ISOLATION AND STRUCTURE ELUCIDATION OF IPOLAMIIDE FROM THE STEM BARK OF *STACHYTARPHETA ANGUSTIFOLIA* MILL VAHL (*VERBENACEAE*)

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ABSTRACT

This research work is aimed at isolating and structural elucidation of the compound from the stem bark extract of S. angustifolia using standard phytochemical and spectroscopic techniques. The grounded powdered material of the stem bark was extracted and partitioned using various solvent of different polarity. Column chromatography, n-BuoH soluble fraction of the ethanol extract of stem bark S. angustifolia was run using silica gel and subsequently pooled fractions from various portions were purified using sephadex LH_{20} to obtain compound 1. Pure isolate of compound 1 was subjected to analysis using FTIR, 1and 2D NMR. Compound 1 was determined as an Iridoid glucoside Cyclopenta [C] pyran-4-Carboxylic acid, 1-(β-D glucopyranosyloxy)-1, 4A, 5,6,7,7A Hexahydro-4A, 7- dihydroxy-7- methyl-, Methyl Ester, (1S, 4AR,7S, 7AR) (Ipolamiide), MP 218-220^{o}C, MW $C_{17}H_{27}O_{12,}$ $[M]^{+}$424(EIMS) on the basis of spectral analysis and Comparism with reference data.

Keywords: Stachytapheta angustifolia, Verbenaceae, Ipolamiide, Spectral data.

INTRODUCTION

Medicinal herbs constitute indispensable components of traditional medicine practiced worldwide due to low cost, easy access and ancestral experience (Marini-Bettolo, 1980). Bacterial and viral resistance to almost all anti-bacterial and anti-viral agents has been reported, this might be attributed to an indiscriminate use of anti-microbial drugs commonly employed for the treatment of infectious diseases (Gbodossou, 2005). Apart from the development of resistance, some antibiotics have serious undesirable side effects which limit their application. Therefore, there is an urgent need to developed new anti-microbial agents that are highly effective with less toxicity from natural sources (Maurer-Grams *et al.*, 1996). Previous phytochemistry of *S.angustifolia* and other species in the genus *Stachytarpheta* revealed the presence of the following Prenyl hydroquinone glycoside as 1 – O – (4" – O – caffeoyl) – β – glucopyranosyl – 1 – 4 – dihydroxy – 2 – (3; 3 – dimethyl allyl) Benzene, Acteoside isolated from the leaf and stem bark of *Stachytarpheta cayemensis* (Cordell, 2000, Ganapathy et al., 1998). Lamiide, Ipolamiide and Samangaoside were all reported to be isolated from the leaf

extract of *Stachytarpheta indica* (Sophon, 2002). While, Lucidemic acid, korolkoside, a Bis – iridoid glycoside, Citrifolinoside, Isorhamnetin as 3-O- β-D- apio -D- furanosyl (1- 2)-β-D-galactopyranoside and Serratoside were all reported to be isolated from *Stachytarpheta* species (Farnsworth, 2000).

The cold infusion of *Stachytarpheta angustifolia* when mixed with natron is taken as a remedy for, gonorrhea and other forms of venereal diseases. It is also taken as a vermifuge or a purging vehicle for other vermifuge. The boiled leaf portion of the plant is taken as a remedy for diabetes (Dalziel, 1999). In Asia and America the aerial part of *Stachytarpheta angustifolia* is boiled and taken traditionally as a remedy for diarrhea, intestinal parasites and skin ulcer. (Eldridge *et al.,* 1975).

In Brazil, the triturated stem bark of *Stachytarpheta angustifolia* is applied locally for the treatment of ulcer and also as a good remedy for rheumatism. The leaves have also been used for the relief of sprain. The plant has been reported to contain a glucosidal substance "Stachytarphine" which is reputed to be Abortificient. In Ghana according to Buntings, the juice from the leaf of this plant is used as a remedy for eye trouble such as cataract and also applied to sores on children's ear. The aqueous leaf extract of *Stachytarpheta angustifolia* are also used to cure heart problems (Burkill, 1995).

