AkiNik

Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

JEZS Journal of Entomology and Zoology Studies

ISSN 2278-4136
ISSN 2349-8234
JPP 2013; 2 (3): 77-85
© 2013 AkiNik Publications
Received: 15-7-2013
Accepted: 9-8-2013

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Bioactive Caffeic Glycoside Ester and Antimicrobial Activity of Various Extracts from the Leaf of *Stachytarpheta angustifolia* Mill Vahl (Verbenaceae).

M. Mohammed, A.M. Musa, A.A. Adeiza, S. H. Musa and L. Lande

ABSTRACT

This study examines the extraction and isolation of the Caffeic glycoside ester Compound 1. [mp 222-224 ^{0}C], $C_{29}H_{26}O_{15}$, $[M]^+$624.594 (EIMS) from the n-BuoH soluble fraction of the ethanolic extract of *S. angustifolia* (*verbenaceae*). It was characterized on the basis of spectral analysis (UV, FTIR, 1and 2D NMR techniques) as –β-(3^1, 4^1- dihydroxyphenyl) -ethyl-O-α-L- rhamnopyranosyl-(1-3)-β-D- (4-O-Caffeoyl) -glucopyranoside. Antimicrobial properties of Compound 1 and other extracts were tested against some microorganisms namely *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgari, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi Bacillus subtilis, Penicillium digitatum, Candida albicans, Aspergillus niger, Fusarium oxysporum and Penicillium notatum.* The antimicrobial sensitivity test indicated that the extract inhibited the growth of *Staphylococcus aureus*, *Streptococcus pyogenes Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Penicillium digitatum, Candida albicans and Penicillium notatum* with 30 mm, 29 mm, 35 mm, 34 mm, 36 mm, 28 mm, 24 mm, 25 mm while the highest activity of caffeic glycoside ester was exhibited by the n-BuoH fraction against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi* with
34mm, 36mm and 36mm respectively.

Keywords: *Stachytarpheta angustifolia,* Caffeic Glycoside Ester, Spectral Data, Anti-microbial Activity.

1. Introduction

Medicinal herbs constitute indispensable components of traditional medicine practiced worldwide due to low cost, easy access and ancestral experience. Traditional herbs are an inspirational part of our health because it is the total sum of knowledge, skill and practice based on theory, belief and experience used in maintaining good health as well as curing diseases using medicinal plant [43]. Infectious diseases are the major cause of death worldwide and in the tropical part of the world, they account for approximately 50% of death cases [52]. This issue might be attributed to poverty and increasing incidence of multiple drug resistance. 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[18]. Apart from the development of resistance, some antibiotics have serious undesirable side effects which limit their application. Therefore, there is an urgent need to develop new anti-microbial agents that are highly effective with less toxicity from natural sources [32]. *Stachytarpheta angustifolia* (mill) vahl (verbenaceae) has been used in Northern Nigeria for the treatments of Sexually transmitted diseases, Dysentery, as anthelmintic agent, purgative agent and as a remedy against diabetes. It is commonly known as “Devils coach whip, verbena, or Bastard vervain. The Hausa’s called it Wutsiyar kadangare while the Yoruba’s called it Iru-Alangba in Nigeria, Africa [23, 1]. The medicinal value of this plant in Nigeria and its environs cannot be over emphasized; the decoction of the whole shrub mixed with natron is taken as a remedy for dysentery and also for similar condition for horses.

The cold infusion of the plant 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 plant is boiled and taken as a remedy for diabetes. The aerial part of *Stachytarpheta angustifolia* in Asia and America is boiled and taken traditionally as a remedy for diarrhea, intestinal parasites, and skin ulcer and as an abortifacient agent [12, 14].
In Brazil, the triturated fresh leaf of the plant is applied locally for the treatment of ulcer and also taken as a good remedy against rheumatism. The leaves have also been used for the relief of sprain by rubbing the juice on the affected part and also as an adulterant in tea. This plant is reported to contain a glucosidal substance (stachytarpheta) which is reported to be abortifacient. In some parts of West Africa, Gold Coast according to Buntings, the juice from the plant herb is used as a remedy for eye trouble such as cataract and also applied to sores on children's ear. The aqueous leave extract of the plant are also used to cure heart problems. In papua (New Guinea), oral infusion of the plant is used as an ant fertility agent [49]. In the Antilles (western part of India), the juice obtained from the fresh leaf is used as an emetic and also as a purgative agent, while the decoction of the whole plant is taken as an antihelmintic agent. The infusion of the whole plant is taken as a remedy for gonorrhea and jaundice [12].
Literature review reveals the presence of citrifolinoside, macrophyloside, triterpenes, friedelin, stigmasterol, ursolic acids and Oleanolic acid in the genus of this family. Although, no other compounds have been reported which could explain the ethno medical uses of this plant.
In our investigations the n-Butanol soluble extract exhibited significant antimicrobial activities against some test microorganisms. We reported here the isolation and structural determination of caffeic glycoside ester by means of chromatographic spectroscopic techniques, so also the antimicrobial properties of the caffeic glycoside ester using some test microorganism.

