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ISOLATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF A FURO-CHROMEN-4-ONE FROM THE SEEDS OF *BRACHYSTEGIA EURYCOMA* HARMS

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ABSTRACT

A bioactive compound identified as 2-(4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one was isolated from the seeds of *Brachystegia eurycoma* Harms by employing column and thin layer chromatographic methods. It was than characterized by IR, ^{1}H NMR and MS spectroscopic data. The compound showed potent free radical scavenging activity at minimum and maximum concentrations of 100 µg/mL and 500 µg/mL, respectively. This investigation suggests the use of the compound in the treatment of diseases and ailments mediated through free radical activities. It also authenticates the use of *B. eurycoma* plant in the treatment of wounds in herbal medicine in Nigeria.

Key words: *Brachystegia eurycoma* Harms, Antioxidant activity, Free radicals, Herbal medicine.

INTRODUCTION

Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring antioxidants. Recently, a large number of natural antioxidants have been isolated from different plants[1]. Although animals are bestowed with antioxidant and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage, completely[2]. Hence antioxidants in the diet are of great importance as possible protective agents to help human body to reduce oxidative damage[3,4].

Free radicals are highly reactive molecules with an unpaired electron and are produced by radiation or as by-products of metabolic process[4]. They initiate chain reactions, which lead to disintegration of cell membranes and cell compounds, including lipids, proteins and nucleic acids[5]. Reactive oxygen species generated through lipid peroxidation

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can oxidatively modify the amino acid residues of low density lipo-protein (LDL) and this can initiate atherosclerotic process[6]. Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation, which ultimately affects the organoleptic properties and edibility of food. Hence, intervention of an antioxidant may have a therapeutic effect and also maintain the freshness of food product[5,6]. Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress-related diseases[7,8].

There were no documented reports found on the antioxidant activity of *B. eurycoma* Harms. Nevertheless, these investigations were undertaken as part of our contributory effort to research on the bioactive compounds obtainable from Nigerian vegetation. *B. eurycoma* is a large plant that is native to tropical Africa and grows along river banks, swamps and well drained soils[8,9]. The exudate of the plant is used in herbal medicine in Nigeria for treatment of wounds and in right combinations with mucin and honey used for prevention of bacteria infection, scar formation and promotes regeneration of hair follicles[10]. Analyses carried out on the seeds of *Brachystegia eurycoma* Harms led to the isolation of 2-(4-ethylphenyl)-5-hydroxy-3-methyl-6-7-dihydrofuro-chromen-4-one. The compound showed antioxidant activities, which are reported herein.

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

Plant materials

Brachystegia eurycoma seeds were bought from Umuahia main market in Abia State, Nigeria. The plant seeds were identified and authenticated by Mr. I. K Ndukwe of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike. Clean and wholesome seeds were selected. The seeds were weighed (1 Kg) and then decoated by soaking in water for 24 hours. The loosened hull was washed with several

changes of water. The dehulled seeds were air-dried and then 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 phytochemical

The powdered seeds of *B. eurycoma* (500 g) was packed into a soxhlet apparatus (2 L) and extracted exhaustively with 100 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 0.57 on TLC (using chloroform and methanol (7 : 3)).

Determination of antioxidant activity

The antioxidant activity of the compound was determined using the ferric thiocyanate method[11]. 2 mL of 100 µg/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^{o}C and kept in a dark cupboard to accelerate oxidation. 0.1 mL of the mixture above was added to 9.7 mL of 95% ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 minutes, 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 hours during incubation. Ascorbic acid was used as standard antioxidant, while a blank of distilled water was run with each assay. The experiment was repeated with 200, 300, 400 and 500 µg/mL of the compound. The inhibition of lipid peroxidation as a percentage was calculated by the following equation:

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

Where, A_1 = Absorbance of control reaction

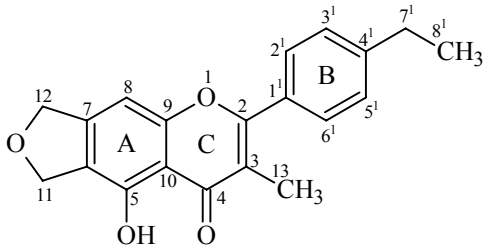
A_2 = Absorbance in the presence of the compound

Statistical analysis

All measurements were replicated three times and standard deviation determined.