MATERIALS AND METHODS

Plant Material

The plant *Stachytarpheta angustifolia* (MILL) Vahl. Verbenaceae was collected from a farm land in Basawa, a village outskirt of Zaria, Kaduna State, Nigeria in the month of October, 2013. The plant was authenticated at the herbarium of the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria, by comparing with the existing Herbarium Sample, (Voucher No. 900188). The fresh plant material was carefully separated into different parts, the leaf, stem bark and root. The stem bark was cut, air-dried and made into powder using pestle and mortar and subsequently referred to as powdered plant material of the stem bark.

Sample preparation

The powdered material of the stem bark (500g) was extracted with petroleum ether 60 – 80^0C (5 x 600 ml) to exhaustion using maceration technique. The defatted marc was air dried at room temperature and exhaustively extracted with 95% ethanol (7 x 500 ml) using the same procedure to obtain the ethanol 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 stem bark 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 portions respectively (Yaching *et al.,* 2004; Shengmin *et al.,* 2003).

The ethanol stem bark extract and the partition portion of *S. angustifolia* were subjected to phytochemical screening using standard protocols (Sofowora, 2008; Trease and Evans, 2002). 2.5g of n-butanol extract from the stem bark 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, Ethyl acetate/ Methanol mixture and finally with methanol by gradient elution technique. The progress of elution was monitored using thin layer chromatography. A total of 465 fractions of 10 ml aliquot were obtained. Fractions were combined based on their TLC profile to afford 10 major fractions Coded as B_1- B_{10}. Fraction B_3 consisting of two major spots was subjected to repeated gel filtration using sephadex LH-$_{20}$, eluted with methanol to afford 36mg of Compound 1.

Sample analyses

Infrared (IR) absorption spectrum was recorded using an infrared spectrophotometer. Proton ^{1}HNMR and ^{13}CNMR spectra both (ID and 2D) were obtained using NMR Spectrometer. ^{1}HNMR and ^{13}CNMR experiments were performed on Bruker spectrometer 400 MH_Z for ^{1}H and 125 MH_Z for ^{13}CNMR. NMR spectra were referenced to the CD_3OD solvent signals at ∂3.30 (^{1}H) and 49.00 (^{13}C) with TMS as an internal standard. Chemical shift values (∂) were reported in parts per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J-values) were given in Hertz,

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.

RESULTS

Plate 1*.Stachytarpheta angustifolia (Mill) Vahl; Verbenaceae*

Table 1: The results of Preliminary phytochemical screening of the ethanolic extract and the partition portion of the stem bark extract of *S. angustifolia* plant was summarized in table 1.

Const.	TEST	Ps	Es	CL	EtOAC	n-But	AQ
Carbohydrate	Molisch	-	+	-	-	-	++
	Fehling's		++	-	-	-	+++
	Barfoed		+	-	-	-	++
	Benedict		+	-	-	-	++
Alkaloids	Mayer's	-	-	-	-	-	-
	Wagner		- -	-	-	-	-
	Dragendorff		- -	-	-	-	-
	Hager's		- -	-	-	-	-
Flavonoids	Lead Acetate	-	++	+	+	+ +	-
	Shinoda		++	+	+	++	-
	Tetraoxosulphate (VI) acid		+	+	-	+ +	-
Glycosides	Borntrager's	-	++	-	+	+	++
	Legal		+	+	+	+	++
Saponin	Froth test	-	++	-	+	+ +	+++
Cardiac (Glycosides)	Keller Killiani	-	+	-	++	+	++
Tannins	Gelatin test	-	+	+	-	+	++
	Alkaline -reagent test		+	-	-	+	++

Key: (+) Present, (-) Absent, Es =Ehanol , Ps=Pet ether, CL=Chloroform, EtOAC=Ethylacetate, n-But= n-butanol and AQ=Residual aqueous

I **II**

Plate 2: TLC Analysis of compound 1 in Different solvent system

Table 2: Showing TLC profile of Ipolamiide

Solvent system	Spraying reagent	Colour of spot	No. spot	R_f Values
(A). EtoAc: MeOH:Water (100:16.5;13.5)	10% H_2SO_4	Bluish green	1	0.48
(B). $CHCl_3$: MeOH:Water (3:3:1)	10% H_2SO_4	Bluish green	1	0.51

Chemical 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 1 and then kept in hot oven for 2-3 min. (Manguro and Lemmen, 2007).