2. Experimental

Infrared (IR) absorption spectra were recorded using an infrared spectrophotometer. Proton NMR and ^{13}CNMR spectra both (ID and 2D) were obtained using NMR Spectrometer, with the residual solvent peaks as internal standard.
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 and NOESY are also obtained. The NMR solvent use for this measurement was deuterated methanol.

2.1 Plant Material: The plant material was collected from Basawa, Zaria, Nigeria in 2011. This was identified by Musa Gallah of the Herbarium Biological Science Department Ahmadu Bello University Zaria, Nigria. A voucher specimen (No. 900188) was deposited.

2.2 Extraction and Isolation: The air dried Powdered leaves (1.5kg) was extracted with 95% ethanol (3L) at 50 ^{0}C for 48hrs using cold maceration techniques. The extract was concentrated to dryness under reduced pressure and the residue suspended in water (800ml) and partitioned successively with n-hexane (3x500ml), chloroform (4x500ml), ethylacetate (3x500 ml) and n-butanol (5x500ml). The various extracts were concentrated using rotary evaporator to afford n-hexane (7.22 gm), chloroform (5.23 gm), ethylacetate (3.38 gm), n-butanol (7.54 gm) and Aqueous residue (5.73 gm) respectively. These extracts were subjected to phytochemical screening using standard protocols [46, 7, 43]. The n-butanol fraction (5.26 gm) was subjected to column chromatography using gradient elution techniques. The progress of elution was monitored using thin layer chromatography with the following solvent system as chloroform: Methanol: water (3:3:1) and Ethylacetate: Methanol: water (100:16.5:13.5). Combination of similar fractions on the basis of TLC analysis affords 580ml fraction of 10 ml aliquot of 10 fractions (F_1-F_{10}). The pooled fraction F_4 (150 mg) with three spots was subjected to a repeated Gel-filtration techniques using sephadex L_H -20 with 100% methanol as the eluent to obtain 85 portions aliquot of 2 ml. Portion (26 – 68) of the eluent with the same chromatographic pattern of two spots were pooled together and concentrated at room temperature. This was then submitted to a Preparative thin layer chromatography technique (PTLC).
PTLC was carried on the concentrated fraction using Fluka Silica gel precoated glass plate (20x20 cm) with layer thickness of 0.25 mm using Ethyl cetate: Methanol: Water (3:7:5) as the solvent system. The extract was uniformly applied along a faintly drawn line of about 1.5cm from the bottom of the plate with the help of a capillary tube. The plate was allowed to dry after which it developed using the solvent system was mentioned above. The chromatogram was air dried in a fume cupboard; the position of the band of interest was marked with pencil under UV light and scraped off the back of the plate on to a foil. The scraped sorbent was size reduced using pestle and morter, transferred in to beaker, washed repeatedly with Acetone 100% and then centrifuge. The supernatant layer was obtained and crystallized using solvent system petroleum ether: Methanol (8:2) to afford (86mg) of a yellowish Crystal coded 1. The TLC of 1 was carried out using the solvent system Ethylacetate: Methanol: Water (3:7:5) and chloroform: Methanol: water (100:16.5:13.5) to afford a single homogeneous spot [17, 38].

3. Chemical Test

3.1 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. This is to test for the presence of phenolics [29].

3.2 Vanillin/Sulphuric Acid Test

4.0g solution of vanillin was dissolved in 100 ml 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 – 10 min after which it was removed to ascertain the colour formed [38].

3.3 Liebermann Burchard's. Test

1ml of anhydrous Acetic acid was added to 1 ml of chloroform, and cooled to 0 ^{o}C in a test tube. Few drops of concentrated H_2SO_4 was added to the test tube containing solutions of compounds 1 [46].

