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Isolation and characterization of a flavonoid from, and antimicrobial evaluation of *Palisota hirsuta*

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Abstract
The leaves and root bark of *Palisota hirsuta* were subjected to phytochemical studies in order to validate the ethnomedicinal claim of the plant. Evaluation of the extracts against some microorganisms was also carried out. The results of the study revealed significant antibacterial activity of the leaf against Gram-positive organisms with no activity against Gram-negative organisms. There was however absence of activity with the root part of the plant. The phytochemical studies of the bioactive leaf extract led to the isolation and characterization of a flavonoid compound. The ethnomedicinal use of the plant as remedy against bacterial infections was justified.

Keywords: *Palisota hirsute*; Commelinaceae; Antibacterial activity; Flavonoid

Introduction

The diversity of plants growing in Nigeria, along with their ethno-pharmacological uses, offer enormous possibilities of finding novel structures with antibacterial activities. One of such medicinal plants with diverse ethnomedicinal usage is *Palisota hirsuta*. *Palisota hirsuta* (K. Schum) is a robust herb belonging to the family Commelinaceae, consisting of 10 genera and approximately 250 species. It grows up to 2 - 4m high and reproduces from the seeds. The stem is rigid, more or less fleshy and covered with soft and dense brown hairs. The leaves are arranged in rosettes, mostly at the terminal of stem. The flowers are whitish to purple and open for 16 hours until dusk. The fruits are glossy and black. (Agyakwa and Akobundu, 1988). It is popularly known as '*Ikpela aturu*' among the Ibos, and '*Ijamgbokun ojo*' in Yoruba. The decoction of the shoots and leaves are used as antiseptic and antipyretic. The leaves along with the roots of *Heurya aestuans*, *Polythia suaveoleus*, *Carica papaya* and the stem bark of *Enantia chlorantha* is used as antimalarial. The poultice of fresh leaves is applied over boils and wounds while the stem is smoked for toothache. (Gills, 1992).

Palisota hirsuta is reported to possess antiviral property against Herpes simplex, Hindbis and Polio virus (Akpagana *et al.*,

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2000; Anani *et al*., 2000). The root juice of the plant is used as a remedy against gonorrhea in the South Eastern part of Nigeria while the fresh leaf extract possesses anti-rheumatoid and wound healing properties (Ibe and Nwufo, 2005; Obute, 2006). The folkloric use of the plant in the treatment of bacterial infections necessitated this study and also the need to justify the claims by traditional usage of the plant through a bioactivity guided isolation technique.

Experimental

Collection of plant materials. The leaf and root samples used for this research work were obtained from the Taboga village, South-East of Edo State, Nigeria in March, 2007. The plant was identified by Mr. A. Sunny of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria, where a voucher specimen was deposited.

Extraction of plant material. The fresh leaves and root of *Palisota hirsuta* were dried at room temperature and powdered with the aid of a mechanical grinder. The powdered samples (400 g leaf; 350 g root) were each extracted with methanol for 48 hours by maceration process. The extracts were concentrated to dryness using a rotary evaporator at reduced pressure, and stored at -4^{o}C until use.

Phytochemical screening. The crude plant material was subjected to phytochemical screening testing for alkaloids, tannins, saponins and flavonoids using standard experimental procedures (Harbone, 1973; Sofowora, 1993, Evans, 1989).

Isolation of chemical constituents. The methanol extracts of *P. hirsuta* leaves were partitioned with *n*-hexane, ethyl acetate and butanol. The ethylacetate-soluble fraction of methanolic extract was subjected to repeated column chromatography to give compound **1**, using solvent systems of n-hexane 100 %, ethyl acetate: methanol 90:10, 80:20 up to methanol 100 %. 30 fractions were collected with fraction 20 - 23 containing one spot on analytical TLC pate.

Antimicrobial assay. The methanolic leaf extract (10g) of *P. hirsuta* was dissolved in 5 ml of Dimethyl sulphoxide (DMSO) and the volume made up to 20 ml with sterile distilled water to give 500 mg/ml stock solution. 50, 100 and 200 mg/ml were aseptically pipetted into three sterile Petri dishes. The dishes were shaken to ensure even distribution of extract and allowed to stand on the bench to allow the agar set after which, they were dried in an oven. 0.2 ml of overnight cultures of *Staphylococcus aureus* (ATCC 25923), clinical isolate of *S. aureus*, *E. coli* (ATCC 25922), clinical isolate of *E. coli*, *Pseudomonas aeruginosa and Bacillus subtilis* were spotted on each nutrient agar plates while *Streptococcus viridans* was spotted on chocolate agar. The plates were allowed to stand on the bench for 30 minutes to allow for agar diffusion process to occur which is the basis of the technique been employed. The plates were then incubated at 37^{o}C for 24 hours. The method of Hugo (1975) was used to determine the zones of inhibition.

