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CHEMICAL AND MEDICINAL PROPERTIES OF SPONDIAS *MOMBIM* (LINN) LEAF HARVESTED FROM THE SOUTH EASTERN NIGERIA

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ABSTRACT: *Spondia Mombin Linn is a very important medicinal plant used in the sourth eastern Nigeria for the treatment of various ailment , Initial phytochemical analysis of the leaf revealed the presence of alkaloids 2.2%, flavonoids 1.34%, saponins 3.73%, tannins 2.4%, cardiac glycosides and steroids .The antimicrobial analysis against certain selected human pathogens revealed activities against Staphylococus aureus by a diameter of 35mm, Streptococcus specie 30mm ,Aspergillis niger 18mm and Mucor specie 19mm but could not inhibit the growth of Klebsiella specie and Pseudomonas aeruginosa .GC-MS of the chloroform extract was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101coated with polymethyl silicon (0.25 mm x 50 m) and Gas chromatography Mass spectrum GC-MS QP 2010 Plus Shimazu Japan, showed that the leaf extract contained 26 compounds: Stigmast-4-en-3-one C29H48O, lupeol C30H50O, tetracontane C29H50O , Heptacosane C44H90, Vitamin E C29H56 , γ-TocopherolC29H50O2, 2H-1-benzopyran-6-ol C28H48O2 ,heptacosane C27H46O, Squalene C27H56 ,methyl [2] 5,11,14,17 eicosatetraenoate C30H501,2-Benzene dicarboxylic acid bisoctylester C24H38O4, Phenol-3 pentadecyl C21H36O,Octadecanoic acid C18H36O2,. 9,12-Octadecadienoylchloride[z,z] C18H31ClO. Phytol C20H40O, n-Hexadecanoic acid C16H32O2,3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol C20H40O. 3,7,11,15-tetramethyl-2-hexadecen-1-ol C20H40O,.5-Octadecene[E] C18H36, Tetradecanoic acid C14H28O2 , Decane -5,6-bis(2,2-dimethyl propylidene)(E,Z) C20H38,1-Hexadecene C16H32 .Cholestan-3-d-2-methylene(3β,5a)C28H48O, Benzocycloheptatriene C11H10, Benzene- 2-ethenyl-1,3,5-trimethyl C11H14 and Napthalene C10H8 . Most of these compounds have marked anticancer, anti-tumour and anti-inflammatory properties making the plant leaf a potent medicine for the treatment of cancer, tumour, inflammation and cardiovascular disease.*

KEYWORDS; spondias mombin, phytochemicals, antimicrobials, anti-inflammation, antitumour, anticancer.

INTRODUCTION

Spondias mombin Linn (Anacardiaceae) also known as Hog plum is of the genus Spondias which is a flowering plant belonging to the cashew family (Anacardiaceae). It comprises of 18 species.

The leaf is said to have anomocytic and tetracytic stomata distributed on abaxial surface.The midrib has convex symmetry with collateral vascular bundles in a close arc disposition ,the leaf has been reported to contain mono and sesquiterpenes, triterpenes and steroids, hydrolysable tannins, phenylpro¬panoid glycosides, cinnamic derivatives and flavonoids (1) and has been reported to have many uses in traditional African Medicinal practices it is believed to be involved in the treatment of many ailment such as ;inflammatory conditions, wounds, and sour throat, and tooth decay .It has been reported to posseses cytotoxic, antioxidant, ulcer protective, anti-dementia, hepato-protective, anti-arthritic properties (2) . The leaves have been reportedly used for child birth aid, cough, sore throat, malaria and stomachic. The fruit has been used to cure dizziness and fibroid. The seed is important in the treatment of fibroid. (3) Spondias mombin is useful in induction of labour, expulsion of afterbirth, and stabilization of women after childbirth.(4) All parts of the plant have medicinally application in traditional healthcare. The fruit extracts serves as a diuretic and febrifuge, the extracts of the bark and the leaves as emetic, anti-diarrhoea and are useful in the treatment of dysentery, haemorrhoids, gonorrhoea and leukorrhea. The plant extracts have documented antimicrobial, antibacterial, antifungal, and the antiviral properties. it has been reported that phenolic acid, 6-alkenyl-salicylic acid from Spondias mombin is responsible for the antibacterial and molluscicidal properties (5). The leaf of the plant has been reported to contain ascorbic acid , Niacin ,Riboflavin and Thiamine while its mineral composition includes Potassium, Sodium, Magnesium, Calcium and Phosphorus Mg .(6) The ethanolic extract has been reported to have activity against Staphylococcus aureus(7) .It has been reported that the supplementation of the food of wistar rats with leaf extracts of Spondias mombin alleviate inflammatory responses which could possibly be via suppression of the production of pro-inflammatory mediators and cytokines (8) .The fruit contains reasonable amount of Potassium and Copper and some amount of carotenoids such as , β cryptoxanthin, lutein, zeinoxanthin, α and β carotene, being β-cryptoxanthin (9) .The decoction of the leaf is used in the treatment of anemia, diarrhea, dysentery, and skin infections and also as an abortifacient . it is also used to treat diabetes mellitus The gum exudates of the plant can serve as an expectorant and also to expel tapeworms A decoction from the root bark can be used to regulate menstruation and for the treatment gonorrhea (3). Though a lot of work has been done on this plant, the constituents has not been fully characterized. This work is aimed at determining the constituents of the leaf and their possible medicinal applications.

