GC-MS Analysis of *Spondias mombin* (Linn) Methanol Leaf Extract

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors CJN and SCO designed the study. Author ASE performed the statistical analysis. Author CIN wrote the protocol and author SCO wrote the first draft of the manuscript. Author POU managed the analyses of the study. Author DMO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The bioactive components of *Spondias mombin* leaf have been evaluated using gas chromatography and mass spectroscopy (GC/MS). The plant were collected washed, shade dried and milled and the methanol extract of the leaf plant were prepared by soxhlet extract method. The methanol extract were analyzed for the identification of phytochemical compounds presenting the *Spondias mombin* plant leaf using GC/MS methods. Result of the GC-MS analysis of *Spondias mombin* leaf extract leads to the identification of 8 compounds. The identification of phytochemicals was based on the Molecular Weight, molecular formula, RT (Retention Time) and peak area % (concentration). These peaks indicated the presence of eight bioactive compounds in the extract. The names, molecular formula, percentage composition and molecular masses of these

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1. INTRODUCTION

Medicinal plant in treatment of diseases is as old as man. Many developing countries of the world look upon medicinal plants as possible substitute to synthetic drug because natural sources of herbal medicines are more protective, safe, biodegradable, fewer side effects than artificial and synthetic remedies because the photochemical in the plants extract target the biochemical pathways [1]. The entire dependence of man on plants and plant products directly for his basic needs make plants vital to his survival and the basis of his continued existence.

Phytochemicals are natural bioactive compounds found in plant materials that work with dietary nutrients to protect our body against disease. They are also non-nutritive plant chemicals, working together with nutrients found in leaves, fruits, vegetables and nuts, may help slow the aging process and reduce the risk of many diseases, including diabetes, anemia, ulcer, cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, urinary tract infections etc [2]. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures [2].

When gas chromatography is coupled to mass spectrometry (GC-MS) additional information takes place about molecular mass, elemental composition, functional groups, and in certain cases, molecular geometry and spatial isomerism of each separated complex [3]. Gas chromatography and Mass Spectroscopy (GC-MS) is broadly used for compound analysis, drug screening and assessment resulting from environmental pollution. Mass spectrometry (MS) measures the mass-to-charge ratio of ions of analysts and is a plot of strength as a function of mass-to-charge ratio [4]. Relying on the database of National Institute of Standards and Technology Chemistry Web Book [5] for diverse compounds, researchers can thus recognize and quantify these potential compounds and components.

**Spondias mombin** L. (Family-Anacardiaceae) also recognized as Hog plum is a small deciduous tree up to 20 m tall, it grows in forest and the coastal part of Africa. It is recognized locally as “iyawe” and “iyeye” by the Hausa and Yoruba people of Nigeria respectively [6]. The leaves are pinnate, with 5-8 leaflets opposite pairs with a terminal leaflet, 10 cm × 5 cm (4 in × 2 in). The flowers bloom January to May and are sweet-scented, in large, lax terminal panicles of small white flowers. Fruits appear July to September and are nearly 4 cm (1.5 in) long, ovoid yellow, acid, wrinkled when dry. The fruits have a sharp, somewhat acid taste and are edible. Their flesh surrounds a single spiny kernel.

In traditional medicine, *Spondias mombin* has had a variety of uses. The fruit has been used as a diuretic and febrifuge [7]. The bark is astringent and used as an emetic and for diarrhea, diabetes [8]. Dysentery, hemorrhoids, gonorrhea, and leucorrhoea [7]. The flowers and leaves are used to make a tea for stomach ache, biliousness, urethritis, cystitis, and inflammation [7]. A tea of the leaves and flowers of *S. mombin* is taken to relieve stomach ache, biliousness, cystitis, urethritis, throat and eye inflammations. Herbalists in Southwest Nigeria use the plant in the treatment of diabetes, psychiatric disorders, typhoid, nervous disorders and tuberculosis [9]. The extract of the powder of the dried leaves and fresh crushed leaves are used for healing inflammation, wounds, varicose ulcers, burn and frost-bite in herbal medicine [10]. The is this study to determine the organic compound present in the leaf extracts of *Spondias mombin* with the aid of GC-MS Techniques which may provide an insight in its use in traditional medicine.

