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Isolation and Characterization of the Methanolic Extract of the Stem of *Adenanthos sericeus* (Woolly Bush)

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ABSTRACT

Natural products or their derivatives form one important avenue for the search of new antibacterial agents. Plants are rich store house of diverse chemical compounds. Drug-like molecules obtained from plants offer an alternate choice over conventional synthetic drugs. In this present study, isolation on the methanolic extract of *Adenanthos sericeus* was performed using column chromatographic technique with 9:1 n-hexane and ethyl acetate mixture. Eluted fraction was further purified via preparative thin layer chromatography with a solvent system of 1:4 ethyl acetate and n-hexane to obtain a greenish–yellow and oily compound. With further characterization using H^1- NMR and GCMS, oleic acid was confirmed. This forms part of an alternate source of antibacterial agents which can be explore from the stem bark of *Adenanthos sericeus*.

Keywords: *Adenanthos sericeus*, oleic acid, antibacterial agents, isolates,H^1- NMR, GCMS.

INTRODUCTION

Natural products are phytochemicals produced as a result of secondary metabolism in plants. They are used by plants as a defence mechanism against the attack of bacteria, fungi, protozoa etc. As a result of such potency, natural products are used in enormous physiological activities on humans and animals e.g. alkaloids, tannins, phenolics, steroids, terpenes etc. Natural products are naturally occurring in the leaves, flowers and vegetables [1].

Natural products represent a major approach for the discovery and development of new drugs. Hence, the need for isolation and characterization from the stem bark of *Adenanthos sericeus* (woolly bush). *Adenanthos sericeus*, commonly known as Woolly Bush, and African-never die, is a shrub native to the south coast of Western Australia. *Adenanthos sericeus* belongs to the family Proteaceae of the genus *adenanthos.* It has bright red but small and obscure flowers, and very soft, deeply divided and hairy leaves [2].

Adenanthos sericeus mostly grows as an upright, spreading shrub but occasionally takes the habit of a small tree up to 5 m (16 ft.) tall. It has erect branches that are covered in short hairs when young, but these are lost with age. Leaves may be up to 40 mm (1.6 in) long, and repeatedly divide by threes into from 5 to 50 narrow laciniae, circular in cross-section, with a diameter of less than 0.5 mm [2]. It is cultivated in domestic gardens rather than in agricultural fields and as such can be used throughout the year.

Information obtained from folklore medicine reveals that this plant was used to relieve pain, detoxify venom of scorpion bite, and as a stimulant across the northern part of Nigeria as claimed by traditional practitioners. A report on the stem bark extracts of *Adenanthos sericeus* revealed the presence of ten out of twelve various phytochemicals in methanol, acetone, n-hexane and aqueous extracts. Quantitative estimation conducted on four phytochemicals revealed terpenoid, having highest, while tannin being the least. The inhibitory concentration (IC_{50}) on acetone, methanol, n-hexane and aqueous extract shows aqueous extract having the best IC_{50} than acetone, methanol, and n-hexane extract. Although α-tocoperol (standard) exhibited the best IC_{50} which implies a better antioxidant potency. The antimicrobial activity conducted on some selected microorganisms revealed the potency of the extract on microorganism. The study concluded that the stem bark of *Adenanthos sericeus* could be explore to make antioxidant and antibacterial drugs. However, there was no report on the active component responsible for the antioxidant and antibacterial activity of the stem bark of *Adenanthos* sericeus. Therefore, this research is aimed at isolating the methanolic extract of the stem bark of *Adenanthos sericieus* was report on the phytochemicals, antioxidant and antimicrobial activities of the crude extracts of *Adenanthos sericeus* [3].

MATERIALS AND METHODS

Collection of Plant Materials

The stem bark of *Adenanthos sericeus* was collected in Hanwa, Zaria, Kaduna State, Nigeria. It was identified and authenticated in the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria with the voucher number 20775.

Drying and Pulverizing

The stem bark of *Adenanthos sericeus* was washed thoroughly with distilled water in order to remove dust, soil particles and any other impurities. The stem bark was air dried for two weeks

under shade to prevent ultra-violet rays from activating the chemical constituents [4, 5]. It was later pulverized (ground into powder form) using laboratory mill.

