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Original Research Article

Constituent of *Spondia mombin* Stem Bark: Phytochemical Screening and GC-MS Profiling of Ethyl Acetate Fraction

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Abstract

Purpose: *Spondia mombin* is a widely cultivated edible plant traditionally used in Southern Nigeria to treat and manage various health disorders. This study aimed to examine the phytochemical contents of the ethyl acetate fraction of *S. mombin* stem bark through chemical screening and GC-MS profiling.
Method: The stem bark of *Spondia mombin* was collected, washed, dried, and ground into powder. The powdered material was extracted with ethyl acetate using a Soxhlet apparatus. Ethyl acetate was selected for its effectiveness in extracting medium-polar phytochemicals, such as flavonoids, terpenoids, and phenolic compounds. The extract was analysed using gas chromatography-mass spectrometry (GC-MS) on a Perkin Elmer Clarus 500 system equipped with an AOC-20i autosampler, interfaced with a mass spectrometer. GC-MS was employed because it enables the separation, detection, and structural elucidation of volatile and semi-volatile phytochemicals, thereby providing a comprehensive profile of the extract's constituents.
Result: Qualitative phytochemical analysis showed the presence of alkaloids, tannins, and flavonoids, while 62 peaks corresponding to 62 bioactive compounds were identified via GC-MS. These include dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, oleic acid, octadecanoic acid, vitamin E, phytol, 7-hexadecene (Z), naphthalene-1-methyl, naphthalene-2-methyl, 1,1-naphthalene, benzene-1,2,4,5-tetramethyl, benzene-1-ethyl-2,3-dimethyl, benzene-1-ethyl-2-methyl, and p-xylene.
Conclusion: Qualitative phytochemical screening of the ethyl acetate fraction of the stem bark of *S. mombin* confirms the presence of alkaloids, cyanogenic glycosides, tannins, phenols, steroids, and flavonoids, amounting to 62 bioactive compounds. These compounds are renowned for their diverse medicinal properties and may, however, explain the ethnomedicinal uses of different plant parts.

Keywords: *Spondia mombin*, Phytochemical compound, Pharmacology, GC-MS analysis.

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INTRODUCTION

It is estimated that 80% of the people living in developing countries, including Nigeria, rely heavily on herbal medicine as an integral part of primary health care and alternative medicine [1, 2]. In Nigeria, many Indigenous plants are used as spices, food, or medicine [3]. These plants possess these features due to a wide range of phytochemicals, including alkaloids, tannins, saponins, flavonoids, and phytate, among others [4, 5]. Throughout the twenty-first century, health science focused primarily on curing diseases with powerful drugs, more sophisticated diagnostic tests, and more effective therapies [6]. Today, the emphasis is on health expectancy rather than life expectancy. Synthetic drugs may have been reputed for serious side effects on health [7, 8], thereby necessitating a paradigm shift to the use of herbal medicine as an alternative [9] owing to their perceived level of safety and minimal toxicity [10]. One such commonly used plant, noted for its vast ethnopharmacological potential, is *Spondia mombin* [11]. *S. mombin* belongs to the family Anacardiaceae; it is a fructiferous tree that thrives in the rainforest and coastal areas of Africa. It also has a wide distribution in Southern America and the West, but grows to a limited extent in the Indian subcontinent and Indonesia [12]. Selected pharmacological properties of *S. mombin* include anti-fertility, antioxidant, anti-malarial, antimicrobial, antidiarrheal, abortifacient, muscle relaxant, sedative, antipsychotic, and anti-inflammatory [13, 14]. Likewise, [15] reported that administering the aqueous leaf extract of *S. mombin* significantly improved the learning and cognitive capacities of Wistar rats. In addition, traditional homoeopaths in Nigeria have reportedly used preparations of the plant to enhance memory and cure some patients with mental illnesses [16, 17]. The plant's cytotoxic feature has also been reported [18]. This study aims to explore the phytochemical constituents of *S. mombin* using GC-MS analysis, to promote the therapeutic use of its bioactive compounds, and to provide scientific support for its ethnomedicinal applications.

