

Phytochemical and anatomical studies of Hog plum (*Spondias mombin* linn-anacardiaceae)

Morah Ifunanya Ann. and Alexander .I. Izundu

Department of Botany Nnamdi Azikiwe University, Anambra State, Nigeria.

ABSTRACT

This study examined the phytochemical constituents as well as anatomical features of *Spondias mombin*. Both aqueous and ethanol extracts were analysed. Analyses were carried out in the leaf, bark and root of the plant. Analytical methods were used for the phytochemicals while plant parts were sections for anatomical study using microtone. Results showed that the concentration of tannins were 3.18 ± 0.02 , 2.94 ± 0.05 , 0.91 ± 0.07 mg/100g for ethanol extract and 3.18 ± 0.02 , 2.79 ± 0.07 and 0.86 ± 0.07 for aqueous extract of the leaf, bark and root respectively. Saponin content was between 4.35 ± 0.02 and 1.31 ± 0.02 for leaf, bark and root. Cyanogenic glycoside under ethanol extraction was 3.46 ± 0.07 mg/100g in the leaf and 0.94 ± 0.07 mg/100g in the root while it was not detected in the bark. The alkaloid content was 3.91 ± 0.02 , 3.54 ± 0.04 , and 1.77 ± 0.04 4.35 ± 0.02 for aqueous extract of leaf, bark and root respectively. The anatomical microscopy showed the distribution of different tissues and cells, arrangement of vascular bundle, epidermis, ground tissue etc. The findings may indicate that the plant is a potential source of phytomedicine considering the diverse ethnopharmacological uses of the plant.

Keywords: Phytochemical, anatomical studies, *Spondias mombin* linn

INTRODUCTION

In recorded history, plants are indispensable source of food and medicine to all kinds of life [1]. They perform dual functions of manufacture of food and purification of the atmosphere which are the monopoly of green plants and are performed by the chloroplasts of the leaf during the day, sunlight being the source of energy [2,3,4,5]. Man depends on plant and plant products directly for his basic needs as food, clothing and shelter [6,7]. It also depends on plant indirectly for their beneficial influence on the climate and maintenance of his immediate and remote environment [8]. *Spondias mombin* is a fructiferous tree having habitat in Nigeria, Brazil and several other tropical forests in the world [9]. It belongs to the family Anacardiaceae. It grows in the rain forest and in the coastal area [10]. The root is used for emergency water source. The stem as living fences in farm land, shelter by artisans and has low quantity wood. The wood in carpentry for match sticks, match boxes, physicians spatulas, stick for sweet, meats, pencils, pen-holder, racking cases, interior sheathing of homes and boats and as a substitute for cork [11,12]. In Brazil, used as bottle

stoppers and to make seal wax. In Tropical Africa, it serves as poles for huts, garden poles and for axes and hoe handles. In Costa Rica, it is used as fuel, wood ashes in Africa and also as indigo-dye. The bark is used in carving figures like amulet, statuettes, cigarette holder and various ornamental objects and also as dyeing agent and gum glue [13]. The young leaves are cooked as green vegetable and fruit edible - eaten out of hand in stewed with sugar, extracted to prepare ice-cream, cool beverages and jelly in Costa Rica and Brazil. It is used as jam in Panama, Peru and Mexico; wine in Guatemala and Mexicans pickle the green fruit into vinegar [14,15]. It is widely valued as feed for cattle and pig. The flowers are used for decoration and the nectar is worked on intensively by honey bees [16,17,18]. *Spondias mombin* is widely relied on various herbal remedies for numerous conditions and virtually every part of the tree is used from its thickly corky bark, to its leaves, root, fruits and even its flower. It has been reported that the plant extract has antibacterial, antiviral, Antihelminthic and molluscicidal activities [19,20]. The leaves have also been shown to exhibit abortifacient and lipid lowering actions

[21, 22, 23]. The reported use of the plants leaf extract as an abortifacient or labour inducing agent on one hand, and also for prevention of miscarriages on another are mutually exclusive sides of a coin. In many villages, pieces of information refers *Spondias mombin* as a front line medicinal plant whose leaves are used to reduce labour pains and induce faster delivery in mammals [24]. The fruits are edible and other

parts could be mixed together, boiled and steamed and the steam inhaled and or used in bath or drink for cure of malaria, stomach problems and general weakness [25]. These unpublished claims can only be ascertained by first identifying the constituents of the plant. The aim and objectives of this research is to determine the phytochemical and anatomical composition of Hog plum (*Spondias mombin* linn -*anarcadiaceae*)

MATERIALS AND METHODS

Source of material

Spondias mombin used in this study was collected from a garden in Science Village of Nnamdi Azikiwe University Awka, Anambra State. They were

certified and authenticated by Professor C.U Okeke of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State.

