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# GC-MS Analysis and Anti-Bacterial Property of *Ptrocarpus santalinoides* Leaf from Abakaliki, Ebonyi State, Nigeria

*[1]P.M. Aja, [1]C.O. Okoro, [2]N. Ogbu Patience, [2]C.O. Egwu,*
*[3]O. L. Nweke, [4]V.E.O. Ozougwu and [4]O.C. Enechi*

[1]Department of Biochemistry, Faculty of Sciences, Ebonyi State University, Abakaliki, Ebonyi State
[2]Department of Medical Biochemistry, Federal University Ndufu-Alike Ikwo, Ebonyi State, Nigeria
[3]Department of Medical Biochemistry, Faculty of Basic Medicine, Ebonyi State University Abakaliki, Nigeria
[4]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

**Abstract:** *Pterocarpus santalinoides* is one of the medicinal plants used in the treatment of various diseases traditionally in Nigeria. The GC-MS analysis and anti-bacterial activity of *Pterocarpus santalinoides* leaf were carried out using standard methods. The result of GC-MS analysis revealed the presence of eight chemical constituents which includes: octa-1-ene (4.57%), nona-1,3-diene, hexadecanoic acid (22.87%), octadeca-9,11-enoic acid (3.66%), octadecanoic acid (14.02%), octadeca-10,12-enoic acid acid (18.90%), octadeca-11,13-enoic acid (6.40%) and octadeca-9-enoic acid (27.43%). The result of antibacterial activity of ethanol extract of *Pterocarpus santalinoides* leaf showed zone of inhibition on *Streptococcus pneumonia*, *klebsiella pneumonia, Escherichia coli* and *Staphylococcus aureus*. This result indicates the use of *Pterocarpus santalinoides* in ethno-medicine for the management of different ailment.

**Key words:** GC-MS Analysis · Chemical constituents · Anti-bacterial activity · *Pterocarpus santalinoides* leaf

## INTRODUCTION

Plant role in the maintenance of good health cannot be overemphasized. Studies have shown that plants play important roles in maintenance of good health [1]. The bases of many modern pharmaceutical used today are plants and plants based product [2]. Plants have been generally utilized for the treatment of diseases Worldwide. WHO (1999) [3] also observed that the majority of the population in the developing countries still rely on herbal medicine to meet their health needs stems from the fact that indiscriminate use of commercial antimicrobial drugs commonly utilized in the treatment of infectious diseases has led to the development of multiple drugs resistance and side effect on host associated with the use of conventional antibiotics [4], the safety and cost effectiveness of the use of plants in traditional medicine as well as in modern medicine [5]. Thus, there has been a need to develop alternative antimicrobial of plants origin which has been found to have enormous therapeutic potential [6].

In Nigeria, over 300 plants are used for treating various diseases including HIV/AIDS, opportunistic infections such as pneumonia, diarrhoea, typhoid fever, candidacies, tuberculosis and other ailment [7, 8]. Medicinal plants are known to owe their curative potentials to a certain biological active principles or phytochemical substances these include terpenes, flavonoids, saponins, anthraquinones, glycosides, etc [9]. Essential oil is important constituents of some higher plants comprising monoterpenes, sesquiterpene, arylpanoids and fatty acid derivatives. They have been recognized long ago to posses antimicrobial activities [10].

*Pterocarpus santalinoides* plant commonly referred to as "Red sandal wood" in English, "Ouokisse" in French, "Uturukpa" in Igbo, "Gunduru gyadar kurmi" in Hausa and "Gbenghe" in Yoruba [11]. various morphological part are use as food (as vegetable among the Igbo's) and its extract are use as an ethno-medicine in many African countries to treat an array of human ailments such as treatment of diarrhea (anti-diarrhea) and

**Orresponding Author:** P.M. Aja, Department of Biochemistry, Faculty of Sciences, Ebonyi State University, P.M.B 053, Abakaliki, Ebonyi State

Fig. 1: Leaves and Flowers of *Pterocarpus santalinoides*

gastro-intestinal disorder by creating a good plat form for combating the diverse underlying factors which triggers off diarrhea, it's also use for management of diabetes syndrome by lowering of triglyceride and glucose level in the blood. The bark and leaves of *P. santalinoides* possesses anti-malarial and anti-bacterial activity [12].

