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Isolation and Characterization of Baphianoside from the leaves of *Baphia nitida.*

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Abstract
A chemical investigation of the bioactive constituents of the leaves of *Baphia nitida*, one of the medicinal plants used widely in Nigeria by the herbalists for the treatment of different ailments such as ringworm, stiff joints, sprains, rheumatic pains and infectious diseases resulted in the isolation of a new compound, *Baphianoside.* The structure was elucidated using a two dimensional spectroscopy, NMR (^{1}H, ^{13}C) spectroscopy in combination with IR and MS spectra data.
Keywords: Baphianoside, Dye, Diseases

1.0 Introduction

Most of the drugs introduced in the market today are derived from medicinal plants. World Health Organization has also recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines (WHO, Geneva). Nigeria is blessed with many of such medicinal plants which include *Baphia nitida. Baphia nitida,* a medicinally important plant of the family leguminosae is also known as camwood or African sandalwood. It is a tree which is about 10 m high with trunk to about 45 cm diameter and slender branches which form an umbrella-shaped crown; usually an under storey tree of wetter parts of the coastal area. It belongs to the family of leguminosae. The tree is often planted in the villages as an ornamental or shade and as a source of medicines and dye. its wood is commonly used to make a red dye. Antimicrobial activity of camwood (*Baphia nitida*) dyes tested on common human pathogens showed that the dyes exerted good inhibitory activity against the gram positive organisms. Another study that looked at the germ-killing (antibacterial property) of camwood extracts, revealed that it has ability to kill some disease-causing germs at high concentrations. In the study, the researchers tested the antimicrobial activity of four aqueous extracts of camwood dyes obtained from different locations in Nigeria against five disease causing germs obtained from inpatients attending the University of Port Harcourt Teaching Hospital. The isolates were *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Proteus vulgaris* and *Pseudomonas aeruginosa.* The results of the test showed that the dyes possess some level of antimicrobial activity and can be used as a remedy for pathogenic infections (Agwa, *et al*). Thus the dyes can be used as an alternative to medicine in the treatment of infectious diseases, most of these dyes are used to cure infectious diseases relating to the skin, urinary tract, enteritis and other gastrointestinal problems which the test isolates are associated with.

Baphia nitida is applied against ringworm, stiff joints, sprains and rheumatic pains. It can equally be used for treating constipation, skin and venereal diseases. Phytochemical analysis carried on the leaves detected tannins, flavonoids, and saponin glycosides [Onwukaeme 1995]. Extracts from the leaves of *Baphia nitida* have been reported to be good inhibitors for mild steel corrosion in both acid media and better performances were obtained in 2 M HCl solutions (Njoku *et al,* 2014). Proximate analysis done on the seeds of *B. nitida* revealed high protein, 20.30 ± 0.70%, carbohydrate and minerals, indicating that the plant may be an economic and alternative protein, oil, mineral and carbohydrate source that could alleviate malnutrition in developing countries and improve overall nutritional status of functional food in the developed countries (Adewuyi, 2009).

2.0 Materials and Method:

2.1 Plant material : the leaves of *Baphia nitida* were harvested from the field of Micheal Okpara University of Agriculture,Umudike ,Abia state, Nigeria. Authentication of plant materials was done by Ibe, Ndukwe of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.

2.2 Extraction and isolation of plant material : The leaves were washed and allowed to dry in the laboratory bench. The dried leaves were milled into fine powder with Thomas Willey milling machine and then stored in air tight bottles for analysis. 2kg of the sample was percolated in 98% ethanol for 48hrs, this was then filtered. The filtrate was concentrated with rotary evaporator at 40^{o}C to a dark brown crude extract (50.5g). The crude extract was partitioned between $CHCl_3$ and water and a $CHCl_3$ - soluble fraction (15.0g) was obtained. 10.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60 – 80^{0}C) and aqueous methanol. 3.0g of the $CHCl_3$ fraction was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100ml petroleum ether, then petroleum ether : $CHCl_3$ (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90;), and 100ml $CHCl_3$; then $CHCl_3$: Methanol (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90) and 100ml methanol to yield ten major fractions Chromatographic (partition chromatography, column chromatography, and TLC) and spectroscopic (IR, ^{1}HNMR, ^{13}CNMR, COSY, DEPT and MS) techniques were employed to isolate, characterize and identify active constituents from $CHCl_3$ extracts of the leaves.