Preparation of Samples

Aqueous Extract

Samples of the leaf, bark and root of *Spondias mombin* were collected and prepared by soaking 200g of the powdered sample in 1.2 liters of distilled water for 24 hours in amber coloured bottles. The contents of the

bottles were filtered with Whatman no.1 filter paper and the filtrate collected was evaporated to dryness with steam on water bath (40°C) and used for the analysis.

Ethanol Extract

Five grams of dried plant powder was extracted for 4-5 hrs with (150ml) ethanol by hot continuous percolation method in Soxhlet apparatus. After the effective extraction, solvent were

concentrated using rotary flash evaporator and water was removed by evaporated to dryness on a hot water bath to yield a soxhlet crude extract

.Phytochemical Screening of *Spondias mombin*

The Phytochemical tests were carried out on the leaf, bark and root of

Spondias mombin using standard procedures as described by [13].

Qualitative Phytochemical analysis

Test for Alkaloids

The presence of alkaloid was tested using Dragendorff's Mayer's, Wagner's reagent. A pinch of the sample was put into a test tube and 5ml of distilled

water was added. The mixture was allowed to boil for 5mins and allowed to cool and filtered.

Mayer's test

To the filtrate 3 drops of Mayer reagent was added to produce a milky

precipitate which indicated the presence of alkaloids.

Wagner's test

To the filtrate 3 drops Wagner's reagent was added. The brownish greenish

colour indicated the presence of alkaloids.

Test for Flavonoids

To the pinch of the sample 10ml of ethyl acetate was added. It was allowed to boil, cooled and filtered. Four millilitre of filtrate was put into two test tubes. To each test tube 1ml of dilute ammonia was added and shaken. There was the formation of two layers and the

lower layer indicated the presence of flavonoids. To the second test tube, 1ml of 1% aluminium chloride was added and shaken. There was formation of two layers and the upper layer showed yellow colour which indicated the presence of flavonoids

Test for glycoside

To a pinch of the sample 5ml of distilled water was added and boiled for 5minutes and filtered. To the 2ml of the

filtrate, 2 drops of Fehling solution A and B was added for 2minutes. The

reddish brown precipitate indicated the

presence of glycosides.

Test for Tannin

To the sample 5ml of 45% ethanol was added and boiled for 5mins. It was allowed to cool, filtered and the filtrate shared into three portions. To the first portion 3 drops of lead sub acetate was added. The formation of gelatinous precipitate showed the presence of tannin. To the second part 3 drops of

bromine water was added, pale brown precipitate showed the presence of tannin.

To the last portion 3 drops of ferric chloride solution was added, brown precipitate showed the presence of tannin.

Test for Saponins

This test was done using froth and emulsion test. To a pinch of the sample 5ml of distilled water was added. It was

allowed to boil for 5mins and filtered while still hot.

Emulsion Test

To the first portion 2 drops of olive oil was added, shaken vigorously and the

formation of emulsion (dispersion of oil on shaking) was observed.

Froth Test

To the second portion 4ml of distilled water was added and shaken vigorously.

The observed formation of froth (foam) showed the presence of saponins

Test for Sterol

The sample was heated with 5ml of petroleum ether. To the filtrate 2ml of 2% perchloric was added and heated at

150°C. The observed brown colour showed the presence of sterol.

Test for Phenol

Ferric chloride test

The sample was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were

added. The formation of a dark green colour indicated the presence of phenol.

Lead acetate test

The sample was dissolved in 5ml of distilled water. To this, 3ml of 10% lead acetate were added. The observed

presence of a bulky white precipitate indicated the presence of phenol compounds

Test for Protein

To the sample 5ml of distilled water was added, shaken and allowed to stand for 3hours. It was filtered and to the 2ml of the filtrate 2 drops of million reagent

were added. The formation of yellow precipitate showed the presence of protein.

Quantitative Phytochemical analysis

The phytochemicals which are present in the aqueous and ethanol extracts of

Spondias mombin were determined and quantified by standard procedures.

Determination of Alkaloids

One gram of the sample was mashed with 10ml of water and filtered. Five millilitre of 5% acetic acid were added to 1ml of the filtrate and centrifuged. The supernatant was collected and warmed to 70°C. Then drop point ammonia was added until the PH is 10. The mixture

was centrifuged again and the residue was collected. To the residue 5ml of 96% ethanol, 20% sulphuric acid was added. It was allowed to stand for 180minutes and absorbance was read at 570nm. All determinations were carried out in triplicates.

$$\frac{(Abs \times D)}{SL}$$

$$\frac{G}{G}$$

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample, SL= slope (graph of absorbance against concentration).