3.4 Determination of Sugar in Compound 1

Compound 1, 4mg were dissolved in H_2O (2.5 ml) and 2N aqueous solution of CH_2F_2-COOH (2.5 ml) was added and then refluxed on a water bath for 3hrs each. After this period, the reaction mixture

was diluted with H_2O (10 ml) and extracted with CH_2Cl_2 (2 x 5 ml). The combined CH_2Cl_2 extract was washed with H_2O and then evaporated to dryness *in vacuo*. The concentrated aqueous layer was passed through a short Amberlite column and evaporated to dryness to give a sugar fraction (1.2 mg). This was analysed by HPLC using CH_3CN/H_2O (85:15). The sugar identified in compound 1 was D-glucose and L-rhamnose based on their HPLC retention time of t_R of 11.65 and 13.57 min respectively (D-glucose t_R-11.55 min). Co-TLC of the sample with authentic samples was carried out. The sugars were analysed by silica gel TLC comparison with standard sugars using solvent system as above [33].

3.5 Acid Hydrolysis
A solution of (3.5 mg) of compound 1, in 0.1M H_2SO_4 (1 ml) was heated at 90 ^{0}C for 30 min under argon atmosphere. After cooling, H_2O (5 ml) was added to the mixture and extracted with $CHCl_3$ (3x5 ml). The combined $CHCl_3$ layer was washed with brine, dried over Na_2SO_4 and evaporated to afford an aglycone fraction (1.4mg) respectively. The sugar fraction was dissolved in CH_3OH/H_2O (2:8) after passing through a sep-pak C_{18} Cartridge, it was analysed by HPLC using CH_3CN/H_2O 85:15. The sugars in compound 1 were identified as D- glucose with $t_{R\ 11.75min}$ and L-rhamnose $t_{R\ 13.43min}$ (D-glucose $t_{R,\ 11.60min}$) respectively [24].

3.6 Methylation
5mg of compound 1 was treated with excess methanol and 2 drop of H_2SO_4 added and then refluxed for 12 hours after which the solution was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O and the temperature reduced to 0°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 [46].

3.7 Antimicrobial Screening
The antimicrobial activities of Compound 1 and the various extract of *Stachytarpheta angustifolia*, was determined using the microbes obtained from the department of medical microbiology A.B.U Teaching Hospital Zaria. These include, *Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgari, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Bacillus subtilis, Penicillium digitatum, Candida albicans, Aspergillus niger, Fusarium oxysporum and Penicillium notatum.* All the Isolates were checked for purity and maintained in slants of Nutrient agar for bacteria and slant of saborand dextrose for the fungi before use.

3.8 Antimicrobial assay
0.8 g of the extracts were weighed and dissolved in 10ml of DMSO to obtain a concentration of 80 mg/ml. This was the initial concentration of the extracts used to check the antimicrobial activities of the extracts [19]. Mueller Hinton agar medium was prepared according to manufacturers Instruction, sterilized at 121 ^{o}C for 15mins and the sterilized medium was then poured into sterile Petri dishes and the plates were allowed to cool and solidify. Diffusion method was used for screening of the extracts. The medium was seeded with 0.1ml of the standard inoculums of the test microbes, the inoculums were spread evenly over the surface of the medium by the use of sterile swab. After setting, the use of a numbers of standard cork borer of a number 4 sterile cork borer of 6mm in diameters were obtained, a well was cut at the centre of each inoculated plate medium. The inoculated medium was then incubated at 37 ^{o}C for 24hr, after which each plate was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result was recorded in millimetres [5].
The minimum inhibition concentrations of the extracts were carried out using the broth dilution method. Mueller Hinton broth was prepared and 10mls was dispensed into test tube, the broth was sterilized at 121 ^{o}C for 15mins and allowed to cool. Mc-farlands turbidity standard number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tubes and the test microbes were inoculated and incubated at 37 ^{o}C for 6hrs. Dilution of the test microbes in the normal saline was done until the turbidity marched that of the Mc-farland's scale by visual comparison, at this point the test microbe has a concentration of about 1.5 x 10^8cfu/ml [47,11]. Two fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml and 5 mg/ml. The initial concentration was obtained by dissolving 0.8 gm of the extracts in 10mls each of the sterile broth. Having obtained the different concentrations of the various extract in the broths, 0.1 ml of the standard inoculums of the test microbe was then inoculated into different concentration of the extract in the broth. Incubation was made at 37 ^{o}C for 24 hr, after which the test tubes were observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration [37].

