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Research Article

ISOLATION, CHARACTERIZATION AND ANTIFUNGAL ACTIVITY OF 5-HYDROXY-3-METHYL-2-PHENYL-6,7-DIHYDROFURO-CHROMEN-4-ONE FROM THE SEEDS OF *BRACHYSTEGIA EURYCOMA* HARMS.

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Abstract: Chemical investigation of the bioactive constituents from the seeds of Brachystegia eurycoma Harms resulted in the isolation of 5 – hydroxy – 3 – methyl – 2 – phenyl – 6, 7- dihydrofuro – chromen – 4 – one. Colum and thin layer chromatographic techniques were employed in separating the compound from ethanolic extract of the seeds. The structure was elucidated using Infrared Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry. Antifungal studies showed that the isolated compound successfully inhibited Aspergillus niger, Penicillium notatum and Fusarium oxysporum. These results authenticate the use of the plant in phytomedicine for disease prevention and treatment of infections.
Key words: Brachystegia eurycoma Harms, Antifungal activity, Herbal medicine, Bioactive compound

INTRODUCTION

Plants and herbs with diversified pharmacological profiles have been shown to be rich sources of phytochemicals with the potential to prevent and or cure human diseases [1]. Food chemists and natural product scientists have identified hundreds of phytochemicals that are being evaluated for the prevention and treatment of human diseases and infections. These include the presence in plant of such potentially bioactive substances as carotenoids, chlorophyll, flavonoids, indoles, isothiocyanates, protease inhibitors, sulfides and terpenes [2].

Herbs and their products are mixtures of different compounds, some of which could be harmful although several medicinal plants have been proved to be free of serious adverse effects when used appropriately. However, only a limited number of phytomedicines conform to the same efficacy and safety criteria established for synthetic drugs [3]. It is against this backdrop that isolation of single-molecule bioactive compounds from herbal plants emerges with appreciable recognition and development. As part of our contributory effort in the light of the above, we report herein the isolation, characterization and antifungal activity of 5-hydroxy-3-methyl-2-phenyl-6,7-dihydrofuro-chromen-4-one from the seeds of *Brachystegia eurycoma* Harms.

Brachystegia eurycoma Harms is a tropical large tree that is native to West Africa. The plant grows mainly in swampy areas and also on well-drained soils [4,5]. The voracious uses of *Brachystegia eurycoma* Harms in South Eastern Nigeria as a soup thickener and condiment and also as a medicinal plant necessitated a probe of its phytoconstituents and antifungal activity. The plant is used in herbal medicine for the treatment of wounds and infections [6]. It has been reported that the ethanolic extracts of the seeds and stem bark of *Brachystegia eurycoma* Harms possess significant anti-inflammatory properties in carrageenan-induced acute and formalin-induced chronic inflammatory models in 36 albino rats [7]. The exudate from *Brachystegia eurycoma* Harms plant is used in faster healing of wounds and prevention of bacterial infections [8]. The fruit ripens from September to January and is released by explosive mechanism. It flowers between April and May. The fruits occur as broad lathery dark purplish brown pods containing between four to six brown shiny flat disc-like seeds [9]. The plant possesses a rough fibrous bark, which peels off in patches and often gives out brownish buttery exudates [10]. The leaves make excellent browse material for cattle, sheep and goats. The tree is also used as ornamental and shade tree. The plant provides good timber sold in international market for building construction. The cones from the plant can be used as fuel. The plant is indeed economical.

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 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

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 (1kg) 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 Plant Materials

The powdered seeds 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 0.62 on Thin Layer Chromatography [using chloroform and methanol (7:3)].

Bioassay

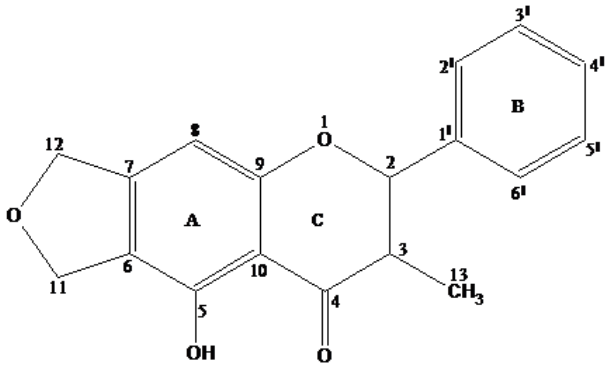
The *in vitro* antifungal activity of the oil was carried out for 24h culture of three selected fungi. The fungi organisms used were *Aspergillus niger*, *Penicillium notatum* and *Fusarium oxysporum*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Central Laboratory services Unit f 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 oil was determined using the Disc Diffusion Technique [11,12] . 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 oil having different zones and selecting the lowest concentration.

Statistical Analysis

All bioassay were replicated three times and means determined [13].

