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Isolation, characterization and antibacterial activity of 3-hydroxy-2,2-bis (6-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl) propanal from the stem exudate of *Brachystegia eurycoma* Harms

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ABSTRACT

A bioactive phytochemical compound identified as 3-hydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl) Propanal was isolated from the stem exudate of Brachystegia eurycoma Harms by making use of Column and Thin Layer Chromatography as separation techniques. It was then characterized by Infrared Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry. The compound exhibited marked antibacterial activities against Pseudomonas aeruginosa, Streptococcus faecalis and Bacillus cereus. These results authenticate the use of the stem exudate of Brachystegia eurycoma Harms in the treatment of wounds and infections in herbal medicine in Nigeria. The results further suggest that a possible development of the compound in pharmaceutical industries would enable the compound to be used in the pharmaceutical treatment of infections and diseases caused by these organisms since it would be safer than synthetic ones.

Keywords: *Brachystegia eurycoma* Harms, Antibacterial activity, Phytochemicals, Exudate, Herbal medicine.

INTRODUCTION

Most plants which are widely used as food in Nigeria have medicinal values. But due to paucity of information on the bioprotective properties of these plants, their uses as medicine have languished in obscurity. *Brachystegia eurycoma* Harms is one of such plants. The plant is an indigenous legume that is widely used traditionally in Eastern Nigeria as condiment and thickening agent. However, its use for medicinal purposes has not been fully reported and documented. Plants have been used for medicinal purposes for centuries, and today about 80% of the world population relies primarily on botanical preparations as medicine [1,2].

The family of *Brachystegia eurycoma* Harms (*Fabaceae*) comprises of herbs, vines, shrubs, trees and lianas found in both temperate and tropical areas of the world [3]. The antifungal properties of ethanol and water extracts of the stem bark of *Brachystegia eurycoma* have been reported [4]. After 43 hours of incubation, the two examples at 2 mg/ml inhibited the growth of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans,*

Epidermophyton floccosium, Fuscarium solani, Mucor mucedo, Microsporum audonii and *Trichophyton verrucasum* [4].

The effects of aqueous extracts of five wood samples *Khaya grandifoliola, Mansonia altissima, Brachystegia eurycoma, Milicia excelsa* and *Terminalia superba* on some cellulolytic bacterial strains have been investigated [5]. The aqueous extracts of the wood samples inhibited the growth and cellulolytic activity of the *Bacillus subtilis*. The inhibitory effect of the extracts of *B. eurycoma* against bacterial strains was highest when compared to other wood types [5]. The wound healing effect of the snail mucin have been evaluated with special attention to the effect when combined with honey in *B. eurycoma* gel preparation [6]. *B. eurycoma* exudate, snail mucin and honey were combine in different concentrations in the treatment of wound made by excision model in rats. It was observed that mucin when combined in different concentrations in the *B. eurycoma* gel heals faster than when used alone [6]. *B. eurycoma* gum was also observed to effect fast healing of wounds when used alone [6]. *B. eurycoma* gum in right combinations with mucin and honey is used for wound healing, prevention of bacteria injection, scar formation and regeneration of hair follicles [6].

Experiments have shown that gums physiologically functions as soluble fibre when ingested and as such are very effective in reducing blood cholesterol levels and moderating glucose response in diabetics [7]. It has also been reported that the supplementation of the diets of diabetic patients or those with impaired glucose tolerance with fibre in form of plants gum resulted in an improvement in blood glucose profiles, reduction in urinary glucose and a decrease in the mean serum cholesterol level [8].

Brachystegia eurycoma affords a lot of medicinal values in herbal medicine in Nigeria. In the light of this, we report herein the isolation, characterization and antibacterial activity of 3- hydroxyl -2, 2-bis (6-methoxy-3-3 methyl-2-2, dihydrobenzofuran-2-yl) propanal from the stem exudate of *Brachystegia eurycoma* Harms.

MATERIALS AND METHODS

Experimental

The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 FT spectrophometer using TMS as internal standard. Chemical shifts were expressed in parts per million. LC-ESIMS spectra were determined in the positive ion mode on a PE Biosystem API 165 single quadruple instrument; HRESIMS (positive ion mode) spectra were recorded on a Thermo Finniga MAT 95 XL mass spectrometer. Column chromatography was carried out with silica gel (200-300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on precoated 0.25 mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck, Damstadt Germany.