3.9 MBC/MFC
The Minimum Bactericidal Concentration and Minimum Fungicidal Concentration were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared and poured into sterile Petri dishes, this was allowed to cool and solidify. The contents of the MIC in the serial dilutions were then sub-cultured onto the prepared medium. Incubation was made at 37 ^{o}C for 24hrs after which each plate was observed for colony growth. The plate with the lowest concentration of the extract without colony growth is considered the MBC for the Bacteria and MFC for the fungi [36, 15, 48].
For the analysis of Caffeic glycoside ester, the method employed was that of Bauer [9]. The organisms used were same as above. Tryptic say Agar (Merck KGa A) was the medium of choice, and was prepared according to the manufacturer's Instructions. This was dispensed into sterile plates in 20ml aliquots after gelling and drying, the plates were seeded with the aforementioned organisms by streaking evenly in a cotton swab. The inoculums were allowed 5min to dry. Sterile filter paper disks (4 mm diameter) already soaked with n-BuoH (4 µl/disk) were placed and gently pressed down to ensure contact. The plates were inoculated at 37 ^{o}C for 24hrs. The zones of inhibition were measured with ruler [35].

4. Result

Table 1: Phytochemical screening of the ethanolic leaf extract of *Stachytarpheta angustifolia.*

Constituets	Test	Observation	Inference
(Carbohydrate) **General test** **Monosaccharide** **Reducing sugar**	Molisch Barfoed's Fehlings	Red colour Red. ppt Red. ppt	+ + +
Tannins	Lead ethanoate Methanol's Iron (III) chloride	White ppt Red. ppt Blue-black	+ + +
Saponins	Frothing	Persist frothing	+
Sterols	Liebermann-Burchard Salkowski	Blue-green Red ring at interphase	+ +
Terpenoids	Liebermann, Burchard	Brown ring with brown interphase	+
Alkaloids	Dragendoff's Mayer's Wagner's	- - -	- - -
Flavonoids	Shinoda Ferric chloride Sulphuric acid	- - -	+ + -
Cardiac glycoside	Keller-Kilanis Legal's	Reddish brown Deep red colour	+ +

Key: + = Present, - = Absent

Table 2: HNMR AND ^{13}CNMR SPECTRAL DATA OF Compound 1 IN (CD_3OD.400 MHz)

H(δ)ppm		C(δ)ppm
2 (7.09)	(s)	1 (127.82)
5 (6.80)	d(8)	2 (114.87)
6 (6.75)	d(8)	3 (146.28)
7 (7.54)	d(16)	4 (148.16)
8 (6.25)	d(16)	5 (144.82)
2^{1} (6.65)		6 (123.35)
5^{1} (6.50)	d(8)	7 (146.98)
6^{1} (6.69)	d(8)	8 (115.39)
7^{1} (2.80)	m(8)	9 (168.46)
3.65	m(8,9)	1^{1} (131.65)
8^{1} (3.84)	m(8,9)	2^{1} (116.46)
1^{11} (4.80)	d(7.5)	3^{1} (144.82)
2^{11} (3.56)		4^{1} (144.20)
3^{11} (3.88)		5^{1} (116.67)
4^{11} (4.80)	t(9.5)	6^{1} (121.41)
5^{11} (3.42	m	7^{1} (36.72)
6^{11} (3.58)		8^{1} (70.56)
(3.51)		1^{11} (103.16)
1^{111} (5.30)	d(1)	2^{11} (74.03)
2^{111} (3.70)	dd(1.2,5)	3^{11} (76.19)
3^{111} (3.30)		4^{11} (70.56)
4^{111} (3.13)	t(9.5)	5^{11} (72.50)
5^{111} (3.39)	m	6^{11} (62.50)
6^{111} (1.05)	d(6)	1^{111} (104.37)
		2^{111} (70.75)
		3^{111} (70.56)
		4^{111} (72.40)
		5^{111} (68.80)
		6^{111} (18.48)