Ethno-medically, leaf-extract of *P. santalinoides* can be combined with other leaves such as *Solanum macrocarpum* (Gorongo) and are used in the management of high blood pressure, as well as an antipyretic to the people of Ntezi in Ishielu Local Government of Ebonyi State, Nigeria and to the people of Ngene in South East of Nigeria [13-16]. Despite the use of *Pterocarpus santalinoides* leaf for the treatment of various diseases, there is still paucity of documented data available regarding Gas chromatography-Mass spectrometric (GC/MS) analysis of the chemical constituents of the fruit. This study therefore evaluates the anti-bacterial activity and Gas chromatography–mass spectrometric (GC/MS) analysis of the chemical constituents of ethanol leaf-extract of *Pterocarpus santalinoides.*

## MATERIALS AND METHODS

**Materials:** The materials used in this research work were *Pterocarpus santalinoides* leaf, microorganisms (*Escherichia coli*, *Staphylococcus*, *Streptococcus*, *Salmonella, Klebsiella* and *Pseudomonas*) and Chlorophenical.

**Plant Collection:** The fresh leaves of *Pterocarpus santalinoides* were collected by hand picking in the month of November, 2014 in Agaga village of Ntezi community in Ishielu Local Government Area of Ebonyi State, Nigeria. The plant was identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. Some parts of the plant were also deposited in the herbarium for reference purpose.

**Preparation of Plant Sample:** The leaves were destalked, washed and shade dried at ambient temperature with constant turning to averts fungal growth. The dried leaves were later milled to obtained the vegetable leaf meals (VLMs) using an electric blender and was stored in 4°C temperature in refrigerator in well labeled air-tight containers for analysis.

**Preparation of *Pterocarpus santalinoides* Ethanol Leaf-Extract:** Exactly 40grams of dried powdered leaves of *Pterocarpus santalinoides* were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extract was filtered using what-man N0.1 filter paper to remove extractable substances at every 3hrs interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C in air-tight sterile container in refrigerator.

**GC-MS Analysis of Ethanol Leaf-Extract of *Pterocarpus santalinoides***

**Procedures:** GC-Ms analysis of the ethanol extract of *Pterocarpus santalinoides* leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming

from 80-200°C held at 80°C for 1minute, rate 5°C/min and at 200°C for 20min. Field ionization detector (FID) Temperature of 300°C, injection temperature of 220°C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220°C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

**Component Identification:** Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literatures.

**Preparation of Growth Medium**

**Broth Nutrient Agar Medium:** Exactly 5g of nutrient broth agar was measured into conical flask. Then, 180ml of distilled water was added and stirred thoroughly and allowed to stand for 15 minutes. The broth was cocked with cotton wool. The broth was autoclaved for 15 minute and allowed to cool. Then it was poured it into test tubes (depending on the amount of micro organisms) and the test tubes were cocked with cotton wool to avoid contamination by other micro organisms.

**Preparation of Nutrient Agar:** Exactly 10g of nutrient agar was measured into a baker containing 400 ml of distilled water and stirred and allowed for 20 minutes. The mixture was cocked with cotton wool and was autoclaved and Poured into plating media and allowed to cool.

**The Test Organisms Used for Antimicrobial Analysis:** The test bacterial were clinically and environmentally isolated and obtained from the Microbiology Laboratory Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria. These include: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, klebsiella pneumoniae* and *Salmonella typhi*.

**Screening of the Extracts for Antibacterial Activity:** One gram of each ethanol leaf- extract was reconstituted in 20% Dimethyl sulphoxide (DMSO) to obtain extract concentration of 200 mg/ml. This was serially diluted in 2-folds to obtain the following lower extract concentrations of 100, 50, 25 and 12.5 mg/ml. The activities of the plant extracts were determined using agar well diffusion techniques. Eighteen (18) hours old standardized inoculums of each test bacterial isolate was inoculated on dried surface of Mueller-Hinton agar by streaking with a sterile cotton-tipped swab to achieve a confluent growth. The inoculated plates were allowed to dry after which wells were punched on the agar at equidistant positions using a sterile standard 6 mm cork borer. Subsequently, 100 ml of different concentrations of the extract were separately introduced into different wells that have been labeled accordingly. Equal volume of 20% DMSO was introduced into the well bored in the centre of the plate as a control. This procedure was repeated in duplicate for all the test organisms and allowed to stay for 30 min on the bench after which they were incubated for 24 hours at 37°C. At the end of incubation, observed zones of inhibition were measured and recorded to the nearest millimeter using meter rule.

## RESULTS

**Result of GC-MS Analysis of Ethanol Leaf-Extract of *Pterocarpus santalinoides*:** Eight peaks were identified from the chromatogram of the Ethanol leaf-extract of *Pterocarpus santalioides* (Figure 2). These peaks (1-8) indicate the presence of eight compounds (Table 1). These compounds comprise mainly hydrocarbons, esters and fatty acids. The composition of the extract comprises of octa-1-ene (4.57%), Nona-1, 3-diene, hexadecanoic acid (22.87%), octadeca-9,11-enoic acid (3.66%), octadecanoic acid (14.02%), octadeca-10,12-enoic acid (18.90%), octadeca-11,13-enoic acid (6.40%) and octadeca-9-enoic acid (27.43%) major chemical constituents.