This study aims to explore the phytochemical constituents of *S. mombin* using GC-MS analysis, to promote the therapeutic use of its bioactive compounds, and to provide scientific support for its ethnomedicinal applications.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

The stem bark of *S. mombin* was collected on 1st June 2018 at Otuoke town (4°49'N 6°20'E), Ogbia Local Government Area, Bayelsa State, Nigeria. It was authenticated at the Niger Delta University Herbarium on Wilberforce Island in Bayelsa State. A voucher number, NDU P008, was assigned to ensure accurate recording and future reference. The stem bark of *S. mombin* was thoroughly cleaned with distilled water to remove dirt and impurities. It was air-dried until reaching a consistent weight, then ground into a fine powder using an electric blender (Saisho, Model S-742). Cold maceration of 247.20 g of coarse powder with 770 mL of 100% ethyl acetate was utilised for extraction, with periodic shaking on an orbital shaker for 48 hours.

Figure 1: Stem bark of *Spondia mombin* under study

Qualitative Phytochemical Analysis of the Ethylacetate Fractions of *S. mombin*

Qualitative phytochemical analysis of the ethyl acetate fractions of *S. mombin* was conducted to assess the presence of alkaloids, flavonoids, phenols, sterols, saponins, tannins, and cardiac glycosides using the method described by [6]. Saponin content was evaluated using the Frothing method, alkaloid by the Dragendorff and Meyer test, flavonoid by Pew's test, and phenols by the Folin-Ciocalteu. At the same time, cardiac glycosides were determined by the Killer-Killarnis method.

GC-MS Analysis of *S mombin* Stem Bark Extract

GC-MS analysis was carried out on a Gas Chromatography system (Clarus 500, PerkinElmer Inc., Waltham, MA, USA), comprising of a AOC-20i auto-sampler interfaced to a mass spectrometer instrument employing the following conditions:

column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 μM df, composed of 100 % dimethyl polydiloxane), operating in electron impact mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 0.5 μL was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature of 280 °C. The oven temperature was programmed to start at 110 °C (isothermal for 2 min), then increased by 10 °C/min to 200 °C, followed by a 5 °C/min increase to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV, with a scan interval of 0.5 s, and Fragments from 40 to 450 Da. The total GC runtime was 28 min. Identification of Components: The mass spectrum GC-MS was interpreted using the database of the National Research Institute of Technology (NARICT), Zaria, Nigeria, which contains more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NARICT Ver. 2.1 MS data library. The names, molecular weights, retention times, and structures of the components of the test materials were determined**.**

Qualitative Phytochemical Analysis of the Ethylacetate Fractions of *S. mombin*

The result of the phytochemical analysis of the ethylacetate fractions of *S. mombin* showed the presence of various phytochemicals, as presented in Table 1.

Table 1: Qualitative Phytochemical Analysis of the Ethyl Acetate Fractions of *S. mombin* Stem bark

Phytochemical	Presence
Alkaloids	+ve
Cyanogenic glycosides	+ve
Flavonoids	+ve
Phenol	+ve
Saponin	-ve
Steroid	+ve
Tannin	+ve

Key: +ve = present, -ve = absent

GC-MS Analysis of *S. mombin*

A GC-MS chromatogram of the ethyl acetate extract of the stem bark of *S. mombin* is presented in Figure 1, and it clearly shows 62 peaks indicating the presence of 62 bioactive compounds.

