

ISOLATION, **STRUCTURAL ELUCIDATION** AND **ANTI-LIPID PEROXIDATION**
ACTIVITY OF 3-(5-HYDROXY-2-(4-HYDROXYPHENYL)-4-METHYL-
CHROMEN-3-YL) PROPANOIC ACID FROM THE STEM BARK OF
BRACHYSTEGLIA EURYCOMA HARMS

O. U. Igwe^{1*} and J. O. Echeme²

Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267
Umuahia, Abia State, Nigeria. +2348063862710; +2347051509517

okesoty@yahoo.com; okenwauigwe@gmail.com

Authors' Contribution

This work is a collective contribution of two authors. Author OUI designed the research and carried out the analyses. Author JOE supervised the research from the beginning to the end.

ABSTRACT

Aim: To isolate, characterize and ascertain the anti-lipid peroxidation activity of a chromene propanoic acid from the stem bark of *Brachystegia eurycoma* Harms.

Study Design: The study was designed to search, isolate and elucidate the structure of a novel compound and then test its anti-lipid peroxidation activity.

Place and Duration of Study: Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, between February 2010 and October 2012.

Methodology: The structure of the compound was elucidated using Infrared Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry. Anti-lipid peroxidation activity was determined using the ferric thiocyanate method.

Results: A novel compound, 3-(5-hydroxy-2-(4-hydroxyphenyl)-4-methyl-chromen-3-yl) propanoic acid was isolated from the ethanolic extract of the stem bark of *Brachystegia eurycoma* Harms. The compound exhibited marked anti-lipid peroxidation activity at minimum and maximum concentrations of 100 µg/ml (29.42±1.42 %) and 500 µg/ml (74.52±1.22 %) respectively. Ascorbic acid was used as a standard anti-lipid peroxidation compound; it gave 94.62±1.18 % lipid peroxidation inhibition activity at 100 µg/ml.

Conclusion: These analyses reveal the use of the compound in the treatment of diseases caused by free radical activities and therefore corroborate the use of *Brachystegia eurycoma* plant in the treatment of wounds in herbal medicine.

Keywords: *Brachystegia eurycoma* Harms, Chromene propanoic acid, Anti-lipid peroxidation activity, Free radicals, Herbal medicine.

1. INTRODUCTION

The health benefits of plants have been recognized for millennia. Clinical studies and basic research on plants and their component chemicals are emerging at an exponential rate. Despite this, there is an oblivion of the herbal medicines available, as well as understanding their beneficial effects. Companies prefer to convert the bioactive natural compound into a modified synthetic analogue that permits it to obtain intellectual property rights [1]. Consequently, only a few phytomedicines have been examined to the same level of scientific scrutiny that is required for synthetic drugs. Nevertheless several promising herbs and plants used in traditional medicines have been examined for their chemistry, and many studies aimed to provide basic assessment efficacy and safety are currently performed [2]. It is in the light of this that the isolation, characterization and antioxidant activity of a novel chromene propanoic acid from the stem bark of *Brachystegia eurycoma* Harms is reported herein.

Brachystegia eurycoma plant belongs to the family, *Fabaceae*. The plant grows mainly along the river banks or swamps of western and eastern Nigeria. It also grows on well-drained soils [11,12]. It has been reported that the extracts from *Brachystegia eurycoma* seeds possess marked anti-inflammatory activity which was due to the presence of bioactive constituents in them [13]. The plant is large with irregular and twisted spreading branches. The seed has a roundish flat shape with brown colour and hard hull. The fruit ripens from September to January and is released by explosive mechanism [13]. The exudate of *Brachystegia eurycoma* is used in herbal medicine in Nigeria for the treatment of wounds and in right combinations with mucin and honey used for prevention of bacterial infection, scar formation and promotes regeneration of hair follicles [14,15]. According to the natives, the edible seed which is used in soup making as a thickener helps in maintaining heat within the body when consumed, in other words, it helps in the control of body temperature [16]. The seeds help in softening bulky stools and have been associated with protection against colon and rectal cancer [17]. The antifungal properties of ethanol and water extracts of the stem bark of *Brachystegia eurycoma* have been reported. After 48 hours of incubation, the two extracts at 2mg/ml inhibited the growth of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Epidermophyton floccosum*, *Fusarium solani*, *Mucor mucedo*, *Microsporium audonii* and *Trichophyton verrucosum* [18]. The seeds and stem bark of *B. eurycoma* have

