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Isolation and characterization of polyphenolic tertiary butyl from the exudate of *Brachystegia eurycoma* (HARMS)

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ABSTRACT

Polyphenolic compounds possess high degree of physiological and pharmacological benefits. They have the ability to scavenge free radicals and microorganisms, hence they possess antioxidant and antimicrobial activities. Other activities shown by polyphenolic compounds are anti-inflammatory, anticancer, antimalaria, immune-stimulating, antiallergic and vasolidatory. Analysis of the bioactive components from the stem exudates of Brachystegia eurycoma Harms resulted in the isolation of bis-6,6-methylenebis (4-tert-butyl-2-methylphenol). The structure was elucidated using IR, NMR and MS spectroscopic data.

Keywords: *Brachystegia eurycoma*, polyphenolic compound, free radicals, antioxidant, metal chelation.

INTRODUCTION

Plants have always played a significant role in maintaining the health and improving the quality of human life. Many conventional drugs owe their origin to plant extracts[1].

As a contributory effort to explore the bioactive secondary metabolites from Nigerian vegetation, *Brachystegia eurycoma* Harms was selected for study because of its myriad of uses as food and medicine. *Brachystegia eurycoma* is a large tree with irregular and twisted spreading branches, the fruits ripen from September to January and is released by explosive mechanism[2,3]. The plant possesses a rough fibrous bark which peels off in patches and often gives out brownish buttery exudates[4,5]. The exudate is used for faster healing of wounds[6]. *B. eurycoma* exudate in right combination with mucin and honey is used for wound healing, prevention of bacteria infection, scar formation and promotes regeneration of hair follicles[6]. 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 the body temperature[7,8]. The stem bark of *B. eurycoma* is macerated for preservation of palm wine in Igbo land of Eastern Nigeria. The plant grows mainly along the river banks or swamps in western and eastern Nigeria. It also grows on well-drained soils[2].

As part of our chemical studies on Nigeria medicinal plants, we describe herein the isolation of polyphenolic tertiary butyl from the stem exudates of *Brachystegia eurycoma*.

MATERIALS AND METHODS

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 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25 mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck, Damstadt Germany.

Plant Materials

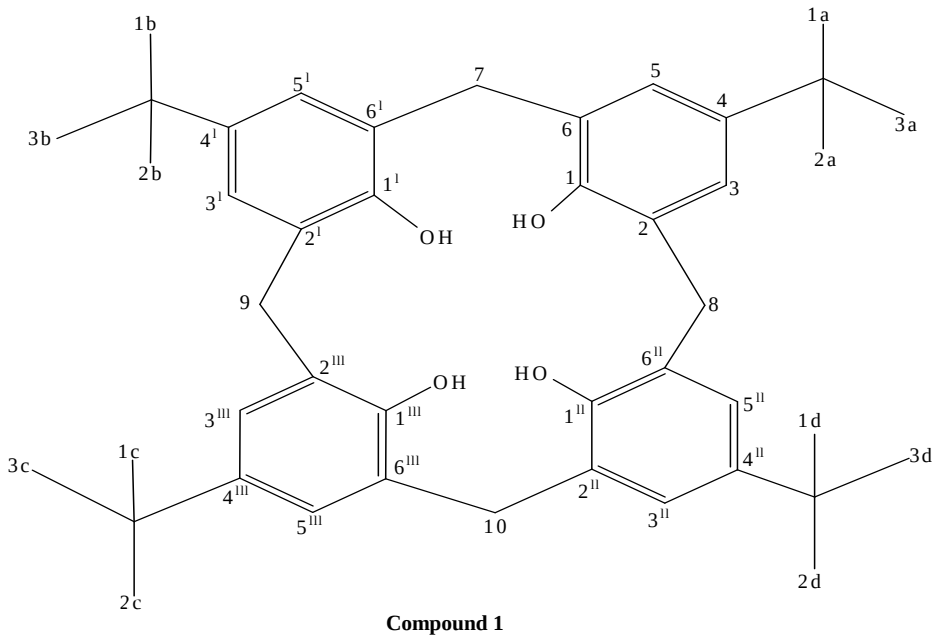
The stem exudate of *Brachystegia eurycoma* was collected from the tree plant at Umuovo Village Stream, Old Umuahia, Abia State, Nigeria. The plant was identified by Mr N. I. Ndukwe of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria.