| Compounds | Molecular Formula | Description |
|-----------|------------------|-------------|
| Hexamethyl-3-termethylsioxysiloxane | (C_6H_{13}O_2Si) | |
2. METHODS

2.1 Plant Materials and Authentication

The leaves of *Spondias mombin* were harvested from a compound bush in Abia State University, Uturu, Abia State, Nigeria. The plant was authenticated at the department of Plant Science and Biotechnology, Abia State University Uturu by a taxonomist and voucher samples deposited in the Departmental herbarium. (Voucher number: ABSU/PSB/00086)

2.2 Preparation of Plant Extract for GC-MS Analysis

The dried extract of leaves of *Spondias mombin* was dissolved in absolute methanol (1 mg/ml), (10ml) of this sample was then injected for GC-MS analysis.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The samples for GC-MS was prepared by dissolving 3g of extracted powder in methanol solvent. For the analysis, GC-MS-QP 2010 SHIMADZU instrument was used. To analyze the sample the column oven temperature and Injector temperature was set at 800°C and 200°C respectively. The flow control mode was maintained in linear velocity with a split injection mode split ratio of 20. The column flow was 1.46 ml/min with a helium carrier gas of 99.9995% purity. The column oven temperature program was set as follows:-

The temperature was set at 80°C with 2 minutes hold time by the rate of 10. The temperature was 300°C with 10 minutes hold time. The column at 5 minutes was used with a length of 30 millimeters and diameter of 0.25mm and its film thickness will be 0.25 μm. The ion source temperature for MS condition was 200°C and interface temperature was 240°C. Starting m/z (Mass to charge) ratio was 40 and ending with m/z ratio of 700 (40-700 m/z).

Identification of phytochemicals and interpretation of mass spectrum GC-MS was conducted using the National Institute of Standards and Technology (NIST) 14 Mass Spectral Library, Washington, DC, USA. The name, molecular weight, retention time and structure of the components of the test materials were ascertained and reported.

2.4 Identification of the Phytochemical Constituents

Identification and interpretation of mass spectrum of GC-MS was conducted using the National Institute of Standard and Technology (NIST) database. The unknown components were
compared with spectrum of known components stored in the NIST library. The name, molecular weight and structures of the components of the test materials were ascertained.

### 3. RESULTS

The result GC-MS analysis of *Spondias mombin* leaf extract leads to the identification of 8 compounds from the GC fractions. The identification of photochemical was based on the MW, molecular formula, RT and peak area % (concentration). These peaks indicated the presence of eight 8 bioactive compounds in the extract. The names, molecular formular, percentage composition and molecular masses of these compounds are shown in Table 1. The 8 bioactive compounds are as follows; 9-oxononanoic acid,3-isopropoxy ,1,1,15,5,5 hexamethyl-3-trimethylsioxytrisiloxane (C_{12}H_{34}O_{4}Si_{4} ),1,4-methanocycloocta(d) pyridazine, 1α4α4α10α(C_{13}H_{23}ON_{2}), Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecadiethyl,1,4 methanocycloocta (d) pyridazine 1,4,4a,5,6,9,10,10a- octaldehyd 1,11-dimethyl-(1α,4α4α10α), Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 dodecamethyl, 1,4 methanocycloocta (d) pyridazine 1,4,4a,5,6,9,10,10a- octaldehyd 1,11-dimethyl-(1α,4α4α10α), Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 dodecamethyl, 3-isopropoxy-1,1,1,5,5,5 hexamethyl-3-(trimethylsiloxy) trisiloxane C_{12}H_{34}O_{4}Si_{4} and 1,1,1,3,5,5,5 Heptamethyltrisiloxane (C_{7}H_{22}O_{2}Si_{3}).

