dunnett's t-test and one-way ANOVA (Table 2).

FTIR (KBr) cm^{-1}study

The IR absorption spectrum of the compound showed absorption peaks at 3275.95 (OH Str.), 2944.78 (CH_2 Str.), 1564.36 (CH=CH Str.), 1391.31 (CH Str.), and 1062.73 (C-O-C Str.).

3.4 ^{1}HNMR (CD_3OD, 500 MHz) study. The ^{1}HNMR study of the compound had given signals at δ 5.41: CH (d, 1H, CH, H-2″), 5.37: CH=C (s, 1H, CH=C, H-14), 5.23: COOH (s, 1H, COOH, H-4a), 5.14: CH (d, 1H, CH, H-6′), 5.11: OH (s, 1H, OH, H-6″), 5.09: OH (s, 1H, OH, H-5″), 5.08: OH (s, 1H, OH, H-4″), 5.04: CH (s, 1H, CH, H-2′, 6′, 3″), 5.01: OH (s, 1H, OH, H-4′, 5′), 4.88: CH (d, 1H, CH, H-6″), 4.85: CH (t, 1H, CH, H-5″), 4.02: CH (d, 1H, CH, H-3′), 3.91: CH (t, 1H, CH, H-4′, 5′), 3.74: CH (t, 1H, CH, H-4″), 3.38: CH (t, 1H, CH, H-10), 3.07: CH (t, 1H, CH, H-14b), 2.34: CH (t, 1H, CH, H-5), 2.30: CH (t, 1H, CH, H-6), 1.98: CH (s, 2H, CH, H-1), 1.91: CH (d, 2H, CH, H-13), 1.77: CH (q, 2H, CH, H-11), 1.63: CH (t, 2H, CH, H-4), 1.52: CH (t, 2H, CH, H-3), 1.43: CH (t, 1H, CH, H-12b), 1.40: CH (t, 2H, CH, H-8), 1.36: CH (t, 2H, CH, H-7,12), 1.22: CH (s, 3H, CH_3, H-6a), 1.20: CH (s, 3H, CH_3, H-9), 6b, 12a, 0.91: CH (s, 3H, CH_3, H-2), and 0.77: CH (t, 1H, CH, H-8a) ppm.

^{13}C NMR ($CDCl_3$, 125 MHz) study

Table 2: Effect of the aqueous extract and major fractions in alloxan induced diabetic rats

Group	Treatments, mg/kg b.w.	Blood glucose levels (mg/dl)							Percentage decrease at the end of 10 hours
		0 hr	1 hr	2 hrs	4 hrs	6 hrs	8hrs	10 hrs	
I	Solvent control (Tween+water)	271.7 ± 10.0	272.5 ± 5.0	282.5 ± 4.9	278.2 ± 9.2	287.3 ± 13.1	269.7± 14.1	268.8 ± 9.8	0.029
II	Glibencamide,5	282.7 ± 2.6	225.2 ± 4.0[b]	176.2 ± 5.2[c]	114.3 ± 6.6[c]	105.8 ± 7.1[c]	88.3 ± 3.3[c]	78.8 ± 7.2[c]	72.1
III	Aqueous extract, 200	264.2 ± 6.7	226.8 ±11.8[b]	174.2 ± 8.0[c]	136.7 ± 8.1[c]	121.2 ± 8.9[c]	109.8 ± 7.9[c]	92.7 ± 4.6[c]	64.9
IV	n-Hexane fraction, 200	274.2 ± 8.5	267.5 ± 6.9	268.3 ± 10.4	271.7 ± 11.9	261.7 ± 13.3	268.3 ± 12.8	257.2 ± 10.2	6.2
V	n-Butanol fraction, 200	268.2± 5.7	272.3 ± 6.7	265.2 ± 9.9	257.7 ± 11.9	246.1 ±13.2[b]	254.5 ± 10.0	212.8 ± 12.6	20.6
VI	Ethyl acetate fraction, 200	289.5 ± 5.8	256.7 ± 8.8	216.5 ± 12.9[c]	188.7 ± 7.3[c]	164.1 ± 6.3[c]	136.7 ±10.5[c]	128.3 ± 9.7[c]	55.7
VII	Ethanol fraction, 200	264.2± 6.7	226.8 ± 11.8 [c]	174.2 ± 8.0[c]	136.7 ± 8.1[c]	111.1 ± 8.9[c]	99.8 ± 7.9[c]	82.7 ± 4.6[c]	68.7
	F-value	1.83	8.32**	28.62**	58.09**	51.79**	65.35**	85.11**	—

