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PHYTOCHEMICAL AND CHARACTERIZATION OF GLUCOSIDE FROM THE LEAF EXTRACT OF *LUFFA CYLINDRICA*

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ABSTRACT

An Iridoid glucoside, mp 218-220°c, $C_{19}H_{26}O_{11,}$ $[M]^+$430 was isolated from the ethanol leaf extract of *Luffa cylindrica.* The structure was elucidated mainly by using *FTIR*, and a combination of 500 MHz & 125MHz 1-D and 2-D NMR technique.Thus, named as 6, 7 – Dihydroxy – 5 – hydroxyl methyl – 7 – (3 – hydroxyl - propyl) – 4a – methyl – 1 – (3, 4, 5 – trihydroxy – 6- hydroxyl methyl tetrahydro – pyran – 2 yloxy) 1, 4a, 5, 6, 7, 7a – hexahydro cyclo penta [c] pyran – 4 – carboxylic acid methyl ester.

Keywords: Cucurbitaceae, Iridoid glycoside, *Luffa cylindrica*, Spectral data (1D and 2D)

INTRODUCTION

Luffa cylindrica is a sub-tropical plant, which requires warm summer temperatures and long frost-free growing season when grown in temperate regions. It is an annual climbing plant which produces fruit containing fibrous vascular system [1]. This is a summer season vegetable plant, with a long history of cultivation in the tropical countries of Asia and Africa. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America [2].

EXPERIMENTAL

Infrared (IR) spectra were recorded on spectrophotometer shimadzu 8400s (Japan). Melting points were determined on GallenKemp apparatus (Britain) and results were uncorrected. ^{1}HNMR and ^{13}C NMR were performed on Bruker spectrometer 500 MHz (Germany), for ^{1}H NMR and125 MHz for^{13}C NMR. Chemical shift values (δ) were reported in part per million in relations to the appropriate internal solvent standard (TMS). The coupling constant (J-values) were given in Hertz while the HMBC, DEPT, COSY and NOESY are also obtained. The NMR solvent use for this measurement was deutrated methanol. Spectra's were referenced to the

CD_3OD solvent signals at δ3.30 (^1H) and 49.00(^{13}C).TLC was carried out on plates precoated with R-P18 gel Merck, (Britain) and silica gel 60-120 mesh were used for column chromatography. Spots on plates were visualized by spraying with 10% H_2SO_4 followed by heating in oven.Gel-filtration techniques were carried out on sephadex L_H20. TLC visualization was obtained by U.V. absorption at 245nm.

PLANT MATERIAL

The plant *Luffa cylindrica* of the cucurbitaceae family was collected from a farm land in Basawa, a village outskirt of Zaria, Kaduna State, Nigeria in the month of October, 2017. The plant was authenticated at the herbarium of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. The plant material was compared with the existing Herbarium Sample, (Voucher No. 1638 DC). The fresh plant material was carefully separated into different parts, leaf, stem bark and root bark. The leafwas cut, air-dried and made into powder using pestle and mortar and subsequently referred to as powdered plant material of the leaf.

Extraction and Isolation

The powdered material of the leaf (500g) was extracted with petroleum ether 60 – 80^OC (3 x 500 ml) to exhaustion using cold maceration technique. The defatted marc was air dried at room temperature and exhaustively extracted with 95% ethanol (5 x 500 ml) using the same procedure to obtain the ethanolic extract. The solvents were removed *in-vacuo* to afford an oily and a dark brown gummy mass referred to as petroleum ether extract coded "Ps" and ethanol extract coded "Es" respectively.

The ethanol extract (30g) of the leaf was suspended in water (500ml) and sequentially partitioned with chloroform (3 x 500ml), ethyl acetate (4 x 500ml), and n-butanol (5x 500ml). These were concentrated using rotary evaporator to afford chloroform, ethylacetate, n-butanol and residual aqueous soluble portion respectively.

The ethanol leaf extract, and the various partition fractions weresubjected to phytochemical screening using standard protocols [3].