MATERIALS AND METHOD

Sample collection

Fresh leaves of *Spondias mombin* were collected from the Department of Forestry and wildlife farm, Federal University of Technology Owerri in Imo State in the Eastern part of Nigeria. The plant was identified by Mr Iwueze Francis a taxonomist of the same department. The plant leaves was room dried , milled into fine particles with the aid of an electric blender and then stored in an air tight container.

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Test for Flavonoids

5g of the sample was soaked with 50cm^3 of water for 2hours and then filtered and to the filtrate was added drops of ammonia and 3cm^3 of concentrated H_2SO_4 was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids.(10)

Test for Alkaloids

5g of the sample was extracted using 20% acetic acid in ethanol .5cm^3 of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloids. (10)

Test for Tannins

5g of the root sample was weighed into a beaker and 50cm^3 of water was added and allowed to soak properly for two hours and extracted. Te extract was treated with drops of ferric chloride. A blue-black precipitate indicates the presence of tannins.(10)

Test for Steroids

5cm^3 of the water extract was treated with concentrated H_2SO_4 in acetic anhydride. The formation of a blue-green color indicates the presence of steroids. (10)

Test for Glycosides

20cm^3 of the water extract was treated with Fehling solutions of A and B in equal amount and boiled. A brownish red precipitate indicates the presence of glycoside. (10)

Alkaloid determination

10 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 20 % acetic acid in ethanol was added and covered to stand for 6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitation was collected by filtration using whatman filter paper, the precipitate was dried and weighed.(11,12)

Saponin Determination

20 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 20 % ethanol was added and stirred using glass rod. The mixture was heated over water bath for 4hrs with continuous

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stirring while the temperature was maintained at 55 °C. The mixture was extracted and the residue was extracted with 200 cm^3 of 20 % ethanol. The combined extract was reduced to 40 cm^3 over water bath at 90 °C. The concentrated extract was transferred into a 250 cm^3 separation funnel and 20cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice. 60 cm^3 of n-butanol was added. The mixture was washed twice with a10 cm^3 of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight. The saponin content was calculated in percentages (11,12)

Flavonoid Determination

10 g of the plant sample were extracted repeatedly with 100 cm^3 of 80% of aqueous methanol at room temperature. The solution obtained was filtered with whatman filter paper no 45. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath and weighed (13,12)

Tannin Determination

0.5 g of the sample was weighed into 250 cm^3 beaker and 50 cm^3 of distilled water was added and stirred vigorously with a glass rod for one hour the solution was filtered into a 50 cm^3 volumetric flask and made up to the mark. 5 cm^3 of the filtrate was pipetted into a test tube and mixed with 3 cm^3 of O.I M $FeCl_3$ in O.IN HCI and 0.008 M Potassium Ferro cyanide. The absorbance was measured with the Jenway digital spectrophotometer model 6303 at 120 nm wave length. The absorbance was compared with those of standard made from tannic acid (12)

Preparation of Samples for GC-MS Analysis

Two hundred grams of the sample was repeatedly extracted with ethanol using soxhlet extractor, another 200 g of sample was soaked in ethanol for 48 hour and extracted. The extracts from the soxhlet extracts and that obtained from cold extracts for the sample were combined and re-extracted using chloroform to obtain chloroform soluble extract. This was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC and GC-MS analysis.

GC-MS Experimental Procedures

GC- analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are as follows: Temperature programming from 80 – 200oC held at 80 ^oC for I minute, the rate is 5 ^oC/min and at 200 ^oC for 20 minutes. FID Temperature of 300 ^oC, injection temperature of 250 ^oC, carrier gas is Nitrogen at a flow rate of 1 cm3/min and split ratio of 1: 75. GC-MS Gas chromatography Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu

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Japan with injector Temperature at 230 ^{o}C and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kv and sampling rate of 0.2seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany . (12)

ANTIMICROBIAL ANALYSIS

Bacterial isolates and identification

Clinical bacterial isolates were obtained from Federal Medical Centre Owerri microbiology laboratory. The bacteria isolates includes, *Klebsiella pneumonia, Staphylococcus aureus and Streptococcus pneumonia.* Each of these bacteria was sub-cultured on a sterile plate of nutrient agar, Mac-Conkey agar, blood agar and chocolate agar according to the type of bacteria. The identification of the bacteria was confirmed using the using the following gram staining and biochemical test such as methyl red test, motility test and indole test