Vanillin/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 inserted in to the oven and heated to 110^oC, for about 10min after which it was removed to ascertain the colour formed (Richard, 1998).

1ml of anhydrous Acetic acid was added to 1ml of chloroform, and cooled to 0^oC in a test tube. Few drops of concentrated H_2SO_4 were added to the test tube containing solution of compound 1 (Harbone, 1984).

Shinoda's TestA 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. Hydrochloric acid (HCl). (Trease and Evans, 2002).

Methylation

3mg of the isolated compound 1 was treated with excess methanol and 2 drops 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^oC. 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 solvent system to obtain compound 1 (Tian-shung *et al.*, 2001).

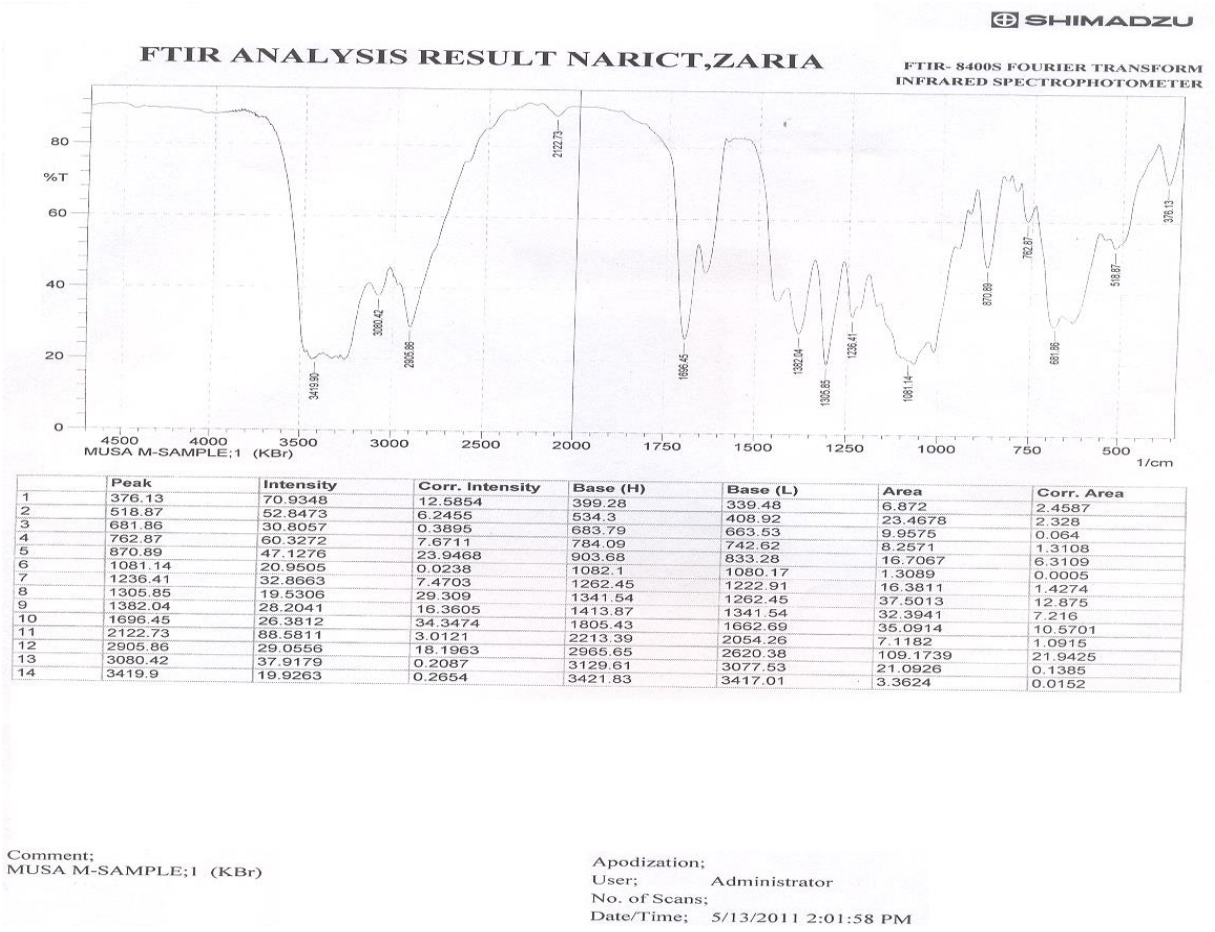
The IR frequency of 3419cm^{-1} observed in MND indicate the presence of a hydroxyl group while, frequency at 1648cm^{-1} (s) could be attributed to esters of aliphatic acid. The intense band observed at 1021cm^{-1} (s) could be attributed to CH_3C-O (kemp, 1991).

