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Isolation and Characterization ofPhenanthrene, phenyl acetate from the Leaves of *Antiarisafricana*

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Abstract
Antiarisafricanaplant commonly known as "false iroko" tree is one of the medicinal plants used in the treatment of mental and nervous disorder such as epilepsy. It is also used to treat respiratory infections, syphilis and skin irritants in Nigeria. An Ester, (1E, 3E)-4-(6, 7, 8, 9, 10, 13, 14, 15, 16, 17-decahydro-17-hydroxy-5, 10, 13, 14-tetra methyl-5H-cyclopenta [α] phenanthrene-16-yl) buta-1,3-dienyl-2-phenyl acetate was isolated and characterized from the leaves of Antiarisafricana using IR, ^{1}H NMR, ^{13}C NMR, MS, COSYand DEPT. The presence of the isolated compound may be responsible for some of the biological activities exhibited by Antiarisafricana plant.

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I. Introduction

Most drugs originated from natural sources, either they are directly isolated from plants or the isolated compound is modified to improve its efficacy. Plants are sources of bioactive compounds which are used directly as therapeutic agents, as well as starting material for the synthesis of drugs. Traditional medicines have played important roles in disease treatment since the emergence of man's evolution. Nigeria is blessed with many medicinal plants which are used traditionally to treat different diseases. The plant *Antiaris Africana* is a highly medicinal and a large attractive deciduous timber tree with blotchy grey and white bark. The small, greenish white flowers yield red, velvety fruits. It belongs to the family Moraceae(Keay,1989). It has white latex and alternate dissymmetric leaves. The heartwood of the plant is whitish to pale yellow; the texture is moderately coarse and the wood is lustrous. Fresh wood has woolly surfaces. The wood is light in weight and soft. It is used for interior joinery, panelling, moulding, shuttering, furniture, strip flooring, boxes and crates, tool handles, toys and carvings. It is fairly commonly used domestically for light construction and canoes. It has a wide usage both in industry (for timber making) and as traditional medicine.

The leaves, stems and barks of the plant are used in the treatment of various diseases such as rheumatic and respiratory infections, epilepsy, skin irritant, syphilis etc.The bark extract is used in traditional medicine for the treatment of chest pains. The latex of the plant is used as a purgative. It is also used as a cure for leprosy and sore throat. The plant is used in Cameroon folk medicine to treat cancer. Decoctions of the leaves are applied in the treatment of syphilis. Tapsell*et al*, 2006 and Kuete*et al*, 2009 reported that *Antiaris Africana* is rich in antioxidant, anti-inflammatory, antimicrobial and anticancer phytochemicals. The leaves and roots are also used to treat mental illnesses. The plant is utilized traditionally in ethnomedicine for epilepsy, lumbago, skin irritant, purgative, nervous disorders (Keay, 1989).

In spite of the various uses of this plant as medicine, its constituents have not been fully documented. In this research, a novel compound was isolated and characterized from the leaves of*Antiarisafricana.*

II. Materials and methods

2.1 Plant Materials: The leaves of *Antiarisafricana* were collected from the Botanical garden of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and authentication of plant materials was done by Mr.NdukaIbe of Taxonomy section, Forestry Department.

2.2 Sample Preparation: The fresh plant sample was washed and air dried on a laboratory bench on a period of two weeks. The dried leaves were milled into fine powder with Thomas Willey milling machine and then stored in air tight bottles for analysis. 2kg each of the sample were used.

2.3 Extraction of the Plant Materials.

The leaves of *Antiarisafricana* were ground into powder. The powdered samples (2kg) were percolated in ethanol for 48 hours and filtered.