Gram staining test

The bacterial colonies were collected using a sterile inoculating loop and emulsified onto a drop of normal saline on clean grease free slides. The smear was allowed to air dry and then heat fixed by passing over flame the slide containing the heat fixed smear was placed on a staining rack and flooded with primary stain crystal violet and allowed to stand for 60 second before being washed with water. After, the smear was flooded with Lugols' iodine and allowed to stand for 60seconds before being washed with water. Acetone was also used to flood the smear in order to decolorize it, after being flooded with acetone; the smear was quickly washed with water. The flood was also flooded with secondary stain safanine and allowed to react for 60secondsbefore being washed with water the back of the slide was cleaned and allowed to air. After drying, the smear, a drop of oil was placed on the surface of the smear and examines using oil immersion objective lens

Motility TEST

There are some bacteria genera that can move actively from one place to another, others that lack locomotive structures such as flagella cannot move. The ability to test this is important in their identification as it helps to differentiate motile from non-motile bacteria. Stab culture technique was used to demonstrate motility of using straight wire, the inoculum were picked up and a straight line stab of the inoculum made into a semi solid medium so that the stab stopped at about the center of the medium. This was done in duplicate for the test bacteria isolates.it was incubated at 39^{O}c for 24hrs and the tube examined non motile bacteria grew only along the line of stabbing

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while the motile bacteria grew along the line of the stabbing and diffused into the medium away from the line of stabbing causing turbidity and rendering the medium opaque. The non-motile bacteria growth was confined to the path of inoculation

Catalase Test

Aerobic bacteria produce varying levels of catalase enzymes that break down hydrogen peroxide to water and oxygen. This test was performed by bringing the test bacteria in contact with hydrogen peroxide. The production of effervescence indicated that the organism is a catalase producer. Two drops of hydrogen peroxide was made on a clean grease free glass slide using a clean glass rod, the bacteria was transferred to the first drop, the second was used as control

Coagulase Test

The test was carried out using a slide test method. A colony of the bacteria was emulsified on a glass of physiological saline on a glass slide to make a thick suspension. A drop of plasma was added to the suspension and mixed gently. Clumping of the of organism which did not re-emulsify indicates the presence of coagulase positive test

Indole Test

2ml of peptone water was inoculated with 5ml of bacterial culture and incubated at 35^{o}C for 48hrs. Kovacs; reagent 0.5ml was added well shaken and examined after 60 seconds. A red colour in the reagent layer indicates indole.

Citrate utilization test

This test assists in the differentiation of entobacteriaceae. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as only its source of nitrogen. This was carried out by inoculating 4ml of sterile Simon citrate medium with 24hrs culture of the test organism using a sterile wire loop. The inoculated agar was incubated at 35^{0} C for 48hrs and observed for colour change from green to royal blue

Methyl Red Test

Colonies of the test bacteria were inoculated in 0.5ml sterile glucose phosphate broth, after overnight incubation at 37^{o}C a drop of methyl red solution was added .a red colouration was positive and indicated an acid phi resulting from fermentation of glucose. A yellow colouration indicates negative result

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Oxidase Test

Oxidase test was used to differentiate bacteria that produce oxidase enzyme from the non-oxidase producing type. Many facultative organisms lack cytochrome C which can be detected using oxidase test. Oxidase catalyze the electron transport between electron donor in the bacteria and a redox dye, di tetraellyl-p-phenylenediaminedehydrochloride is produced which is reduced to a purple colour

A piece of filter paper was placed on a clean petri-dish .3 drops of oxidase reagent was added on the filter paper using a piece of glass rod, the test bacteria was smeared on the filter paper. Appearance of a bluish –purple colour within 10 seconds indicates positive reaction

Mycological Examination of Fungal Isolates

Two fungal isolates were sub-cultured on sterile plates of sabouraud dextrose agar. The two fungi include *Aspergillus niger* and *Mucor specie.* The identity of the fungus was determined using the needle mouth technique. With the help of a sterile inoculating needle, small portion of each fungal structure was collected and dropped at the center of a sterile grease free glass slide. With the help of Pasteurs' pipette, a drop of ethanol was made on the fungal structure .the ethanol was allowed to evaporate. A drop of lactophenol cotton blue stain was made on the fungal structure using Pasteurs' pipette. Two inoculating needles were used to tease the fungal structure. The preparation was carefully covered with cover slip. It was examined using low and high power magnification; the fungal isolates were identified using types of conidia, chlamydosperm and hyphae

Evaluation of Antimicrobial Activity

Preparation of Bacterial and Fungal Suspensions

2mls of normal saline was aseptically poured into sterile 5ml test tube. The test tube. The test tubes were labeled with the names of each isolate; bacteria and fungi. Two gram positive bacteria , *Staphylococcus aureus and Streptococcus pneumonia,* one gram negative bacteria (*Klebsiella specie* , two fungi specie *Aspergillus niger* and *Mucors specie.* These five test tubes were set up on test tube rack. With the help of a sterile wire loop, each fungal and bacteria were transferred to each tube bearing each isolates name according to the previous labeling of the test tubes. The test tubes were swirled after each inoculation until the isolate suspension becomes turbid. The color of each tube was marched with that of a 5% Marc Farlard standard. Bacterial suspension had four test tubes while the fungal suspensions had two test tubes

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Dilution of the plant extract