Table 3: FTIR data of IPOLAMIIDE

Absorption bands (cm^{-1})	**Intensity**	**Vibrations**
3419	Broad	OH
2848	M	Stretch
1648	S	C-H
1486	M	C=O
1021	S	-C=C Stretch C-O or OH (def)

Proton Nuclear magnetic resonance of Ipolammide

The ^{1}HNMR spectrum of compound ipolamiiderevealed resonance at δ_H 5.8(1H, d,1.1H$_Z$, H-1), 7.4 (1H, S, H-3), 2.2 (H-5), 1.9 (1H, m, H-6), 1.6 (IH,m, H-7), 2.5 (1H, brs, H-9), 1.2 (3H, S, H-10), 3.7 (3H, S, OCH_3), 4.6 (1H, d, 7.8H$_Z$ H-1^1), 3.2 (3H,m, H-2^1), 3.3 (3H,m, H-3^1), 3.4 (3H,m, H-4^1), 3.4 (3H,m, H-5^1), (1H,dd, H-6^1).



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	376.13	70.9348	12.5854	399.28	339.48	6.872	2.4587
2	518.87	52.8473	6.2455	534.3	408.92	23.4678	2.328
3	681.86	30.8057	0.3895	683.79	663.53	9.9575	0.064
4	762.87	60.3272	7.6711	784.09	742.62	8.2571	1.3108
5	870.89	47.1276	23.9468	903.68	833.28	16.7067	6.3109
6	1081.14	20.9505	0.0238	1082.1	1080.17	1.3089	0.0005
7	1236.41	32.8663	7.4703	1262.45	1222.91	16.3811	1.4274
8	1305.85	19.5306	29.309	1341.54	1262.45	37.5013	12.875
9	1382.04	28.2041	16.3605	1413.87	1341.54	32.3941	7.216
10	1696.45	26.3812	34.3474	1805.43	1662.69	35.0914	10.5701
11	2122.73	88.5811	3.0121	2213.39	2054.26	7.1182	1.0915
12	2905.86	29.0556	18.1963	2965.65	2620.38	109.1739	21.9425
13	3080.42	37.9179	0.2087	3129.61	3077.53	21.0926	0.1385
14	3419.9	19.9263	0.2654	3421.83	3417.01	3.3624	0.0152

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Figure 2: FTIR of the Ipolamiide

Signal observed at δ_H 4.6ppm is a characteristic of an anomeric proton while δ_H 7.4ppm (s) signal could be attributed to an iridoid proton presnt on the aglycone moiety.

