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Isolation, Characterization and Antibacterial activity of 4-(4-phenyl-1, 4-dihydronaphthalen-1-yl) Pentenoic Acid from the Stem Bark of *Brachystegia eurycoma* Harms

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Abstract

A new napthalene pentenoic acid identified as 4–(4–phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid was isolated from ethanol extract of the stem bark of *Brachystegia eurycoma* Harms. The structure of the compound was elucidated using Infrared Spectroscopy, Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry. The isolated compound showed marked antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus fecalis*. These results provide a corroborative evidence for the use of *Brachystegia eurycoma* stem bark in the treatment of gonorrhea and other infections including healing of wounds in herbal medicine in Nigeria.

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Brachystegia eurycoma, Antibacterial activity, Herbal medicine.

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INTRODUCTION

Nigeria is endowed with a variety of medicinal and nutritional plants. The rainforest vegetation of South Eastern Nigeria affords myriad of medicinal benefits to the people. Most of these herbal plants have not received research attention. This prompts dynamic research on daily basis by natural products chemists and other scientists to explore their bioprotective and phytotherapeutic potentials. A large portion of these plants used in phyto-medicine, however, continues to languish in obscurity. *Brachystegia eurycoma* Harms is one of such plants.

Brachystegia eurycoma is an economically very valuable tree crop mostly grown in the tropical rain forest of West Africa[1]. Its uses range from food to medicine. In West Africa, the edible seed is used in soup making as a thickener. According to researchers, it helps in maintaining heat within the body when consumed, in other words, it is a good source of nutrient and helps in the control of body temperature[1,2]. The seeds help in softening bulky stools and have been associated with the protection against colon and rectal cancer[1]. The phytochemical composition of the seeds and stem bark of *Brachystegia eurycoma* shows that they are good sources of flavonoids, tannins, terpenes, alkaloids, saponins and phenols[3,4]. The plant has been reported to contain antioxidant phytochemicals suggesting the use of the plant in the management or treatment of tissue inflammation, arthritis, cancer, cardiovascular disorders, wounds and arthrosclerosis[5]. *Brachystegia eurycoma* is a large tree with an irregular bole and huge-twisted, spreading branches which forms a generous canopy[6]. It also possesses a rough fibrous bark which peels off in patches and often gives out brownish buttery exudates[7]. The exudate from *Brachystegia eurycoma* stem bark is used in faster healing of wound and in right combination with mucin and honey is used for wound healing, prevention of bacteria infections, scar formation and promotes regeneration of hair follicles[8,9]. In agro-forestry, the tree is suitable as a shade tree and ornamental plant especially in the dry season when the tree produces masses of coloured young foliage. The fruit pods make good fuel wood[7]. Timber known in the international timber market is produced from *Brachystegia eurycoma*. It is brown in colour, very dense, moderately strong, fairly durable and used to produce plywood cones and faces, flooring frames, stairway tool handles, furniture and cabinet works, roof trusses, rafters and shuttering[4,7].

In spite of the various uses of *Brachystegia eurycoma* in herbal medicine in Nigeria and as food thickeners, the phytoconstituents of this plant have not been fully documented. In this research article, the details of the isolation and structure elucidation of a new naphthalene pentenoic acid; 4-(4-phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid and its antibacterial activity are hereby reported.

MATERIALS AND METHOD

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 spectrophotometer 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 95XL mass spectrometer. Column chromatography was carried out with silica gel (200-300 mash) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on a precoated 0.25 mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck, Darmstadt Germany.

Plant Materials

Brachystegia eurycoma stem barks were harvested from the tree plant located at Umuovo village stream in Old Umuahia, Umuahia South Local Government Area of Abia State, Nigeria. The plant material was identified by Mr. N. I Ndukwe of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The harvested barks (2kg) were then dried on the laboratory bench for 30 days. They were thereafter milled into a uniform and fine powder by a mechanically driven attrition mill. The powdered plant material was dried and kept properly for further use.

Extraction and Isolation of Plant Materials

The powdered stem bark of *Brachystegia eurycoma* (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 obtain the compound. It gave R_f value of 0.88 on thin layer chromatography [using chloroform and methanol (7:3)].

Bioassay

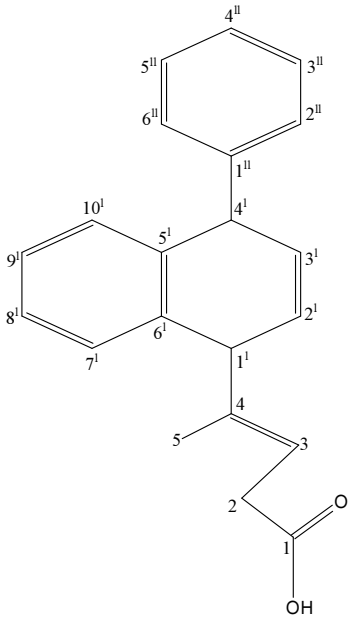
The *in vitro* antibacterial activity of the isolated compound was carried out for 24h culture of three selected bacteria. The bacteria organisms used were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Streptococcus fecalis*. All the test organisms were clinical isolates of human 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 aluminium 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[10,11]. 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 extract bearing paper discs was carefully place 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.