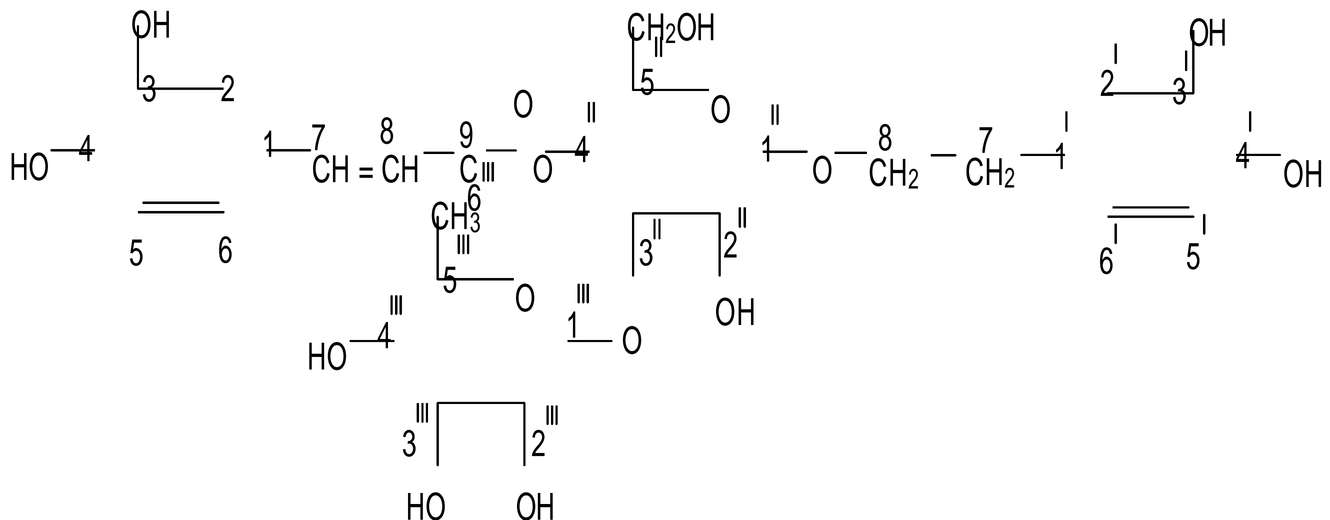

Table 3: Antimicrobial Assay of some microorganisms against Leaf Extracts with their Zone of Inhibition (mm)

Extract	S.a	S.p	P.v	P.a	K.p	E.c	S.t	B.s	P.d	C.a	A.n	F.o	P.n
n-hex (80mg/ml)	12	14	-	10	-	5	13	-	6	3	-	-	6
$CHCl_3$ (80mg/ml)	25	23	-	22	-	19	15	-	10	14	-	-	20
EtoAc (80mg/ml)	25	22	-	21	-	18	14	-	12	17	-	-	19
N – BuoH (80mg/ml)	30	29	-	35	-	34	36	-	28	24	-	-	25
Aq (80mg/ml)	21	23	-	19	-	23	19	-	14	17	-	-	14
Caffeic glycoside (4ul/disk)	34	28	-	36	-	38	36	-	20	22	-	-	25
Ampiclox Ug/ml	35	31	-	38	-	37	35	-	-	-	-	-	-

Key:- n-hex ⟶ n-hexane, $CHCl_3$ ⟶ Chloroform, EtoAc ⟶ Ethylacetate, n - BuoH ⟶ n-Butanol, Aq ⟶ Aqueous, S.a ⟶ *Staphylococcus aureus*, S.p ⟶ *Streptococcus pyogenes*,

P.v ⟶ *Proteus, vulgaris* P.a ⟶ *Pseudomonas aeruginosa*, K.p ⟶ *Klebsiella pneumonia*, E.s ⟶ *Escherichia coli*, S.t ⟶ *Salmonella typhi*, B.S ⟶ *Basillus subtilis*, C.a ⟶ *Candida albicans*, A.n ⟶ *Aspergillus nigar*, F.O ⟶ *Fusarium oxysorum* and P.n ⟶ *Penicillium notatum*.

Table 4: Minimum Inhibition Concentration of the Extracts against Test Micro Organisms

Test Organisms	n-hex(mg/ml) 80 40 20 10 5	$CHCl_3$(mg/ml) 80 40 20 10 5	EtoAc(mg/ml) 80 40 20 10 5	n-BuoH(mg/ml) 80 40 20 10 5	Aq(mg/ml) 80 40 20 10 5
(+) S. aureus	- 0x + ++ +++	- - 0x + ++	- - 0x + ++	- - 0x + ++	- - - 0x +
(+) S. pyogenes	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++	- - 0x + ++	- - 0x + ++
(-) P. vulgaris	-	-	-	-	-
(-) P. aeruginosa	- 0x + ++ +++	- - 0x + ++	- - - 0x +	- - - - 0x	- - - 0x ++
(-) K. pneumoniae	-	-	-	-	-
(-) E. coli	- 0x + ++ +++	- - - 0x +	- - 0x + ++	- - - 0x +	- - 0x + ++
S. typhi	- 0x + ++ ++	- - - 0x +	- 0x + ++ +++	- - 0x + ++	- - 0x + ++
B. subtilis	-	-	-	-	-
P. digitalis	- - 0x + ++	- - - 0x +	- - 0x ++ +++	- - 0x + ++	- 0x + ++ +++
C. albicans	- 0x + ++ ++	- - 0x + ++	- - 0x + ++	- - - 0x +	- 0x + ++ +++
A. niger	-	-	-	-	-
F. oxysonum	-	-	-	-	-
P. notatum	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++

Table 5: Min. Bactericidal/Fungicidal Concentration of the Extracts against Test Micro Organisms