Determination of phenolic compounds

One hundred grams of the extract of the sample was weighed accurately and dissolved in 100ml of triple distilled water (TDW). One millilitre of this solution was transferred to a test tube, then 0.5ml 2N of the Folin- Ciocalteu

reagent and 1.5ml 20% of Na₂CO₃ solution was added and ultimately the volume was made up to 8ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was read at 765nm.

$$\frac{(Abs \times D)}{G}$$

$$\frac{SL}{G}$$

G

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample, SL= slope (graph of absorbance against concentration).

Determination of Flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. One hundred gram of the plant extracts in ethanol (10 mg/ml) was mixed with 20% aluminium trichloride in

ethanol and a drop of acetic acid, and then diluted with ethanol to 5ml. The absorption at 415nm was read after 40 minutes. All determinations were carried out in triplicates.

$$\frac{(Abs \times D)}{G}$$

$$\frac{SL}{G}$$

G

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample, SL= slope (graph of absorbance against concentration).

Determination of Glycosides

To one gram of the sample 10ml of water was added and shaken frequently for an hour. The 1.5ml of 15% lead acetate solution was also added, shaken and allowed to settle. The filtrate was extracted with 5ml of chloroform twice and evaporated to dryness in a water

bath. To the dried residue 3ml of glacial acetic acid, 0.1ml of 5% ferric chloride and 0.25ml of concentrated H₂SO₄ was added and stirred and was put in the dark for 3 hours. Absorbance was read at 530nm.

$$\frac{(Abs \times D)}{G}$$

$$\frac{SL}{G}$$

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample, SL= slope (graph of absorbance against concentration) and convert to percentage.

Determination of Tannin

The 0.1% of the sample was mixed with 10ml of ethanol. The mixture was shaken vigorously and allowed to stand for 16hours. It was filtered and to 1ml of the filtrate, 0.2ml of lead acetate solution was added. It was further warmed at 75oC until precipitate forms. The mixture was centrifuged for

5minutes. To the residue 2 drops of 1 in 20 H₂SO₄ was added and mixed thoroughly. 3ml of water was added and centrifuged for 5 minutes. To the mixture 2ml of H₂SO₄ and 1ml of water was added. Absorbance was read at 570nm.

$$\frac{(Abs \times D)}{G}$$

$$\frac{SL}{G}$$

G

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample used, SL= slope (graph of absorbance against concentration).

Determination of Saponins

To 0.1g of the sample 30ml of 1 normal sulphuric acid was added and boiled for 12hours. The mixture was filtered and

concentrated to dryness. It was dissolved with 5ml of methanol. One millilitre from the solution was collected

and titrate with 0.1ml methanolic potassium hydroxide until PH reached

10.

$$\frac{(\text{Abs} \times \text{D})}{\frac{\text{SL}}{\text{G}}}$$

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample used, SL= slope (graph of absorbance against concentration).

Determination of Sterol

Ten millilitre of ethanol was used to dissolve residue (fats and oil residue). The 1ml of the sample was collected and put in a test tube and another 1ml of ethanol added. Another 2ml of colour

reagent was added and the mixture was allowed to stand for 30minutes, thereafter the absorbance was read at 550nm.

$$\frac{(\text{Abs} \times \text{D})}{\frac{\text{SL}}{\text{G}}}$$

Abs = absorbance of the test sample, C = concentration of the standard in mg/ml, D = dilute factor, G = gram of stock

sample used, SL= slope (graph of absorbance against concentration).

ANATOMICAL STUDIES

The Anatomical study was carried out on the leaf, bark and root of *Spondias mombin* using temporary sections. Sledge microtone was used for the cross sectioning of the specimen between 10-15microns. The sections were transferred into staining jar and stained in 1% safranin for 10 minutes. The safranin was drained off and sections washed 3 times with H₂O. They were washed again with 97% absolute alcohol

2 times each respectively. The sections were counterstained in 1% fast green for 10 minutes and washed with absolute alcohol for 4 times. They were transferred into staining jar containing 50/50 alcohol and xylene and washed until they are clear. Pure xylene was finally used to clear the sections. Canada balsam was used to mount the slide for microscopic examination.

Statistical analysis

Each treatment or assay was repeated or replicated three times. Data were analysed statistically using analysis of variance to compare variations. Results

were reported as means ± standard deviation and test of significance were carried out at 95% level of probability.

RESULTS

The qualitative phytochemical screening of the aqueous and ethanol extract of leaf, bark and root of *Spondias mombin* showed the presence of most of the phytochemicals tested. The leaf, bark and root contained alkaloid, flavonoids, phenol, sterol, tannin and saponins. Notably, the bark did not show the presence of cyanogenic glycoside both in the aqueous and ethanol extract but it

was present in the leaf and root. The result also indicated the presence of tannin and saponin in large quantity in the aqueous and ethanol extract of the leaf. Alkaloid was present in large quantity in aqueous extract of the leaf and bark but in small quantity in the ethanol extract of the leaf and bark (Table 1).

