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Isolation and Characterization of Palmitic Acid from Ethyl Acetate Extract of Root Bark of Terminalia Glaucescens

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Abstract
The aim of this study is to identify and characterized the bioactive compounds from the root bark of the plant. Preliminary phytochemical screening of the root bark extract of *Terminalia glaucescens* revealed the presence of steroids, terpenoids, saponins, flavonoids, tannins and cardiac glycoside. The plant has wide folk medicinal use in traditional medicine. The air dried root bark was pulverized to powder, subjected to hot extraction (soxhlet) with methanol, and fractionated into n-hexane, ethyl acetate, and n-butanol fractions. Ethyl acetate as bioactive fraction based on sensitivity test was subjected to TLC and column chromatography. The isolated compound was colourless powder, which was further subjected to IR, UV, ^{13}CNMR and ^{1}HNMR for proper characterization and elucidation of the structure. The compound was concluded as palmitic acid.

INTRODUCTION

Pharmaceutical industries started to use crude extracts of medicinal plants for manufacturing drugs (Ali and Azhar, 2000). The acceptance of traditional medicine as an alternative form of health care couple with development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Lis-Balchin and Deans, 1996, Maoz and Neeman, 1998, Hammer *et al.,* 1999). Presence of tannins, flavonoids, terpenoids, saponins , steroids, cardiac glycosides, volatile oils, alkaloids, anthraquinones and other phenolics have been reported to have antimicrobial activities (Hostettman and Nakanishi, 1979, Hostettman *et al.,* 1995, Isaac and Chinwe, 2001). *Terminalia glaucescens* is belonging to family Combretaceae, is a tree up to 20 m high. It is commonly found in West Africa especially in Savannah regions. The plant is the most important medicinal species of the genus T*erminalia* (Ndukwe *et. al*., 2005). *Terminalia glaucescens* is belonging to family Combretaceae, is a tree up to 20 m high. It is commonly found in West Africa especially in Savannah regions. The plant is the most important medicinal species of the genus T*erminalia* (Ndukwe *et. al*., 2005).It is abundant in Nigeria and is commonly called *baushe* (Hausa Language) Idi Odan (Yoruba Language), Edo (Igbo Language) while the local dilate where the plant was collected is called palma (Bura – Babur Language). The roots bark of the plant has not been thoroughly evaluated for antibacterial activity. Therefore, the organisms used in this study are known to cause dysentery, fever, diarrhea, wound, tooth decay, ulcers, typhoid fever and various stomach related problems (Richard *et al*, 2004 and WHO 1997). The purpose of this study is to identify and characterize the bioactive compound (s) from the root bark of *Terminalia glaucescens*. In this paper, we report the isolation and characterization of known compounds from the plant namely palmitic acid.

EXPERIMENTAL

Collection, Identification and preparation of plant materials.

The plant materials were collected from Hyera Road of Shaffa District, Hawul Local Government Area, Borno State of Nigeria. The plant was identified and the herbarium (voucher) specimen number UDUS/Bio/12/113 was prepared and deposited at the Herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, by A. M. Umar (Taxonomist). The root bark of the plant was air dried under shed, then pulverized into powder with the aid of pestle and mortar. The powder obtained from the plant was then sieved and stored in polythene bags until required for use (African pharmacopoeia, 1985).

Extraction and Isolation

Six hundred gram (600g) of powdered root bark was extracted by soxhlet extractor used 1500ml of methanol as solvent at temperature of 85 ^{0}C, was concentrated using hot air sterilizing cabinet at 60^{0}C and yield 123.11gram of methanol crude extract. Split method of separation was adopted according to (Abubakar, 2009). The n- hexane was directly added to crude methanol extract and was vigorous stirring before filtration and the filtrate are all n-hexane soluble portion, which is the n-hexane fraction while the residue was allowed to dry and same method was repeated with ethyl acetate, n- butanol and finally the residue obtained is methanol fraction. N-hexane, ethyl acetate, n - butanol, and methanol fractions were obtained and were concentrated at 60^{0}C in hot air sterilizing cabinet. 100 ml burette was use as a Column with 50g of silica gel as a stationary phase while mobile phase was