Extraction and Isolation of Plant Materials

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 0.50 on TLC [suing chloroform and methanol (7:3)].

RESULTS AND DISCUSSION

The molecular formula of the compound was established as $C_{44}H_{56}0_4$ based on its HREIMS and NMR data. The IR spectrum revealed hydroxyl, aliphatic, aromatic and ether bands at 3356.52, 2932.99, 1595.16 and 1058.45 cm^{-1} respectively.



Compound 1

Table 1: IR absorptions of Compound 1

IR Absorption (cm^{-1})	Functional group	Compound Type
3356.52	O-H	Alcohol
2932.99	C-H	Alkane
1595.16	C=C	Aromatic
1058.45	C-O	Ether
1377.48	C-H	Alkane(CH_3)
668.95	C-H	Aromatic

In the ^{1}H NMR spectra of the compound, all the methyl protons were in the same chemical environment. The coupling of the thirty six protons of the methyl groups gave a singlet peak at δ0.9245. The four –CH_2- protons were also chemically equivalent and as a result gave a singlet peak at δ1.3124. The four –OH protons coupled because of their chemical equivalence to give another singlet peak at δ4.9322. Each benzene ring in the compound has two protons, but all the benzene protons were in the same chemical environment, hence they coupled to give an eight proton singlet peak at δ7.6145.

Table 2: Proton NMR chemical shifts and multiplicities of Compound 1

Position	Chemical shift (δ)	Multiplicity
1a, 2a, 3a, 1b, 2b, 3b, 1c, 2c, 3c, 1d, 2d, 3d	0.9245	36Hs
7, 8, 9, 10	1.3124	8Hs
1, 1^{I}, 1^{II}, 1^{III}	4.9322	4Hs(OH)
3, 5, 3^{I}, 5^{I}, 3^{II}, 5^{II}, 3^{III}, 5^{III}	7.6145	8Hs