The n-butanol extract (1.8g) was mounted over glass column (100cm×4cm) packed with silica gel (60-120 mesh). The column was eluted continuously using chloroform, chloroform ethylacetate mixture, ethylacetate, ethylacetate methanol mixture and finally with methanol by

gradient elution technique. The progress of elution was monitored using thin layer chromatography. A total of 60 fractions of 10ml aliquot were obtained. Similar fractions were pooled together on the basis of their TLC profile to afford 21 major fractions F_1- F_{21}. The pooled fractions F_{8-14} consisting of two major spots was subjected to repeated gel filtration using sephadex LH-20 and eluted with methanol to afford 5 fractions (A_1-A_5). FractionA_3 gave a single homogeneous spot on the TLC using Chloroform: Methanol: Water (3:3:1) as solvent system. This was concentrated to afford (37mg) of a light brown amorphous solid coded as compound 1.

Identification Test

Ferric Chloride Test

5.0% ferric chloride in 0.5N HCl was sprayed on the chromatogram, fluka-silica gel precoated glass plate of compound 1and then kept in hot oven for 2-3 min [4].

Anillin/Sulphuric Acid Test

4.0g solution of vanillin was dissolved in 100ml of Tetraoxosulphate (VI) acid (H_2SO_4). This was spread on the chromatogram precoated glass plate of compound 1 in a fume chamber with the aid of a spray canister. The plate was taken to the oven and heated to 110^{o}c, for about 5 min after which it was removed to ascertain the colour formed [5].

Liebermann Buchard's Test.

1ml of anhydrous acetic acid was added to 1ml of chloroform, and cooled to 0^{o}C in a test tube. Few drops of concentrated H_2SO_4wasadded to the test tube containing solution of compound A [6].

Shinoda's Test

Little portion of the compound 1 was dissolved in ethanol. This was further warmed and filtered. Three to four pieces of magnesium chips was added to the filtrate, followed by the addition of few drops of Conc. (HCl). [7].

Methylation

The isolated compound 1 (3mg) was treated with excess methanol, 2 drop of H_2SO_4 added and then refluxed for 12 hours after which the solution was evaporated to dryness in vacuum. The residue was dissolved in H_2O and the temperature reduced to 0^{o}C. 5ml each was extracted with

CH_2Cl_2 (10ml x 2). The methylated compound was chromatographed on silica gel with Pet-ether: $CHCl_3$ (8:2) as the eluent to obtain each compound 1[8].

RESULTS AND DISCUSSION

Table 1: The results of phytochemical screening of the partition portion of the leaf extract of the plant Luffa cylindrica.

CONSTITUENTS	TEST	OBSERVATION	PORTIONS OF EXTRACTS					
Carbohydrate			Ps	Es	Cl	ETA	N-But	AQ
General Test	Molisch	Red colouring	-	+	-	-	-	++
Sugar Test	Aniline	Red colour	-	-	-	-	-	+++
Sugar (Monosaccharide)	Barfoed's	Red ppt	-	+	-	-	-	++
Red Sugar	Fehling's	Red ppt	-	+	-	-	-	++
Tannins	Lead Ethanoate Iron (III) Chloride Ethanoic acid Methanol's	White ppt Blue – Black White ppt Red ppt	- - - -	+ + + + + +	- - - -	- - - -	++ ++ - ++	++ + - +
Saponins	Frothing Liberman B.	Persist frothing Blue or green	- -	+ + + +	- -	+ +	++ ++	- +
Saponin Glycoside	Fehlings Solution Tetraoxosulphate(iv) acid	Red ppt Brick red	-	+ + + +	- -	+ +	++ ++	- -
Phlobatannins	Hydrochloric Acid	Red ppt	-	+ +	-	-	+	-
Carotenoids	Carr price's	Blue to red colour	-	+ +	-	-	-	++
Emodol	Borntrager's	Red colour	-	-	-	-	-	++
Flavones aglycones	Shibata's	Red to Orange	-	-	-	-	-	-
Terpenoids	Lieberman B. Dragendoff's	Pink to Red colour Orange red ppt	++ +	+ + + +	+ +	+ +	+ +	- -
Alkaloids	Mayer's Wagner's	Buff ppt Dark brown ppt	- -	- -	- -	- -	- -	- -
Flavonoids	Shinoda Tetraoxosulphate (vi) acid	Dee red Deep Yellow	- -	- -	- -	- -	- -	- -
Cardiac glycoside	Legal's Kedd's Keller – kilanis Baljet	Deep red colour Violet colour Reddish brown Orange to Deep red	+ + + +	+ + + +	+ + + +	++ + ++ +	++ + ++ ++	- - - -

