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Original Research Article

Comparative Phytochemical and Mineral Element Analysis of Leaf and Stem Bark of *Spondiasmombin*

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ABSTRACT

The leaves and stem bark of *Spondias mombin* have been acclaimed to be used by local populace to treat diseases and as food supplements. The research determined and compared the phytochemicals and mineral composition of the plant leaves and stems bark. The phytochemical analyses were done using standard methods. The mineral element analysis was done using multiple parameter photometer. The result of phytochemical analysis revealed the presence of alkaloids (8.3±0.5%, 1.5±0.4%), flavonoids (11.67±1.4%, 9.8±0.2%), saponins (8.65±0.4%, 5.3±1.2%), tannins (0.96±0.1, 0.88+0.1%) and phenol (1.14±0.01, 1.2±0.01%) for leaves and stem bark, respectively. While the specific phytochemicals result showed the presence of proanthocynanin, naringenin, flavan-3-ol, anthocyanin, sapogenin, phenol, flavonones, steroids, kaempferol, flavones, catechin and resveratrol at specific concentrations for each extract. Ribalinidine, naringenin, spartein and oxalate were present in the leaves extract and absent in the stem bark extract. Rutin, quinine, ephedrine and epicatechin were present in the stem bark extract and absent in the leaves extract at various concentrations. The mineral element composition of the plant components revealed the presence of aluminum, silicon, phosphorus, sulphur, potassium, calcium, iron, zinc and copper at different concentration for both leaves and stem bark extract. Magnesium, manganese and cobalt were absent in the leaves extract and slightly present in the stem bark extract. Their various concentrations were not harmful as they are within the permissible range.
These compounds contained in the plant have been reported in literatures to possess therapeutic properties against pathogens which have supported the ethnomedical claims and functions of this plant.

Keywords: Phytochemical, Mineral Element, Comparative analysis, Gas chromatogram-Flame ionization detector (GC-FID), *Spondias mombin*

Introduction

Medicinal plants also called medicinal herbs have been applied in the treatment of various diseases in African, Asia, South American and other diverse cultures of the world as traditional medicine[1]. Higher world's population ratio still depends mostly on traditional or herbal medicine for treatment of diseases, especially in Africa and other developing nations. These plants have long been used even in prehistoric days[2]. The widely use of medicinal plants are as a result of its usefulness in non-industrialized societies, due to their availability and affordability than modern medicines. The continuous practice of traditional systems of medicine may be as a result of population rise, prohibitive cost of treatments, inadequate supply of drugs, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases which may have led to increased awareness on the use of plant materials as a source of medicines for human ailments.[3] Some of the reported compounds that have been identified from *S. mombin* include gallocatechin, kaempferol and quercetin glycosides and their methoxy products, gallic and ellagic acid derivatives.[4]

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It has been reported that hundreds of chemical compounds (Phytochemicals) can be synthesized from medicinal plantswhich have medicinal values. Many phytochemicals with medicinal potentials and good biological activity have been identified. However, a plant contains diverse phytochemicals, therefore the effects of using a whole plant as medicine are uncertain because one cannot state the compound responsible for the healing activities. Furthermore, the phytochemical content and pharmacological actions of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety.[3] Though treatment with medicinal plants have been considered safe. Since they are natural, this is an advantage. Researchers have shown that most of the potent medicinal plants have relatively no or low toxic or adverse effects when used by humans. The golden fact is that, the use of herbal treatments is independent of any age groups and sexes. Among such plants used medicinally for treatment of different illnesses is *Spondias mombin*, a flowering plant from *Anacardiacea* family.
*Spondias mombin (*hog plum*)* belongs to the flowering plant family of *Anacardiaceae* which is a fruit bearing tree.[1]The plant is found in tropical area of America, Brazil, Nigeria, West indies and other tropical rain forests of the world. [3-8] *S. mombin* have a height of over 10 m girth of over 1 m. Its bark is thick, corky, and deeply fissured. Branches are low and branchlets are glabrous. The leaves are pinnate. The flowers bloom January to May and are sweet-scented, in large, lax terminal panicles of small white flowers. Fruits appear July to September. The fruits have a sharp, somewhat acid taste and are edible [7]. It hangs in numerous clusters of more than a dozen on the tree. Very rich in vitamins B_1 and C, the fruit mostly exists as an oval seed. Other common names, according to different ethnic groups in Nigeria