Compound 1 was identified as Octa-1-ene and has molecular formula of $C_8H_{16}$ (m/z 112) with base peak at m/z 41 which was due to loss of propane group ($C_3H_5$) from the parent molecule. The fragmentation peak at m/z =111.20 was due to loss of octane radical while the loss of propyl molecule gave weak peak at m/z=40. It constitutes 4.57% of the extract. Compound 2 constitutes 2.13% of the extract with molecular formula $C_9H_{16}$ (m/z 124) and base peak at m/z 43 which occurred due to the detachment of a fragment $C_3H_5$ fragment (m/z 43) from the compound. It was identified as None-1, 3-diene. Compound 3 has molecular formula $C_{16}H_{32}O_2$ (m/z 256) and base peak at m/z 43 which was due to the loss of propanone group. The compound was identified as hexadecanoic acid constituting 22.87% of the extract. Compound 4 is octadeca-9, 11-enoic acid with molecular formula $C_{18}H_{38}O_2$ (m/z 280) and base peak at m/z 41 which was due to the loss of $C_4H_7$ group. The constituent was 3.66% of

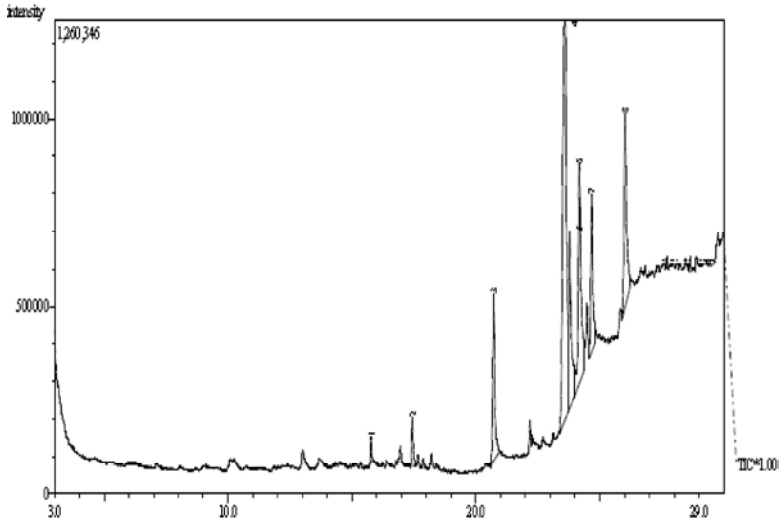


Fig. 2: Chromatogram from GCMS analysis of *P. santalinoides* leaf.

Table 1: GC-MS Analysis and Mass Spectral Data of Ethanol Fraction from the Leaf of *Pterocarpus santalinoides* Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time, Mass Peaks and Base Peaks

| Peaks | Compounds | Molecular formular | Molecular Weight | Retention time | Percentage Content (%) | Mass peaks | Base peaks |
|---|---|---|---|---|---|---|---|
| 1 | Oct-1-ene | $C_8H_{16}$ | 112 | 15.78 | 4.57 | 29 | 41 |
| 2 | none-1,3-diene | $C_9H_{16}$ | 124 | 17.44 | 2.13 | 32 | 43 |
| 3 | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 20.73 | 22.87 | 52 | 43 |
| 4 | Octadeca-9,11-eoic acid | $C_{18}H_{32}O_2$ | 280 | 23.60 | 3.66 | 77 | 41 |
| 5 | Octadecanoic acid (stearic acid) | $C_{18}H_{36}O_2$ | 286 | 23.80 | 14.02 | 67 | 43 |
| 6 | Octadeca-10,12-enoic acid | $C_{18}H_{32}O_2$ | 283 | 24.18 | 18.90 | 68 | 67 |
| 7 | Octadeca-11,13-enoic acid | $C_{18}H_{16}O_2$ | 280 | 24.68 | 6.40 | 66 | 67 |
| 8 | Octadeca-9-enoic acid | $C_{18}H_{16}$ | 281 | 27.43 | 27.43 | 77 | 55 |

Table 2: The Mean MIC Value of Ethanol Leaf-Extract of *Pterocarpus Santalinoides*

| Organism | Stock | 50% | 25% | 12.5% | Control |
|---|---|---|---|---|---|
| *Streptococcus pneumonia* (Strep) | 17 | 10 | No inhibition | No inhibition | No inhibition |
| *Klebsiella pneumonia* (Kleb) | 15 | 12 | 10 | No inhibition | No inhibition |
| *Escherichia coli* (*E. coli*) | 16 | 13 | 10 | No inhibition | No inhibition |
| *Staphylococcus aureus* (Staph) | 18 | 10 | 7 | No inhibition | No inhibition |
| *Pseudomonas aeruginosa* (Ps) | No inhibition | No inhibition | No inhibition | No inhibition | No inhibition |
| *Salmonella typhi* (Sal) | No inhibition | No inhibition | No inhibition | No inhibition | No inhibition |