2.3.1 Isolation of the Constituents of leaves of *Antiarisafricana*

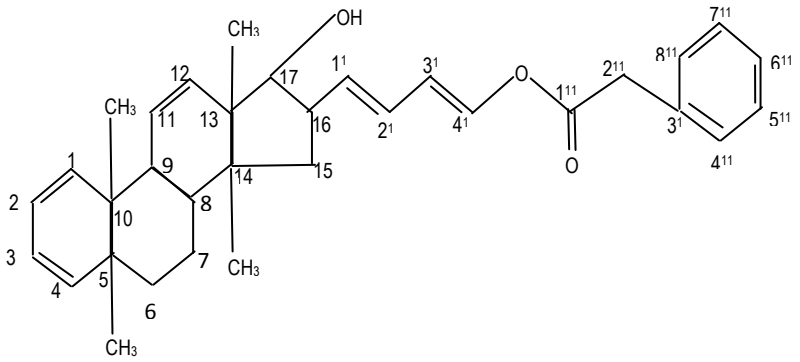
The filtrate from the leaves was concentrated with Rotary evaporator at 40^{o}c to a dark brown crude extract (8.0g). The crude extract was partitioned between $CHCl_3$ and water. 4.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60-80^{o}c) and aqueous methanol. 3.0g of the chloroform fraction was subjected to column chromatography over silica gel (200 mesh) and eluted gradually with petroleum ether, petroleum ether : $CHCl_3$ 90:10,80:20,70:30,60:40,50:50,40:60, 30:70,20:80,10:90), $CHCl_3$ then $CHCl_3$– Methanol (90:10,80:20,70:30,60:40,50:50, 40:60,30:70,20:80,10:90) and methanol to yield eight major fractions labeled : F(light yellow oil, 0.5g), G_1 (yellow oil ,0.4g), H (golden yellow oil,0.80g), I (light yellow oil, 0.3g), K (yellow oil, 0.5g), c_2 (yellow oil, 0.40g), g (brown oil 0.1g).\

2.4 Thin-layer Chromatography

A thin layer of the adsorbent, silica gel (60G) was spread onto the glass plate and allowed to dry. The different sample solutions were spotted each as a small spot near the base of the plate using capillary tube and placed upright in a TLC tank which contains a shallow layer of solvent, the solvent ascends the layer of the adsorbent on the plate. When the thin layer plate has been developed, the plate was removed from the developing tank and allowed to dry until it is free of solvent. The plate was placed in a chamber containing iodine crystals and left to stand for a short time (Uchegbu *et al*, 2015; Uchegbu *et al*, 2019). Most of the fractions gave a coloured spot each that was clearly visible and their R_f values were calculated.

Chromatographic (partition chromatography, column chromatography, and TLC), spectroscopic (IR, ^{1}HNMR, ^{13}CNMR and MS), 2DNMR techniques were employed to isolate, characterize and identify active constituent from $CHCl_3$ extracts of the leaves.

III. Results and Discussion



Compound [1]

Compound [1] was eluted with chloroform and methanol at the ratio of 70:30. It was isolated as yellow oil (0.89g). TLC carried on the compound showed one spot (Rf 0.753). The molecular formula of compound [1] is $C_{33}H_{40}O_{13}$, m/z 644 calculated based on combined analysis of IR, ^{1}HNMR, ^{13}CNMR and COSY spectra. It was found to be an Ester and named, *(1E, 3E)-4-(6, 7, 8, 9, 10, 13, 14, 15, 16, 17-decahydro-17-hydroxy-5, 10, 13, 14-tetra methyl-5H-cyclopenta [α] phenanthrene-16-yl) buta-1,3-dienyl-2-phenyl acetate*

Table 1 shows the Infrared absorption data of compound [1]. The Infrared spectrum depicted the presence of ester carbon-oxygen doubled bond (C = 0) at 1733 cm^{-1}, carbonyl carbon – oxygen single bond (C – 0) at 1377 cm^{-1} , and 1242 cm^{-1}, aromatic ring carbon – carbon double bond at 1463 cm $^{-1}$, carbon-hydrogen bond at 2916 cm^{-1} and 2849 cm^{-1} . The Infrared spectrum showed anabsorption at 3500 cm^{-1} indicating the presence of hydroxyl (OH) which was confirmed in ^{1}HNMR spectrum.