Statistical Analysis

All bioassay were replicated three times and means determined[12].

RESULTS AND DISCUSSION

Compound **1** isolated from the stem bark of *Brachystegia eurycoma* gave R_f value of 0.88. The IR spectrum of the compound showed a strong broad band at 3399.48cm^{-1} due to O-H stretching vibration. A strong absorption at 2920.71cm^{-1} was due to C-H stretching vibration characteristic of a tetrahedral carbon. The absorptions at 1506.22cm^{-1} and 1460.22cm^{-1} were characteristic of C=C stretching of aromatic bonds while the out-of-plane C-H bending of aromatic ring gave absorption at 665.80cm^{-1}. An absorption at 1049.25cm^{-1} was due to C-O stretching vibration in the compound. Another absorption at 1653.43cm^{-1} was indicative of an alkene double bond. The spectrum also showed absorption at 1699.44cm^{-1} which was typical of a carbonyl group.



Compound 1

Table 1: IR Absorptions of Compound 1

IR Absorption (cm^{-1})	Functional group	Compound type
3399.48	O-H	Alcohol
2920.71	C-H	Alkane
1506.22	C=C	Aromatic
1460.22	C=C	Aromatic
665.89	C–H	Aromatic
1049.25	C–O	Ether
1699.44	C=O	Carbonyl
1653.43	C=C	Alkene

The proton NMR spectrum of compound **1** showed a doublet peak observed at δ1.3132 due to $-CH_2-$ protons of C_2. The two protons should have given a singlet peak since they were in the same chemical environment but this was not so because of spin-splitting caused by the proton of C_3 causing a doublet peak. A triplet peak observed at δ6.1145 was due to C_3 proton. The proton at C_3 was split by the two C_2 protons and the absorption occurred downfield (δ1.6341) because of deshielding caused by the alkene double bond. The two protons of the methyl group of C_5 coupled to give a three proton singlet peak at δ1.8112. The proton of $C_1{}^1$ appeared as a doublet peak at δ1.6341. The doublet peak was caused by $C_2{}^I$ proton (splitting). $C_2{}^I$ proton appeared as a triplet peak at δ5.9011. The triplet peak observed was as a result of spin-splitting caused by $C_1{}^I$ and $C_3{}^I$ protons. The proton at $C_4{}^I$ was split by $C_3{}^I$ proton to give a doublet peak at δ1.5224. The protons of $C_7{}^I$, $C_8{}^I$, $C_9{}^I$ and $C_{10}{}^I$ were equivalent. Because of this equivalence in chemical nature, the protons coupled to give a four-proton singlet peak at δ7.5272. Protons of $C_2{}^{II}$, $C_3{}^{II}$, $C_4{}^{II}$, $C_5{}^{II}$ and $C_6{}^{II}$ were also equivalent. They also coupled to give a five-proton singlet peak at δ7.1317. A singlet peak at δ11.2146 was characteristic of O-H proton of a carboxylic acid which is present at C_1.