Four sterile test tubes were set up on a test tube rack and labeled accordingly. 1 ml of the plant extract was added to the first test tube that is the stock. I ml of distilled water was added into each test tube from test tube 2-4. I ml of plant extract. 1ml of plant extract was added into test tube 2 (1/20).the 1ml distilled water and 1 ml extract were carefully shaken to obtain homogenous mixture. 1 ml of this mixture was transferred to the third test tube and well shaken. With a new pipette 1ml of the content of test tube 3 was transferred to test tube 4 and carefully shaken. 1ml of its content was discarded using another sterile pipette. With this method four different concentrations of plant extracts were obtained in each test tube

ANTIBACTERIAL ACTIVITY USING WELL-IN- AGAR METHOD

4 plates of sterile Muller Hinton agar plates were prepared according to the manufacturer's instruction. The plates were allowed to cool and solidify. With the help of a pipette 0.1ml. *Klebsiella specie* suspension was introduced on the surface of the plate of prepared Muller Hinton agar. In like manner, equal volume of other bacterial suspensions were made on the remaining agar plates. Using a sterile 6mm cork borer, four wells were made on each agar plate. The wells were labeled according to the dilution of plant extract made (stock, 1/10, 1/20, 1/40). With different pipette, each extract of the plant extract was filled into each well as labeled until each well is filled to the brim. This was left for 1 hour to ensure that the extract has been absorbed by the agar. The plates were incubated at 37^{o}C for 24 hours. Zones of inhibition of the different the plant extract on each bacteria were observed and recorded. The process was repeated with each of the bacteria isolates.

ANTIFUNGAL ACTIVITY USING WELL IN AGAR METHOD

Two plates of sabouraud dextrose agar were freshly prepared. Two plates of the agar were smeared with *Aspergillus niger* while the other two plate were also smeared with *Mucor specie*, four well agar were also bored on each plate. Each well containing different dilution of each extract just as was done to the bacteria isolates earlier. The plates were incubated at 37^{o} C temperature for 48 hours. Plates were observed and zones of inhibition of different plant extracts on the two fungi plates were recorded.

GC-MS EXPERIMENTAL PROCEDURE

GC-MS analysis was carried out with Gas Chromatography 5975 7890 with a fixed GC column OV101 coated with polymethyl silicon (0.25mm x 50mm) and the conditions are as follows: Temperature programming from 80-200^{o}C held at 80^{o}C for 1 minute, the rate is 5^{o}C/min and at 200^{o}C for 20minutes. FID temperature of 300^{o}C, injection temperature of 250^{o}C, carrier gas is Nitrogen at a flow rate of 1cm3/min and split ratio 1:75. GC-MS Gas chromatography Mass

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spectrum analysis was conducted using GC-MS QP 2010 with injector Temperature at 230^{o}C and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. The elements were automatically passed into the Mass spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 seconds. The Mass spectrometer was also equipped with a computer fed Mass spectra data bank. Reagents and solvents such as Ethanol, chloroform, Diethyl ether, hexane all of analytical grade was obtained from Merck Germany

RESULTS AND DISCUSSION

Table.1.RESULTS OF THE PHYTOCHEMICAL TEST

Phytochemical constituents	Inference
Alkaloids	++
Cardic glycoside	++
Flavonoid	++
Saponin	++
Steroids	++
Tannins	++

KEY +; present

Table 2.RESULTS OF THE QUANTIFICATION

Phytochemical components	Solvent extracts	Percentage yield (
Alkaloid	Acetic acid + Ethanol	2.2
Flavonoid	80% Methanol	1.34
Saponin	20% Ethanol	3.73
Tannin	Water	2.4

Alkaloids have been reported to be present in most green leafy plants and they have many therapeutic properties. Most alkaloids have found their way in medicinal application as antimalarial, antimicrobial , antibacterial, antifungal and antiparasitic activities . Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever (14,12). Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and thensynthetic derivatives are used as the basic medicinal agent because of their analgesic antispasmodic and bacterial properties (15) . They show marked physiological effects when administered to animals.

Flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. The family encompasses flavonoids, flavones, may provide protection against a number of chronic

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diseases over the long term consumption (16).The include chalcones, catchins, anthocyanidins and isoflavonoid. They exhibit free radical scavenging activities(17) Flavonoids have multiple biological activities including – vasodilatory, anti-carcinogenic,anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholpase H2, cycloxygenase, glutathione reductase and xanthine oxidase. (18), they support lactogenecity.These properties therefore support the use of plant extract in cancer therapy (19) . Flavonoids in intestinal tracks lower the risk of heart diseases. As anti-oxidant, favonoids provide anti-inflammatory actions. The biological function of flavonoids include protection against allergies, inflammation, platelets aggregation microbes, ulcer, viruses and tumors (20,21).

Saponins was found to be available in the leaf of *Spondias mombin* Some of the general characteristic of saponins includes; formation of forms in\aqueous solutions, hemolytic activity and cholesterol binding properties(22) Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungal and yeast infections. These compounds serve as natural antibiotic, helping the body to fight infections and microbial invasion.(12) . Saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk (20). Saponins are responsible for numerous pharmacological properties (23) They lower the cholesterol level; have anti-diabetic and anti-carcinogenic properties (24).