Signal at δ_H 3.7ppm (3H,S) is assign to the methoxy group while signal at δ_H 1.2ppm (3H,S) is assign to the only methyl proton of compound ipolamiide.

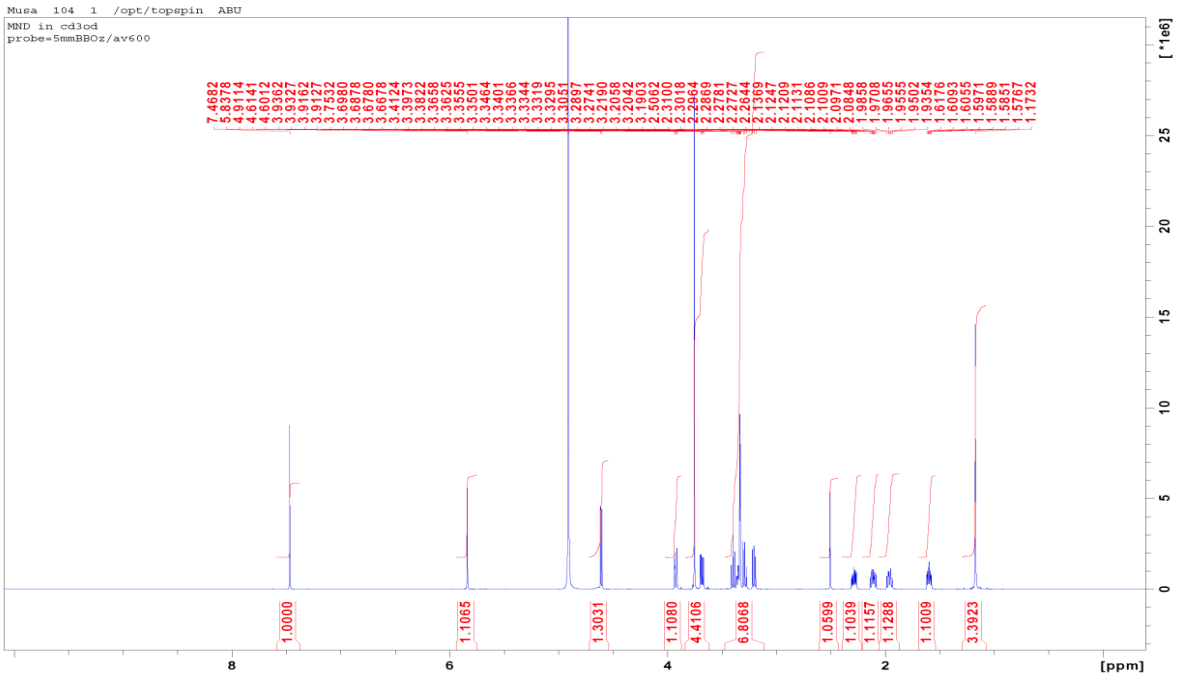

Figure 4: Proton Nuclear Magnetic Resonance Spectrum of ipolamiide in CD_3OD

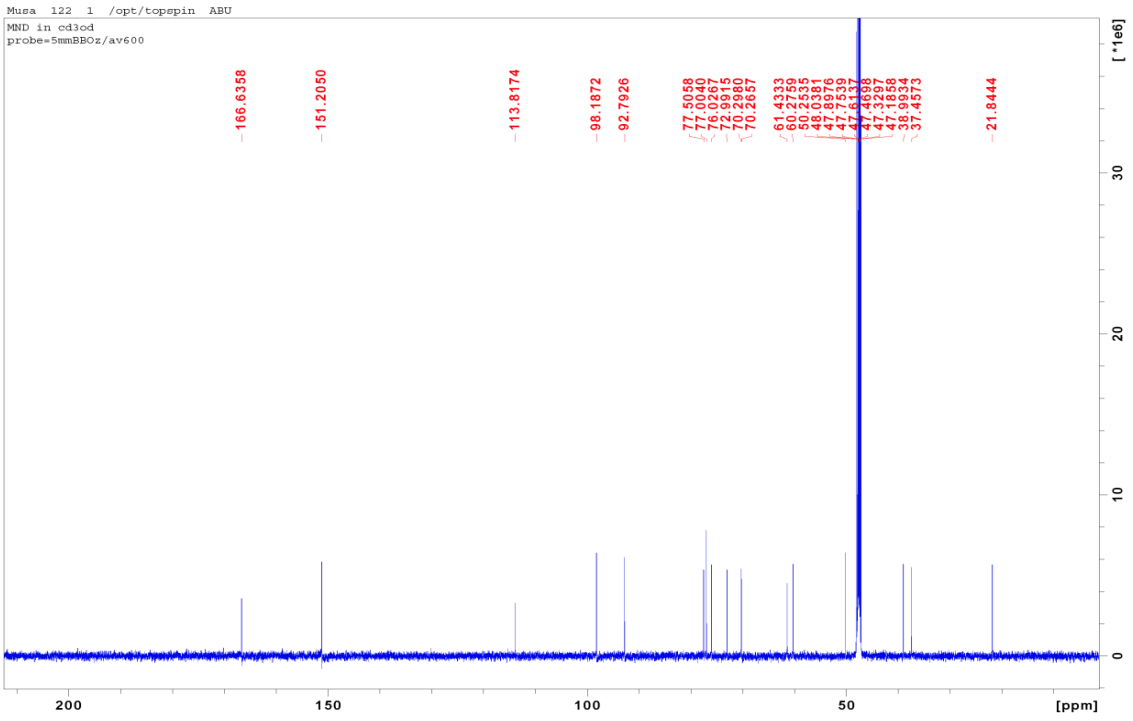

Figure 5: Carbon -13 Nuclear magnetic resonance Spectrum of MND in CD_3OD

Carbon-13 Nuclear magnetic resonance

The ^{13}CNMR and ^{13}C-DEPT spectrum of MND exhibited resonance at δ_c 93.0ppm(C-1), 151.2 (C-3), 114.0 (C-4), 70.3(C-5), 38.0 (C-6), 39.0 (C-7), 78.0(C-8), 60.5 (C-9), 22.0 (C-10), 167.0(C-11), 50.3 (OCH_3), 98.2 (C-1^1), 73.0 (C-2^1), 76 (C-3^1), 70.3 (C-4^1), 77.0(C-5) and 61.4 (C-6^1). ^{13}CNMR spectra and DEPT experiment of MND indicated the presence of 17 carbon atoms. Resonance at δ_c 98.2ppm exhibited the presence of an anomeric proton. The spectrum showed 2 quaternary carbon atom at δ_c 114.0, 78ppm and δ_c 167ppm. The resonance around δ_c 77-61.4ppm exhibited the characteristic of sugar nucleus and methyl carbon atom on δ_c 22.0ppm.

DISCUSSION

The phytochemical screening of the stem bark ethanol extract and the partition portions revealed the presence of the following secondary metabolite such as Saponins, Tannins, Cardiac glycosides, Flavonoids and Sterols while Alkaloids were found to be absents.

Column chromatographic separation of the n-butanol fraction of the stem bark extract followed by a repeated gel filtration using sephadex L_H 20 led to isolation of an amorphous brownish solid. The ferric chloride test on the isolate gave bluish-green coloration on the chromatogram. The bluish green coloration observed on subjecting the compound 1 to $FeCl_3$ test indicates the presence of phenolics OH (Francis, 2003).