Values are expressed in Mean ± SEM of six animals. Dunnett's t-test and one-way ANOVA were done. The t-value denote statistical significance at [a]p <0.05, [b]p <0.01 and [c]p <0.001 respectively, in comparison to Group-I. F-value denotes statistical significance at *p <0.05, **p <0.01.

The ^{13}C-NMR study has given signals at δ 37.7 (C-1), 24.7 (C-2), 35.2 (C-3), 23.1 (C-4), 180.3 (C-4a), 24.0 (C-5), 26.1 (C-6), 42.4 (C-6a), 39.6 (C-6b), 29.8 (C-7), 18.3 (C-8), 49.5 (C-8a), 33.0 (C-9), 77.5 (C-10), 23.7 (C-11), 28.3 (C-12), 30.2 (C-12a), 48.5 (C-12b), 21.1 (C-13), 23.9 (C-14), 37.8 (C-14a), 30.1 (C-14b), 90.6 (C-2′), 70.0 (C-3′), 63.1 (C-4′), 74.3 (C-5′), 90.9 (C-6′), 92.5 (C-2″), 72.1 (C-3″), 65.0 (C-4″), 74.0 (C-5″), 90.9 (C-6″) ppm.

3.6 LC-MS m/z study

The LC-MS m/z study yielded, 752.38 $(M)^+$, 734, 692, 604, 602, 586, 456, 296, 148, 132, 131, 115, 60. Mass values are expressed as m/z values. The LC-MS spectroscopy showed the molecular ion peaks at 752.38 m/z value that corresponded to a molecular formula**,** $C_{40}H_{64}O_{13,}$ which was consistent with the theoretical value of 752.43. Fragmentation ion peaks at m/z 734 corresponded to the loss of hydroxyl unit, and other fragmentation ion peaks were also observed at m/z 692, 604, 602, 586, 456, 296, 148, 132, 131, 115 and 60.

Interpretation of data

The isolated compound (Fig. 2) was white and crystalline with the melting point, 322-324 ^{0}C. It responded positively to Salkowski and Lieberman-Burchard tests, indicating the presence of triterpenoids. An addition of a few drops of cerric ammonium nitrate reagent to the compound, the red coloration was produced, suggesting the presence of alcoholic groups[19]. The elemental analysis with Elementar, Vario EL III revealed that the compound contains 38.46 % C, 61.54 % H, and 0 % N. The FTIR spectroscopic analysis exhibited the characteristic broad peak, centered at 3275 cm^{-1}, which is characteristic of O-H stretching; this suggested the presence of a hydroxyl group, which was substantiated by chemical identification tests. The absorption band at 2944.78 cm^{-1} corresponded to CH_2-stretching, at 1564.36 cm^{-1} as a result of CH=CH stretching; the absorption peak at 1391.31 cm^{-1} was due to an aliphatic C-H stretching, and the band at 1062.73 cm^{-1} was due to the presence of C-O-C stretching. The ^{13}C-NMR spectrum revealed the presence of approximately 30-32 numbers of C atoms, including seven methyl groups, ten methylene groups, thirteen methane groups and seven quaternary carbons. The ^{13}C-NMR result elucidated a recognizable sharp signal at δ 180.3 ppm, which corresponded to the presence of –COOH group, which should be assigned to C-4a position. The obtained absorption peaks were at δ 90.6 and 92.5 ppm, which should correspond to an angular C-O-C structure at 2′ and 2″ positions. Absorption peaks at δ 63.1, 74.3, 90.9, 72.1, 65.0, 74.0 and 90.9 ppm illustrated the presence of multi-hydroxyl groups, corresponding to the carbon positions, 3′, 4′, 5′, 3″, 4″, 5″ and 6″, respectively. Signals at δ 23.9 and 37.8 ppm are assigned to the C-14 and C-14a double bonds, respectively. The peaks at δ 90.9 ppm corresponding to both carbon positions at 6′ and 6″ each with same value may be because of the presence of electron withdrawing groups, oxygen (C-O). But, alkene carbons appeared at δ 23.9 and 37.8 ppm. From the antidiabetic activity of aqueous extract and 4 major fractions of aerial parts of *Mollugo pentaphylla* examined by OGTT, monitored for 4 h. In alloxan induced hypergycemic rats too, the aqueous extract as well as its ethanolic fraction were highly effective, in comparison to the rest other solvent fractions. In the 10 h monitoring arm of animals the n-hexane fraction was the least effective in reducing glucose levels. However, the standard drug glibenclamide was consistently more effective than the aqueous plant-extract or its fractions. The favorable results in response to the blood glucose lowering ability of the extract and fractions suggest the pancreatic and or extra pancreatic action of the test drugs.