	Liberman	Bluish green	+	+ + + +	+	+	++	-

Key: += fairly present, ++= Moderately present, +++ =Highly present, Ps= Pet ether, CL= Chloroform, ETA= Ethylacetate, N-But= n-butanol,Es.= Ethanol and AQ.= Residual aqueous.

Table 2: ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectra data of Compound 1 in CD_3OD, (δ ppm, J in H_Z)

POSITION	δc (PPM)	δ_H (ppm)	J=H_Z
1	93.4	5.6	
3	152.0	7.3(s)	
4	114.0	-	
5	106.3	-	
6	36.0	1.90(d)1.70,1.4	
7	38.0	-	
8	104.5	3.7	
9	61.10	0.96 (s)	
10	23.5	-	
11	167.1	OCH_3	
12	51.9		
1'	98.6	4.4	9.85
2'	73.5	3.5	
3'	70.8	3.1	
4'	77.7	3.2	
5'	76.4	3.4	
6'	61.8	3.6	9.12
1''	152.0	-	
2''	82.8	3.7	
3''	74.8	3.4	

Table3: ID and 2D NMR Spectral Summary for compound 1 in CD_3OD (500MH_Z, 125MH_Z).

Position	$\delta_{H\ ppm}$ (J= Hz)	$\delta_{C(ppm)}$	DEPT	HSQC	NOESY
1	5.60	93.4	CH	H-1	H-1'
3	7.30	151.6	CH	H-3	
4	-	114.6	C	-	
5	-	106.3	C	-	
6	1.90(d)	36.0	CH_2	H-6	
7	1.70,1.4	38.0	CH_2	H-7	
8	-	104.5	C	-	
9	3.4	61.1	CH	H-9	
10	0.96	23.5	CH_3	H-10	
11	-	167.1	C	-	
12	3.65(S)	51.9	OCH_3	12	
1'	4.40 (9.85Hz)	98.6	CH	H-1'	H-2'
2'	3.50	73.0	CH	H-2'	
3'	3.10	70.8	CH	H-3'	H-3"
4'	3.20	77.7	CH	H-4'	
5'	3.40	76.4	CH	H-5'	
6'	3.70	61.8	CH_2	H-6'	
1"	-	152.0	C	-	
2"	3.6	82.8	CH	H- 2"	
3"	3.5	74.8	CH	H-3"	

Compound 1 was isolated as a light brown amorphous solid. This produced a positive result with Vanillin/ H_2SO_4 test but gave a negative result with perchloric acid and ferric chloride test. The IR frequency of 3419cm^{-1} observed on compound 1 indicated the presence of a hydroxyl group, while resonance of 1648cm^{-1}(s) could be attributed to C=O in esters of aliphatic acid. An intense band at 1027cm^{-1}(s) could be attributed to C-O while absorption band at 2848cm^{-1} could be attributed to C – H. The absorption band at 1486cm^{-1} indicated the presence of -C=C- (stretch) [9]. The ^{1}H NMR spectrum of compound 1 showed signal at δ_H5.6ppm corresponding to H – 1,δ_H 7.3ppm/ H – 3, δ_H 0.96ppm/ H – 10 and δ_H3.65ppm/ H – 12. The signal at δ_H1.40ppm corresponds to H – 7, δ_H 1.9 ppm/ H – 6 and δ_H 3.7 ppm/ H – 9. The signal at δ_H 7.3/ H – 3 could be attributed to an Iridoid proton while signal at δ_H 4.4ppm (1H, d, J = 9.9Hz) /H – 1' could be attributed to an anomeric proton respectively [10]. The over lapping signals ranging from δ_H 3.4ppm - 3.6ppm / H – 2' – H – 6' could be attributed to protons on the sugar nucleus. Signal observed at δ_H 0.96ppm/ H – 10 and δ_H 3.65ppm/(OCH_3) could be attributed to a tertiary methyl and a methoxy protons respectively. The signals observed at δ_H 1.9 ppm (d)/ H – 6, δ_H 1.4ppm / H – 7 could be attributed to methylene and a methine proton while signal observed at δ_H3.6ppm