been reported to contain alkaloids, flavonoids, tannins, phenols and saponins [13]. A compound, 2-(4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one has been reported to have been isolated from the seeds of *B. eurycoma* Harms [14].

Many phytochemicals have potent antioxidant activity owing to the presence of hydroxyl groups that can readily donate one electron [3]. These antioxidants have the potential to protect LDL particles from oxidation, and thus lessen their atherogenicity [4,5,6]. Reactive forms of oxygen are involved in the pathogenesis of a broad range of diseases [7]. Oxygen free radicals such as superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen can damage DNA, RNA, proteins, carbohydrates and unsaturated lipids in cell membranes [8]. Antioxidant defense system is essential for protection of cell structures and macromolecules from damage by free radicals, which largely result from normal metabolic processes [9]. This defense system generally declines with age and can be compromised by various forms of oxidative stress resulting from exposure to smoke, drugs, environmental pollutants, radiation and physical exercise [9,10].

2. MATERIALS AND METHODS

2.1 Experimental

The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ¹H 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 quadrupole 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 mesh) 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 F₂₅₄ aluminum plates 20 x 20 cm Merck, Darmstadt Germany.

2.2 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 (2 kg) 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.

2.3 Extraction of Plant Materials

The powdered stem bark of *Brachystegia eurycoma* (500 g) was packed into a soxhlet apparatus (2 L) and extracted exhaustively with 1 L ethanol for 24 h. The ethanol extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 2 days. Compound elution was performed by column chromatography with different solvents of different polarity namely chloroform, petroleum ether and methanol. 100 ml of chloroform: petroleum ether mixture was used in the following respective ratios, 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. Next was petroleum ether: methanol mixture in the following respective ratios, 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. The compound gave R_f value of 0.81 on Thin Layer chromatography [using chloroform and methanol (7:3)].

2.4 Determination of Anti-lipid Peroxidation Activity

The anti-lipid peroxidation activity of the compound was determined using the Ferric Thiocyanate method [19]. 2 ml of 100 μ g/ml of the compound, 2 ml of 2.5 % (w/v) linolenic acid in 95 % ethanol (v/v), 4 ml of 0.05 M of phosphate buffer (pH 7.0) and 2 ml of distilled water were mixed in 50 ml test tubes covered with rubber band. A blank sample was prepared using 4 ml of distilled water, 2 ml of 2.5 % (w/v) linolenic acid in 95 % ethanol and 4 ml of 0.05 M of phosphate buffer (pH 7.0). The test tubes were placed in water bath at 37 °C and kept in a dark cupboard to accelerate oxidation. 0.1 ml of the mixture above was added to 9.0 ml of 95 % ethanol and 0.1 ml of 30 % (w/v) ammonium thiocyanate. After 5 min., 0.1 ml of 0.02 M ferrous chloride solution in 3.5% (v/v) HCl was added to the mixture and stirred. The amount of peroxide formed was determined by reading absorbance at 500 nm at intervals for 24 h during incubation. Ascorbic acid was used as a standard anti-lipid peroxidant. The experiment was repeated three times with each concentration of 200, 300, 400 and 500 μ g/ml of the compound. The inhibition of lipid peroxidation as a percentage was calculated by the following equation:

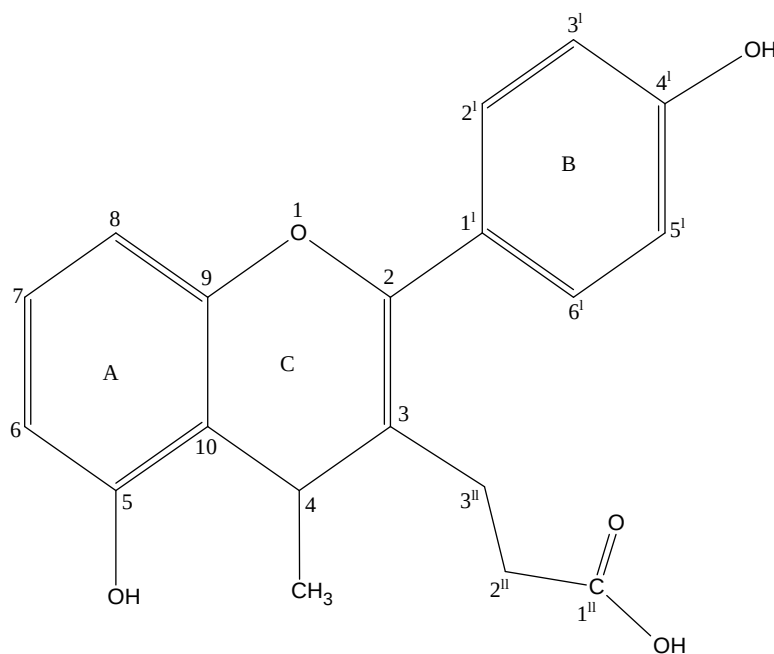
$$\% \text{ Inhibition} = \frac{A_1 - A_2}{A_1} \times 100$$

Where, A_1 = Absorbance of control reaction

A_2 = Absorbance in the presence of the compound

3. RESULTS AND DISCUSSION

The molecular formula of the compound was established as $C_{19}H_{18}O_5$ based on its HREIMS and NMR data. The IR spectrum revealed a strong, broad band at 3368.06 cm^{-1} which was due to O-H stretching vibration. There were absorptions at 1270.07 cm^{-1} , 1159.66 cm^{-1} and 1073.96 cm^{-1} characteristic of C-O stretching vibration characteristic of a tetrahedral carbon. Strong absorptions at 1604.73 cm^{-1} and 1460.93 cm^{-1} were characteristic of C=C stretching of aromatic bonds while 1639.93 cm^{-1} absorption was for alkene double bond. The out-of-plane C-H bending of aromatic ring gave absorption at 721.61 cm^{-1} . The spectrum also showed absorption at 1710.33 cm^{-1} which was typical of a carbonyl group.



3-(5-hydroxy-2-(4-hydroxyphenyl)-4-methyl-chromen-3-yl) propanoic acid

Compound 1

Table 1: IR Absorptions of compound 1

IR Absorption (cm ⁻¹)	Function Group	Compound Type
3368.06	OH	Alcohol
2922.78	C-H	Alkane
1270.07	C-O	Ether
1159.66	C-O	Ether
1073.96	C-O	Ether
1604.73	C=C	Aromatic
1460.93	C=C	Aromatic
1710.33	C=O	Carbonyl
721.61	C-H	Aromatic
163.93	C=C	Alkene

In the ¹H NMR spectrum of compound **1**, a doublet peak at δ1.2122 was due to the methyl group protons of C₁₁. The three protons coupled, but the doublet peak observed was as a result of splitting caused by the heterocyclic proton at C₄ which itself appeared as quartet at δ1.6277. A quartet peak observed for the heterocyclic proton at C₄ was due to the spin-splitting caused by the three protons of C₁₁ methyl group. The –CH₂– protons of C₃¹¹ appeared as triplet at δ1.4276 due to the adjacent –CH₂– protons that caused the splitting to give a triplet peak. The same reverse phenomenon occurred with the –CH₂– protons of C₂¹¹ to give another triplet peak at δ1.337. A singlet peak at δ4.2128 was due to OH proton of C₄¹ while another singlet peak at δ4.5132 was due to the OH proton of C₅. The meta protons of ring B coupled because of their chemical equivalence, but they appeared as a doublet peak at δ7.1181 due to spin-splitting caused by the ortho protons. The same reverse process occurred