petroleum ether hundred percent followed by 9:1 ratio of petroleum ether and ethyl acetate as eluting solvent. The column was parked by wet parking method, after parking was allowed overnight with 3g of concentrated ethyl acetate fraction was dissolved in pet ether solution and soaked with cotton wool was placed on top of silica gel in the column. Between the cotton and the top of silica gel there was disc made of filter paper and the bottom of the column there was also another cotton wool. 2.4ml per minute each were collected in collection bottles range from 1 to 50. The column fraction's profiles were monitored by TLC to confirming the similarities of elutes based on the number and color (s) of the spot (s).

Thin Layer Chromatography

Commercially pre-coated TLC silica gel plate was used a line was drawn with a pencil 2cm at the bottom from one end of the plate. The sample(s) were dissolved in little ethyl acetate solution and was spotted on the line drawn on the plate by capillary tube and then allowed to dry. The dry plates were placed into the chroma tank contained (9:1) ratio of chloroform and methanol, the tank was covered. The solvent rose up on the plate by capillary action, when the solvent front was just about 2cm to the upper end of the plate, the plate was removed and a line was drawn to mark the position of the solvent front. The plates were allowed to dry and the spots were developed by spread with 5% H_2SO_4 as spraying reagent. The R_f value of the spots were measured using meter rule.

Tests for steroid

Salkowski reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to thesolution. A reddish color was seen in the upper chloroform layer (Harbone, 1984).

Liebermann burchardreaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2 - 3 drops of acetic anhydride.Solution turned violet blue and finally green (Harbone, 1984).

RESULTS AND DISCUSSION

Table 1. TLC Result of the ethyl acetate fraction of *Terminalia glaucescens*.

Fraction	Solvent System	No of component	R_f values
Ethyl acetate	Chloroform and methanol ratio 9:1	6	0.18 0.35 0.38 0.64 0.76 0.82
Column fa	Chloroform and methanol 9:1	1	0.76

Table 2. IR value of the isolated compound (A) from ethyl acetate fraction of *Terminalia glaucescens*

Vibration Frequency (cm^{-1})	**Assignment**	**Types of Vibration**	**Functional Group**
1838.22	C=O	stretch	Carbonyl group
2353.23	(CH_2)n	Bending	Overtone bands
2864.39	CH_3	stretch	Methyl group
3186.51-838.47	O-H	Free	OH attached to acid.

Table 3. Comparative chemical shifts of ^{1}HNMR of the isolated Palmitic Acid.

Position	Group	δ_H (ppm)	
		Obtained	ACD/ChemSketch (Product version:10.00)
1	CH_3	0.91	0.96
2	CH_2	1.39	1.33
3	CH_2	1.31	1.29
4	CH_2	1.31	1.29
5	CH_2	1.31	1.29
6	CH_2	1.31	1.29
7	CH_2	1.31	1.29
8	CH_2	1.31	1.29
9	CH_2	1.31	1.29
10	CH_2	1.31	1.29
11	CH_2	1.31	1.29
12	CH_2	1.31	1.29
13	CH_2	1.31	1.29
14	CH_2	1.40	1.56
15	CH_2	2.32	2.23
16	C	-	-
17	OH (at C17 position)	9.78	9.00

Table 4. Comparative chemical shifts of ^{13}CNMR of the isolated Palmitic Acid.

Position	Group	δ_C (ppm)	
		Obtained	ACD/ChemSketch (Product version:10.00)
1	CH_3	24.67	14.10
2	CH_2	31.78	22.80
3	CH_2	31.93	31.90
4	CH_2	29.13	29.70
5	CH_2	29.13	29.70
6	CH_2	29.13	29.70
7	CH_2	29.13	29.70
8	CH_2	29.13	29.70
9	CH_2	29.13	29.70
10	CH_2	29.13	29.70
11	CH_2	29.13	29.70
12	CH_2	29.13	29.70
13	CH_2	29.13	29.70
14	CH_2	25.96	24.80
15	CH_2	34.07	36.10
16	CH	179.21	177.30