Table 1: Qualitative Phytochemical screening of the leaf, bark and root extracts of *Spondias mombin*.

Plant Part	Extraction media	Tannin	Saponin	Alkaloid	Flavonoid	Phenol	CYG	Sterol
Leaf								
	Aqueous	++	++	++	+	+	+	+
	Ethanol	++	++	+	+	+	+	+
Bark								
	Aqueous	+	+	++	+	+	-	+
	Ethanol	+	+	+	+	+	-	+
Root								
	Aqueous	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+	+

+ Present in small quantity, ++ Present in large quantity, - absent

Quantitative phytochemical assay showed that in general most of the phytochemicals were high using ethanol extraction medium than with aqueous extraction. However, the differences between these phytochemical concentrations of the two extraction media were not significant in most cases. Again the different phytochemicals were highest in the leaf followed by the bark and then the root. The tannin content was 3.18 ± 0.02 ,

2.94 ± 0.05 and 0.91 ± 0.07 mg/100g for the ethanol extract of the leaf, bark and root respectively (Table 2). The aqueous extract of phenol had 0.28 ± 0.03 , 0.12 ± 0.01 and 0.095 ± 0.01 mg/100g for the leaf, bark and root parts respectively. Cyanogenic glycoside under ethanol extraction was 3.46 ± 0.07 mg/100g in the leaf and 0.94 ± 0.07 mg/100g in the root while it was not detected in the bark (Table 2).

Table 2: Quantitative Phytochemical composition of the leaf, bark and root extracts of *Spondias mombin* (mg/100g).

Phytochemical Composition %								
Plant Part	Extraction Media	Tannin	Saponin	Alkaloid	Flavonoid	Phenol	CYG	Sterol
Leaf	Aqueous	3.18±0.02	4.35±0.02	3.91±0.02	1.88±0.01	0.28±0.03	3.66±0.04	0.24±0.02
	Ethanol p-value	3.18±0.02 Ns	4.51±0.07 **	3.77±0.03 **	1.84±0.02 **	0.35±0.01 **	3.46±0.07 Ns	0.29±0.02 **
Bark	Aqueous	2.79±0.07	2.70±0.02	3.54±0.04	1.41±0.02	0.12±0.01	Nd	0.15±0.02
	Ethanol p-value	2.94±0.05 **	2.94±0.05 **	3.53±0.07 Ns	1.33±0.01 **	0.15±0.02 Ns	Nd Na	0.17±0.02 Ns
Root	Aqueous	0.86±0.07	1.31±0.02	1.77±0.04	0.87±0.07	0.095±0.01	0.87±0.07	0.13±0.01
	Ethanol p-value	0.91±0.07 **	1.45±0.02 **	1.91±0.04 **	0.89±0.06 Ns	0.14±0.01 Ns	0.94±0.07 Ns	0.17±0.02 **

**p<0.05 Ns: not significant, Nd: not detected Na: not applicable

Leaf anatomy

Transverse section of the leaf of *Spondias mombin* revealed the presence of dorsiventral epidermis, phloem, xylem, spongy mesophyll and palisade types. The structures were more or less uniform from one surface to the other. Viewed from the surface, the cells of the epidermis were somewhat irregular in outline but closely fitted together

without intercellular spaces. The mesophyll is seen to be differentiated into spongy parenchyma in the centre and palisade on either side. The vascular bundle bordered parenchyma and sclerenchymatous sheath with each bundle made up of xylem and phloem (plate 1).