Tannins has astringent properties, hastens the healing of wounds and inflamed mucous membrane. Tannins are astringent, bitter plant polyphenol compounds that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants where they play a role in protection from predation and perhaps also as pesticides and in plant growth regulation. The astringency from tannin is what causes the dry puckery feeling in the mouth following the consumption of unripe fruits or red wine. Tannins are important ingredients used in process of making tannin leather. Medicinally, tannins are used as anti-diarrhea, haemostatic and anti-hemorrhoid compounds Plant leaves with high tannin content has been used successfully as hops alternative in beer. (25,26) Plants that contain tannins are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery (27). The high tannin content may be partly responsibility for the bitter principle associated with the leaves. Tannins has astringent properties, hastens the healing of wounds and inflamed mucous membrane. The presence of tannins in the leaves of Spondias Mombin can support its strong use for healing of wounds, varicose ulcers, hemorrhoids, frost-bite and burn in herbal medicine . (28)

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tannins in the leaves of Spondias Mombin can support its strong use for healing of wounds, varicose ulcers, hemorrhoids, frost-bite and burn in herbal medicine . (28)

Glycosides are molecules in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plant store chemicals in form of inactive glycosides. Many such plant glycosides are used as medications. Some glycosides have shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes but concluded that further study was required to determine the proper dosages (26) Cardiac glycoside acts on the heart muscles and increase renal flow (diuresis), while phlobatannins on the other hand, have astringent or styptic properties(30)

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Table 3..Antimicrobial analysis

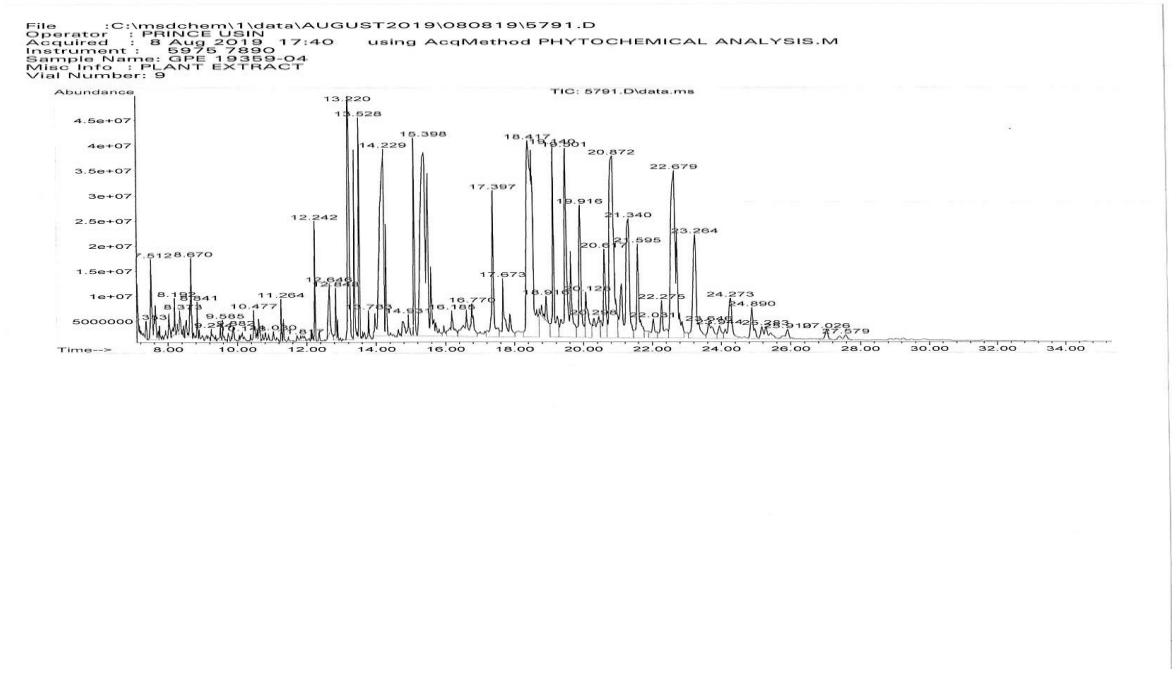
Conc	*Streptococcus specie*	*Staphylococcus auerus*	*Klebsiella pnuemonia*	*Pseudomonas aeroginosa*	*Aspergillus niger*	*Mucor specie*
Stock	30	35	R	R	18	19
1/10	20	25	R	R	10	12
1/20	15	11	R	R	8	10
1/40	R	R	R	R	8	10