RESULT AND DISCUSSION

Knowledge of the phytochemical properties of plants is advantageous not only for the discovery of therapeutic agents but also for identifying new sources of economic raw materials for the synthesis of complex chemical substances [19, 20]. Qualitative analysis of *S. mombin* identified tannins, alkaloids, and flavonoids. Each of these phytochemicals has been documented to play a crucial role in the antimicrobial, anti-inflammatory, and antioxidant properties of *S. mombin* extract, whether in biological organisms or in humans upon ingestion. Tannins have been widely used topically to treat tender bruises and superficial wounds. It can be inferred that tannins and other phenolic compounds from *S. mombin* are responsible for the observed broad-spectrum antimicrobial activities. Tannins are a group of polymeric phenolic substances found in nearly all parts of the plant: stem bark, wood, leaves, fruits, and roots. They are reported to inactivate microbial adhesins, enzymes, and cell envelope transport proteins due to their astringent properties. Tannins have been shown to exert physiological effects, including anti-irritant, anti-secretolytic, anti-inflammatory, antimicrobial, and anti-parasitic actions [21, 22, 23]. Alkaloids, among the earliest bioactive compounds, are heterocyclic nitrogenous substances derived from the stem bark of *S. mombin*. Their mechanism of action primarily involves their ability to interact with DNA and inhibit enzymes such as esterases, DNA polymerase, and RNA polymerase, thereby disrupting cellular functions and potentially leading to cell toxicity. The metabolites, including alkaloids, steroids, glycosides, saponins, and tannins, identified in the root extract of *S. mombin* are known to possess activity against various pathogens [10]; consequently, the plant's stem bark is traditionally employed in the treatment of diverse illnesses [21, 24]. Flavonoids are known to be synthesized by humans in response to microbial infection. Therefore, it is unsurprising that in vitro studies have demonstrated their efficacy as antimicrobial agents against a broad spectrum of microorganisms [14, 25]. Flavonoids, antioxidant phytochemicals present in *S. mombin* extract, have been linked to antimicrobial and anti-inflammatory effects in numerous studies [26, 27, 28]. It is reported that flavonoids play a significant role in managing oxidative stress. Their antioxidative capabilities are attributed to several mechanisms, including free radical scavenging, metal ion chelation (e.g., iron and copper), and inhibition of enzymes involved in radical generation [29]. In this study, flavonoids were detected in appreciable quantities

within the stem bark. *S. mombin* extract could thus function as an effective oxidizing agent to eliminate free radicals in the human body. As phenolic compounds, flavonoids' activity is likely due to their capacity to form complexes with extracellular and soluble proteins, bacterial cell walls, and potentially disrupt membranes, especially in highly lipophilic flavonoids [25, 30]. In humans, flavonoids are recognized for their potential to reduce the risk of cardiovascular disease, underscoring their significance as valuable phytochemicals in *S. mombin* extracts. These compounds are potent water-soluble antioxidants and free radical scavengers that prevent oxidative cellular damage, exhibit substantial anticancer activity, and inhibit multiple stages of carcinogenesis [29, 31]. The GC-MS analysis of the stem bark of *S. mombin* revealed bioactive compounds, as shown in Table 2. The gas chromatogram displayed the relative concentrations of various compounds as they eluted over time, with peak heights indicating the relative abundance of each component. The mass spectrometer examined the compounds eluted at different times to determine their nature and structure. Identification of the bioactive compounds was performed by comparing their mass spectra and retention times with those of reference standards and published data. The compounds identified through GC-MS include acids, hydrocarbon esters, and alcohols. The hydrocarbons isolated include benzene, 1-ethyl-2-methyl; benzene, 1,2,3-trimethyl; benzene, 1-ethyl-2,3-dihydro-5,6-dimethyl; and naphthalene. Acids identified in the stem bark of *S. mombin* comprise dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, oleic acid, and octadecanoic acid. Also, alcohols such as phytols were detected. The compounds identified in the stem bark of *S. mombin* possess numerous biological properties and have been reported to exhibit anti-inflammatory, anti-cancer, anti-ageing, antimicrobial, and anti-infertility activities. Most of Nigeria's population lives in rural areas with limited access to modern family planning methods, often relying on herbal remedies for birth control, such as inducing abortion, preventing conception, and sterilizing the couple. Administration of *S. mombin* caused estrogen suppression, likely due to its antiestrogenic properties. [32] also suggested that the reduction in the weight of the ovary and uterus indicates the plant's antiestrogenic nature, as antiestrogenic substances are known to decrease the uterus's wet weight. Progesterone and oestrogen are recognized as the most vital hormones for maintaining pregnancy and facilitating blastocyst implantation in humans and other mammals [33]. The extract of *S. mombin* has also been shown to significantly reduce these hormonal levels in a dose-dependent manner [34, 35, 36]. Oleic acid inhibits oncogenic action in breast cancer [37]. It has been shown to lower blood pressure and reduce the development of cardiovascular disease (CVD) [6], [35, 38]. Oleic acid also synergistically enhances the effectiveness of cancer drugs [39]. Linoelaidic acid, an isomer of linoleic acid, has been extensively studied for its ability to modulate cancer, atherosclerosis, obesity, immune function, and diabetes [40]. Additionally, linoleic and linolenic acids are reported to possess anti-inflammatory, insecticidal, hypocholesterolaemic, cancer-preventive, hepatoprotective, and antihistaminic properties [41, 42, 43]. Tetradecanoic acid, one of the bioactive compounds, opposes oxidation or inhibits reactions caused by dioxygen or peroxide [27]. Similarly, the GC-MS analysis identified compounds such as n-hexadecanoic acid, which exhibits antioxidant properties and cyclooxygenase activity [44, 45, 46]. The essential fatty acid 9,12,15-octadecatrienoic acid (alpha-linolenic acid) is reported to have neuroprotective effects against soma-induced neuropathology [47]. Vitamin E functions primarily as an antioxidant, protecting cellular membranes from oxidative damage and red blood cells from haemolysis. It is also believed to enhance vitamin A utilisation and, at high doses, may inhibit platelet aggregation [48]. Through its scavenging activity, vitamin E defends cell membranes and polyunsaturated lipids against reactive oxygen species (ROS) by activating various signal transduction pathways [49] and is thus mostly recognised for its antioxidant role. Indeed, studies have focused mainly on its antioxidant properties, although the vitamin's role is broader and more varied [50]. Phytol (3,7,15-tetramethylhexadec-2-en-1-ol) is a diterpene and belongs to the group of branched-chain unsaturated alcohols [51, 39]. It is a product of chlorophyll metabolism in plants; therefore, phytol is abundantly available in nature. It is considered a reasonably effective antimicrobial agent. Additionally, it exhibits low toxicity and high stability. The antimicrobial efficacy of phytol is comparable to that of other traditional disinfectants [52]. According to report [53], phytol isolated from the mangrove plant *Avicennia marina* has antibacterial activity against *Staphylococcus aureus*, with a zone of inhibition of 12mm. It can also block the teratogenic effects of retinol [54]. Phytol is known to reduce the production of free radicals, a property attributed to its structural features.