Test Organisms	n-hex (mg/ml) 80 40 20 10 5	$CHCl_3$ (mg/ml) 80 40 20 10 5	EtoAc (mg/ml) 80 40 20 10 5	n-BuoH (mg/ml) 80 40 20 10 5	Aq (mg/ml) 80 40 20 10 5
(+) S. aureus	0x + ++ +++ +++	0x + ++ +++ +++	0x + ++ +++ +++	- - 0x + ++	- 0x + ++ +++
(+) S. pyogenes	0x + ++ +++ +++	- 0x + ++ +++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++
(+) P. vulgaris	-	-	-	-	-
(-) P. aeruginosa	0x + ++ +++ +++	- 0x + ++ +++	- 0x + ++ +++	- 0x + ++ +++	0x + ++ +++ ++++
(-) K. pneumoniae	-	-	-	-	-
(-) E. coli	0x + ++ +++ ++	0x + ++ +++ +++	- 0x + ++ +++	- - 0x + ++	- 0x + ++ +++
S. typhi	0x + ++ ++ +++	0x + ++ ++ +++	0x + ++ ++ +++	0x + ++ ++ +++	0x + ++ ++ +++
B. subtilis	-	-	-	-	-
P. digitalis	0x + ++ +++ +++	0x + ++ +++ +++	0x + ++ ++ +++	0x + ++ +++ ++++	0x + ++ +++ ++++
C. alnilans	0x + ++ ++ +++	- 0x + ++ +++	- 0x + ++ +++	- 0x + ++ +++	- 0x + ++ +++
A. Niger	-	-	-	-	-
F. oxysonum	-	-	-	-	-
P. notatum	0x + ++ ++ +++	0x + ++ ++ +++	- 0x + ++ +++	- 0x + ++ +++	0x + ++ ++ +++

Key: - No turbidity (No growth), 0x MIC, + ⟶ light turbid (light growth), ++ ⟶ Moderate turbid, +++ ⟶ High turbidity.

5. Discussion

The phytochemical screening of the ethanolic leaf extract reveals the presence of Tannins, Glycoside, Flavonoids, Steroids, Saponins and Terpenoids.

Extraction of the whole leave extract of *S. angustifolia* followed by an extensive column chromatography of n-butanol extract on silica gel, purification over sephadex LH – 20 and subsequent (PTLC) resulted in the isolation of compound 1. The isolate gave a positive colour test with ferric chloride test suggesting compound 1 to be phenolics, It also gave a red colour at the interphase indicating the presence of glycoside [29, 46]. The I.R Spectrum displayed absorption at 2447cm^{-1} indicating a hydroxyl group. The absorption at 2954cm^{-1} indicate a C – H (stretch) for a methyl group, while absorption at 1462cm^{-1} (C=C) indicates several bands on aromatic ring with many peaks [28]. Absorption band at 2924 – 2853 cm^{-1} (C-H) is a characteristic of a methylene group, while signal at 1713-1720 cm^{-1} could be attributed to rhamnose unit, a sugar moiety [30].

The proton (NMR) of Compound 1 (Table 2) shows signal at δ_H1.05ppm, suggested to be a tertiary methyl group on H-6^{III}. The signals observed at δ_H6.50 ppm, δ_H6.80 ppm, δ_H 6.25 ppm, δ_H 6.65ppm and δ_H 6.56ppm are all characteristic for aromatic protons on C-5, C-6, C-8, C-2^1, C-5^1 and C-6^1 respectively [30]. The signals with δ_H4.50 ppm and δ_H5.30ppm are characteristic for anomeric protons attributed to sugar moieties i.e. Glucose and Rhamnose [40]. The signal with value δ_H1. 05ppm is a major signal found on Methyl group of the rhamnose sugar H-6, ^{III} while the Multiplet signals on δ_H2. 80ppm and δ_H3.84ppm are characteristics of an α and β Methylene assigned to protons on C-7^I and C-8^I of the Caffeoyl moiety signifying the presence of a vinyl protons [24]. Signals found at δ_H4.50ppm and δ_H4.80 ppm are characteristic of sugar protons which is in conformity with a β–Configuration of an anomeric proton. The glucosyl anomeric proton δ_H4.50ppm is linked to a caffeoyl moiety while δ_H4.80ppm was also linked to the aglycone by a glycosidic bond [22].