RESULTS AND DISCUSSION

The molecular formula of the compound was established as $C_{18}H_{16}O_4$ based on its HREIMS and NMR data. The IR spectrum of compound 1 showed a strong, broad band at 3376.36cm^{-1} due to O-H stretching vibration. Absorption at 1462.33cm^{-1} was characteristic of C=C stretching of aromatic bonds whiles the out-of-plane C-H bending of aromatic ring gave absorption at 722.43cm^{-1}. Absorptions at 1070.72cm^{-1} and 1171.71cm^{-1} were characteristic of C-O stretching vibration in the compound. Absorption at 1710.64cm^{-1} was typical of a carbonyl compound.



5-hydroxy-3-methyl-2-phenyl-6,7-dihydrofuro-chromen-4-one.

Compound 1

Table 1: IR Absorptions of Compound 1

IR Absorption (cm^{-1})	Functional Group	Compound Type
3376.36	OH	Alcohol
2920.48	C-H	Alkane
1710.64	C=O	Carbonyl
1070.72	C-O	Ether
1171.71	C-O	Ether
1462.33	C=C	Aromatic
722.43	C-H	Aromatic

The proton NMR spectrum of compound 1 showed a doublet peak observed at δ0.9133 which was due to -CH_3 protons of C_{13}. The three protons instead of appearing as singlet because of their chemical equivalence occurred as doublet due to spin-splitting caused by the proton of C_3. A quartet peak at δ1.7270 was due to C_3 proton which split as a result of the three protons of C_{13}. Peaks at δ3.6247 and δ3.7113 were as a result of C_{12} and C_{11} protons respectively. The –CH_2- protons of C_{12} coupled to give a singlet peak while the –CH_2- protons of C_{11} also coupled to give a singlet peak. A singlet peak at δ3.9211 was due to O-H proton. A doublet peak at δ4.0817 was as a result of C_2 proton. The proton at C_2 appeared as doublet due to C_3 proton which caused spin-spin splitting. The ortho protons of the benzene ring B coupled top give a doublet peak at δ7.6242. The peak was doublet because of C_5^1 and C_3^1 protons that caused spin-splitting. The meta protons (C_5^1 and C_3^1) also coupled to give a triplet peak at δ7.1224. The triplet was due to spin-splitting caused by the neighbouring protons. The para proton of ring B also gave a triplet peak at δ7.3182 due to

spin-splitting caused by protons at C_5^1 and C_3^1. The only para proton of ring A gave a peak at δ7.8376.

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

Position	Chemical Shift (δ)	Multiplicity
2	4.0817	1Hd
3	1.7270	1Hq
5	3.9211	1Hs (OH)
8	7.8376	1Hs
11	3.7113	2Hs
12	3.6247	2Hs
2^1, 6^1	7.6242	2Hd
3^1, 5^1	7.1224	2Ht
4^1	7.3182	1Ht

From MS data, compound 1 was assigned the molecular mass m/z 296.0014 (M^{o+}) calculated for $C_{18}H_{16}O_4$ (m/z 296) with the base peak at m/z 77.1034 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 29.0123, 44.1320, 49.0191, 56.1300, 73.0899, 85.0399, 90.0886, 134.1334 and 162.0387. The fragmentation pattern of compound 1 is shown in Figure 1.

The results of the antifungal activities of the compound from *Brachystegia eurycoma* seeds are shown in Table 3. The results of the bioassay reveal that the compound possesses potent inhibition on the three fungi (*Aspergillus niger, Penicillium notatum* and *Fusarium oxysporum*)