indicated the presence of an oxymethylene of the sugar moiety [11]. The structural elucidation of Compound 1 was also accomplished with the aid of ^{13}CNMR spectrum and DEPT experiment. ^{13}CNMR spectrum experiment, indicated the presence of 19 carbon atom signals, with 13 carbon atoms assigned to the aglycone, 6 to the sugar moiety and 3 carbon signals attributed to the cyclo propyl ring. The signal observed at δc 151.6ppm(C-3) and signal at δc 152.0 (C-1") were found to resonate within the same region with a single peak. The down field signal observed at δc 167.1ppm could be attributed to a carbonyl group in esters while signal at δ_H 23.50ppm could be attributed to the methyl group [12-13]. The DEPT experiment indicated the presence methylene carbon at δc 36.0ppm/C-6 and δc 38.0 ppm/C-7 while signal at δc 61.8 ppm/C-6' could be attributed to an oxymethylene of the sugar moiety. The methine carbon at δc 151.6 ppm/C-3 confirms the presence of an iridoid carbon on the aglycone moiety [14]. The signal at δc 23.50ppm/C-10 could be attributed to the presence of a methyl group while signal at δc 51.9ppm/C-12 is for a methyl carbon due to methoxy considering its chemical shift. The HSQC has further assisted in the assignment of the protons to the respective carbons. The signals at δc 93.4ppm (C - 1) corresponds to δ_H 5.60ppm (H-1), δc 152.0ppm (C – 3)/ δ_H 7.30ppm (1H, s), δc 36.0ppm (C– 6)/ δ_H 1.90ppm (1H, d), δc 38.0ppm (C – 7)/ δ_H 1.4ppm (H – 7), δc 104.5ppm (C - 8), δc 61.1ppm (C – 9)/ δ_H 3.7ppm, δc 23.5ppm (C – 10)/ δ_H 0.96ppm (3H, S, H – 10) and δc 51.9ppm (OCH_3)/ δ_H 3.70ppm (3H, s) are all attributable to the aglycone moiety[15].

The signals at δc 98.6 ppm (C – 1')/ δ_H 4.40 ppm (1H, d, j=9.85 H – 1'), δc 73.5 ppm (C – 2') /δ_H 3.50 ppm (3H, m, H – 2'), δc 70.8 ppm (C – 3')/ δ_H 3.10 ppm (3H, m, H – 3'), δc 77.7 ppm (C – 4') /δ_H 3.20 ppm (3H, m, H – 4'), δc 76.4 ppm (C – 5')/ δ_H 3.4 (3H, m, H – 5') and δc 61.8 ppm (C – 6')/ δ_H 3.60 ppm/ (1H, dd, 6.0 H – 6') are all attributed to the sugar moiety [16].The signals at δc 152.0 ppm/C-1", δc 82.8 ppm(C-2")/ δ_H3.6 ppm/H-2"and δc 74.8 ppm(C-3")/ δ_H 3.5 ppm/ H-3" could be attributed to the cyclo propyl ring [17].The resonance observed at δc 98.6 ppm (C- 1') which correlates with δ_H 4.40 ppm (1H, d, j= 9.85Hz/ H – 1") suggest the presence of an anomeric carbon while signals between C – 2'– C – 6' are for the sugar nucleus. The resonance at δc 61.8ppm (C – 6')/ δ_H 3.6 (H – 6') is a signal due to oxymethylene of the sugar moiety [18].