Spectroscopic Characterization and Elucidation of the Structure

The GC-MS result showed the peak with the highest M/Z value of 256 which suggested the molecular mass of the compound. By Nitrogen Rule, this implies that the compound does not contain nitrogen or it contains even number of nitrogen atoms. The base peak has M/Z 43 which suggested the presence of oxalium ion that is ketones, carboxylic acid, etc may be present.. Peaks were also observed at M/Z of 256, 239, 227, 213, 199, 185, 171, 157, 143,129, 115, 97, 83, 73, 60, 43, and 31. From M/Z 83 to 227 we have peaks differing from their predecessor and successor by mass of 14 suggesting serial cleavage of methylene groups in the compound. In addition going by these observations and the molecular mass of the compound is (256.241), we could proposed a molecular formula of $C_{16}H_{32}O_2$ which belongs to the series of compound $C_nH_{2n}O_2$ that is fatty acid (Palmitic acid). In a similar way the GC-MS machine through its library gave a possible structure as given in Fig 1.

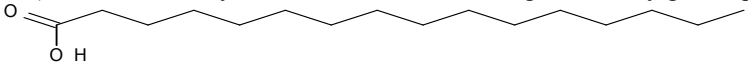

Fig. 1 Palmitic Acid (Hexadecanoic acid or Saturated Fatty acid)

The UV spectroscopy of our is a colourless oilly compound, gave λ_{max} in $CHCl_3$: 220 nm which suggested the absorption of carboxylic acid. Infrafred (IR) spectroscopic analysis, absorptions bands at 3570.36

– 3186.51 cm^{-1} which is characteristic of O ‑ H stretching , 2864.39 cm^{-1} is due aliphatics (CH_3) stretching, 1833.22 cm^{-1} due to carbonyl (C=O) stretching and 2353.23cm^{-1} due to overtone of the long chain $(CH_2)n$ bending frequency. Other absorption frequencies are having no significant to structure. These absorption frequencies resemble the absorption frequencies observed for saturated fatty acid as resembled data published by Lunn and Theobald, (2006).

The ^{1}HNMR spectrum (300MHz, $CDCl_3$) of the compound (Fig. 1) revealed a long peak of multiplet at δ 1.31, the typical 28H of the long chain was evident as a multiplet that integrated for one proton. The ^{1}HNMR spectrum showed a triplets centered at δ 2.23 (J = 6.7Hz) which could be attributed to two methylene group at C-2. The singlet at δ 9.8 was demonstrative of an OH in COOH. On the other hand, the triplet of three proton intensity at δ 0.91 could be assigned to the terminal methyl group at C- 16. This compound is having one methyl, fourteen methylene and one quaternary carbon with a hydroxyl group. The above spectral features are in close agreement to those observed for palmitic acid according to Guillen and Ruiz (2001).

The ^{13}C ‑ NMR has shown recognizable signal 179.21 which is assigned C16 double bonds of the acidic group as singlet, the alkane carbon or terminal C-1 appeared at δ24.67 as a triplet, From C-4 to C-13 appeared as a single long peak multiplet at δ29.70, alpha and Beta carbon to the acidic group appear at δ34.07 and δ25.96 are doublet respectively. Also alpha and Beta carbon to the alkane group or terminal carbon appeared at δ31.78 and δ31.93 is doublet respectively. Spectra showed sixteen carbon signal including fourteen methylenes, one methyl and one quaternary carbon. The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. On comparison the standard data matched with the simulated data which supports the proposed structure of this compound as palmitic acid.

Conclusion

This suggests that it could be used in the treatment of infections commonly associated with the microorganisms. Fatty acid (Palmitic acid) was isolated and characterized, the work also confirmed the possibility of isolating more bioactive compounds from the plant. The findings in this work have justified the traditional potency of this plant in ethno - medicinal treatment of oral infection of dysentery, fever, diarrhoea, wound, tooth decay, malaria, ulcers, typhoid fever and various stomach related problems which are caused by some of these organisms used in this study though the study is ongoing.

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