Extraction of Plant Sample

The ground stem was subjected to exhaustive extraction using soxhlet extractor. It was weighed, and 100 g of it with 99.5% methanol were used in the extraction process. The crude methanolic extract of the sample was concentrated in an oven. The methanolic extract was packed in plastic bottle with proper labeling for further analysis [6].

Characterization of the Crude Methanolic Extract

Thin Layer Chromatography

The methanolic extract was run on silica gel precoated aluminium plate. The optimal solvent for the identification of compound was determined by varying the ratios of the solvents for developing the solvents. Visualization was carried out by spraying the plate with vanillin-sulphuric acid reagent [7].

Purification of Bioactive Compound(s) using Silica Gel Chromatography

About 5.00 g of methanolic extract of *Adenanthos sericeus* was subjected to a column chromatography using silica gel (70-230 mesh size). The column was packed and eluted with 9: 1 n-hexane: ethyl acetate mixture to obtain 32 fractions of 50.00 cm^3 each.

The collected fractions were spotted on a thin layer chromatographic (TLC) plate and a single spot was observed for A10 and further purification was carried out using preparative TLC with a solvent system of 1:4 ethyl acetate: n-hexane to obtain a homogenous A10 compound. This was a greenish –yellow oily compound [8].

Characterisation

Nuclear Magnetic Resonance Spectrometry (NMR)

Nuclear magnetic resonance experiment was performed at 500.1 MHz on a Varian NMR Systems Inc. spectrometer using a Varian probe (model PFG MR0803P007) in Shanghai Jiaotong University, China. The transmitter frequency was adjusted to the Larmor frequency. The probe temperature was stabilized for at least 30 min prior to each experiment. The magnetic field stability was adjusted using a deuterium lock. The magnet was shimmed to optimize field homogeneity.

Proton NMR spectra was obtained by using a standard PRESAT sequence to suppress the residual water signal. In this sequence, a long, selective rf-pulse is initially applied at the water frequency to saturate the water signal followed by a non-selective π/2 rf-pulse and the NMR signal is acquired. The length of the π/2 pulse was 10 μs for all experiments. To increase the signal-to noise ratio (SNR), the signal was averaged over 976 scans for each spectrum. To confirm that the SNR was sufficient, the spectrum, normalized for the number of averages, was compared to one measured with 4096 scans. These signals were found to be the same within experimental error [9].

Gas Chromatography Mass Spectrometry (GC-MS)

The phytochemical investigation of methanolic extract of the isolated compound performed in the research laboratory; American University of Nigeria, Yola on a GC-MS equipment (Thermo scientific co.), Thermo GC-TRACE ultra-version: 5.0, Thermo MS DS Qii in American University of Nigeria, Yola.

The experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non polar column, Dimensions: 30 mts ID: 0.25 mm, Film thickness: 0.25 μm. Flow rate of mobile gas phase (carrier gas: He) was set at 1.0 cm^3/min. In the gas chromatography part, temperature programme (oven temperature) was 40 ^oC raised to 250 ^oC at 5 ^oC/min and injection volume was 1μL. The isolated sample was dissolved in chloroform and was run at a range of 50 -650 m/z. The result was compared using NIST library search programme [10].

RESULTS AND DISCUSSION

The ^1H -NMR spectrum of isolated compound in Fig.1 showed some characteristics signals which are similar to those reported for oleic acid in Table 1. The signals at chemical shift 5.33 ppm correspond to olefinic protons of unsaturated fatty acids. The signals at 2.00 ppm were characteristics of allylic methylene protons in all unsaturated fatty acids including mono unsaturated fatty acids. The signal at 1.64 ppm is due to methylene protons beta to the carbonyl carbon while at chemical shift 1.24 ppm is due to the chain of methylene protons $(CH_2)_n$ of the aliphatic chain. The terminal methyl proton signal was 0.86 ppm, and this chemical shift is common for all terminal methyl protons [11].