with the ortho protons to give another doublet peak at $\delta 7.8345$. In ring A, the proton at C₆ appeared as a doublet at $\delta 8.2214$ due to spin-splitting caused by C₇ proton. The C₇ proton appeared as a triplet at $\delta 7.4252$ due to the C₈ and C₆ protons. C₈ proton appeared as a doublet peak as a result of spin-splitting caused by C₇ proton. A singlet peak at $\delta 11.5671$ was characteristic of OH proton by a carboxylic acid

Table 2: Proton NMR chemical shifts and Multiplicities of compound 1

Position	Chemical shift (δ)	Multiplicity
4	1.6277	1 Hq
5	4.5132	1 Hs (OH)
6	8.2214	1 Hd
7	7.4252	1 Ht
8	7.6127	1 Hd
11	1.2122	3 Hd
2 ¹ , 6 ¹	7.1181	1 Hd
3 ¹ , 5 ¹	7.8345	1 Hd
4 ¹	4.2128	1 Hs (OH)
1 ¹¹	11.5671	1 Hs (OH)
2 ¹¹	1.33337	2 Ht
3 ¹¹	1.4276	2 Ht

s= singlet; d= doublet; t= triplet; q= quartet

From MS data, compound **1** was assigned the molecular mass m/z 326.0301 (M^+) calculated for C₁₉H₁₈O₅ (m/z 326) with base peak at m/z 93.0138 calculated for C₆H₅O (m/z 93). The base peak occurred as a result of the detachment of the phenol portion of the compound. Other important peaks occurred at m/z 28.0886, 45.1235, 49.1101, 61.1413, 68.0777, 75.0869, 92.0133 and 141.1911. The fragmentation pattern of compound **1** is shown in Figure 1.

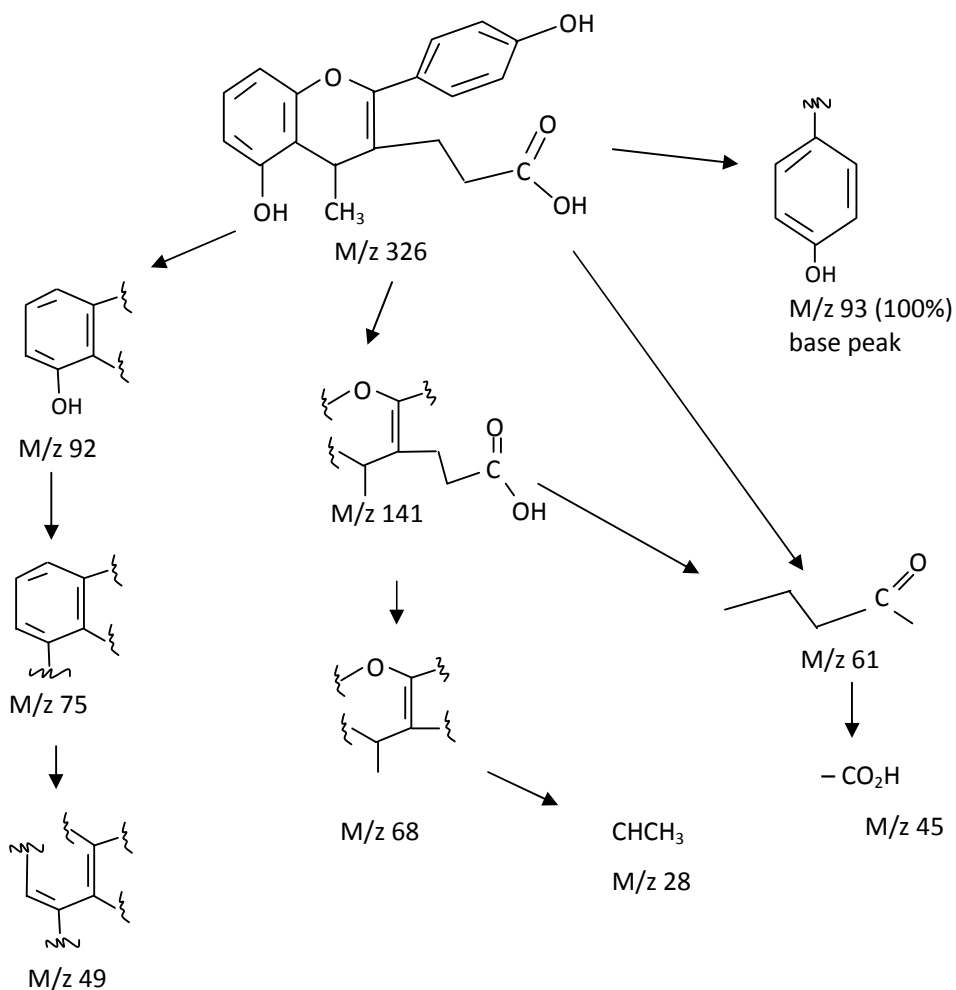
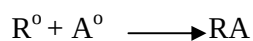
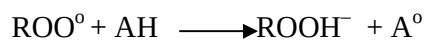
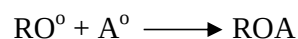