RESULTS AND DISCUSSION

The molecular formula of the compound was established as $C_{20}H_{18}O_4$ based on its HREIMS and NMR data. The IR spectrum revealed hydroxyl, aliphatic, aromatic, carbonyl and ether bands at 3405.62, 2920.00, 1607.43, 1708.64 and 1156.59 cm^{-1}, respectively.



Compound 1

Table 1: IR Absorptions of Compound 1

IR Absorption (cm^{-1})	Functional group	Compound type
3405.62	OH	Alcohol
2920.00	C-H	Alkane
1607.43	C=C	Aromatic
1708.64	C=O	Carbonyl
1156.59	C-O	Ether

The ^1H NMR spectrum of compound 1 showed the presence of $-CH_3$ protons of the ethyl group at δ 0.9413. The absorption appeared as a triplet due to spin-spin splitting caused by the adjacent $-CH_2-$ of the ethyl group. The $-CH_2-$ of the ethyl group had its own absorption at δ 1.4414 and appeared as quartet as a result of spin splitting caused by the adjacent $-CH_3$ group. An absorption at δ 1.7011 was due to $-CH_3$ protons attached to C_3. The three protons coupled, since they were in the same chemical environment to give a three-proton singlet peak at δ 1.7011. The $-CH_2-$ protons attached to C_{12} and C_{11} had their absorptions at δ 3.4233 and δ 3.6766, respectively. Each of these $-CH_2-$ protons appeared as a two-proton singlet peak. A singlet peak at δ 3.9188 was as a result of the –OH proton. The two ortho protons of the benzene ring (ring B) coupled, since they were in the same chemical environment. However, they were split by the meta protons to give a doublet peak at δ 7.5336. The same reverse phenomenon was also observed for the meta protons, hence they gave a doublet peak at δ 7.4182. The only para proton in the benzene ring A gave a singlet peak at δ 7.9862.

Table 2: Proton NMR Chemical shift and Multiplicities

Position	Chemical shift (δ)	Multiplicity
5	3.9188	1 Hs
8	7.9862	1 Hs
11	3.6766	2 Hs
12	3.4233	2 Hs
13	1.7011	3 Hs
2'	7.5336	1 Hd
3'	7.4182	1 Hd
5'	7.4182	1 Hd
6'	7.5336	1 Hd
7'	1.4414	2 Hq
8'	0.9413	3 Ht

From MS data, the compound was assigned the molecular mass m/z 322.01030 (M^{+}) calculated for $C_{20}H_{18}O_4$ (m/z 322) with base peak at m/z 105.0714 calculated for C_8H_9 (105). The base peak occurred due to the detachment of the phenylethyl portion of the compound. Other peaks occurred at m/z 29.1080, 44.1243, 73.1051, 76.0081, 83.0336, 90.1313, 134.1101 and 188.0677. The fragmentation pattern of compound 1 is shown in Fig. 1.

The results of the antioxidant activities of the compound from *Brachystegia eurycoma* 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 manner.

Table 3: Free radical scavenging activities of Compound 1

Concentration (µg/mL)	Radical Scavenging (%)
100	21.65 ± 1.32
200	42.82 ± 1.12
300	57.71 ± 1.60
400	65.55 ± 1.72
500	71.22 ± 1.40
Ascorbic acid (100 µg/mL)	94.62 ± 1.18