Evaluation of minimum inhibitory concentration. Three wells were made with sterile cork borer on each of the six nutrient agar plates previously seeded with the standard organisms and their clinical isolates. The wells in the nutrient and chocolate agar plates were inoculated with 0.2 ml, 0.3 ml, and 0.4 ml of the stock solution equivalent to 100 mg, 150 mg and 200 mg of the extract. They were incubated at 37^{o}C for 24 hours; the chocolate agar was placed in carbon dioxide jar (oxygen free) for 30 minutes and later incubated at 37^{o}C for 24 hours. The same procedure was used for the root extract.

Results and Discussion

Results of phytochemical screening of *P. hirsuta* (root and leaf) are shown in Table 1. The plant contains saponins, alkaloids, tannins and flavonoids. A copious amount of silica was also found as precipitate during the extraction.

The result of the antimicrobial evaluation of the root and leaf extracts of the plant is presented in Tables 2 and 3. The leaf extract at a concentration of 100mg/ml – 200mg/ml was observed to produce zones of inhibition of 17 – 22 mm on the Gram positive organisms. *E. coli* and *P. aeruginosa* (Gram negatives) did not show any activity (no zone of inhibition). The result showed significant antibacterial activity of the leaf extract against the tested Gram positive organisms *S. aureus* and *Bacillus subtilis.* The extract inhibited the growth of *Streptococcus viridans* (obtained from sputum) with zones of inhibition between 18 and 20 mm at the concentrations used. This also supports the ethnomedicinal claim of the plant as remedy against cough. There was no activity against Gram negatives (*E. coli* and *P. aeruginosa*). The high antibacterial potency on *S. aureus* could also be responsible for its use in wound healing. This probably is due to the inhibition of the luxuriant proliferation of *S. aureus* commonly implicated in wounds.

The root of *P. hirsuta* and the isolated compound showed no antibacterial activity against the tested organisms at the concentrations used. Table 4 shows the minimum inhibitory concentrations (MIC) of the extracts against the Gram positives and Gram negatives organisms used in this study. The MIC of 150mg/ml, 150mg/ml and 200mg/ml for *S. aureus,* and *B. subtilis* respectively. The high MIC of *B. subtilis* is due to the spore forming activity of the organism.

Compound **1** was isolated from the ethylacetate-soluble part of methanolic leaf extract of *P. hirsuta*. The compound was found to be fluorescent under UV light and appeared yellow after spraying with cerium (IV) sulphate reagent.

The IR spectrum displayed a broad absorption band at 3450 cm^{-1} due to hydroxyl functional group. The same spectrum also showed absorption at 1660 and 1580 cm^{-1} ascribed to carbonyl and aromaticity respectively. The UV absorptions at λ_{max} 350, 266 and 256 nm were diagnostic of a flavonoid skeleton. The molecular formula was deduced from the HR EI MS as $C_{16}H_{12}O_6$ corresponding to the m/z 300.0642. The fragmentation pattern (Fig. 1) showed *m/z* 272.0688, 166.0266 and 134.0362.

The ^{1}H NMR spectrum of compound **1** (Table 4) showed a broad singlet of one proton at δ 7.30 (H-$2^{/}$), a doublet at δ 6.99 (H-$5^{/}$) and a broad doublet at δ 7.38 (H-$6^{/}$), each integrating for one proton and ortho coupled to each other (J = 7.8 Hz). The broadness of singlet and doublet at δ 7.30 (H-$2^{/}$) and δ 7.38 (H-$6^{/}$), respectively, was due to their meta coupling. The same spectrum also revealed two additional doublets of one proton integrating at δ 6.39 (H-6) and 6.58 (H-8) with equal coupling constant of 2 Hz indicating a meta coupling. A singlet of three protons at δ 3.87 in the ^{1}H NMR spectrum and its associated carbon at δ 57.5 in the ^{13}C NMR spectrum showed the presence of a methoxy group in the compound. The interpretation of the spectra was corroborated by HMQC and HMBC experiments.

The ^{13}C NMR spectrum (Table 4) displayed 16 signals in the broad band showing one methyl, 6 methines and nine quaternary carbons. The carbonyl carbon resonated at δ 181.7 characteristic of a flavanol derivative. The structure of the compound was deduced as 2-(3,4-dihydroxyphenyl)-5-hydroxymethoxy-7-methoxychromen-4H-one

Table 1: Phytochemical Screening of *P. hirsuta*

Compound class	Root bark	Leaf
Alkaloids	-	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	++

+ = present - = absent

Table. 2: Antimicrobial screening of leaf extract of *P. hirsuta* (zones of inhibition)

Organisms	Conc. of extracts		
	100 mg/ml	150 mg/ml	200 mg/ml
S .aureus (ATCC25923)	22	22	22
S. aureus (clinical isolate)	18	18	18
E. coli (ATCC 25922)	-	-	-
E. coli(clinical isolate)	-	-	-
P. aeruginosa	-	-	-
Bacillus subtilis	17	18	18
S. viridans	18	18	20