3.0 Results and Discussion

Compound [1] was isolated using a mixture chloroform and petroleum ether in the ratio of 80:30. The thin layer chromatography carried out on compound [1] showed one spot. Based on the chromatographic spectra, IR, NMR, MASS, COSY and DEPT, the compound was proposed as *Baphianoside* with molecular formula C_{44} H_{66} O_{16} m/z 846 calculated for m/z 845.4 and its base peak at m/z 180.6 calculated for m/z 180 ($C_aH_8O_4$). IR spectrum revealed V_{max} (2980, 1720, 1600 and 1360 cm^{-1}) for aliphatic, carbonyl, aromatic and ether respectively. Analysis of IR is shown in Table 1.

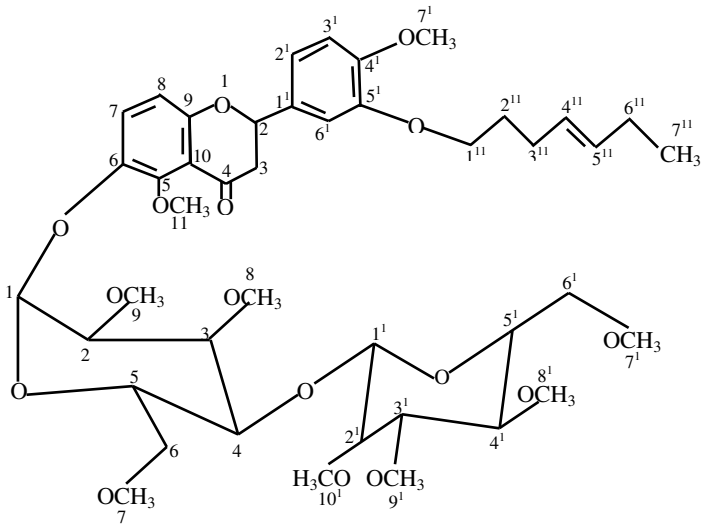

Fig 1: Baphianoside [1]

The ^{1}H NMR spectrum showed the presence of aromatic protons at δH 5.029 - δH 5.126, methoxy protons at δH 4.232 - δH 4.705, methyl proton at δH 0.732 and methylene protons at δH 1.954 - δH 2.205. The anomeric protons were fully substituted with methoxy groups. The analysis of ^{1}H NMR is shown in Table 2.

^{13}C NMR spectrum revealed the presence of aromatic carbons whose absorptions were seen at δC 105.94, δC 122.04, δC 128.82, δC 130.90, δC 132.46 and δC 157.25. The spectrum also revealed methoxy carbons at δC

50.02 - δC 68.17, methyl carbon at δC 10.97 and methylene carbons at δC 23.75 - δC 27.10. The anomeric carbons showed their absorptions at δC 36.58 - δC 37.99. The analysis of is shown in Table 2.

The isolated compound is a flavonoid glycoside and its presence in the plant indicates that *Naphia nitida* has biological activities. Flavonoids have been reported to have beneficial effects against atherosclerosis, osteoporosis, diabetes mellitus and certain cancers such as breast cancer (Uchegbu,*et al*). Many flavonoids have been isolated from plants and most of them have been reported to have antibacterial, antioxidant and anti-inflammatory activities (Veitch and Grayer, 2008). Thus the presence of this isolated compound in the plant may be the reason why *Naphia nitida* is used in traditional medicine to treat elephantiasis and infections. It also implies that *Naphia nitida* can be used in the treatment of other diseases such as diabetes mellitus and certain cancers.

Conclusion

The result of this analysis revealed that the plant, *Baphia nitida* has a lot of biological activities and thus can be used as raw material by pharmaceutical industries for drug formulation. Thus this contributes to the scientific evidence for the use of this medicinal plant in traditional medicine for the treatment of rheumatic pains, constipation, skin, venereal diseases, e.t.c in Nigeria.

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Table 1: IR analysis of compound [1]

IR Absorption (cm^{-1})	Functional Group	Compound Type
2980	$-CH_2$	Aliphatic
1720	C= O	Carbonyl
1600	C = C	Aromatic
1360	C = O	Ether

Table 2: ^{1}H NMR and ^{13}C NMR analysis of compound [1]