Table 2: GC–MS Analysis Results of *Spondia mombin* Stem bark

S/N	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Retention Time (RT)	Area %
1	Xylene	C_6H_{10}	106.00	5.27	0.33
2	Benzene, 1-ethyl-2-methyl	C_6H_{12}	120.00	6.495	18.00
3	Benzene-1,2,3-trimethyl	C_9H_{12}	120.00	6.58	15.40
4	Benzene, 1,2,4-trimethyl	C_6H_{12}	120.00	7.204	15.20
5	Benzene, 1-ethyl-2,3-dimethyl	C_6H_{14}	134.00	7.759	16.50
6	Benzene, 1,2,4,5-tetramethyl	$C_{10}H_{14}$	134.00	8.149	10.10
7	Naphthalene	$C_{10}H_8$	128.00	8.824	25.40
8	Indene-2,3-dihydro-4,7-dimethyl	$C_{11}H_{14}$	146.00	8.91	14.00
9	Indene-2,3-dihydro-5,6-dimethyl	$C_{11}H_{14}$	146.00	9.361	8.01
10	Naphthalene-2-methyl	$C_{11}H_{10}$	142.00	9.90	24.00
11	Naphthalene-1-methyl	$C_{11}H_{10}$	142.00	9.63	21.20
12	7-Hexadecene (Z)	$C_{16}H_{32}$	224.00	12.20	4.81
13	Tetradecanoic acid	$C_{14}H_{28}$	228.00	14.02	62.05
14	n-Hexadecanoic acid	$C_{16}H_{32}$	256.00	16.22	8.51
15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}$	270.00	15.79	67.40
16	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}$	284.00	16.21	59.60
17	Octadecanoic acid, ethyl ester	$C_{20}H_{40}$	312.00	18.49	8.85
18	Phytol	$C_{20}H_{40}$	296.00	17.75	44.90
19	Methyl Stearate	$C_{19}H_{38}$	298.00	17.85	27.00
20	9,12-Octadecadienoic (Z,Z)	$C_{18}H_{32}$	280.00	18.22	33.10
21	Linoelaidic acid	$C_{18}H_{32}$	280.00	17.99	10.10
22	Octadecanoic acid, 17-methyl-methyl ester	$C_{20}H_{40}$	312.00	18.49	9.20
23	Phenol, 3-pentadecyl	$C_{21}H_{36}$	304.00	21.31	68.00
24	Vitamin E	$C_{29}H_{50}O_2$	430.00	27.54	2.35
25	Oleic acid	$C_{18}H_{34}O_2$	282.00	18.01	5.96