The IR spectrum (1) displayed absorption attributable to a hydroxyl group at 3401.58cm^{-1}, a conjugated carbonyl group at 1648.23cm^{-1} and the presence of a glycosidic linkage at 1130-1025cm^{-1}(IK, Kwi *et al.*, 2004). The ^{1}HNMR at signal δ_H (7.4, S) indicated the presence of a 4-substituted enol ether system typical of an Iridoid proton (Kemp 1991). Signal at (δ_H 3.8, 3H, S) is typical of a methoxy proton and also another signal at (δ_H 1.2,3H, S) is for a tertiary methyl group. The signal at (δ_H 4.6 d, j= 7.8Hz) was assigned to the anomeric proton of a - β-glucopyranose unit. The integral signal at (δ_H 5.8378, d), which was shifted downfield due to glycosidation, indicated the attachment of glucopyranose unit to the iridoid moiety. Signals observed at δ_H 4.6141, δ_H 3.2190, δ_H 3.3366, δ_H 3.3464, δ_H 3.5551 and δ_H 3.6780ppm are all protons attributable to glucopyranose (Shu-hua, *et al.*, 2004). The point of attachment for the β-glucopyranose unit (C –1) was confirmed by HMBC and correlation between (H – 1^I/C – 1) of δ_H 4.6141ppm / δ_C 94.2578ppm to (H – 1/C - 1^I) of δ_H 5.8378 / δ_C 99.6268ppm (Masaki, *et al.*, 2001).

The H-H COSY exhibited the correlation of H-1 of the cyclopentano pyran ring system to H-1^I, H-12 and H-10. The H-1^I was also found to be correlated to H-5^I, H-4^I and H-1^I respectively (Nan-zhang *et al.*, 2008). The HSQC correlation shows that δ_C 94.2579ppm/δ_C 99.6268ppm C/C^1 are coupled to δ_H 5.70ppm/δ_H 4.50ppm of H/H^I (Dharma, *et al.*, 2001).

The ^{13}CNMR spectrum of (1) exhibited 17 carbon signals, six of which could be attributed to a β-glucopyranosyl moiety and 11 to the aglycone. Signals at δ_C 98.6268ppm, δ_C 72.9915ppm, δ_C

77.5058ppm, δ_C 70.2980ppm, δ_C 76.0267ppm and δ_C 61.4333ppm are all characteristic carbon signals for glucopyranose unit (Yong and Peng 2003). The β – anomeric configuration for the glucose was judged from its (J_{HZ} 8.00ppm) coupling constants. The correlations between C – 1/H – 1, H – 1/C – 1^I and H – 1/C – 1^I suggested that the β – glucopyranose unit was attached to (C – 1) position of the aglycone unit (Kim, *et al.*, 2004). The chemical shift values, the splitting patterns of H-3 (δ_H 7.4,s) and H-9 (δ_H 2.5, s) were suggestive of C-4, C-5 and C-8 to be positioned at C-4, due to the high desheilded signal of the H-3 proton, and the quaternary carbon resonance at δ_C 70.2657ppm attributed to C-5 (Zuhal *et al.*, 2005). The Complete analysis of the ^1H and ^{13}C NMR spectral data of Compound 1 allowed the assignment of the Multiplet signals observed at δ_H 2.2/2.3 and δ_H 1.6/2.1 to the methylene protons at C-6 (δ_C 37.4) and C-7 (δ_C 38.9934), respectively. The multiplicity of H-9 was also an indicative of a totally substituted C-8. Therefore, the chemical shift value of the tertiary methyl group (δ_H 1.2, S) suggested its attachment at C-8. However, the chemical shift values of both C-8 (δ_C 77.5058ppm) and H-10 also indicated the presence of a tertiary hydroxyl function at C-8 position (Robert, 1989). From the spectra it can be deduced that the glucose is a D- glucopyranose. while the proton (NMR) and ^{13}CNMR spectra has assisted in ascertaining the molecular formula as $C_{17}H_{26}O_{12}$ with molecular weight of424[$M]^+$. The anomeric position of the glucose moiety as a – β – origin was acertain from the coupling constant (7.8Hz) with anomeric proton at δ_H4.60ppm for δ_C 98.1892ppm. By the complete analysis of NMR data of (1), and comparison with data given in the table above.