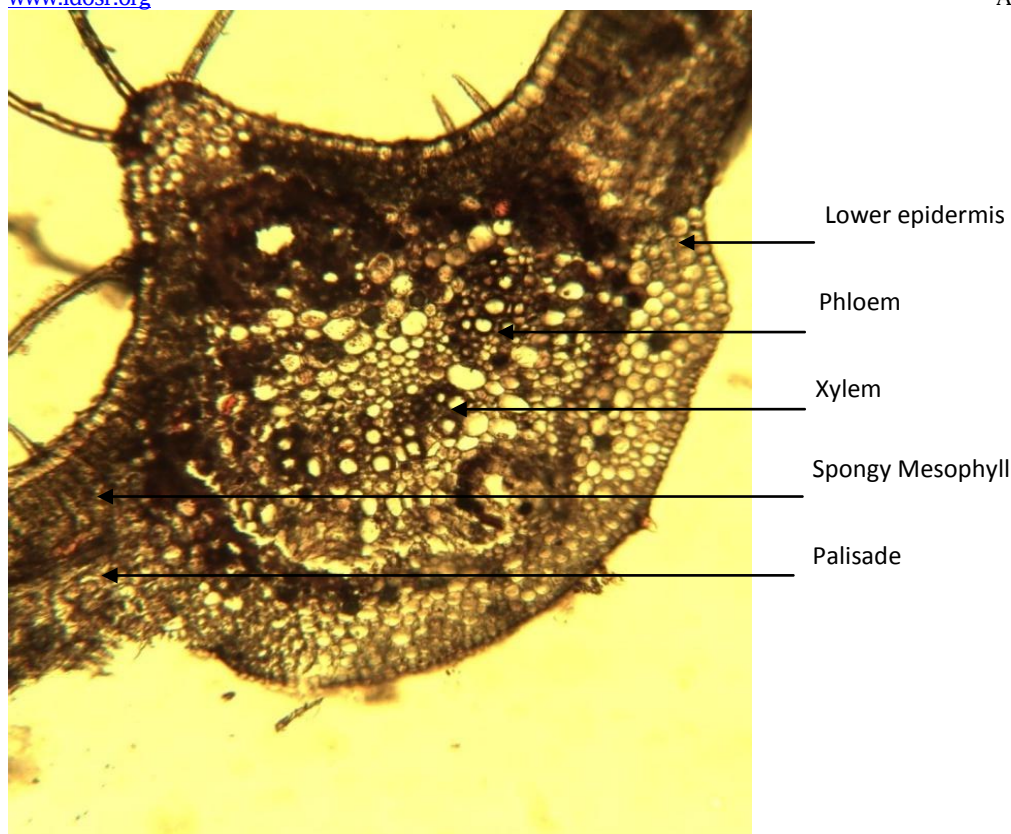
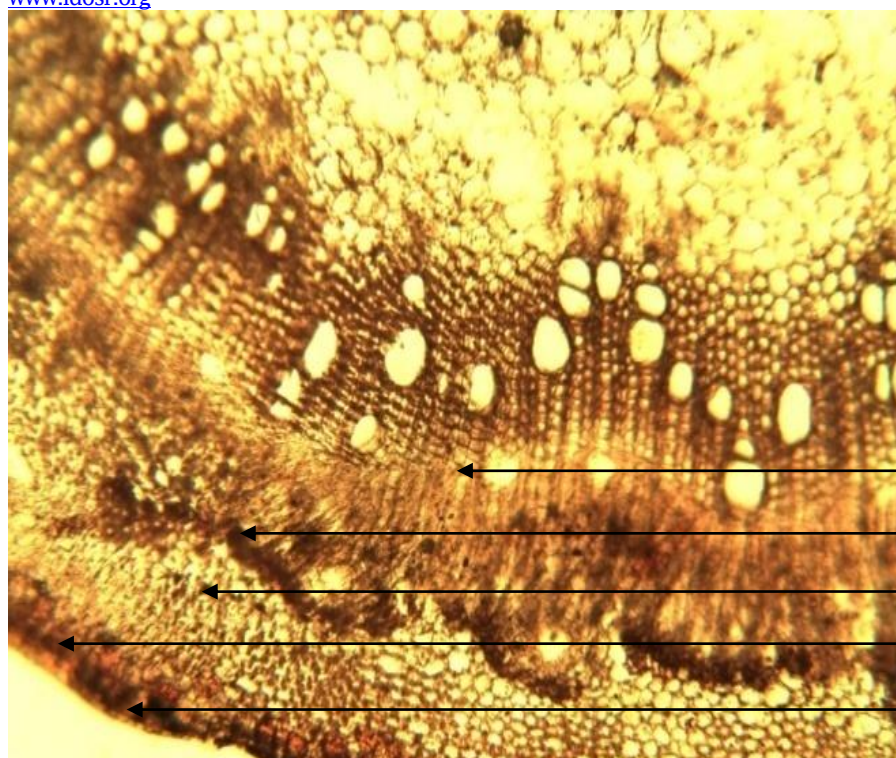


Plate 1: Transverse section of the leaf of *Spondias mombin* x100

The transverse section of the bark revealed the presence of cork cells (replacing the tearing epidermis), the outermost the outermost layer of the cell, flattened tangentially and fitting closely along their radial walls. The cells that cut off on the inner side were

parenchymatous in nature constituting the secondary cortex or phelloderm. Externally, small protrusions on the surface of the stem showed the presence of lenticels- a loose mass of small, thin walled cells. Lenticels may be closed in harmattan hence the presence of cork cells which gets ruptured as the new active season begins (plate 2).