The aforementioned values are in conformity with the –β–D- glucopyranosyl moiety. The anomeric proton resonating at δH 4.4 ppm (1H, d, J = 9.85 H_Z) is in accordance with – β – configuration, because J – values > 7H_Z signifies a – β – configuration at the anomeric proton.

Signal at δ_H 7.3 (1H, s, H – 3) also indicated the presence of an Iridoid proton [19]. Signal observed at δ_H 0.96ppm (3H, s, H - 10) suggest the presence of a tertiary methyl proton [20]. The relative down field chemical shift of C-1 (δ_C93.4ppm) confirms that, the glycosidation of the sugar unit is attached to the carbon at C-1' [21-22].

The glycosidation of the ether linkage between C-5 and C-8 were observed from the COSY spectroscopy correlation, so also the esterfication at C – 4 (HSQC), were indicated by the down field shift respectively. The COSY correlation between C-3" and C-3' of the sugar were observed to be in the same environment [23-24].

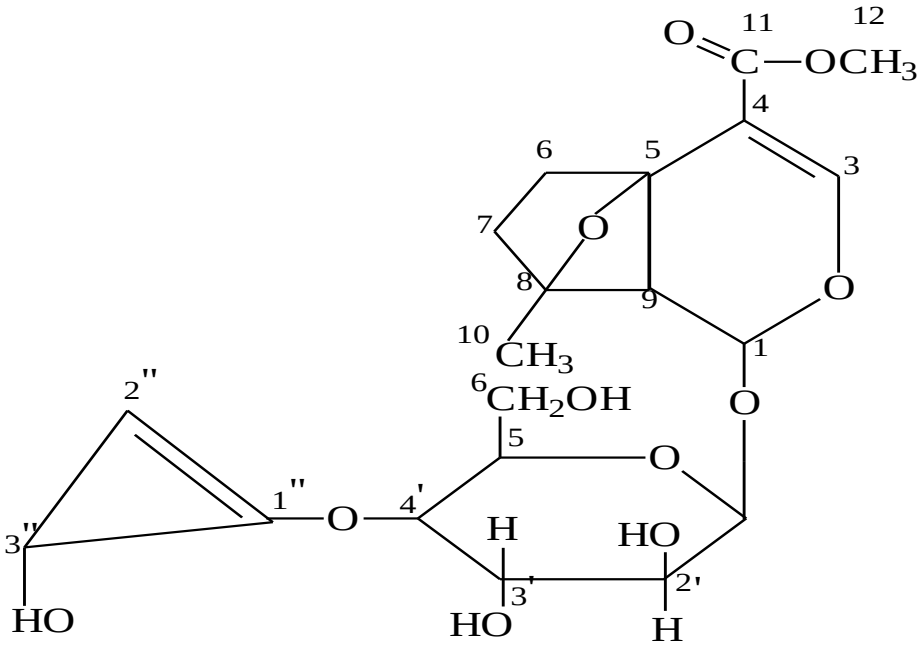

Figure 1: Compound 1.

CONCLUSION

On the basis of 1D and 2D spectrum analysis, compound 1 was proposed to have the molecular formula of $C_{19}H_{26}O_{11}$. Thus, named as 1-[3,4-dihydroxy-5-(3-hydroxy- cycloprop-1-enyloxy)-6-hydroxymethyl-tetrahydro- pyran- 2-yloxy]- 7-methyl-1, 4a, 5, 6, 7, 7a-hexahydrocyclopenta [c] pyran -4- carboxylic methyl ester and 4a, 7 – dihydroxy – 7 – methyl – 1 – (3, 4, 5 – trihydroxy – 6– hydro methyl – tetrahydropyran – 2 – yloxy) – 1 -, 4a, 5, 6, 7, 7a, hexahydro – cyclopentan [C] pyran – 4 – carboxylic acid methyl ester.

Authors' contributions

M. Mohammed, A.Abubakar and A.A. Dan mallam participated in the study, design, and critical revision of manuscript for important intellectual content. M. Mohammed, B. Suleiman, D. Ibrahim and Y.Y. Jibrin drafted the manuscript and participated in analysis and interpretation of the available data.

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