The anomeric proton on H – 1^{III} and the methyl group of the rhamnose Sugar were easily recognized. The first at δ_H5.30ppm, with a small coupling constant (J_{HZ} 1.00ppm) while the latter at δ_H1.05ppm (J_{HZ} 6) as a doublet are in conformity with the α -L- configuration of the rhamnopyranoside[2]. The coupling of the anomeric proton δ_H5.30 ppm with glucopyranosyl proton at δ_H3.88ppm suggests that, the methyl proton δ_H1.05ppm is part of the sugar moiety, a rhamnose. Thus, the two sugars are resolved as glucose and rhamnose (Masaki *et al.,* 2004; Rong, *et al.,* 2003). The signal at δ_H3.88 ppm assign to C-3^{II} was found to couple with the proton at δ_H5.30 ppm (HMBC) and from these observations the methyl signal can be assigned to H-1^{III} of rhamnose [31, 39]. The signals at δ_H3.56ppm, δ_H3.70 ppm, δ_H3.30ppm and δ_H3.13ppm (t) could be attributed to the sugar protons of C-2^{II}, C-2^{III}, C-3^{III} and C-4^{III} associated with glucose and rhamnose moiety [4, 10]. Thus, these assignments are in conformity with the observed values in α-rhamnose 1 – β - 3 – Glucose. The correlation of δ_C 18.4626ppm/1.05ppm and C-1^{III}/1^{III} C104.2324ppm/5.30 ppm could be attributed to rhamnose moiety, while C-1^{II}/H-1^{II} (103.0565 ppm/4.50 ppm) attributed to glucose moiety was observed [50].

In ^{13}CNMR (table 2), peaks were assigned on the basis of Chemical shift consideration and comparison with data for glucose and rhamnose. The two chemical shift (J_{HZ} 7.5 and J_{HZ} 1.00 ppm) on C– 1^{II} and C - 1^{III} could be attributed to δ_C103.0565, δ_C104.2324 ppm for glucose and rhamnose moiety [2]. ^{13}CNMR spectrum was found to exhibit 29 Carbon signals, 9 corresponding to Caffeoyl Moiety, 8 to aglycone, 6 to glucose while 6 to rhamnose sugar respectively [31].

Signals observed at δ_C 146.8685ppm, δ_C148.0262ppm and δ_C144.7106ppm suggested for C-3, C-1 and C–5 are characteristic of an aromatic nucleus. Signals at δ_C115. 2093 ppm and δ_C117.1120ppm indicate the presence of an – αand β Vinylic Carbons coupled to δ_C168.2885ppm which signify the presence of a carbonyl Carbon on C– 9 [21]. Signals on δ_C146.8685ppm, δ_C148.0262ppm, δc144.7106ppm and δ_C146.1605ppm are desheilded carbon signals down field as a result of Para/meta hydroxyl substituent on Benzene ring at C – 3, C – 4, C – 3^1 and C – 4^1 position respectively. The hydroxyl substituent on C-3 and C – 4 are similar to the corresponding – β – (3^I, 4^I dihydroxy phenyl on the aglycone Unit (Yoshiyasu *et al.,* 2004). The deshielding effect on C- 3^{II} with respect to the glucose must be attributed to C-1^{III} as rhamnose (1 →3) glucose bond (+δ) effect and to β – Caffeoyl effect (-δ) effect (Lius *et al.,* 2001). The coupling constant of the anomeric carbons of glucose and rhamnose are completely in consistence with the – β – configuration for the former and α for the latter. The signals on δ_C 146.1605 and δ_C 144.7106 ppm are desheilded down field as a result of hydroxyl group substitute corresponding to –β-(3^I, 4^I dihydroxyphenyl) [50, 2].

In conclusion, it can be deduced that the glucose is a D-glucopyranose while the rhamnose is of L- origin from the acid hydrolysis. The I.R spectrum absorption at [1713 – 1720] signifies the presence of a rhamnose Unit. The proton (NMR) Spectral data has enabled us to resolve the aromatic proton as two ABX systems. One of the attributed to Caffeic acid derivative as Caffeoyl moiety, while the other as 3^I, 4^I dihydroxyphenyl ethyl here after designated as aglycone [16]. The ^{13}CNMR has assisted us in ascertaining the molecular formula as $C_{29}H_{36}O_{15}$ with molecular weight of 624.594. The anomeric position of the glucose moiety as a – β – Origin from the coupling constant (7.5Hz) with anomeric proton at δ_H4.50ppm for δ_C 103.0565ppm. While the rhamnose Unit Conforms with those of α – L- rhamnopyranoside. The nature of sugar was confirmed by the comparative data studies of ^{13}CNMR for caffeic glycoside to be – β – D – glucopyranoside [25].