include "oheeghe" Edo, "nsukakara" Efik, "tsadarmasar" Hausas, 'iyeye'(fruit) and akika (tree) Yoruba . 'ungwu' Tivs, 'ngwu' Kakkas 'ogogo' Ijaws and 'ijikara' Igbo.[6]The fruit has been used as a substance that increases the rate of urine excretion and as an antipyretic medication. The bark is an astringent and used as an emetic and for the treatment of diarrhea, dysentery, hemorrhoids, gonorrhoea, leukorrhea, dyspepsia and can also be used as antiscorbutic.[9] The flowers and leaves are used to make a tea for stomach ache, biliousness, urethritis, cystitis, inflammation as well as to ameliorate inflammation of the eye and throat. Both the bark and flowers are used in folk medicine to make cure-all teas for digestive tract ailments, lower back pain, rheumatism, angina, sore throat, malaria, fever, congestion, diarrhoea, urethritis, metrorrhagia, and as a contraceptive [8].The bark is used in a remedy to treat gonorrhoea, diarrhoea, coughs and colds, haemorrhages. Stomach-achesand to ameliorate or relieve fatigue.[8,10]

The plant extracts of *S. mombin* exhibit antibacterial properties, and a decoction of the bark or root bark is considered antiseptic [11].*S. mombin* plant leaves are also used to expel calcifications from the bladder and for treatment of wounds. Fresh leaves of *S. mombin* is widely used by the natives to aid delivery and to expel the placenta in small ruminants (sheep and goats), especially during difficult labour. In traditional medical practice in southern Nigeria, the freshly boiled aqueous leaf and bark extracts of SM is used to treat dizziness, especially after childbirth.[7] *S.mombin* has also been use in treatment of peptic and gastric ulcer, disease of digestive and respiratory system, rheumatism, angina and infectious diseases.[12] The aim of this presence study is to compare the phytochemicals and minerals of the leaves and stem bark of the *S.mombin* plant and relate it with previous report from literature to ascertain the ethnomedical claims.

Materials and Methods

Plant collection

Fresh leaves and stem barks of ***S. mombin*** were collected from a local farm in Atta Ikeduru Local Government of Imo State on August 2021, and were identified and authenticated by a professional taxonomist from Imo State University Owerri and was deposited at Imo State University Herberium (IMSUH) with voncher number IMSUH-449.

Preparation of the sample for analysis

S. mombin leaves and stem bark samples were washed with water and then dried for 2 weeks under shade. The plant materials following dryness was powdered with a corona mechanical grinder landers yola model South Africa. The powdered leaves (2.0 kg) and stem barks (2.2kg) were weighed and stored in amber coloured Winchester bottle.

Ethanol extract

The powdered *S. mombin* leaves and stem bark (500 g) were percolated with 1000 mL of redistilled ethanol (99%) for 24 hours. The extracts were filtered and concentrated in a water bath at 55^0C.

Qualitative Phytochemical screening of the plant sample: leaves and stem bark

Qualitative Phytochemical analysis of the plant extracts were carried out to determine the phytochemical present in the extracts using the methods used by Ugariogu *et al*.[13]

Quantitative analysis of the phytochemical (Gravimetric method)

Estimation of Alkaloids

Alkaloid determination was done using Harborne method. One gram of each sample (that is: leaves and stem bark) were weighed into a 250 mL beaker each and 200 mL of 10% acetic acid in ethanol were added, covered and allowed to stand for 4 hours. It was filtered and the extracts were concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added by drop wise to each extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate were filtered and washed with dilute NH_4OH. The residue is the alkaloid, which were dried and weighed.[14]