Figure 1: Fragmentation pattern of compound 1

The results of the antioxidant activities of the isolated compound from *Brachystegia eurycoma* stem bark are shown in Table 3. The results of this investigation reveal that the compound possesses significant capacity to inhibit free radical scavenging activity in a dose dependent pattern. Compound 1 gave antioxidant activity of 29.42 % and 74.52 % at minimum and maximum concentrations of 100 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ respectively. Standard reference ascorbic acid gave 94.62 % at 100 $\mu\text{g/ml}$ concentration. The compound showed increase in antioxidant activity with increase in concentration. The compound maybe functions by interrupting the free-radical chain mechanism through the following ways:





In the above scheme, AH represents the antioxidant compound.

Table 3: Free radical scavenging activities of compound 1

Concentration (µg/ml)	Radical Scavenging (%)
100	29.42±1.42
200	48.26±1.28
300	61.71±1.10
400	69.68±2.11
500	74.52±1.22
Ascorbic acid (100 µg/ml)	94.62±1.18

Data are means ± standard deviation of triplicate determination

The isolated compound is new but has a common skeleton of flavonoids which consists of diphenyl pyrons, two benzene rings linked through a heterocyclic pyran or pyrone ring. Consequently, compound **1** could possess the properties of flavonoids. Flavonoids have multiple biological activities including vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and estrogenic effects [20,21,22]. The ability of compound **1** to exhibit high anti-lipid peroxidation which is an anti-oxidative process is an important pharmacological profile of flavonoids. It is believed that the antioxidant properties of phenolic derivatives and phenolics in general are a result of their ability to act as reducing agents, hydrogen donors, and free radical quenchers and can also act as metal chelators in the process of initiating radicals [14]. The compound could be used in the treatment of atherosclerosis which is a condition that results from the gradual build-up of fatty substances, including cholesterol on the walls of the arteries [23]. This reduces blood flow to the heart, brain and other tissues and can progress to cause a heart attack or stroke [23]. Reactive oxygen species generated through lipid peroxidation can oxidize the amino

acid residues of low density lipoprotein (LDL) and this can initiate the atherosclerotic process [24].

4. CONCLUSION

Compound **1** isolated from the stem bark of *Brachystegia eurycoma* Harms has demonstrated appreciable *in vitro* anti-lipid peroxidation activity and could be used in the treatment and or management of diseases mediated through free radical activities in the body. This research has also provided a corroborative evidence for the use of *Brachystegia eurycoma* plant in the treatment of wounds and infections in herbal medicine in Nigeria.

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