On the basis of this discussion it was determined that, the rhamnose to be (- O - α – L- rhamnopyranosyl) while the glucose, to be (β – D - glucopyranose). The two sugars are linked to each other on C – 1^{II}↔ C – 3^{III} by a glycosidic bond.

Therefore, the comparative studies of the data obtained from (HMBC.HSQC and NOESY). Thus compound 1 is named as – β – (3^I, 4^I – dihydroxyphenyl)- ethyl- O - α - L – rhamnopyranosyl (1→ 3) – β – D – (4 – O – Caffeoyl) – glucopyranoside.

The Antimicrobial sensitivity test indicated that, the extracts inhibited the growth of *Staph aureus Streptococcus pyogenes Pseudomonas aeruginosa, E. coli, Salmonella typhi, Candida albicans, Penicillium digitatum and Penicillium notatum.* The extracts were found not to inhibit the growth of *proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilis, Aspergillus niger and Fusarium oxysorum.* The increase in concentration of the extract also increases the zone of growth inhibition of some of the micro-organism. The highest growth inhibition of 31mm and 29mm diameter, was exhibited by (4µl/ disk) 80mg/ml of verbascoside and n-BuoH extract against *Escherichia coli* and *Staphylococcus aureus* respectively. The lowest zone of growth inhibition was observed with 20mm and 3mm diameter as against *Penicillium digitatum* and *Candida albicans* from the extracts of verbascoside

(4 µl/disk) and (80 mg/ml). The highest minimum inhibition concentrations of the extracts on the test isolate was exhibited against *Pseudomonas aeruginosa, Escherichia coli* and *S. aureus* from n-BuoH (10 mg/ml). The lowest MIC was recorded against the test Microbes from the n-hexane fraction of the ethanolic extracts. The observed antimicrobial effects of the extracts on the micro- organisms could be attributed to the presence of aforementioned secondary metabolites contain in the plant [13]. The presence of tannins in plant has been shown to exhibit great potential in phytomedicine as an astringent as well as a strong anti-parasitic agent. The terpenes in the other hand, are been use as an effective anti-tumor and an antiviral agent, as some terpenes are known to be cytotoxic to tumor cells. Although, the eudesmane sesquiterpenes have been reported to exhibit strong antibacterial properties. The saponins, are known to have an anti-oxidant, anti-cancer, anti- inflammatory as well as an anti-viral recipe properties while the Flavonoids are known to be an anti-inflammatory agent [6]. The large zone of inhibition exhibited by the extracts against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi* justified the various uses of this plant by the traditional healers.

P. aeruginosa has been implicated in the cases of boils, sores and wounds [8]. The moderate growth inhibition against *E. coli* could also be attributed for the use of the leaf portion for the treatment of diarrhea and dysentery. Although, *E. coli* have been known to be a common cause of travellers' diarrhoea, infant death and other diarrheagenic infections in humans especially in developing countries. The low MIC of *S. aureus* is of a great significance in the health care sector, this is because it could be used as a substitute or an alternative to the orthodox antibiotics in the treatment of infections caused by the microbes, especially as they frequently develop resistance to the available known antibiotics [42]. *S. aureus* is also known to play a significant role in skin diseases including superficial and deep follicular lesion. The MIC exhibited by the extract against *S. aureus* is of great significant in the health care delivery system, since it could be used as an alternative to Orthodox antibiotics in the treatment of infections caused by these microbes, especially as they frequently develop resistance to known antibiotics [44]. The use of those extract against *S. aureus, Escherichia coli and salmonella typhi* would reduce the cost of obtaining healthcare, this for the obvious reason of its availability, affordability and toxic free compared to the synthetic drugs [12].

The presences of these metabolites in the plant suggest great potential for the use of the extract as a source of phytomedicines. The zone of inhibition exhibited by the extract against *S. aureus, P. pyogenes, E. coli and S. typhi* justified its use by the traditional medical practitioners in the treatment of sores, boils, open wounds, sexually transmitted diseases and dysentery. The strong activity of the chloroform and n-butanol extract on the test microbes, indicates that the plant can be a source of compound that can be effective against related infectious diseases.

The caffeic glycoside ester has shown an impressive activity against some of the test microbes, especially when the isolate is less combine with other secondary metabolites. Therefore, the observed antimicrobial properties of this plant *S. angustifolia* corroborate its use in the ethnomedicine.

6. Conclusion: The result from the finding could now be concluded that, the leaf extract of *S. angustifolia* provides a promising solution in the ethnomedicine practice of some disease control. The caffeic glycoside ester isolated from the n-BuoH could also be used to replace the synthetic antibiotics use in the treatment of the related ailments.

7. Reference:

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