Key: MIC= minimum inhibitory concentration

the extract. Compound 5 was identified as octadecanoic acid (stearic acid) with molecular formula $C_{18}H_{36}O_2$ (m/z 286) and base peak at m/z 43. The base peak occurred as a result of the detachment of $C_4H_9$ (m/z 57) fragment from the compound. It constitutes 14.02% of the extract. Compound 6 is octadeca-10, 12-enoic acid with molecular formula $C_{18}H_{35}O_2$ (m/z 286) and base peak at m/z 67 which was due to loss of $C_5H_5O$ radical. It constitutes 18.90% of the extract. Compound 7 was identified as octadeca-11, 13-enoic acid and with molecular formula $C_{18}H_{32}O_2$ (m/z 280). It constitutes 6.40% of the extract. Compound 8 was identified as octadeca-9-enoic acid and with molecular formula $C_{18}H_3O_2$ (m/z 281) and it constitutes 27.43% of the extract. The base peak occurred as a result of the detachment of $C_5H_7$ (m/z 67.10) fragments from the compound.

**Antimicrobial Result of Some Tested Micro Organism:** Six bacterial are used to test for the antibiotic efficacy of ethanol extract of *Pterocarpus santalinoides.*

These bacterial are gram negative bacterial such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, klebsiella pneumonia* and gram positive bacterial as *Streptococcus pneumonia* and *Staphylococcus aureus.* It was active against some of the bacterial at varying concentrations. However, the extract was inactive against *Pseudomonas aeruginosa* and *Salmonella typhi* even at higher concentrations but still showed susceptibility to *klebsiella pneumonia and Escherichia coli* at a lower percentage concentration of 25% (Table 2). The extract was more active on *E. coli, S. pneumonia* and *K. Pneumonia* than *S. pneumonia* as revealed by the zones of inhibition (Table 2). This can be explained by the differences in the cell wall permeability of the organisms to antimicrobial agents. The cell wall of gram negative organisms makes them less permeable to antimicrobials because of its high lipid content and that the extract was inactive on the other bacterial species probably because of innate resistance.

## DISCUSSION

The ethanol leaf-extract of *Pterocarpus santalinoides* showed eight peaks from the GC-MS chromatogram. These peaks indicated the presence of eight compounds (1-8) in the extract (Figure 2). The composition of the extract comprises of octa-1-ene (4.57%), nona-1, 3-diene, hexadecanoic acid (22.87%), octadeca-9, 11-enoic acid (3.66%), octadecanoic acid (14.02%), octadeca-10, 12-enoic acid (18.90%), octadeca-11,13-enoic acid (6.40%) and octadeca-9-enoic acid (27.43%). This study also revealed that octadeca-9-enoic acid (27.43%) constitutes the major chemical constituents of the ethanol leaf-extract of *P. santalinoides*. The chemical constituent are long chain aliphatic carboxylic acids, (saturated and unsaturated) and their derivatives including, aldehydes as well as carboxylic acid esters and a steroidal compounds. The result of this study was in correlation with the report of Aja *et al.,* (2014) [7] and Nweke *et al*., (2015) [14] that identified sixteen and ten chemical constituents of GC-MS in *Moringa oleifera* and *Vitex doniana* leaves. The study also correlated to the report of Uraku *et al*., (2015) [16] on the G.C-MS constituents of essential oil from *Hyptis spicigera* leaf.

Oleic acid is an unsaturated fatty acid present in several plants and being unsaturated is considered as a healthy source of fat in the diet. Many uncommon (secondary metabolite) fatty acids are known to have antibacterial and antifungal. A research work done on *Moringa oleifera* by Aja *et al.,* 2014, showed that dodecanoic, tetradecanoic, hexadecanoic, octadecanoic and oleic acids are among the fatty acids known to have potential antibacterial and antifungal properties. Oleic acid has been found to be anti-fungi against a wide spectrum of moulds and yeasts. For example, it was observed to cause a delay of 6-8 hours in the germination of fungal spores and was also found to be effective at low concentrations [7]. It has also been proposed that these fatty acids have potential antibacterial and antifungal principles for clinical application [7].

## CONCLUSION

GC-MS analysis of ethanol leaf-extract of *Pterocarpus santalinoides* showed eight chemical constituents with octadeca-9-enoic acid (27.43%) as the major constituents. This study also showed that ethanol leaf–extract of *Pterocarpus santalinoides* has anti-bacterial activity against *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa and Samonela typhi.*

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