Cortex

Phelloderm

Cork cells

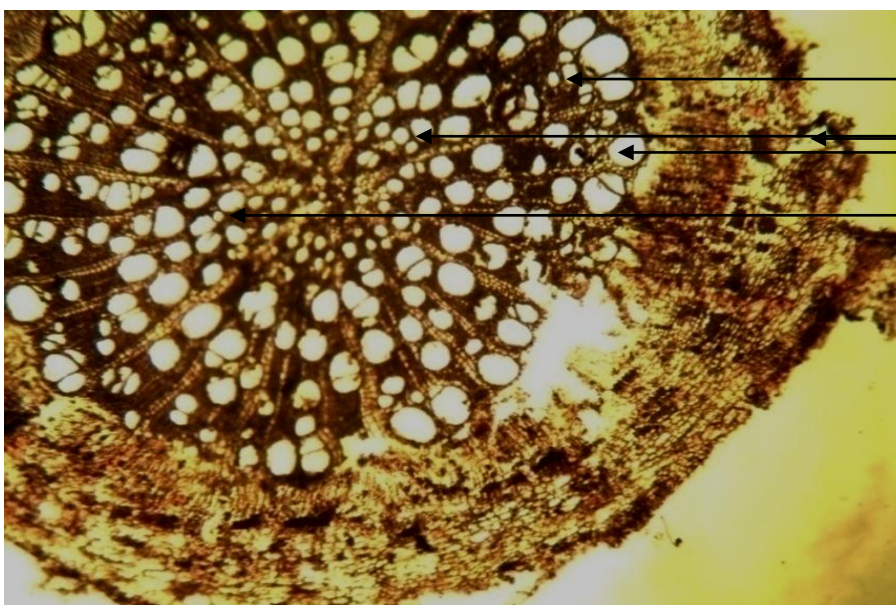
Tearing Epidermis

Lenticels

Plate 2: Transverse section of the bark of *Spondias mombin* x40

The root in transverse section showed secondary growth in thickness due to the addition of new tissues cut off by the cambium and the cork-cambium in the interior as well as in the peripheral region. The section revealed the presence of Epiblema or Piliiferous layer which was a single, outermost layer of the thin-walled cells used for absorption

of water and solutes from the soil. The epiblema in some cases, only short lived and becomes cutinized and form the exodermis of the root. Internally to the endodermis was a single, circular layer- the pericycle that contained abundant protoplasm. The pith occupied a small area in the centre of the root and the medullary ray larger and more prominent in the root enclosing numerous intercellular spaces (plate 3).



Pith rays or medullary ray

Pith

Epidermis

Pericycle

Cortex

Plate 3: Transverse section of the root of *Spondias mombin* x40



Plate 4: *Spondias mombin* leaves in alternate view



Plate 5: Adaxial side of the leaf



Plate 6: Habit photograph of *Spondias mombin*



Plate 7: Fruits of *Spondias mombin* in clusters

DISCUSSION

The study had used phytochemical and anatomical indices to determine the biochemical compounds of *Spondias mombin*, a local plant used in herbal medicinal practice in Nigeria. The results indicated that some of the

phytochemical and nutritive constituents vary ($p < 0.05$) significantly. Alkaloid is one of the main compounds found in hog plum, more on the leaf with appreciable amount in the root and bark. Most of the known functions of

alkaloids were related to protection and to repel parasites and predators. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. The plant contained flavonoids which represent the most common and widely distributed groups of plant phenolics. They are present in the leaf, bark and root. Flavonoids have been reported to be performing biological function of protection against allergies, inflammation, platelets aggregation microbes, ulcer, vineses and tumors [26,27,28,29]. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti- cancer activity. As antioxidants flavonoids provide anti-inflammatory actions this may be the reason behind the use of *Spondias mombin* in the treatment of intestinal troubles in herbal medicine [30]. The plant is also rich in saponin with highest percentage seen in the leaf, bark and root.. Saponins although non toxic can generate adverse physiological responses in animals that consumes them. They exhibited growth inhibition against a variety of cells making them have anti-inflammatory and anti-cancer properties [31,32,33]. That may be the reason why the leaves of *Spondias mombin* are given to expectant ruminant animals and those that delivered without the release of their placenta. Tannin was present in the aqueous and ethanol extract of the leaf. [34], emphasized astringent properties of tannin and their physiological role in the treatment of injuries. [35], reported that tannin found in tropical green leafy vegetables are used for the treatment of intestinal disorders such as diarrhoea and dysentery. *Spondias mombin* contained small amount of phenol both in the leaf, bark and root. [22], studied the total phenolic content of selected Jordanian plant species and established that antioxidant activity was closely correlated with phenolic content. He reported that there is linear relation between antioxidant activity and total phenolic contents. Past research suggests that cyanogenic glucosides can also be found in the fruit, seeds and leaves which can be highly toxic and inconsumable if in a