Estimation of Flavonoids

One gram of each plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The mixtures were filtered through a Whatman No1 filter paper into a pre-weighed 250mL beaker. The filtrates were transferred into a water bath and allowed to evaporate to dryness and weighed.[14]

Saponin determination

Each pulverized samples of (20g)were weighed into a conical flask and 100 cm^3 of 20% aqueous ethanol added. Then the flask was heated on a hot water bath for 4 hours with constant stirring at about 55°C. The mixtures were then filtered and the residues were again extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL on a water bath at about 90°C. The concentrates were transferred into a 250 mL separatory funnel, added 20 mL diethyl ether in it followed by vigorous shaking. The aqueous layers were recovered while the ether layers were discarded. The purification processes were repeated. 60 mL of n-butanol were added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.[14]

Preparation of Fat free Sample

The leaves and stem bark (2 g) sample were defatted with 100 mL of diethyl ether using a soxhlet apparatus for 2 hours.

Determination of Total Phenols by Spectrophotometric Method

The fat free samples were boiled with 50 mL of ether for the extraction of the phenolic component for 15 min. 5mL each of the extracts were pipetted into a 50 mL flask, and then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 mins for colour development. It was measured at 505 nm.[15]

Tannin Determination by Van-Burden and Robinson method

Each of the samples were weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to mark. Then 5mL of the filtered was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins.[15]

Extraction of Natural Products

The extraction of the natural products was done by homogenizing 400g dry pulverized plant with 1000mL of ethanol for 24 hours and it was filtered with 110mm whatmann filter paper. Then the filtrates were evaporated to 1/10 volume at less than 70^0C

Gas Chromatogram-Flame Ionization Detector phytochemical determination

The identification of the phytochemicals in the crude was performed on a Buck M910 Gas chromatography equipped with a flame ionization detector (GC-FID) was used as reported by Duru[16]

Mineral Element Analysis

*Digestion of sample:*5 mL of 65% HNO_3 was added to 0.5 g of the sample in a beaker, the mixture was boiled gently for 30–45 min. After cooling, 2.5 mL of 70 % $HClO_4$ was added, and gently boiled until dense white fumes appeared. The mixture was allowed to cool, 10 mL of deionized water was added followed by further boiling.[13]

*Mineral element (Micro nutrients) Analysis:*All elements were determined using Hanna 8300 multiple parameter photometer to know the concentrations of the trace metals present in the leaf. The principle and methods for the determination of the element was done using the specification and direction of the instrument manual.[17]

Statistical Analysis

The standard error for the gravimetric determination of the phytochemical was made by done using standard deviation.

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Results and Discussion

The results and discussions of the analysis are recorded below with appropriate tables

Phytochemical screening of the crude leaves and Stem barks samples of *S. mombin* were done and the results (shown in Table 1) revealed the presence of alkaloids, flavonoids, saponins, terpenoid, phenol, and tannins for both the leaves and stem barks. These compounds are known to show curative activity against several pathogens and therefore could explain the use of plant traditionally for the treatment of wide array of illness. The phytochemicals present in the plants may be responsible for preventing disease and promoting health. Mamta *et al* revealed that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Phytochemicals may detoxify substances that cause cancer, they appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens.[18] Herbal preparation containing .tannin are used to accelerate blood clothing, reduce blood pressure, decrease the serum lipid level, produce liver necrosis and modulate immune responses. Tannin also act as antioxidant scavenging and neutralizing free radicals and combating oxidative stress.[19] It also prevent the onset of degenerative disease such as cancer and cardiovascular disease, heal wounds and stop diarrhea.[19]Saponinsaffect the immune system by helping to protect the human body against cancer and lower cholesterol levels, decreases blood lipid, and lower blood glucose response [20.] It inhibits dental carriers and platelet aggregation. Saponin is used in the treatment of hypercalciuria and as antidote against acute lead poisioning, it counteract oxidative stress and protect against cell death.[21] Flavonoids decrease risk of disease through various physiological mechanism which include antiviral, anti-inflammatory, cytotoxic, antimicrobial and the antioxidant effects, it also decrease cancer risk and cardiovascular diseases, this can trigger detoxification, decrease inflammation and reduce the risk of tumors spreading.[22] Alkaloids have a wide range of pharmacological activities including antimalaria, anti-asthma, anti-cancer, anti-bacterial, analgesic, vasodilatory and anti-hyperglycemic activities.[23] They are pharmacological active compounds that affect the central nervous system, reduce appetite and act as diuretic. Alkaloids are also important compounds in organic synthesis for searching new synthetic compound with possibly better biological activity than parent compounds.[23]