Position	Chemical shift (δ)	Carbon	Chemical shift	Multiplicity	Proton
1	-	-	-	-	-
2	27.22	CH	2.354	1H	CH
3	23.75	CH_2	1.954	2H	CH_2
4	167.79	C = O	-	-	-
5	105.94	C	-	-	-
6	122.04	C	-	-	-
7	128.82	CH	-	-	-
8	130.90	CH	5.029	1H	CH
9	132.46	C	-	-	-
10	157.25	C	-	-	-
11	-	OCH_3	4.232	3H	OCH_3
$1^{/}$	-	C	-	-	-
$2^{/}$	105.94	CH	5.064	1H	CH
$3^{/}$	122.04	CH	5.080	1H	CH
$4^{/}$	128.82	C	-	-	-
$5^{/}$	130.90	C	-	-	-
$6^{/}$	132.46	CH	5.126	1H	CH
$7^{/}$	157.25	OCH_3	4.254	3H	OCH_3
$1^{//}$	24.48	CH_2	1.975	2H	CH_2
$2^{//}$	24.81	CH_2	2.134	2H	CH_2
$3^{//}$	25.14	CH_2	2.154	2H	CH_2
$4^{//}$	27.76	CH	5.361	1H	CH
$5^{//}$	28.94	CH	5.373	1H	CH
$6^{//}$	25.47	CH_2	2.172	2H	CH_2
$7^{//}$	10.97	CH_3	0.732	3H	CH_3
GLU					
1	36.58	CH	4.080	1H	CH
2	37.11	CH	4.094	1H	CH
3	37.30	CH	4.115	1H	CH
4	37.44	CH	4.128	1H	CH
5	37.99	CH	4.138	1H	CH
6	25.75	CH_2	2.179	2H	CH_2
7	59.46	OCH_3	4.283	3H	OCH_3
8	50.02	OCH_3	4.293	3H	OCH_3
9	68.17	OCH_3	4.306	3H	OCH_3
$1^{/}$	38.73	CH	4.148	1H	CH
$2^{/}$	39.38	CH	4.162	1H	CH
$3^{/}$	39.38	CH	4.181	1H	CH
$4^{/}$	40.85	CH	4.193	1H	CH
$5^{/}$	42.16	CH	4.202	1H	CH
$6^{/}$	27.10	CH_2	2.205	2H	CH_2
$7^{/}$	44.35	OCH_3	4.319	3H	OCH_3
$8^{/}$	59.46	OCH_3	4.654	3H	OCH_3
$9^{/}$	50.02	OCH_3	4.689	3H	OCH_3
$10^{/}$	44.65	OCH_3	4.705	3H	OCH_3

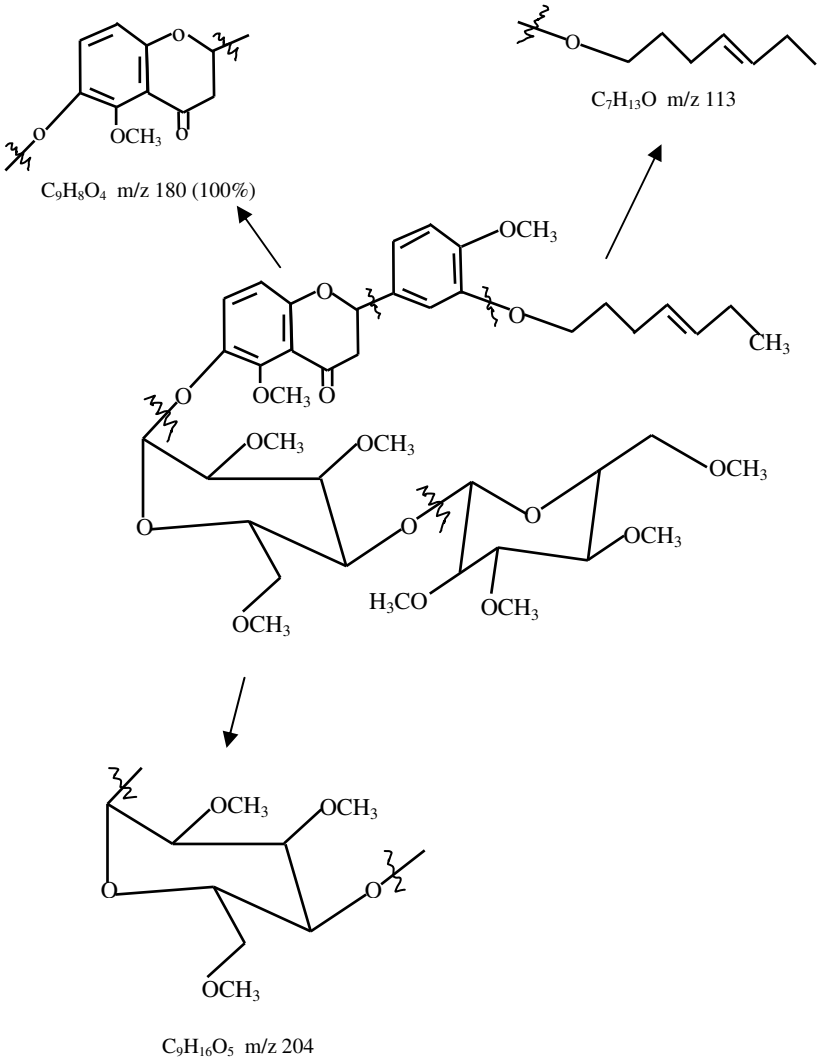

Fig 2: Fragmentation pattern of compound [1]

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