The plant extract showed marked inhibitory potentials against certain selected human patogens, the main stock inhibited the growth of Staphylococus aureus by a diameter of 35mm, streptococcus specie 30mm similarly, other micro-organisms were not left out with inhibitions recorded against Aspergillis niger 18mm and Mucor specie 19mm but could not inhibit the growth of Klebsiella specie and Pseudomonas aeruginosa . Most of these pathogens have been implicated to be the main causes of some human ailments. . *Staphylococcus aureus* is a gram positive coccus that causes skin infection such as; pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, abscesses, pneumonia, toxic shock syndrome, bacteremia and sepsis. (26). Pseudomonas *aureginosa* is a gram negative gamma-proteobacteria which belong to the family *Pseudomonaceae*. It causes bacteremia, pneumonia, foliculitis, swimmer ear which is an ear infection accompanied with swelling, ear pus. Itching, discharge and difficulty in hearing, eye inflammation with associated pains, pus, swelling redness and impaired vision. Klebsiella, a non-motile gram negative, oxidase rod shaped bacteria which causes infectious wounds, pneumonia,

blood stream infections and urinary tract infections(30,26) *Staphylococcus aureus* is a gram positive coccus that causes skin infection such as; pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, abscesses, pneumonia, toxic shock syndrome, bacteremia and sepsis. Pseudomonas *aureginosa* is a gram negative gamma-proteobacteria which belong to the family *Pseudomonaceae*. It causes bacteremia, pneumonia, foliculitis, swimmer ear which is an ear infection accompanied with swelling, ear pus. Itching, discharge and difficulty in hearing, eye inflammation with associated pains, pus, swelling redness and impaired vision. Klebsiella, a non-motile gram negative, oxidase rod shaped bacteria which causes infectious wounds, pneumonia,(26), blood stream infection and urinary tract infection. *Proteus mirabilis* is a gram negative facultatively anaerobic rod shaped bacterium implicated in urinary tract infection (UTIs), bacteremia, type 2 diabetes, cystitis, pyelonephritis, urosepsis and urinary stone (urolithiasis). *Stachyterpheta cayennensis* has been used as a remedy for syphilis, gonorrhea, catarrh condition, skin wounds and sores in children (25). The extracts exhibited some level of inhibitory effects against some of the studied pathogens which have been implicated in one bacterial infection to the other in human and plant. Klebsiella specie causes pneumonia. In plant, *Pseudomonas spp* causes bacterial blight in guinea corn. These microorganisms are inhibited by the leaf extract of *S. cayennesis* implying that the extract may be used to treat diseases associated with these organisms.(26)

GC-MS ANALYSIS OF *SPONDIAS MOMBIN*

Fig 1 GC/MS spectrum of the leaf extract of spondias mombin



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The interpreted values of the chromatogram are in table 4 and the structures of the compounds obtained are in fig 2. Peak 1 occurred at m/z 412, with molecular formula $C_{29}H_{48}O$ and is named Stigmast-4-en-3-one .Peak 2, occurred at m/z 426 with molecular formula $C_{30}H_{50}O$ and is named Lupeol . Similarly other peaks are interpreted as follows; Peak 3, m/z 414, molecular formula $C_{29}H_{50}O$, name Tetratetracontane. Peak 4, molecular formula $C_{44}H_{90}$,m/z 618, name Heptacosane. Peak 5 molecular formula $C_{29}H_{56}$ m/z 380, name Vitamin E. Peak 6,molecular formula $C_{29}H_{50}O_2$, m/z 430,.. name γ-Tocopherol .Peak 7, molecular formula $C_{28}H_{48}O_2$, m/z 416, name 2H-1-Benzopyran-6-ol. Peak8 ,molecular formula $C_{27}H_{46}O_2$,m/z 402, name, 2H-1-Benzopyran-6-ol. Peak 9 molecular formula $C_{27}H_{56}$,m/z 380, name, Heptacosane Peak 10 , molecular formula $C_{30}H_{50}$, m/z 410, name, Squalene. Peak 11, molecular formula $C_{21}H_{38}O_4$, m/z 318 ,name.. Methyl[2]-5,11,14,17-eicosatetraenoate Peak 12, molecular formula $C_{24}H_{38}O_4$, m/z 390, name,1,2-Benzene dicarboxylic acid bisoctylester. Peak13, molecular formula, $C_{21}H_{36}O$, m/z 304, name, Phenol-3-pentadecyl ,. Peak14, molecular formula $C_{18}H_{36}O_2$, m/z 284 name, Octadecanoic acid. Peak15, molecular formula $C_{18}H_{31}ClO$. m/z 298. name, 9,12-Octadecadienoyl chloride[z,z] . Peak 16, molecular formula $C_{20}H_{40}O$, m/z 296,name Phytol. Peak 17, molecular formula $C_{16}H_{32}O_2$,. m/z 256, name, n-Hexadecanoic acid. Peak 18, molecular formula $C_{20}H_{40}O$, m/z 296,name, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol .Peak 19, molecular formula $C_{18}H_{36}$, m/z 252, name, 5-Octadecene[E] ,Peak 20 , molecular formula $C_{14}H_{28}O_2$, m/z 228 name Tetradecanoic acid. Peak 21 , molecular formula $C_{20}H_{38}$, m/z 278 name, Decane,5,6-bis(2,2-dimethyl propylidene),(E,Z). Peak 22, molecular formula $C_{20}H_{38}$, m/z 278,name 1-Hexadecene. Peak 23 ,molecular formula $C_{28}H_{48}O$, m/z 400 name, Cholestan-3-d,2 methylene(3β,5a). Peak 24 , molecular formula $C_{11}H_{10}$, m/z 142 ,name Napthalene. Peak 25 , name Napthalene molecular formula $C_{11}H_{14}$ m/z146. Peak 26, molecular formula $C_{10}H_8$, . m/z 128 name Napthalene