The quantitative phytochemical result (shown in table 2) obtained showed that the different classes of secondary metabolites were present in the plant in varying proportions (percentages) both for the leaves and stem barks.

Table 1: Qualitative Phytochemical Analysis result for Spondiasmombin Leaves and Stem Bark

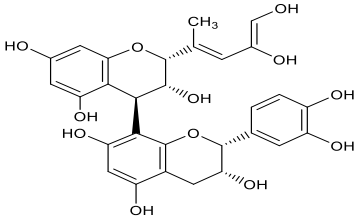
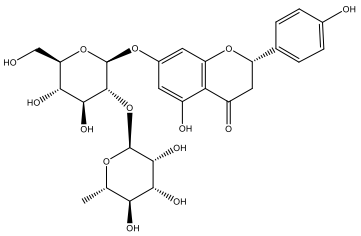
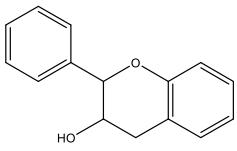
Phytochemicals	Leaves	Stem bark
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phenols	+	+

Present = +

Table 2: Quantitative Phytochemical Analysis result for *Spondiasmombin (Gravimetric Methods)*

Phytochemicals	Leaves	Stem bark
Alkaloid	8.3 ± 0.5 %	1.5 ± 0.1 %
Flavonoid	11.67 ± 1.4 %	9.8 ± 0.2 %
Saponin	8.65 ± 0.4 %	5.3 ± 1.2 %
Tannin	0.96 ± 0.1 %	0.88 ± 0.1 %
Phenol	1.14 ± 0.1 %	1.2 ± 0.1 %

Table 3: Result of the Quantitative Phytochemical Composition Analysis of *S. mombin*Leaf (GC-FID)

Components	Retention time	Concentration(mg/kg)	Structure
Proanthocyanin	0.12	22.78	
Naringin	2.70	4.12	
Flavan-3-ol	5.79	7.80	

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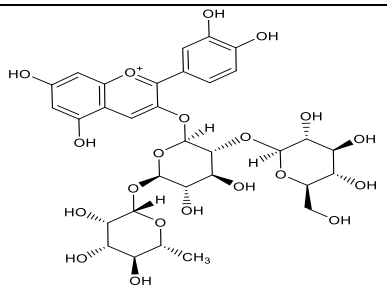
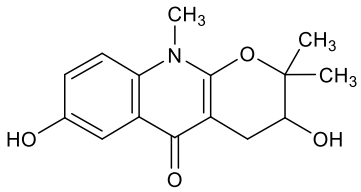
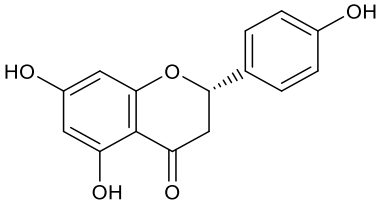
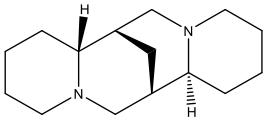
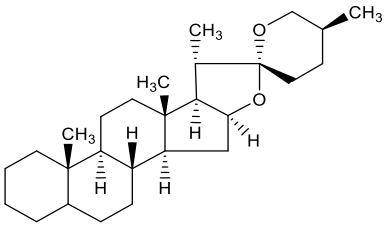
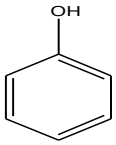
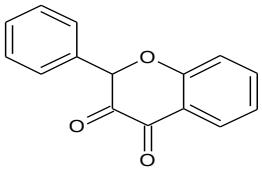
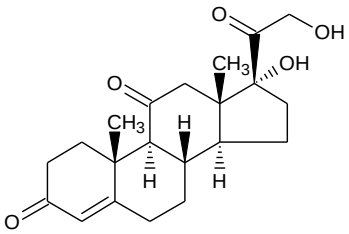
Anthocyanin	8.44	79.35	
Ribalinidine	10.79	6.20	
Naringenin	12.45	19.30	
Spartein	14.04	19.86	
Sapogenin	15.08	3.56	
Phenol	19.76	20.34	
Flavonones	21.55	15.63	
Steriods	24.58	10.35	