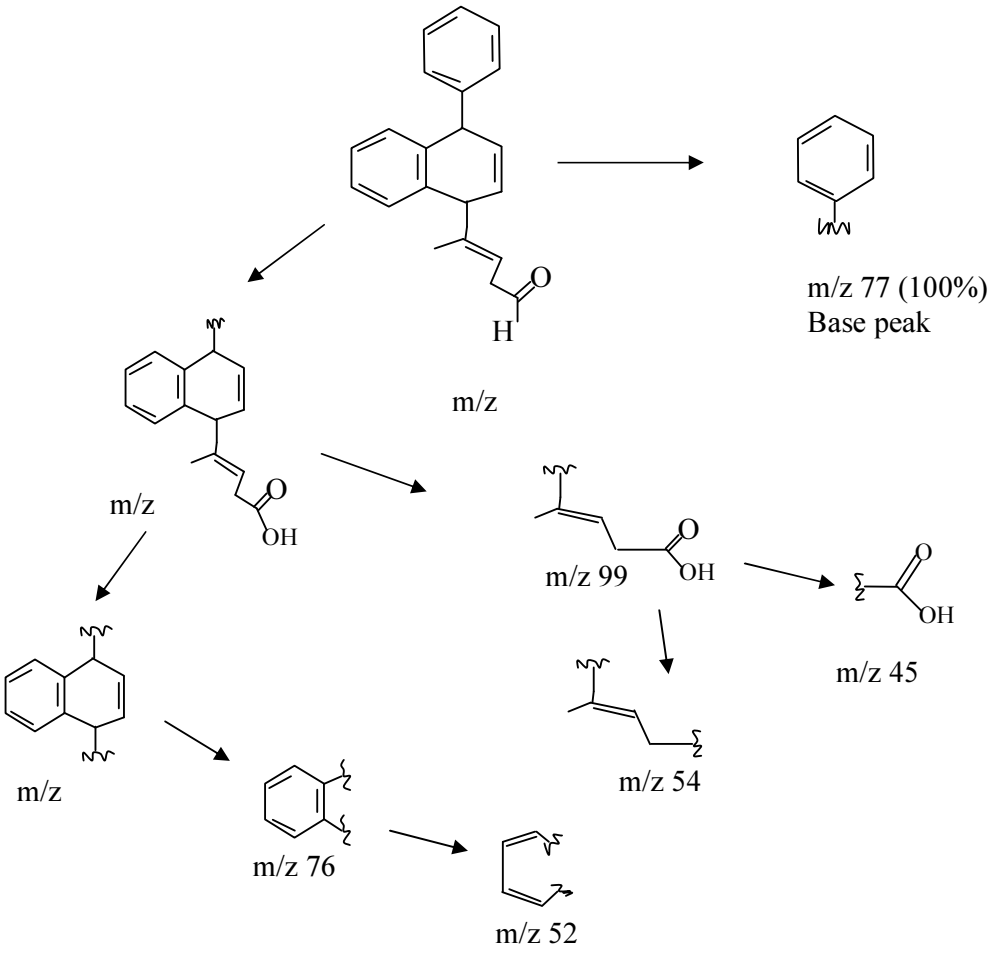


Figure 1: Fragmentation Pattern of Compound 1

From MS data, compound **1** was assigned the molecular mass m/z 304.0211 calculated for $C_{21}H_{20}O_2$ (m/z 304) with base peak at m/z 77.1223 calculated for C_6H_5 (m/z 77). The base peak occurred as a result of the detachment of a benzene ring portion of the compound. Other important peaks occurred at m/z 45.0181, 52.1212, 54.1212, 76.2154, 99.0810, 128.0508 and 227.1034. The fragmentation pattern of compound **I** is shown in Figure **1**.

Table 2: Proton NMR Chemical Shifts and Multiplicities of Compound 1

Position	Chemical Shift (δ)	Multiplicity
1	11.2146	1Hs
2	1.3132	2Hd
3	6.1145	1Ht
5	1.8112	3Hs
1^I	1.6341	1Hd
2^I	5.9011	1Ht
3^I	5.3084	1Ht
4^I	1.5224	1Hd
7^I, 8^I, 9^I, 10^I	7.5272	4Hs
2^{II}, 3^{II}, 4^{II}, 5^{II}, 6^{II}	7.1317	5Hs

s = singlet, d = doublet, t = triplet

The compound isolated from the stem bark of *Brachystegia eurycoma* Harms exhibited antibacterial activity *in vitro* against a wide range of pathogenic microorganisms. This is shown in Table **3**.

Table 3: Inhibitory Effects of Compound 1

Pathogen	Concentration (%)				
	25	50	75	100	MIC (%)
Staphylococcus aureus	6.37	8.33	12.33	15.67	25
Escherichia coli	7.67	10.00	14.67	17.00	25
Pseudomonas aeruginosa	7.33	9.67	13.33	16.33	25
Streptococcus fecalis	6.67	8.67	12.33	16.00	25

Figures are in mm and include the diameter of the paper disc (5mm). Data are means of triplicate determinations.

MIC = Minimum Inhibitory Concentration

The compound successfully inhibited *S. aureus*, *E.coli, P. aeruginosa* and *S. fecalis*. The compound exhibited highest anti-bacterial activity against *E. coli*. In general the order of activity of compound **1** against the bacteria organisms was: *E.coli* > *P. aeruginosa* > *S. fecalis* > *S. aureus* at 25, 50, 75 and 100% concentrations of the compound (Table 3). The minimum inhibitory concentration (MIC) of the compound was 25%. Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membrane's infections of man[13, 14]. These microorganisms have been incriminated in the infection of wounds, sores and boils[15]. The stem bark of *Brachystegia eurycoma* from which compound **1** was isolated possesses the capability of inhibiting the growth of microbial wound contaminants, accelerate wound healing and consequently result in cell proliferation when used in herbal medicine. The existence of compound **1** in the stem bark of *Brachystegia eurycoma* could be the reason behind the utilization of the plant in traditional herbal medicine in Nigeria for the treatment of wounds and infections. The observed inhibitory role of compound **1** on these pathogens also suggests the use of the compound in the treatment of wounds, sores, boils, gonorrhea and other urinary tract infections (UTIs) in human. The mechanism of inhibitory action of this compound on these microorganisms may be due to impairment of a variety of enzyme systems, including those involved in energy production, interference with the integrity of cell membranes and structural component synthesis[16,12]. This research therefore authenticates the use of *Brachystegia eurycoma* in herbal medicine in Nigeria for the treatment of diseases and microbial infections.

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