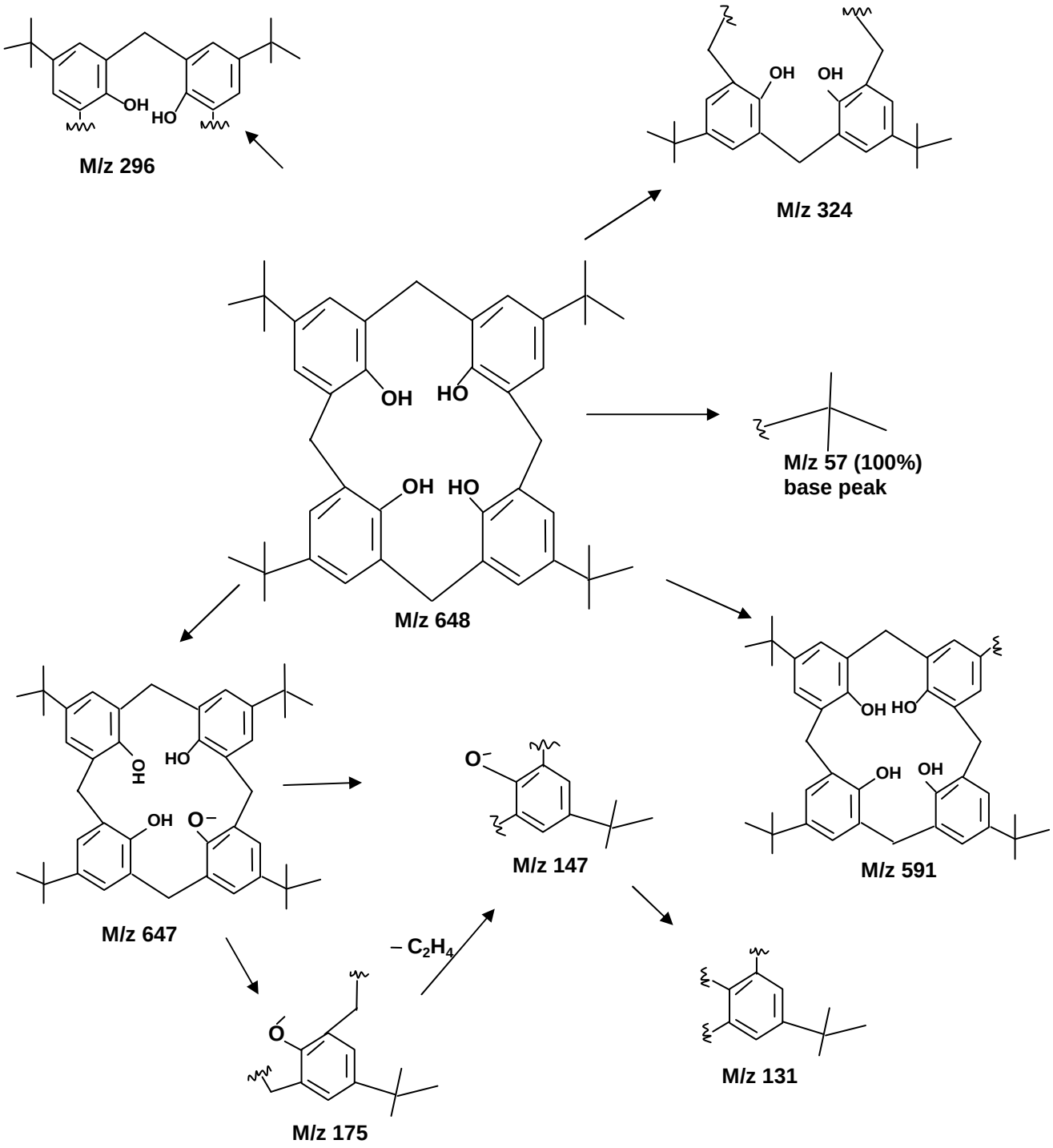


Figure 1: Fragmentation pattern of compound 1.

From Ms data, the compound was assigned the molecular mass m/z 648.0356 ($M^{\cdot+}$) calculated for $C_{44}H_{56}O_4$ (M/Z 648) with base peak at m/z 57.0170 calculated for C_4H_9 (m/z 57). The base peak occurred as a result of the cleavage of a butyl group from the compound. Other important peaks occurred at m/z 131.0852, 147.1901, 175.0038, 296.1099, 324.0810, 591.0335 and 647.1312. The fragmentation pattern of compound **1** is shown in Figure 1.

The isolated compound was identified as a polyphenolic compound. Polyphenolic compounds have multiple biological activities including antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, vasolidatory, antiallergic, immune-stimulating and estrogenic effects[9,10,11]. The hydroxyl groups in the compound are proximally located and could cause transition metal (usually iron) chelation. The conformation of the compound provides a binding capability that can lock metal ions in an active state. This prevents those metal ions from catalyzing the oxidative reactions that damage the cell and its bio-molecules. The conformation of the compound may also permit free radical chelation by the hydroxyl groups present. Because of the high reactivity of hydroxyl groups of polyphenolic compounds, radicals are made inactive[12]. When a polyphenolic molecule acting as a natural antioxidant grabs a free radical, it becomes a weak free radical itself, and another antioxidant will help regenerate it[13]. In the light of the above discussion, the compound could be a strong antioxidant. The proposed mechanism of metal and free radical chelation by compound **1** is elucidated in Figure 2 and 3. In the mechanism, M and R represent metal ion and free radical respectively.