Table. 3: Minimum inhibitory concentrations (MIC) of *P. hirsuta*

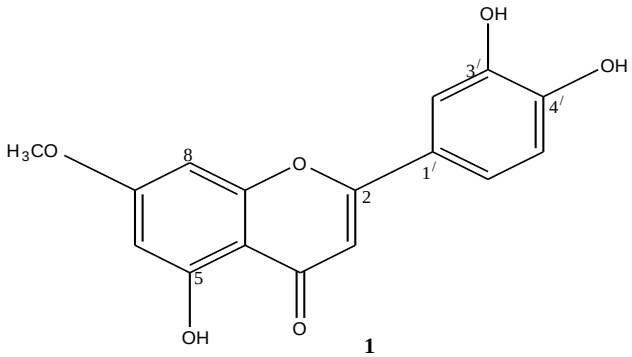
Organisms	Conc. of extracts		
	100mg/ml	150mg/ml	200mg/ml
S. aureus (ATCC25923)	+	-	-
S. aureus (clin. isolate)	+	-	-
E. coli (ATCC 25922)	+	+	+
E. coli(clin. Isolate)	+	+	+
P. aeruginosa	+	+	+
Bacillus subtilis	+	+	-
S. viridans	-	-	-

+; presence of growth -; absence of growth

Table 4: ^{1}H NMR and ^{13}C NMR (DMSO-d_6, 300 MHz) spectral of compound **1**

Carbon No.	δ_C	δ_H
2	164.2	
3	104.5	6.57, s
4	181.7	
5	161.2	
6	97.9	6.39 (d, J = 2.0 Hz)
7	165.1	
8	93.5	6.58 (d, J = 2.0 Hz)
9	157.5	
10	104.8	
1′	121.7	
2′	113.3	7.30, br s
3′	145.7	
4′	162.6	
5′	116.1	66.99 (d, J = 7.8 Hz)
6′	119.0	7.38 (br d J= 7.8 Hz))
7′	57.6	3.87, s
C-4′	55.8	3.89, s

Assignments based on ^{1}H, ^{13}C, DEPT, 2D-COSY, HMQC, and HMBC spectra



1

Fig. 1: Structure of Compound **1**

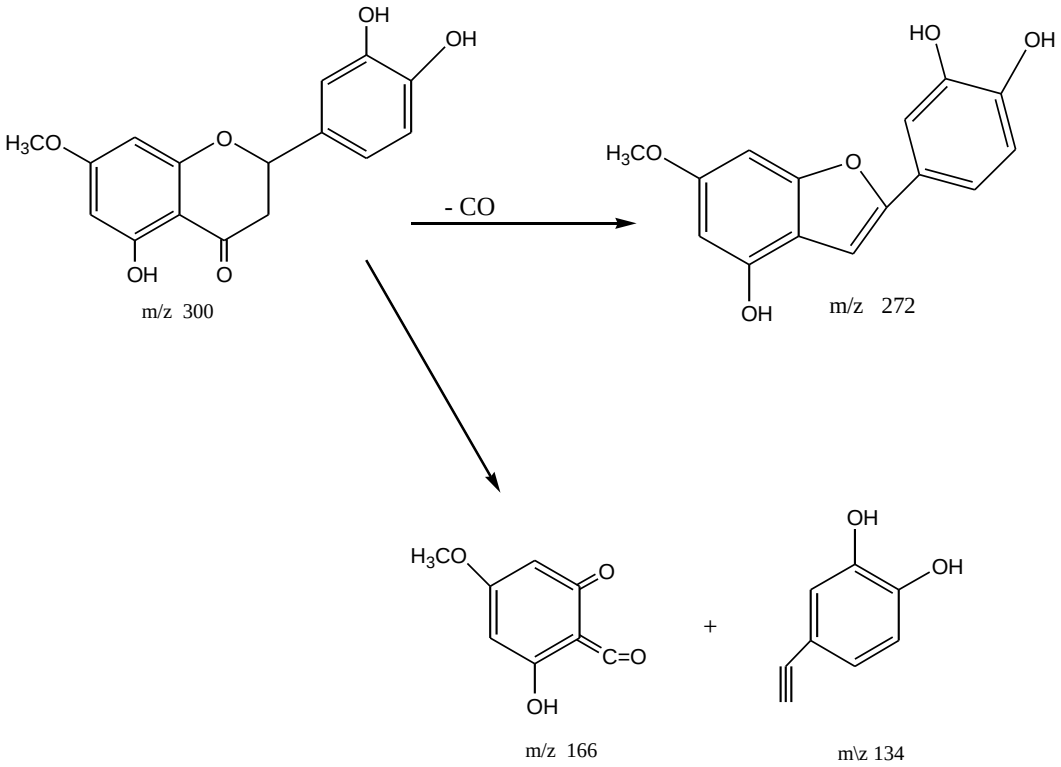


Fig.2: Fragmentation pattern of compound 1

Conclusion

Chemical and biological evaluation of extracts of *P. hirsuta* revealed the presence of some secondary metabolites in the root and leaf parts. The antimicrobial evaluation showed marked activity against Gram positive organisms while there was absence of activity in the root extract. The chemical investigation, for the first time, led to the isolation and characterization of the flavonoid compound with no antibacterial activity. The significant antibacterial activity of leaf extract against Gram positive organisms gives justification for the ethnomedicinal use of the plant by traditional healers.

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