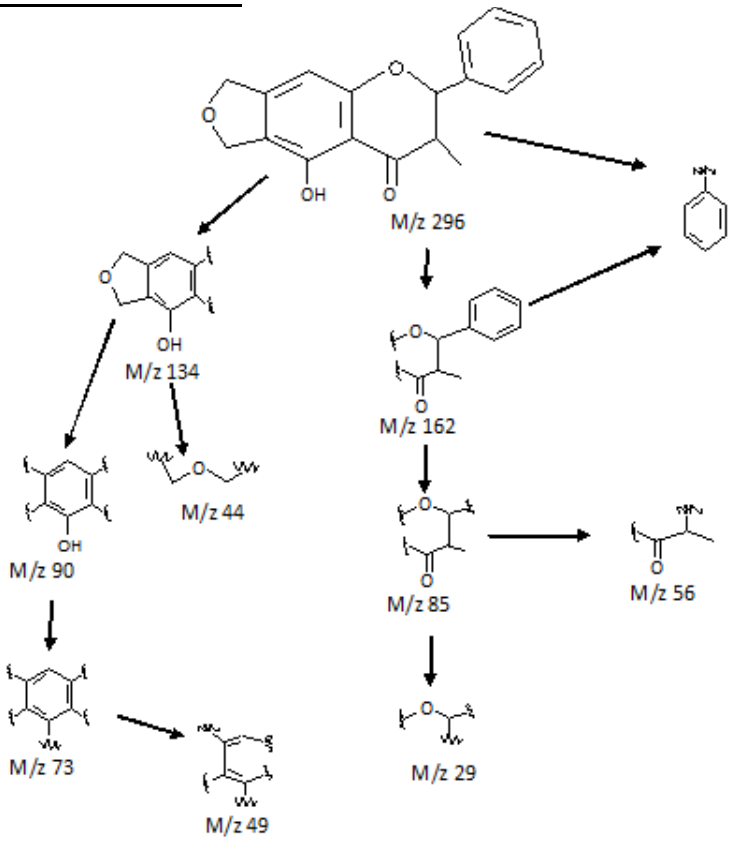


Figure 1: Fragmentation pattern of Compound 1

Table 3: Inhibitory Effects of Compound 1

Pathogen	Concentration (%)				
	25	50	75	100	MIC (%)
Aspergillus niger	-	7.33	11.67	13.67	50
Penicillium notatum	-	8.36	11.33	12.67	50
Fusarium oxysporium	-	6.33	8.67	11.33	50

MIC = Minimum Inhibitory Concentration

\- = Zone of no inhibition.

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

The compound from the seed of *B. eurycoma* Harms successfully inhibited *A. niger, P. notatum* and *F. oxysporum.* It exhibited highest antifungal activity against *A. niger*. The minimum inhibitory concentration (MIC) of the compound was 50%. The bioactive compound isolated might be responsible for the marked medicinal properties of the plant. The mechanism of inhibiting 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 [14,15]. The microorganisms tested are capable of causing diseases in human and have been confirmed to be involved in causing infections in man [16]. These findings

suggest the use of the compound in the treatment of diseases and infections caused by these organisms. The antifungal activity of the compound on these pathogens could be the reason why the plant is used in the treatment of infections in herbal medicine in Nigeria.

CONCLUSION

By this research, the therapeutic potentials of *B. eurycoma* plant are revealed. A possible development of the synthetic analogue of the compound in pharmaceutical industries would provide the world population with antifungal and antibiotic drug with little or no side effects. As stated earlier, several medicinal plants have been proved to be free of serious adverse effects when used appropriately. Therefore, the provision of a single-molecule bioactive compound such as compound 1 from a medicinal plant devoid of myriad side effects is a welcome development. Compound 1 is hereby recommended for more research.

REFERENCES

1. Boone CW, Bacus JW, Bacus JV, Steel VE, Kelloff GJ. *J. Cell Biochem. Suppl*, **1997**; 28: 1–10.
2. Goodman GE. *Proc. Soc. Exp. Biol. Med*, **1997**; 216: 253 – 260.
3. De Smet PAGM. Adverse reactions of herbal drugs, Springer-Verlag, New York, **1999**.
4. Okwu DE, Okoro N. Phytochemical Composition of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds. *J. Med. And Arom. Plant Sc. And Biotech*, **2006**; 26: 1 – 4.
5. Uzomah A, Ahiligwo RN. Studies on the rheological properties and functional potentials of achi(*Brachystegia eurycoma*) and ogbono(*Irvingia gabonensis*) seed gums. *Food Chemistry*, **1999**; 67: 217 – 218.
6. Igwe OU, Okwu DE. Isolation, characterization and antioxidant activity of a furo-chromen-4-one from the seeds of *Brachystegia eurycoma* Harms. *Int. J. of Chem. Sc*, 2013; 11(1): 121 – 128.
7. Igwe OU, Okwu DE. Phytochemical composition and antiinflammatory activities of *Brachystegia eurycoma* seeds and stem bark. *Der pharma chemica.* **2013**; 5(1): 224 – 228.
8. Adikwu MU, Enebeke TC. Evaluation of snail mucin dispersed in *Brachystegia eurycoma* gum gel as a wound healing agent. *Animal Research International*, **2007**; 4(2): 685 – 697.
9. Keay RWJ, Phil D, Bios FT. Trees of Nigeria. Oxford University Press, New York, **1989**.
10. Enwere NJ. Foods plant origin. Afro Orbis Publication Ltd. Nsukka, Nigeria, **1998**.
11. Cheesbrough M. Medicinal laboratory manual for Tropical Countries. Educational Publishers, **2000**.
12. Pelczar M, Chan ECS. Laboratory exercises in Microbiology. Black Dot Inc. New York, **1997**.
13. Steel RGD, Torrie JH. Principles and procedure of statistics with special references to Biological Sciences. McGraw-Hill New York, **1980**.
14. Okwu DE, Ukanwa N. Isolation, characterization and antibacterial activity screening of anthocyanidine glycosides from *Alchornea cordifolia* (Schumach. and Thonn.) Mull. Arg. Leaves. *E-journal of chemistry*, **2010**, 7(1), 000 – 000.
15. Igwe OU, Echeme JO. Isolation, characterization and antibacterial activity of 4-hydroxy-2-(1-p-tolylethylidene)-5,6-dihydrofuro-3-one from the seeds of *Brachystegia eurycoma* Harms. *International Journal of Drug Development & Research*, **2013**; 5(2): 329 – 334.
16. Okwu DE, Morah FNI. The potentials of *Garcinia kola* seed as source of nutraceuticals. *Med. Arom. Plant Sc*, 2006; 28: 605 – 611.