Plant Materials

The stem exudate of *Brachystegia eurycoma* was collected from the tree plant at Umuovo village stream, Old Umuahia, Abia State, Nigeria. The plant was identified by Mr. N. I Ndukwe of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria.

Extraction of Phytochemical

The exudate (500g) was packed into a soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24 hours. The ethanol extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 2 days. The column was packed with silica gel and the extract eluted with different fractions of chloroform, petroleum ether and methanol to afford the compound. It gave R_f value of 0.46 on TLC [using chloroform and methanol (7:3)].

Determination of Antibacterial Activity

The *in vitro* antibacterial activity of the compound was carried out for 24h culture of three selected bacteria. The bacteria organisms used were *Pseudomonas aeruginosa, Streptococcus faecalis* and Bacillus cereus. All the test organisms were chemical isolates of human pathogens obtained from stock cultures at the Central Laboratory Services Unit of National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminum foil and sterilized in an autoclave at $121^{O}C$ for 15 minutes. They were however used within 48 hours of production. The sensitivity of each test microorganism to the compound was determined using the Disc Diffusion Technique [14,15]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the innoculeum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the compound bearing paper discs was carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 hours in an incubator at $37^{O}C$. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimeter rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the compound having different zones and selecting the lowest concentration.

RESULTS AND DISCUSSION

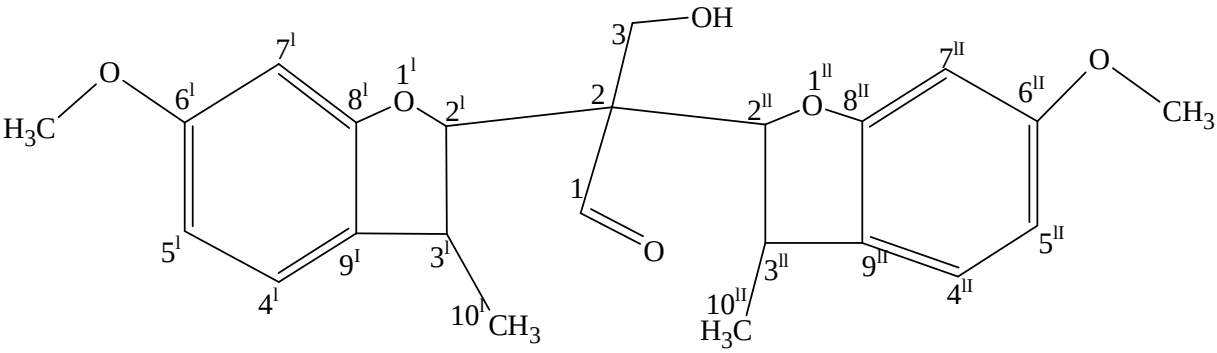
Compound **1** was isolated from the stem exudate of *Brachystegia eurycoma* Harms. It gave R_f value of 0.46. In the IR spectrum of the compound, a strong, broad band at 3381.07 cm^{-1} was due to –OH functional group. Absorption at 2926.85 cm^{-1} was due to C-H stretching vibration of an alkane. The spectrum also showed a carbonyl absorption typical of a ketone at 1708.64 cm^{-1}. Absorption at 1267.00 cm^{-1} and 1049.43 cm^{-1} were typical of ether functional group. The C=C stretching vibration of aromatic bonds showed absorption at 1561.43 cm^{-1} while the out-of-plane C-H bending of the aromatic group gave absorption at 724.16 cm^{-1}. There were absorptions around 1370.00 cm^{-1} which was indicative of C – H bond of a $–CH_3$ group.