The ^{1}HNMR in Table 2 showed a peak at δ 2.00 depicting esther (CH_2 –COOR) which was labelled H – 7^{1}. Aromatic protons were observed at δ 6.40, δ 6.50, δ6.60, δ 6.65, and δ6.70 labelled H – 2^{1}, H – 3^{2}, H – 4^{1}, H – 5^{1}, and H – 6^{1}, respectively and olefinic proton showed at δ 5.00, δ 5.10, δ 5.15, δ 5.21, δ 5.25, δ5.30, δ 5.33, δ 5.40, δ 5.45,and δ 5.49 labelled H – 1, H – 2, H – 3, H – 4, H – 11, H – 12, H – 18, H – 19, H – 20, H – 21 respectively. Chemical shifts of methyl protons were observed at δ0.50, δ0.65, δ0.76 and δ0.80 labelled H – 18, H – 19, H – 20, and H – 21 respectively. Methylene protons were seen at δ 1.00 , δ 1.10 , δ 1.20 , δ 26.72

were labelled H – 6, H – 7, H – 15, and H – 7^1 respectively. Methine protons signals were depicted at δ 1.50 , δ 1.60 , δ 1.80 , δ 1.85 and labelled H – 8, H – 9, H – 16, and H – 17b respectively.

^{13}CNMR spectrum has resonance peaks depicting the presence of aromatic carbons at δ 131.24 , δ 134.97 , δ 135.23 , δ 135 39 , δ 135.23 , δ 135 39, δ 135.78 and δ 135 72. The carbons were labelled at C – 1^1, C – 2^2, C – 3^1, C – 4^1, C – 5^2 and C – 6^1 respectively. The signal for carbonyl carbon was observed at δ 150.96 and was labelled at C – 8^1. The spectrum shows the chemical shifts of the olefinic carbons at δ 109.35, δ 118.13 , δ 118.14, δ 118.17, δ 119.36, δ 121.64, δ 122.59, δ 124.26, δ 124.41 and δ 125.03. These were labelled at C – 1, C – 2, C – 3, C – 4, C – 12, C – 18, C – 19, C – 20, and C – 21 respectively. Signals of quaternary carbons were seen at δ 55.26, δ 55.39, δ 55.05, and δ 56.70 labelled C – 5, C – 10, C – 13, C – 14 respectively. Methyl carbon were observed at δ 14.51, δ 16.03, δ 17.48 and δ 18. 15 and were labelled C – 22, C – 23, C – 24, and C – 25; while Methylene carbons signals were depicted at δ 39 38 , δ 39.62 , δ 39.66, and δ 26.72. The carbons were labelled at C – 6, C – 7, C – 15, and C – 7^1. Methine protons were seen at δ 43.00 , δ 45.85, δ 48.01 and δ 48.30 and labelled C – 8, C – 9, and C – 16, C – 17a respectively.

COSY (Correlation Spectroscopy)

This spectrum shows protons that are coupled to each other. The cosy spectrum takes the ^{1}H spectrum and spreads it out into two dimensions. Here, the peaks are displayed along one axis and the same peaks are also displayed along the other axis.

In the Cosy data of *Antiarisafricana*, there are peaks that appear along the diagonal stretching from the upper right to the lower left through the middle of the plot. The interesting peaks are the peaks that do not appear along the diagonal. These peaks indicate which protons are coupled to which other protons.

The signal at δ 2.00 has a cosy cross peak to a doublet at δ 4.90. Signal at δ 2.4 has a cross peak to δ 4.80 showing triplet. Proton at δ5.10 has a cross peak to a multiplet at δ 2.0.The proton δ 2.0 are coupled to the protons at δ 4.90 and that gives a "cross peak'' at (2.0, 4.90).

Derivatives of phenanthrene have been reported to possess some biological activities such as anticancer activity,antileukemic activity, antitumor activity.The isolated compound is also an ester. Esters have many uses in both the living world and industries. Esters also known as parabenes are used as food and drug preservatives because they can prevent the growth of microorganisms such as molds and yeast.

IV. Conclusion

Reports showed that the leaves, stems and barks of *Antiarisafricana* are used in the treatment of various diseases such as rheumatic and respiratory infections, epilepsy, skin irritant, syphilis etc.The novel compound isolated from the leaves of this plant being an Ester and a phenanthrene derivative possesses biological activities and thus the isolated compound may be the reason why the leaves of*Antiarisafricana*is useful in treatment of many diseases by the Herbalists.