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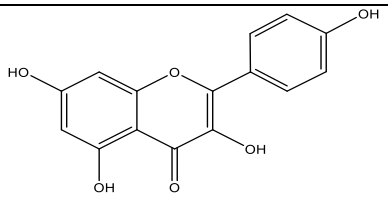
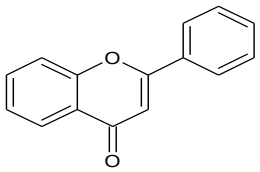
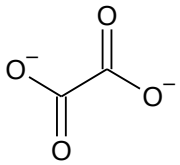
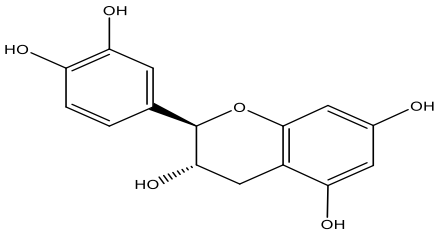
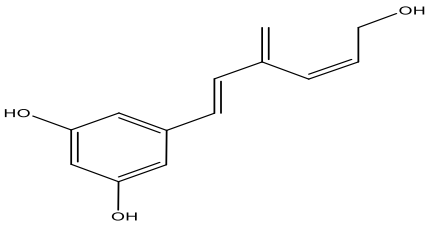
Kaempferol	31.35	3.40	
Flavone	34.12	3.18	
Oxalate	36.01	1.30	
Catechin	39.90	11.23	
Resveratol	41.84	39.22	

Flavonoid gave highest yield for both leaves and stem barks at 11.67 % and 9.8% respectively followed by saponin 8.65% for leaves, and 5.3 % for stem barks. Alkaloid was 8.3% for leaves 1.5% for stem barks, Phenols was 1.14% for leaves and 1.2% for stem barks, while tannins 0.96% and 0.88 % for leaves and stem bark respectively. From the result, it was observed that the leaves of *Spondias mombin* contained more phytochemical percentages than the stem bark. The presence of all these secondary metabolites contributed to the ethnomedical uses of the plant by local populace. The result of the specific phytochemicals reported in Table 3 and 4 showed the presence and concentrations of specific compounds.

Proanthocyanidins are condensed tannins with various pharmacological properties. These phytochemicals are considered as defense molecules because of their human health benefits. The validation of their diverse health aspects, namely, antioxidant, anticancer, antidiabetic, neuroprotective, and antimicrobial has earned them repute in thermochemistry. Proanthocyanidins are oligo- or polymers of monomeric flavan-3-ols produced as an end product of flavonoid biosynthetic pathway.[24] Tannin compound (Proanthocyanin) was present in the leaf and stem bark with the following concentration of 27.8 mg/kg and mg/kg respectively. Flavonoid compounds which includenaringin, flavan-3-ol, anthocyanin, flavonoid, flavonones, kaempferol, flavone, and catechin were present in both leaves and stem bark in different concentrations. Naringenin was present in leaves and absent in stem bark while epicatechin was present in Stem bark but absent in leaves.