Table 4 .Compounds obtained from GC-MS spectrum the leaf of Spo*ndias mombin*

Peak	Chemical name	Molecular formula	Molecular weight
1	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412
2	Lupeol	$C_{30}H_{50}O$	426
3	γ-Sitosterol	$C_{29}H_{50}O$	414
4	Tetratetracontane	$C_{44}H_{90}$	618
5	Heptacosane	$C_{29}H_{56}$	380
6	Vitamin E	$C_{29}H_{50}O_2$	430
7	γ-Tocopherol	$C_{28}H_{48}O_2$	416
8	2H-1-Benzopyran-6-ol	$C_{27}H_{46}O_2$	402
9	Heptacosane	$C_{27}H_{56}$	380
10	Squalene	$C_{30}H_{50}$	410
11	Methyl[2]-5,11,14,17-eicosatetraenoate	$C_{21}H_{38}O_4$	318

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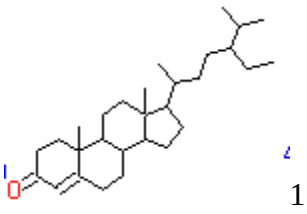
12	1,2-Benzenedicarboxylic acid, bisoctylester	$C_{24}H_{38}O_4$	390
13	Phenol,3-pentadecyl	$C_{21}H_{36}O$	304
14	Octadecanoic acid	$C_{18}H_{36}O_2$	284
15	9,12-Octadecadienoyl chloride[z,z]	$C_{18}H_{31}Cl0$	298
16	Phytol	$C_{20}H_{40}O$	296
17	n-hexadecanoic acid	$C_{16}H_{32}0_2$	256

18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
19	5-Octadecene[E]	$C_{18}H_{36}$	252
20	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
21	Decane,5,5-bis(2, dimethylpropylidene(E,Z),	$C_{16}H_{32}$	224
22	1-Hexadecene	$C_{20}H_{38}$	278
23	Cholestan-3-d,2 methylene,(3β,5α)	$C_{28}H_{48}O$	400
24	Benzocycloheptatriene	$C_{11}H_{10}$	142
25	Benzene, 2,ethenyl-1,3,5-trimethyl	$C_{11}H_{14}$	146
26	Napthalene	$C_{10}H_8$	128

FIG 2 Chemical structures of compounds from leaf of Spondias mombin



1.Stigmast-4-en-3-one

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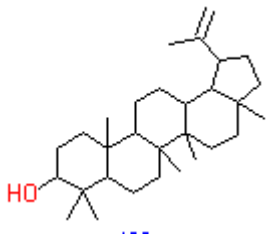
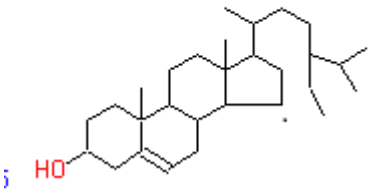


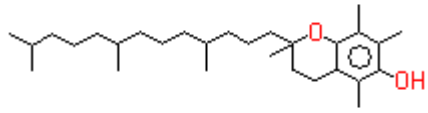
2.Lupeol



3.γ-sitosterol

4. Tetratetracontane

5. Heptacosane



6.Vitamin E

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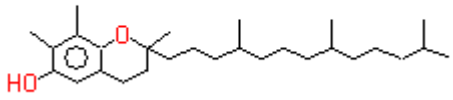
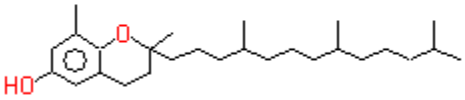
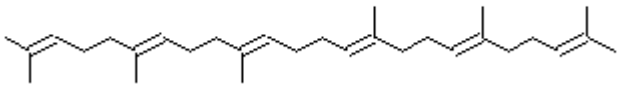


7. γ-Tocopherol



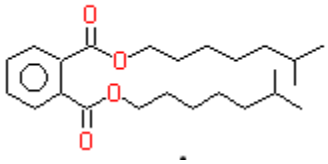
8. 2H-1-benzopyran-6-ol

9.Heptacosane



10.Squalene

11..Methyl[2]-5,11,14,17-eicosatetraenoate



12.1,2-Benzenedicarboxylic acid, bisoctylester

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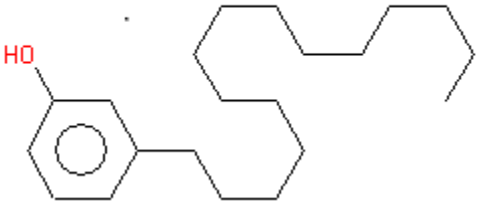
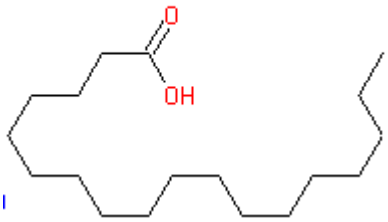
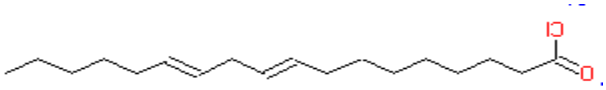