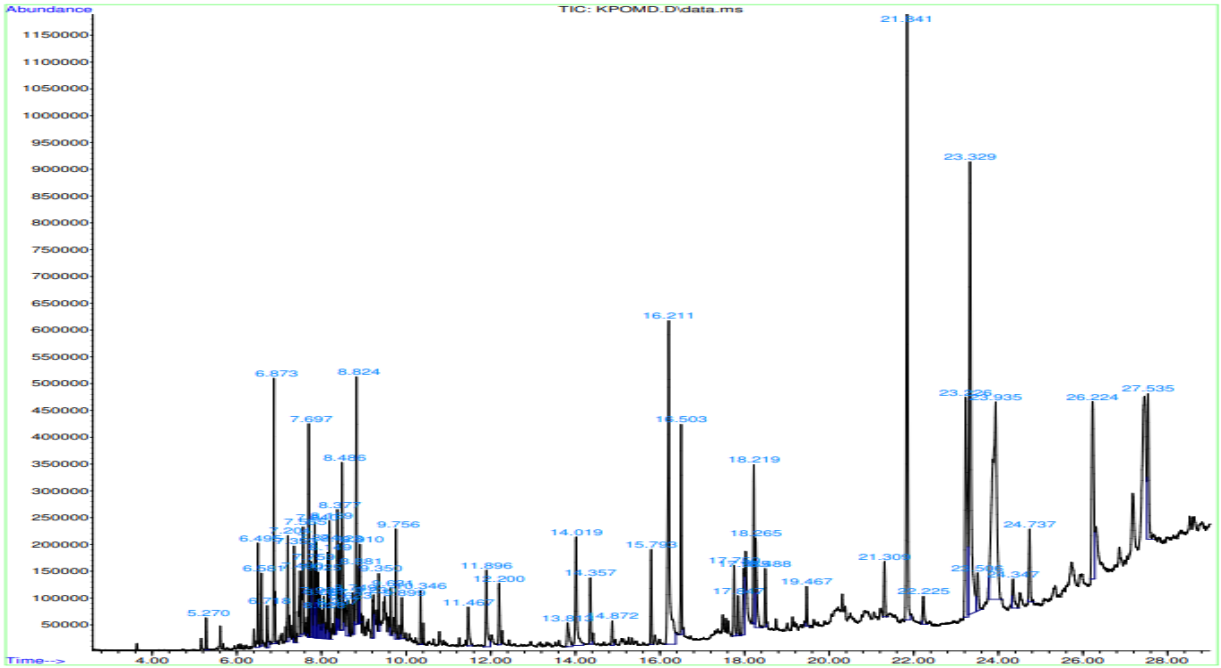

Figure 2: Chromatogram of the bioactive compounds in the ethyl acetate fraction of the stem bark of *S. mombin*

As a branched-chain unsaturated alcohol, its antioxidant activity may be related to the hydroxyl group (OH) in its molecule. Phytol likely reacts with free radicals by donating hydrogen atoms (H) with unpaired electrons, thereby converting them into less reactive species [55]. Phytol significantly reduced the duration and severity of pain. It has a sedative effect and can sometimes produce effects comparable to morphine. Some analgesic effects may also stem from anti-inflammatory activity, as pain is one of the four signs of inflammation [56]. One study found that phytol irreversibly inhibits the enzyme succinic semialdehyde dehydrogenase (SSADH), preventing its breakdown of the neurotransmitter GABA and thereby increasing its levels. Neurotransmitters are chemicals produced by the nervous system to facilitate communication between brain and nerve cells; they can be excitatory, stimulating nervous system activity, inhibitory, reducing it, or both. Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter, and certain drugs such as Valium exert their effects by enhancing GABA activity [56]

CONCLUSION

Qualitative phytochemical screening of the ethyl acetate fraction of the stem bark of *S. mombin* confirms the presence of alkaloids, cyanogenic glycosides, tannins, phenols, steroids, and flavonoids. Simultaneously, 62 peaks were identified in the GC-MS result, corresponding to 62 bioactive compounds. The presence of these bioactive components supports the use of this plant for numerous health challenges by practitioners of complementary and alternative medicine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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