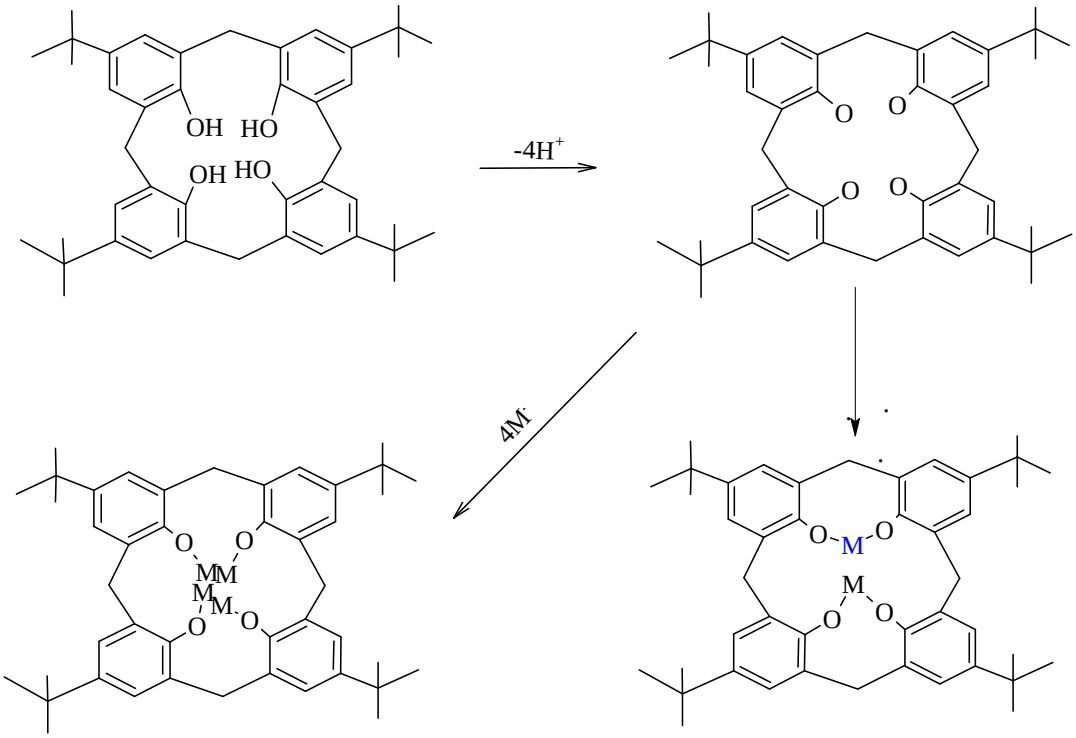


Figure 2: Metal ion chelation Activity of Compound l

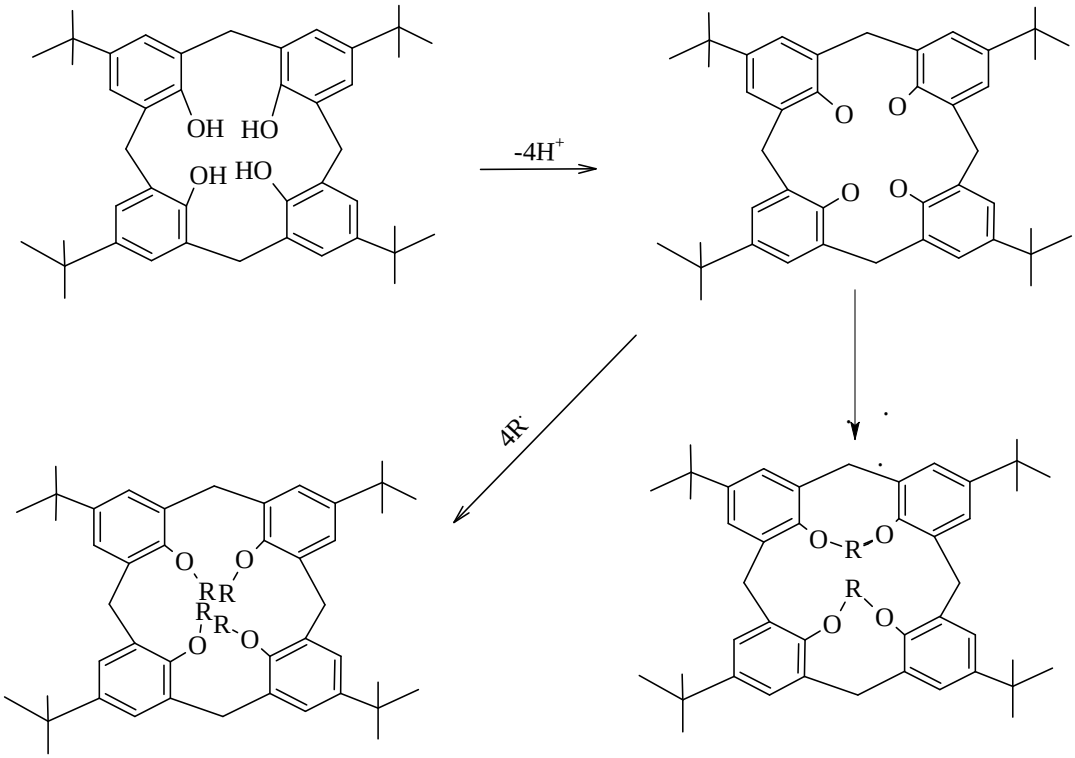


Figure 3: Free radical Scavenging Activity of Compound l

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