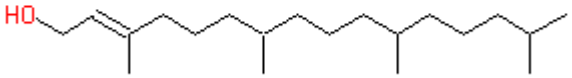
13. Phenol,3-pentadecyl



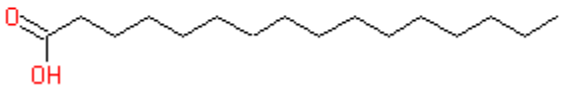
14. Octadecanoic acid



15. 9,12-Octadecadienoyl chloride



16. Phytol



17. n-Hexadecanoic acid

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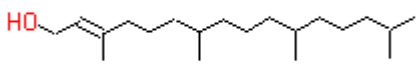
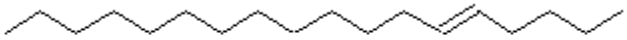
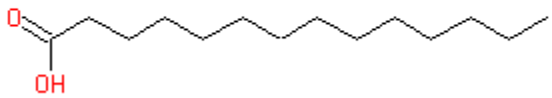


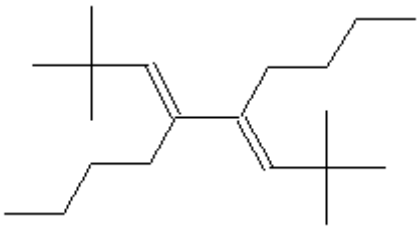
18. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol



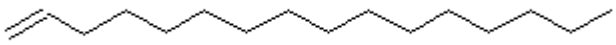
19. 5-Octadecene[E]



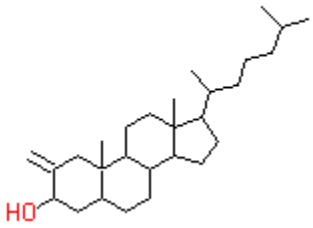
20 .Tetradecanoic acid



21.Decane,5,5-bis(2, dimethylpropylidene),(E,Z)



22. 1-Hexadecene



23. Cholesta-3-d,2 methylene,(3β ,5α)

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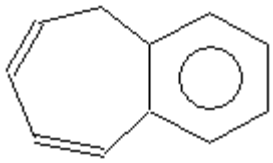
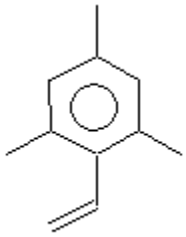
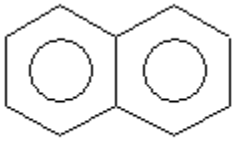


24. Benzocycloheptatriene



25. Benzene, 2,ethenyl-1,3,5-trimethyl



26. Napthalene

Some of the compounds so identified include lupeol, a triterpenoid which is a strong anti-inflammatory and ant-cancer agent, the compound is also known as fagarsterol. The plant is useful in the treatment of cancer and inflammations. Squalene is a natural product that reduces skin damage by ultra violet radiation, it also reduces the cholesterol level in the blood and prevents cardiovascular diseases, having antitumor, anticancer against ovarian, breast, lungs and colon cancer 31. Phytol is a precursor of vitaminK1 and E . It is a schistosomicide drug. It is used in making synthetic vitamin E and K. It has antimicrobial, antioxidant anti-inflammatory cytotoxic effects. Vitamin E was detected in the extract. vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols.. vitamin E functions for strong immunity and healthy skin and eyes. it is an antioxidant is believed to help in healing and reduction of scarring when applied to the skin .it is a lipid soluble component in the cell antioxidant defense system, it can serve to prevent ageing, cancer, arthritis and cataracts, it helps to reduce prostaglandins such as thromboxane which causes clumping of platelets.(32) .Sistosterol is a phytosterol used in lowering cholesterol level and improving benign prostatic hyperplasia, it reduces the risk of cancer and prevent coronary heart disease . Stigmasterone or stigmasterol is a

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plant steroid lowers cholesterol level and prevents heart disease and heart attack. It is a precursor to chemical compound that limits inflammatory processes

CONCLUSION

The analysis of the leaf of *Spondias mombin* has revealed that the leaf of the plant is a potent source of anti-tumour, anti-cancer, anti-inflammation drugs as a result of the identified compounds. 26 compounds has been identified from the leaf and they are responsible for its medicinal uses. These compounds show anticancer, anti-tumour , anti-inflammation, antiageing, cardiovascular protection and antioxidant properties and include phytol, Vitamin E, sistosterol, stigmasterone tocopherol, squalene, lupeol, sisterol and stigmsterones.

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