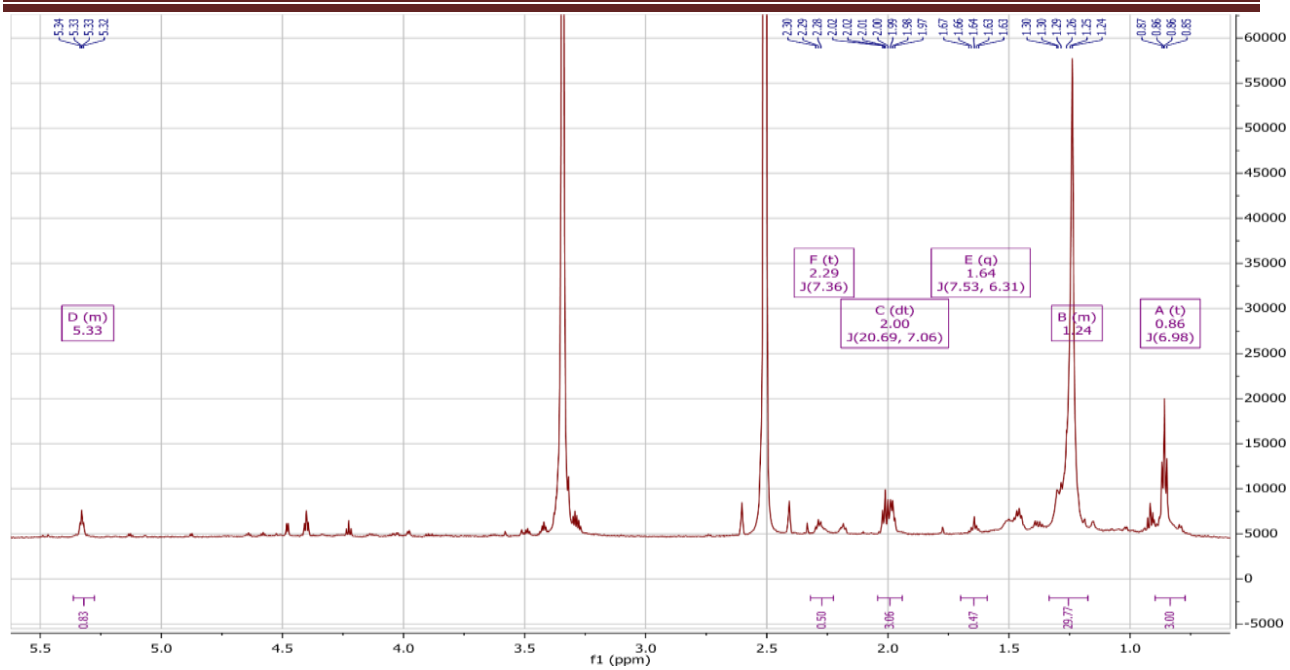

Figure 1: ^{1}H- NMR spectrum for the isolated compound

Table 1: ^{1}H- NMR data for isolated compound

Experimental	Literature	Assignment	Types of protons
5.33	5.34	-CH=CH-	Olefinic protons
2.29	2.29	$-CO_2-CH_2-$	Methylene (α to acyl group)
2.0	2.01	$-CH_2-CH_2-$ CH=CH	Methylene (protons attached to the allylic carbons)
1.64	1.58	$-CH_2-CH_2-$	Methylene (β to acyl groups)
1.24	1.27	$-(CH_2)_n-$	Methylene chains
0.86	0.87	$(CH_2)_n-CH_3$	Terminal methyl

To further confirm the structure of the proposed compound, the mass spectral analysis of the compounds revealed m/z 282 (M^+, 4%), 264 (1%), 222 (4%), 180 (4%), 138 (8%), 97 (48%), 55 (96%), and 13 (1%). The molecular peak ions were in agreement with oleic acid with a molecular weight of 282 g/mol as shown in Figures 2 and 3; and in Table 2.