higher level as in the case of cassava plants [12]. In *Spondias mombin* lower levels of cyanogenic glycoside was traced in aqueous and ethanol extract of the leaf and root but not detected in the bark. *Spondias mombin* contain lower sterol level in the plant parts studied. Various studies on *Pterocarpus Soyansii* and *Pterocarpus santalinoides* had shown lower sterol content with their relationship with such compounds as sex hormones [22]. The transverse section of leaf as shown in plate 1 revealed the presence of dorsiventral epidermis, phloem, xylem, spongy mesophyll, and palisade. The upper epidermis a single layer of cells checks excessive evaporation of water from the surface and protects the internal tissue from mechanical injury. The lower epidermis was meant for the exchange of gases (O_2 , CO_2). The vascular bundle which consists of xylem and phloem lies towards the upper epidermis. The transverse section of the bark as shown in plate 2 revealed the presence of cork cells (replacing the tearing epidermis). The cortex primarily functions as a protective tissue in stems. Its secondary functions are storage, photosynthesis etc. The lenticels may be closed in harmattan by the formation of cork but gets ruptured as the new active season begins. The transverse section of the root as shown in plate 3 revealed epiblema or piliferous layer which was the single, outermost layer of the thin-walled cells. The outer walls of the most of these cells extend outwards and form unicellular root-hairs. This layer was used for absorption of water and solutes from the soil and therefore it has no cuticle. Root hairs increased the absorbing surface of the root [11]. Cortex was also the pumping station of the root where the individual cells act as microscopic pumps by their alternate expansion and contraction, forcing the water absorbed by the root-hairs into the xylem vessels [4]. The medullary ray also transmits water and food material outwards to the peripheral tissues, and was the seat of origin of a strip of cambium prior to secondary growth [5]. All parts of the plants studied have been reported to be medicinally useful and its traditional use in reproduction has been reported. This research had provided comprehensive information on the

phytochemical and anatomical structures with a view to deepen more knowledge of ethnobotanical and non-

medicinal uses of the plant in human and animals.

CONCLUSION AND RECOMMENDATION

The present study has shown the phytochemical and anatomical composition of aqueous and ethanolic extracts of leaf, root and bark of *Spondias mombin*. The presence of flavonoids, alkaloids, saponins, sterols, phenols and tannins were determined. *Spondias mombin* is a powerful plant with many biological compounds that should be respected and taken with caution or according to labelled dosages. The previous reported use of

the plant leaf extract as an abortifacient or labour inducing agent on one hand, and also for prevention of miscarriages on another are mutually exclusive sides of a coin. Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds as well as evaluation of the chemical compounds from the plant for industrial drug composition, indigenous spices and flavouring formulation.

REFERENCES

1. Abo, K.A., Ogunleye, V.O. and Ashidi, J.S. (1999). Antimicrobial potential of *Spondias mombin*, *Croton zambesicus* and *Zygotritonia crocea*. *Journal of Phytotherapy Research* 13: 494-497.
2. Achoba, I., Choba, J.A., Lori, J.A., Elegbede, J.A., Kagbu, J.A (1992). Nutrient composition of black (African) velvet tamarind (*Dialium guineense* willd) seed and pulp from Nigeria. *Journal of Food Biochem.* 16(4): 229-233
3. Adam, K.P. (1999). Phenolic constituents of the fern *Phegopteris connectilis*. *Phytochemistry* 52 (5): 929-934
4. Ademola, I.O., Fagbemi, B.O. and Idowu, S.O. (2005). Antihelmintic activity of extracts of *Spondias mombin* against gastrointestinal nematodes of sheep; studies in vitro and in vivo. *Tropical Animal Health and Production.* 37(3):223-235
5. Burkill, H.M. (1994). *The useful plants of West Tropical Africa*. Vol. 2 Families E-I Royal Botanic Garden, Kew. 605pp.
6. Carnachan, S. M and Harris, P. J. (2000). Ferulic acid is bound to the primary cell walls of all gymnosperm families. *Biochemical Systematics and Ecology* 28 (9): 865-879.
7. Cazarolli, L.H., Zanatta, L., Alberton, E.H., Figueiredo, M.S., Folador, P., Damazio, R.G., Pizzolatti, M.G and Silva, F.R. (2008). Flavonoids: Prospective Drug Candidates. *Mini-Reviews in Medicinal Chemistry* 8 (13): 1429-1440.
8. Choudhary, M. I., Naheed, N., Abbaskhan, A., Musharraf, S. G., Siddiqui, H and Atta-Ur-Rahman (2008). Phenolic and other constituents of fresh water fern *Salvinia molesta*. *Phytochemistry* 69 (4): 1018-1023.
9. Cohn, R and Cohn, A.L. (1996). *The by-products of fruit processing in: Fruit processing*. Chapman & Hall, London, U.K. 220pp.
10. Corthout. J., Pieters, L.A., Claeys, M., Vanden Berghe, D.A. and Viletinck, A.J. (1994). Antibacterial and molluscidal phenolic acid from *Spondias mombin*. *Planta Medica. Journal Chemical Society.* 51 (5): 1237-1241
11. Galeotti, F., Barile, E., Curir, P., Dolci, M and Lanzotti, V. (2008). Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. *Phytochemistry Letters* 1: 44.
12. Han, X., Shen, T and Lou, H. (2007). *International Journal of Molecular Science.* (8),950-988.
13. Harbone, J.B. (1973). *Phytochemical methods; a guide to modern techniques of plant analysis*. Chapman and Hall, New York. 534pp.
14. Khan, Z.I., Hussain, A., Ashraf, M. and McDowell, L.R. (2006). Mineral Status of Soil and Forages in South Western Punjab, Pakistan. *Asian-Australian.*