Table 1: IR Absorptions of Compound 1

IR Absorption (cm^{-1})	Functional group	Compound type
33.81.07	O-H	Alcohol
2926.85	C-H	Alkane
1708.64	C=O	Carbonyl
1267.00	C-O	Ether
1049.43	C-O	Ether
1561.43	C=C	Aromatic
724.16	C-H	Aromatic
1370.00	C-H	Alkane

Table 2 shows the ^{I}H NMR chemical shifts and multiplicities of compound **1**. The spectrum showed the presence of methoxy protons at δ3.5512. The peak appeared as a sharp six-proton singlet. This was as a result of the coupling of the protons of the two methoxy groups since both of them were in the same chemical environment and were chemically equivalent. On the two benzene rings, C_7^1 and C_7^{11} protons were chemically equivalent. C_5^1 and C_5^{11} protons, and C_4^1 and C_4^{11} protons were also chemically equivalent. As a result of this homogeneity in chemical nature, C_7^1 and C_7^{11} protons coupled to give a two-proton singlet at δ7.9081. There was splitting of C_5^1 proton due to the C_4^1 proton and vice versa. However, the coupling of C_5^1 and C_5^{11} protons appeared as a doublet at δ7.5014. In the same vain, C_4^1 protons coupled with C_4^{11} protons to give a doublet peak at δ7.2223. The C_7^1 protons were deshielded by the electronegative oxygen atoms and therefore appeared downfield. The effect of deshielding was least on C_4^1 protons and therefore they appeared at a lower chemical shift than C_5^1 and C_5^{11} protons. The two $–CH_3$ protons were

in the same chemical environment. They coupled to give a six proton singlet peak at δ1.3035. The proton at C_3^1 split into quintet due to the three protons at C_{10}^1 and one proton at C_2^1. Proton C_3^1 then coupled with proton C_3^{11} because of their chemical equivalence to give a two-proton quintet at δ1.6118. Proton of C_2^1 split into a doublet due to C_3^1 proton and then coupled with C_2^{11} proton to give a two-proton doublet at δ3.4058. The two protons of C_3 appeared as a two-proton singlet peak at δ3.8019. The –OH proton appeared as a singlet peak at δ4.9028 while proton of C_1 appeared at δ9.812 as a singlet peak.



Compound 1

Table 2: Proton NMR Chemical Shifts and Multiplicities Compound 1

Position	Chemical Shift(s)	Multiplicity
1	9.8123	1Hs
2	3.8019	2Hs
3	4.9028	1Hs (OH)
2^I, 2^{II}	3.4058	$2H_d$
3^I, 3^{II}	1.6118	$2H_m$
4^I, 4^{II}	7.2223	$2H_d$
5^I, 5^{II}	7.5014	$2H_d$
6^I, 6^{II}	3.5512	$6H_s$
7^I, 7^{II}	7.9081	$2H_s$
10^I, 10^{II}	1.3035	$6H_d$

From MS data, the compound was assigned the molecular mass m/z 398.0233 ($M^{\cdot+}$) calculated for $C_{23}H_{26}O_6$ (m/z 398) with base peak at m/z 106.0811 calculated for C_7H_6O (m/z 106). The base peak occurred due to the detachment of a phenylmethoxy portion of the compound. Other important peaks occurred at m/z 31.1088, 41.0231, 72.1091, 91.1074, 163.0606 and 235.0355. The fragmentation pattern of Compound **1** is shown in Figure 1.