Data are means ± standard deviations of triplicate determinations

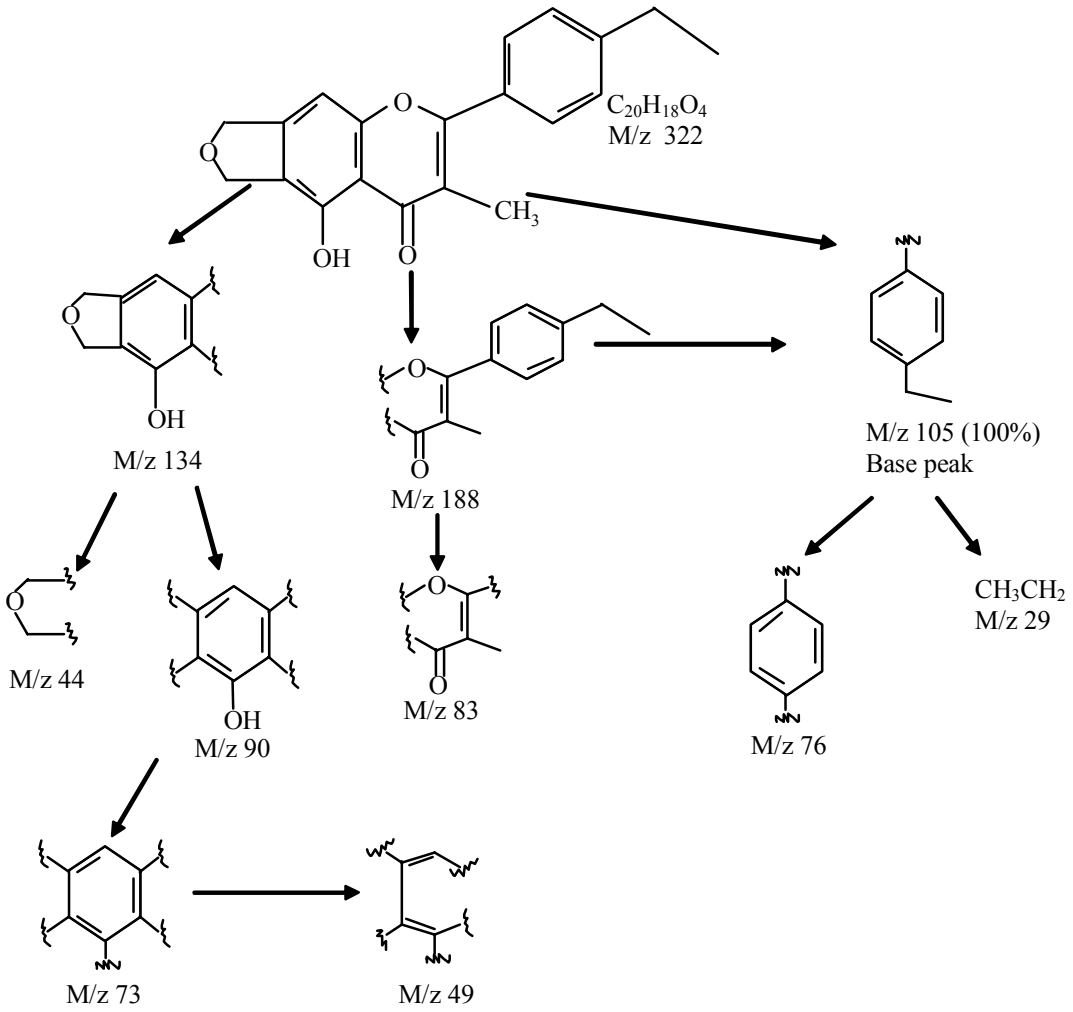


Fig. 1: Fragmentation pattern of compound 1

Compound 1 gave antioxidant activity of 21.65% and 71.22% at minimum and maximum concentrations of 100 µg/mL and 500 µg/mL, respectively. Standard reference ascorbic acid gave 94.62% at 100 µg/mL concentration. The compound showed increase in antioxidant activity with increase in concentration. Plant phenolics and phenolic derivatives in general are effective free radical scavengers and antioxidants[12,13]. It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors and free radical quenchers and phenolics can also act as metal chelators, which prevent the catalytic function of metal in the process of initiating radicals[7,8]. The compound isolated from the seeds of *B. eurycoma* showed potent antioxidant activity and could be used in pharmacy for the treatment of diseases and ailments mediated through free

radical operations and oxidative stress. As noted earlier, the synthetic antioxidants show toxic and mutagenic effects, which necessitates a shift of attention to naturally occurring antioxidants. The discovery of this compound is a right step in the light of this quest. The compound could be used in the management of tissue inflammation, wounds, arthritis, cancer, cardiovascular disorders and arthrosclerosis. This research therefore provides a springboard for the possible development of the drug in pharmaceutical industries. It also authenticates the use of *Brachystegia eurycoma* plant in the treatment of wounds in herbal medicine in Nigeria. The plant is indeed an asset to the people of south eastern Nigeria and other places where it is found. Its cultivation is hereby encouraged.

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