- Journal of Animal Sciences* 19(8): 1139-1147.
15. Lawson, J.R. (1967). A key to the *Discorea* species in Nigeria. *Journal of West African Science Association*. 12:1-9.
16. Le Bot, J., Adamowicz, S and Robin, P. (1998). Modeling plant nutrition of horticultural crops: a review. *Scientia Horticulturae*. 74, 4782.
17. Leland, J. C. (2006). Natural Products from Plants. Second Edition. - Journal CRC.1-30
18. Marion Kite, R. (2006). *Conservation of leather and related materials*. Butterworth-Heinemann press ltd. 23p.
19. Ostlund, R.E, Racette, S.B and Stenson, W.F (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. *Am. J. Clin. Nutr.* 77 (6): 1385-9.
20. Pedersen, J. A and Ollgaard, B.(1982). Phenolic acids in the genus *Lycopodium*. *Biochemical Systematics and Ecology* 10: 3.
21. Raffauf, R.F (1996). Plant Alkaloids. A guide to their discovery and distribution. Haworth press, Inc., Newyork. 275pp.
22. Tawaha, K., Alali, F.M., Gharaibeh, M.M and El-Elmat. T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem.* 104: 1372-1378.
23. Taylor, L.N.D. (2006). A Handbook of Bioactive Compound from Plants. Database Entry for Ubos- Phytochemical Dictionary. Taylor and Francis, Washington, D.C., U.S.A. 237pp.
24. Thompson, L.U. (1994). *Antioxidant and hormone mediated health benefit of grain critical reviews in food science and nutrition*. Macmillan press London 34:437-497.
25. Tressler, D.K., Van Arsdell, W.B and Copley, M.J (1980). The freezing preservation of foods. AVI Publishing Co. Westport, Conn. 4th Edn. Vol. 23.
26. Waller, G.R and K. Yamasaki. (1996). Saponins used in food and Agriculture; *Advances in experimental medicine and biology*, plenum press, New York. 441pp.
27. Wheeler, G. L., Jones, M. A and Smirnoff, N. (1998). The biosynthetic pathway of vitamin C in higher plants *J. Nutr. Sci* 393: 365-369
28. William, J. (1999). *Invitation to Organic Chemistry*. Jones and Bartlett press . 433pp.
29. Yoo, H. D., Ketchum, S.O., France, D., Bair, K and Gerwick, W. H. (2002). Vidalenolone, a Novel Phenolic Metabolite from the Tropical Red Alga *Vidalia* sp. *Ann. Bot.* 55: 355-380.
30. Zhao, H., J., Grossman, H.B., Hernandez, L.M., Dinney C.P., and Wu, X. (2007). Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. *Int. J. Cancer*, 120: 2208-2213.
31. Zhu, J. K. (2001). Plant salt tolerance. *Trends in Plant Science* 6 (2): 66-71

APPENDIX 1: PHYTOCHEMICAL COMPOSITION (%)

N	MEAN						
	TANNIN	SAPONIN	ALKALOID	FLAVONOID	PHENOL	CYG	STEROL
LEAF	3.17	4.38	3.92	1.86	0.28	3.62	0.23
AQU	3.19	4.32	3.89	1.90	0.28	3.70	0.25
	3.18	4.35	3.91	1.88	0.28	3.66	0.24
ETH	3.16	4.50	3.74	1.82	0.34	3.50	0.29
	3.19	4.52	3.80	1.85	0.36	3.42	0.29
	3.18	4.51	3.77	1.84	0.35	3.46	0.29
BARK	2.78	2.70	3.48	1.39	0.12	ND	0.15
AQU	2.80	2.70	3.60	1.42	0.12	ND	0.15
	2.79	2.70	3.54	1.41	0.12	ND	0.15
ETH	2.94	2.92	3.45	1.32	0.13	ND	0.17
	2.94	2.96	3.60	1.34	0.16	ND	0.16
	2.94	2.94	3.53	1.33	0.15	ND	0.17
ROOT	0.86	1.29	1.73	0.84	0.09	0.84	0.12
AQU	0.86	1.32	1.80	0.89	0.10	0.90	0.14
	0.86	1.31	1.77	0.87	0.095	0.87	0.13
ETH	0.90	1.45	1.92	0.88	0.14	0.95	0.17
	0.92	1.45	1.89	0.90	0.14	0.92	0.17
	0.91	1.45	1.91	0.89	0.14	0.94	0.17