Table 1: Infrared Analysis of Compound [1]

ABSORPTION FREQUENCY (cm^{-1})	FUNCTIONAL GROUP	
3500	Alcohol	0H
2196	Alkanes	CH
2849	Alkanes	CH
1733	Carboxyl group ((Ester)	C = 0 (ester)
1363	Aromatic	C = C
1377	Carbonyl	C – 0
1242	Carbonyl	C – 0

Table 2: ^{1}H and ^{13}CNMR Analysis of Compound [1]

Position	^{13}C signals(ppm)	Type of Carbon	^{1}H signals (ppm)	Type of Proton	Multiplicity
1	109.35	= C	5.00	= CH	1Hd
2	118.13	= C	5.10	= CH	1Hd
3	118.14	= C	5.15	= CH	1Hd
4	118.17	= C	5.21	= CH	1Hd
5	55.26	Cq	-	-	-
6	34.38	CH_2	1.00	CH_2	2Ht
7	39.62	CH_2	1.10	CH_2	2Hq
8	43.00	CH	1.50	CH	1Ht
9	45.85	CH	1.60	CH	1Hd
10	55.39	Cq	-	-	-
11	119.36	= c	5.25	= CH	1Hd
12	121.64	= c	5.30	= CH	1Hd
13	56.05	Cq	-	-	-
14	56.70	Cq	-	-	-
15	39.66	CH_2	1.20	CH_2	2Hd
16	48.01	CH	1.80	CH	1Ht

17a	80.93	C – OH	4.00	C – OH	1HS
17b	48.30	CH	1.85	CH	1HS
18	14.51	- CH_3	0.50	- CH_3	3Hs
19	16.03	- CH_3	0.65	- CH_3	3Hs
20	17.48	- CH_3	0.76	- CH_3	3Hs
21	18.15	- CH_3	0.80	- CH_3	3Hs
1^{I}	122.59	= C	5.33	= CH	1Hd
2^{I}	124.26	= C	5.40	= CH	1Hd
3^{I}	124.41	= C	5.45	= CH	1Hd
4^{I}	125.03	= C	5.49	= CH	1Hd
1^{II}	131.24	- C = C -	-	-	-
2^{II}	134.97	- C = C -	6.400	= CH	1Hd
3^{II}	135.23	- C = C -	6.500	= CH	1Ht
4^{II}	135.39	- C = C -	6.600	= CH	1Ht
5^{1}	135.78	- C = C -	6.65	= CH	1Ht
6^{1}	139.72	- C = C -	6.70	= CH	1Hd
7^{1}	26.72	CH_2	2.00	CH_2C00R	2Hs
8^{1}	150.96	C = 0	-	-	-

S = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

References

[1]. Keay, R. W. J. (1989). *Trees of Nigeria.Revised version of Nigerian Trees*. ClarendonPress, Oxford.

[2]. Kuete, V., Vouffo, B.,Mbaveng, A.T.,Vouffo, E.Y.,Siagat, R. M. and Dongo E. (2009). Evaluation of *Antiarisafricana* methanol extract and compounds for antioxidant and antitumor activities.*Pharmaceutical Biology*, 47(11):1042-1049.

[3]. Tapsell, L. C., Hemphill, L., Lynne, C., David, R., Michael, F., Craig, S., Steven, R., Jennifer, B., Peter, M., Peter, G., Virginia, A. and Karen, E. (2006). Health benefits of herbs and spices: The past, the present, the future. *Faculty of Health and Behavioural Sciences Papers*, 185:4-24.

[4]. Uchegbu, Rosemary I.; Echeme, John Bull O.; Ngozi-Olehi, Lynda C.; Njoku,Peter C.&Mbadiugha,Cliff N. (2015). Isolation and Characterization of 5, 7 – dimethoxy, $4^{/}$– propoxyflavone from the seed of *MucunaflagellipesAmerican Journal of Chemistry and Applications*, 2(4): 104 – 107.

[5]. Uchegbu, R.I., Njoku, C. P., Ukpai, K.U. and Ibe, C.O. (2019). Isolation and Characterization of Methoxy, PropoxyDimethylamine from *Detariumsenegalense*Seed.*Bioequivalence& Bioavailability International Journal*(MEDWIN PUBLISHERS),(3)1.

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