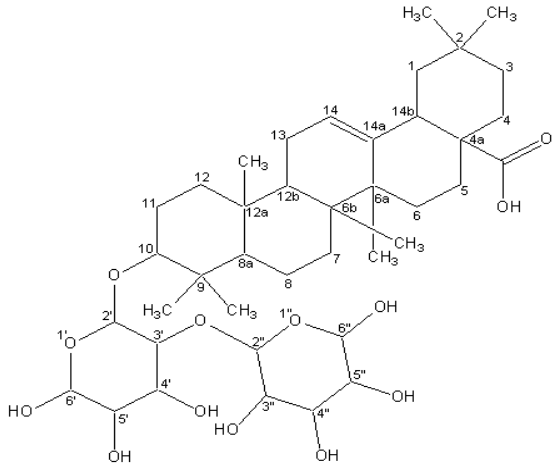

Figure 2: Structure of the compound: 2,2,6a,6b,9,9,12a-Heptamethyl-10-(4′,5′,6′-trihydroxy-3′-(3″,4″,5″,6″-tetrahydroxytetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy)-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2*H*-picene-4a-carboxylic acid.

The ethanolic fraction possesses highest degree of reduction of blood glucose level suggesting the polar nature of the phytochemical responsible for the stated activity. Obviously, for monitoring antidiabetic effectivity of any test chemical, alloxan induced rodent-model is regarded as the ideal procedure. Alkene carbons appeared at δ 23.9 and 37.8 ppm here were as described elsewhere[20,21]. The ^{1}H-NMR spectra displayed sharp signals for seven tertiary methyl groups, viz., at δ 0.91 ppm that may be assigned to the methyl groups attached at the positions of H-2, H-2 and H-6b (i.e., proton containing carbon positions); a sharp peak at δ 1.20 ppm occurred, which may be attributed to two methyl groups attached at positions, H-9 and H-9; and the observed δ 1.22 ppm may be assigned to the position of H-6a. Moreover, two singlet protons at δ 1.98 and 5.04 ppm were ascribable to methane positions, H-1 and H-2′, respectively; five doublet protons at δ 1.91, 4.02, 4.88, 5.14 and 5.41 ppm were assignable to the methane positions, H-13, H-3′, H-6″, H-6′ and H-2″, respectively; thirteen triplet protons at δ 0.77, 1.36, 1.40, 1.43, 1.52, 1.63, 2.30, 2.34, 3.07, 3.38, 3.74, 3.91 and 4.85 ppm were noted, which were ascribable to the methane positions of H-8a, H-(7 & 12), H-8, H-12b, H-3, H-4, H-6, H-5, H-14b, H-10, H-4″, H-(4′ & 5′) and H-5″, respectively; one quadruplet proton at δ 1.77 ppm was assigned to the methane position at H-11. The presence of seven hydroxyl groups were supported by the sharp singlet proton peak signals at δ 5.01, 5.04, 5.08, 5.09 and 5.11 ppm, which were attributed to position at H-(4′ & 5′), H-(6′ & 3″), H-4″, H-5″ and H-6″, respectively. The singlet proton signal observed at δ 5.23 ppm could be attributed to the presence of the functional carboxyl group (–COOH) at the position of H-4a, and the singlet peak at δ 5.37 ppm, due to the presence of carbons of conjugated alkene (CH=C) was assigned to the position of H-14, which is also supported by the IR stretching at 1564.36 cm^{-1}, as seen elsewhere[22]. The LC-MS spectroscopy elucidated the molecular ion $(M)^{+}$ peak value at 752.38 m/z that corresponds to the molecular formula, $C_{40}H_{64}O_{13}$, which was consistent with the theoretical value of 752.43, and this was further supported by the molecular weight calculated by Rast's procedure. The fragmentation ion peaks at m/z 734 that corresponded to the loss of hydroxyl unit from the 6′-carbon position of the parent molecule. The dehydration of fragment at m/z 734 ($M-H_2O$), on successive fragmentation would yield an ion at m/z 