Alkaloid compounds: ribalinidine, spartein were present in leaves while quinine was present in stem bark.

Saponin compound sapogenin were present in both leaves and stem bark with the concentration of 3.6 and 37 mg/kg. steroid was detected in both leaves and stem bark at 10.3 and 20.8 mg/kg concentration. Phenol was also detected in both leaves and stem bark with the following concentration of 20.3 and 82 mg/kg respectively. Stilbene compound: Resveratrol was detected in both leaves and stem bark with concentration of 39.2 and 119.3 mg/kg respectively. Epihedrine was detected in stem bark with 5.4 mg/kg concentration. Epihedrine is a medication and stimulant used often to prevent low blood pressure during spinal anesthesia. It has been used for asthma, narcolepsy and obesity.[25] These phytochemical results supported the ethnomedical claims of these plant due to the present of alkaloid likeRibalinidine and quinine used pharmaceutically in treatment of malaria and fever related illness and steroids which are useful for pharmaceutical application that are related to menstrual irregularities, urinary tract infection etc. This may justify the use of *S. mombin* in treatment of urethritis and gonorrhea.

The distribution of the mineral composition of the *S. mombin* leaves and stem bark (shown in Table 5) showed that they can be used as food mineral supplements. The quantity of the trace elements (Mg, Fe, Zn, K, Ca, P and Cu) which are the elements the body required in a minute quantity are not much in both plant leaves and stem bark. The stem bark contains better quantity and distribution of the essential elements (Mg, P, Fe, K and Ca) when compared with the leaves. The high quantity of calcium in both the leaves and stem bark of this plant indicates its necessity in the diet of both nursing mothers and infants. This result also justifies the ethnomedical use of *S. mombin* in treatment or management of anemia due to the presence of iron in both leaf and stem bark of the plant.

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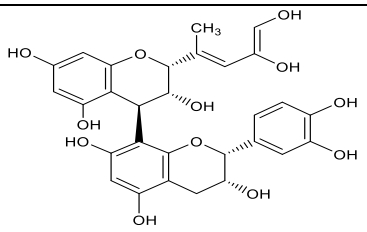
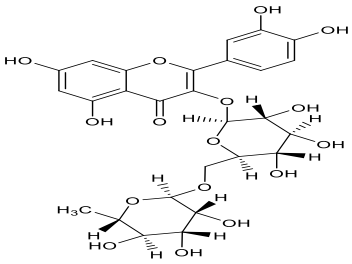
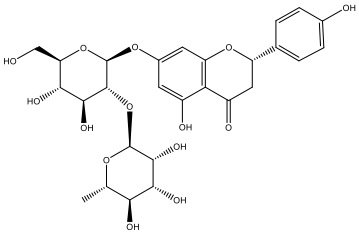
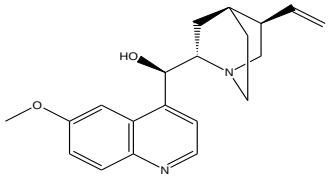
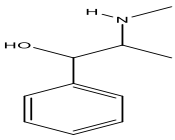
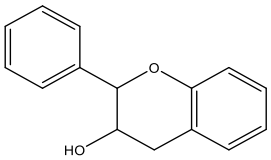
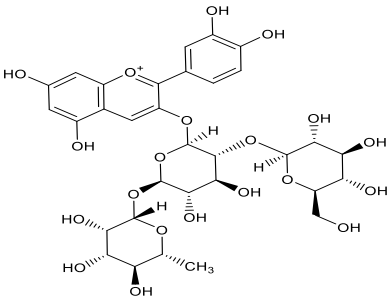
Table 4: Result of Quantitative Phytochemical Composition Analysis of *S. mombin* Stem bark (GC-FID)