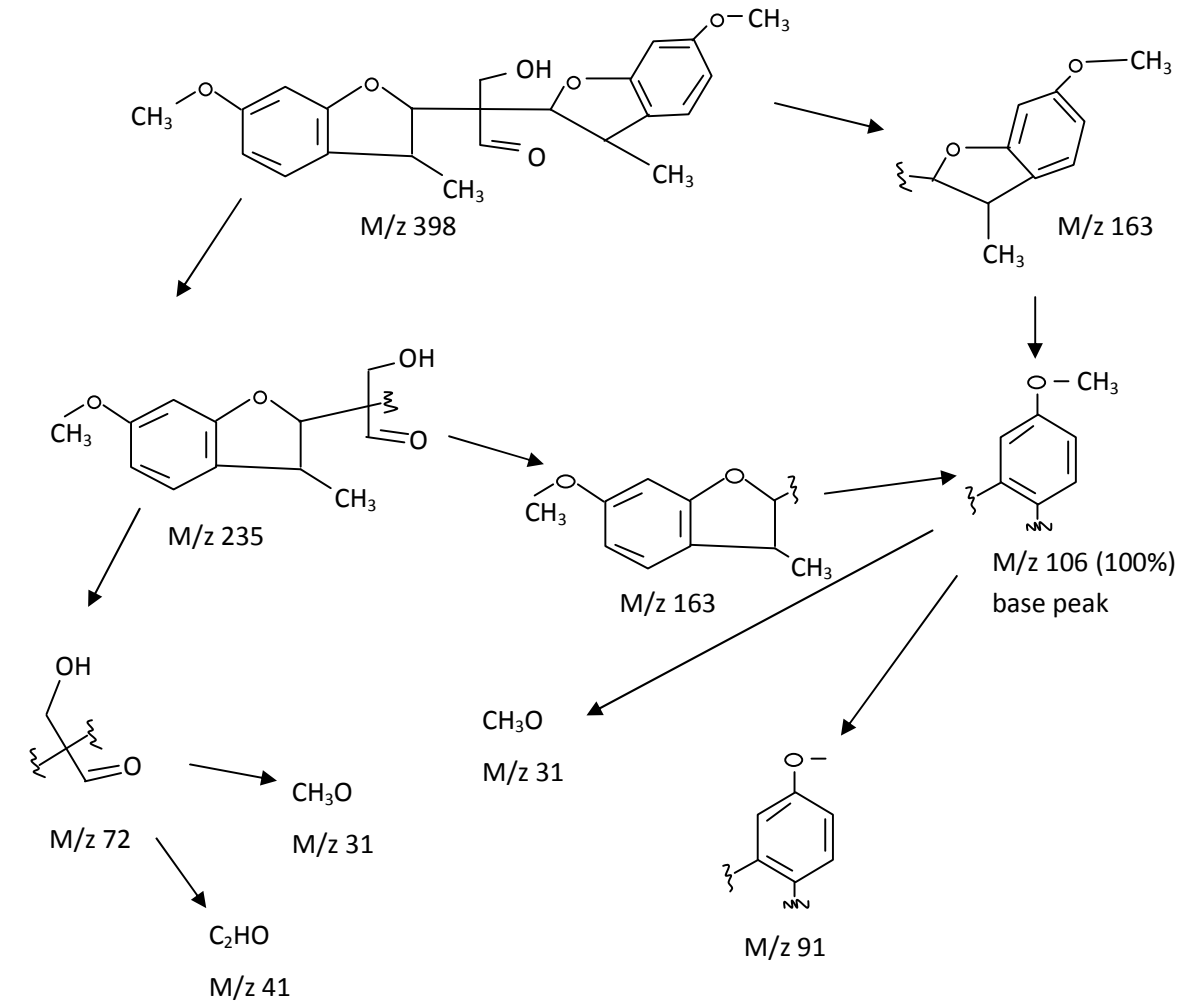


Figure 1: Fragmentation Pattern of Compound 1

Antibacterial Activity

Table 3: Inhibitory Effects of Compound 1 from the Stem Exudate of *Brachystegia eurycoma* Harms

Pathogens	Concentration (%)				MIC (%)
	25	50	75	100	
Pseudomonas aeruginosa	12.33	14.67	17.33	25.33	25
Streptococcus faecalis	-	7.65	9.67	12.33	50
Bacillus cereus	-	9.70	15.33	18.67	50

Figures are in mm and include the diameter of the paper disc (5 mm). Data are means of triplicate determinations.
MIC = Minimum Inhibitory Concentration

- = No inhibition

The compound from the stem exudate of *Brachystegia eurycoma* Harms successfully inhibited *P. aeruginosa, S. faecalis* and *B. cereus*. It exhibited highest antibacterial activity against *P. aeruginosa*. The minimum inhibitory concentration (MIC) of the compound was 25-50%. The microorganisms tested are capable of causing diseases in human and have been confirmed to be involved in the infection of wounds and also could cause urinary track infections [9]. These findings suggest that the compound could be used in the treatment of wounds and urinary tract infections like gonorrhea. Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membrane infections of man [10,11,12]. Wounds and boils provide conducive environment for the growth of microbial organisms [12]. Microbial infection of wounds delays healing which can lead to further tissue injury and damage [12,13]. The antimicrobial activity of the compound on these wound pathogens could be the reason why the stem exudate of the plant is used in the treatment of wounds in herbal medicine. The compound is hereby recommended for development in pharmaceutical industries to be used as drugs for the treatment of wound and infections.

Statistical Analysis
All bioassay were replicated three times and means determined [16].

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