692 with the loss of $C_2H_4O_2$. Moreover, the signal exhibited at m/z 604 could be attributed to the cleavage of one pyran moiety from the 2″-carbon atom position, with the loss of $C_5H_8O_5$ from the parent molecule; this on successive fragmentation would yield ions at m/z 602 that corresponded to the loss of $C_5H_{10}O_5$, m/z 586, and which further corresponds to the loss of $C_5H_{10}O_6$, at m/z 456, due to the loss of $C_{10}H_{16}O_{10}$. on the other hand, it was evident from the mass spectra that the peak exhibited at m/z 296 was equatorial with the agreement of cleavage of a picene ring (the triterpenoid glycoside) from the 10th-carbon position with the loss of $C_{30}H_{48}O_3$ from the parent molecule; this on successive fragmentation would yield ions at m/z 148, due to the loss of $C_{35}H_{56}O_8$; the intense peak at m/z 132, due to loss of $C_{35}H_{56}O_9$, and a sharp peak at m/z 131 due to loss of $C_{35}H_{57}O_9$ were recorded. Its further fragmentation would yield ions at m/z 115 due to loss of $C_{35}H_{57}O_{10}$, and m/z 60, due to the loss of $C_{38}H_{60}O_{11}$. It was evident that the isolated molecule contains one heptamethyl-octadecahydro-picene moiety attached to a carboxyl group at C-4a position, and one pyran moiety at the C-10 position, which could have been attached to another pyran ring at C-2″ position. The presence of the triterpenoid nucleus in the isolated molecule was further supported by the spectral data of the triterpenoid saponin that has been isolated and characterized, as described[5,23]. The reported experimental results and ^{13}C-NMR, ^{1}H-NMR and the mass spectra described herein led us to formulate the molecular formula of the compound as, $C_{40}H_{64}O_{13}$, bearing the IUPAC nomenclature as: 2, 2, 6a, 6b, 9, 9, 12a-Heptamethyl- 10-(4′, 5′, 6′-trihydroxy-3′-(3″, 4″, 5″, 6″-tetrahydroxy-tetrahydro-pyran-2-yloxy)-tetrahydro-pyran-2-yloxy)-1, 3, 4, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-octadecahydro-2H-picene-4a-carboxylic acid.

CONCLUSION

The β-amyrin triterpenoid from the aqueous extract of the aerial plant parts was isolated, which has not been reported earlier. The presence of a carboxyl group of the oleanolic acid in the present compound at the 'C-4a position', configurating it a new β-amyrin type triterpenoid is the novelty (Fig. 2), but in the previously reported triterpenoid compound, the acidic group of the oleanolic acid was associated with a glucose moiety at the 'C-4a position' (Hambugrer *et al.,* 1989; Chopin *et al.,* 1982). However in this isolated compound, the glucose moieties form O-glycosidic linkage at –OH group at C-10 position of the oleanolic acid, rendering its polar nature, which ought to have diversified biological potentials.

Obviously, it was worthwhile to characterize the isolated compound.

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