Components	Retention time	Concentration(mg/kg)	Structure
Proanthocyanin	0.32	49.78	
Rutin	1.37	18.01	
Naringin	3.19	12.35	
Quinine	4.09	19.48	
Epihedrine	5.49	5.37	
Flavan-3-ol	7.09	23.90	
Anthocyanin	9.03	168.59	

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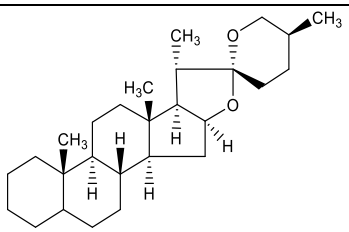
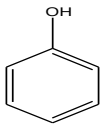
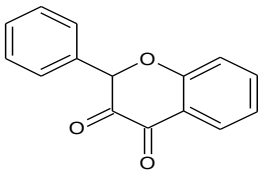
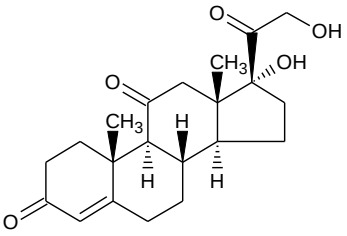
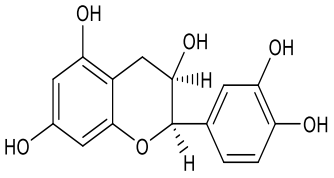
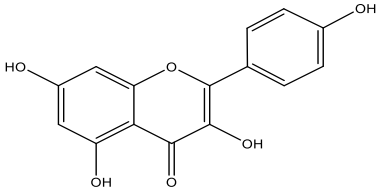
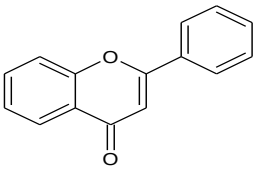
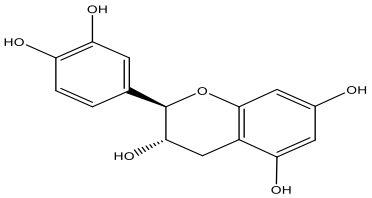
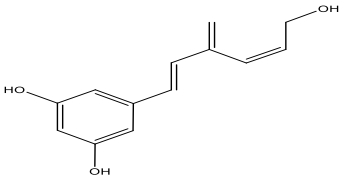
Sapogenin	17.03	37.08	
Phenol	19.11	81.93	
Flavonones	23.30	11.31	
Steriods	25.08	20.76	
Epicatechin	28.59	1.69	
Kaempferol	30.99	4.99	
Flavone	34.12	10.66	
Catechin	39.03	23.12	
Resveratol	43.98	119.29	

Table 5: Result of mineral elemental composition of *S. mombin*leaves and stem bark

Element	Leaves (mg/g)	Stem barks (mg/g)
Magnesium (Mg)	0.00	1.6
Aluminium (Al)	0.56	0.55
Silicon (Si)	0.15	0.45
Phosphorus (P)	0.51	0.49
Sulphur (S)	2.68	1.89
Potassium (K)	4.36	6.91
Calcium (Ca)	17.69	20.67
Iron (Fe)	0.35	0.48
Zinc (Zn)	0.14	0.09
Copper (Cu)	0.00	0.01
Manganese (Mn)	0.00	0.01
Cobalt (Co)	0.00	0.01

Conclusion

The results of the study have showed that *S. mombin* may be potent medicinal plant due to the composition of the phytochemicals and mineral element contained by the plant which some of them have been showed to be medicinal. The results suggested that the plant may be used in prevention of some disease caused by lack of minerals and activities of phytochemicals in the plant as acclaimed ethnomically due to the presence of secondary metabolites. The results conform with the findings of Alex *et al* for the phytochemical and mineral element composition and Esua et al except for flavonoid which was present in this study but absent in their result. The result also showed that the stem bark had higher amount of the phytochemical and mineral element concentration than the leaves. This research has successful validate the ethno medical claim of the plant *Spondias mombin* by the local populace as a medicinal plant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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