CONCLUSION

In conclusion, it could be observed that, the compound isolated is a new compound in this species of *S. angustifolia* but not in the genus of *Stachytarpheta*. Compound 1 was determined to be the same as Ipolamiide termed as Cyclopenta [C] pyran-4-Carboxylic acid, 1-(β-D glucopyranosyloxy)-1, 4A, 5,6,7,7A Hexahydro-4A, 7-dihydroxy-7- methyl-, Methyl Ester, (1S, 4AR,7S, 7AR)(Ipolamiide), mp 218-220^{o}c, $C_{17}H_{27}O_{12}$, $[M]^+$424(EIMS) on the basis of spectral analysis and Comparism with reference data. (Zuhal, *et al.*, 2005; Tayfun *et al., 2001*).

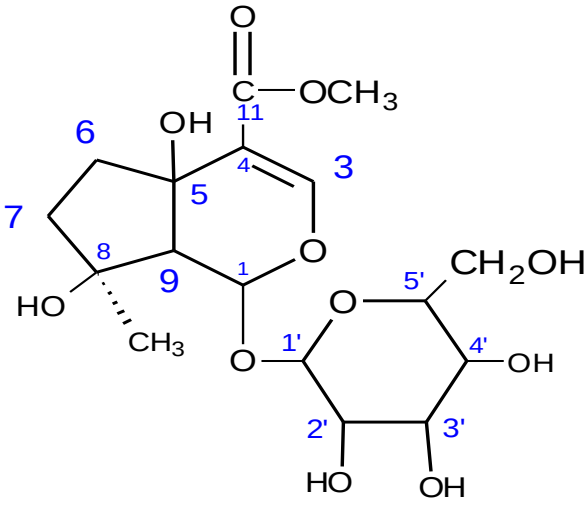

Fig 6: Compound 1

Cyclopenta [C] pyran-4-Carboxylic acid, 1-(β-D glucopyranosyloxy)-1, 4A, 5,6,7,7A Hexahydro-4A, 7- dihydroxy-7- methyl-, Methyl Ester, (1S, 4AR,7S, 7AR)

Table 4: ^{13}C NMR (100 MHz) and ^{1}HNMR (400MHz) Spectral Data for Compound 1 and Reference Compound in CD_3OD (δ in ppm)

Position	δ_C of Cpd 1	δ_H of Cpd 1 J (Hz)	δ_C of Ref Cpd	δ_H of Ref Cpd
1	CH 92.7926	5.8 (1H, d, 1.1H_Z)	93.0	5.7 (1H, d, 1.1H_Z)
3	CH 151.2050	7-5 (1H, S)	151.4	7.4 (1H, S)
4	C 113.8174	-	114.0	-
5	C 70.2667	-	70.6	-
6	CH_2 37.4573	1.9 (1H,m) ,	37.6	2.0
7	CH_2 38.9934	1. 6 (1H,m), 2.05 (1H,	39.6	1.56 (1H,m), 2.05
8	C 77.0040	m)	77.7	(1H, m)
9	CH 60.2769	-	60.5	
10	CH_3 21.8444	2.5 (1H, brs)	22.0	2.79
11	C 166.6558	1.2 (3H, S)	166.8	1.14 (3H, S)
Ome	50.2635	-	50.4	-
1	CH 98..1872	3.8 (3H, S)	98..4	3.72
2’	CH 72.9915	4.6 (1H, d, 7.8)	73.2	4.5
3’	CH 76.0267	3.2 (1H, dd, 8.0)	76.2	3.17
4’	CH 77.0040	3.3 (3H, m)	70.6	3.23
5’	CH 70.2980	3-4 (3H, m)	77.1	3.32
6’	CH_2 61.4333	3.4 (3H, m)	61.5	3.38
		3.7 (1H, dd, 6.0)		3.65

Key: Ref. Compound. Zuhal Guvenalp, Hilal O., Turesin U., Cavit k., and Omur D (2005). Iridoid (Ipolamiide) Flavonoids and phenylethanoid glycoside from *Wiedemannia orientalis*. Turk J. Chem. 391- 400.

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