| S/No | RT     | Compound name                                      | Formular                  | MW     | Area (%) | Molecular structures             |
|------|--------|----------------------------------------------------|---------------------------|--------|----------|----------------------------------|
| 1    | 15.006 | 9- oxononanoic acid                                | C_{9}H_{16}O_{3}          | 172    | 4.825    | ![Image](image1.png)             |
| 2    | 17.577 | Cis,cis and cis, trans-1,9 dimethylspiro[4.5]decane| C_{12}H_{22}            | 166    | 4.747    | ![Image](image2.png)             |
| 3    | 23.211 | Pyrimidine, 4,5-dimethyl                            | C_{6}H_{8}N_{2}          | 108    | 4.940    | ![Image](image3.png)             |
| 4    | 25.918 | 3-Isopropoxy-1,1,15,5,5-hexamethyl-3- (trimethylsiloxy) trisiloxane | C_{12}H_{34}O_{4}Si_{4} | 354    | 7.675    | ![Image](image4.png)             |
| 5    | 26.045 | 1,4-methanocycloocta(d) pyridazine,1,4, 4a,5,6,9,10,10a-octaldehyd 1,11-dimethyl-(1α,4α4α10α) | C_{13}H_{23}ON_{2}      | 204    | 41.551   | ![Image](image5.png)             |
| 6    | 26.326 | 1,1,1,3,5,5,5- Heptamethyltrisiloxane               | C_{7}H_{22}O_{2}Si_{3}   | 222    | 7.011    | ![Image](image6.png)             |
| 7    | 29.550 | Silicic acid,diethylbis(trimethylsilyl) ester       | C_{10}H_{28}O_{4}Si_{3}  | 296    | 4.679    | ![Image](image7.png)             |
| 8    | 30.397 | Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl | C_{13}H_{38}O_{5}Si_{6}  | 430    | 24.572   | ![Image](image8.png)             |

**Key:** RT: Retention Rate; MW: Molecular Weight
Table 2. Biological activity of components in the methanol leaf extract of *Spondias mombin*

| S/N | Compound name | Pharmacological properties | Reference |
|-----|---------------|----------------------------|-----------|
| 1   | 9-oxononanoic acid | Antibacterial, platelet aggregation inducer, atherothrobosis | [11] |
| 2   | Cis,cis and cis, trans-1,9 dimethylspiro[4.5]decane | Antiplasmodial activity exhibited by this compound | [11] |
| 3   | Pyrimidine, 4,5-dimethyl | Analgesic and anti-inflammatory activities, methylation, anticancinogenic nervous system disorder, inducer of ovulation | [12,13] |
| 4   | 3-Isopropoxy-1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane | Anticancer, anti-inflammation properties | [13] |
| 5   | 1,4-methacnycloocta[d]pyridazine,1,4,4a,5,6,9,10,10a-octahydro-11.11-dimethyl-(1α4α4aα10α) 1,1,3,5,5,5-Heptamethyltrisiloxane | Antibacterial activity | [14] |
| 6   | Silicic acid,diethylbis(trimethylsilyl) ester | Antibacterial activity | [14] |
| 7   | Hexasiloxane, 1,1,3,5,5,7,7,9,9,11,11-dodecamethyl | Anti-microbial, antibacterial, anti-septic, hair conditioning agent, emollient | [15,16,17] |

Fig. 2. Chromatogram of GC-MS Analysis *Spondias mombin* leaf showing the retention time % area and various peaks of the bioactive compounds
4. DISCUSSION

The GC-MS analysis of the leaf extracts revealed different bioactive compounds which accounts for the various medicinal properties of the Spondias mombin plants leaf. The Spondias mombin leaf aqueous extract yielded eight 8 compounds which include; 9- oxononanoic acid, which has been shown to possess platelet aggregation inducer which is associated with atherothromosis [13], 3-isopropoxy, 1,1,5,5,5 hexamethyl-3-trimethylsioxylsiloxane (C_12H_24O_4Si_4), this has been shown to have anti-cancer and anti-inflammation properties [13]. Therefore, the data that the identified phytochemicals are medicinally very important and they are helpful for the cure of many infections.

5. CONCLUSION

The results obtained in this study thus suggest that the identified phytochemical compounds are bioactive constituents. Therefore, the data generated from these experiments provide the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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