Table 2: Proposed GCMS data for isolated compound

Fragment ions (m/z)	Abundance (%)	Compound
282	4	$[C_{18}H_{34}O_2]^+$
264	16	$[C_{18}H_{32}O]^+$
222	4	$[C_{16}H_{30}]^+$
180	4	$[C_{13}H_{24}]^+$
138	8	$[C_{10}H_{18}]^+$
97	48	$[C_7H_{13}]^+$
55	96	$[C_4H_7]^+$
14	1	$[CH_2]^+$

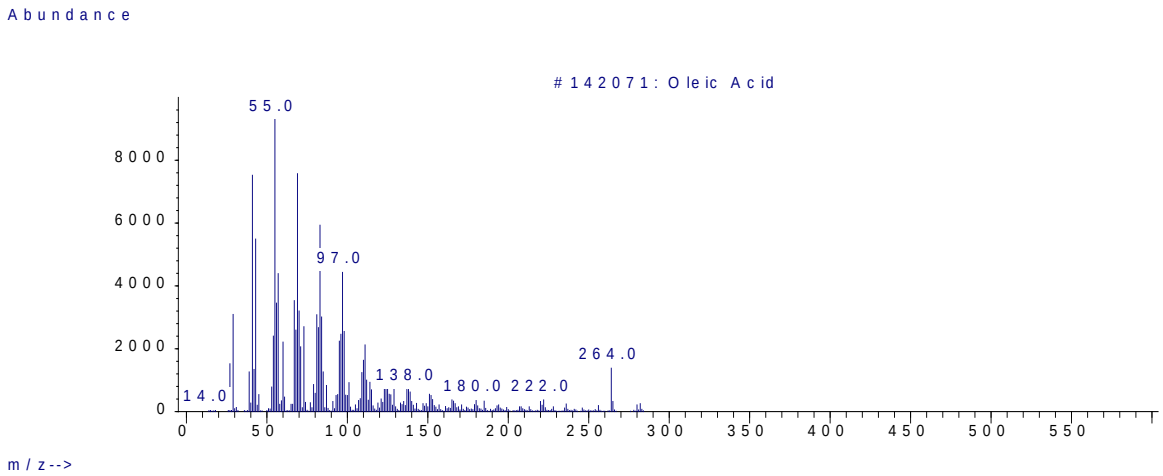

Figure 2: Mass spectrum for the isolated compound

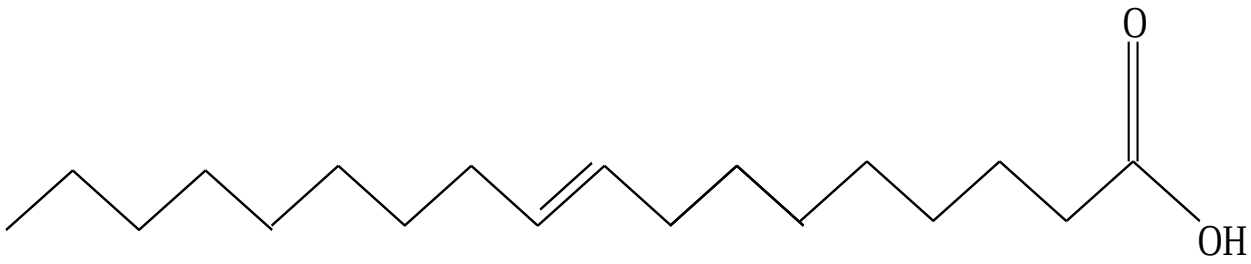

Figure 3: Common name: Oleic acid, IUPAC name; Cis-9, 10 octadecanoic acid: being a mono unsaturated fatty acid. Proposed structure of methanolic extract of *Adenanthos sericeus*

Studies carried out on cetyl alcohol and oleic acid sophorolipids indicated that oleic acid exhibited an anticancer activity [12]. The use of oleic acid as an antibacterial for treating eye infections was also reported [13]. It was also confirmed that oleic acid has beneficial effect on cancer, anti-immunal and anti-inflammatory effect on disease. Besides, it has the ability to heal wound [14].

CONCLUSION

From the present study, it was found that crude extract express good biological ability which indicated that the substance with powerful biological effect exists in this extract. This justifies its use in folklore medicine. It should be isolated and purified to confirm its pharmacological and medical uses. Novel therapeutic approach in future could be geared towards this plant as it may possess antimicrobial effect.

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