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Phytochemical Analysis of Spondias Mombin

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Abstract:
Objective: Evaluation of Spondias mombin leaves for photochemical, vitamins and minerals constituents.
Methods: phytochemical analysis was carried out by method of Sofowora(1993),quantitative identification of tannins Bungard(1998),test for saponins by method of scott(2000),cyanogenic glycoside by alkaline colourimeter method.
Results:Phytochemical screening and subsequent quantification using standard methods revealed the presence of bioactive compounds saponins (4.80%), alkaloids (18.32%), flavonoids (12.84%), tannins (1.24mg/100ml) and phenol (0.53mg/100ml), Vitamin results showed the plant leaves contained ascorbic acid 18.75mg100^{-1}g; Niacin 2.75mg100^{-1}g. Riboflavin 0.35 mg100^{-1}g and Thiamine 0.06 mg100^{-1}g. Mineral analysis revealed in the plant leaves, K 2.66%, Mg 0.455%, Na 0.100%, Ca, 1.410% and P, 0.300%. in leaves and the fruit showed the presence saponins (4.80%), alkaloids (9.0%), flavonoids (2.84%), tannins (1.24mg/100ml) and phenol (0.08mg/100ml). The results showed that the leaves and fruits are rich in nutrients, especially carbohydrate.
Conclusion: The findings indicate that S. mombin leaves are a potential source of highly nutritious feed stuff and useful drug formulation. They are of nutritional, clinical and veterinary relevance considering the diverse ethno pharmacological uses of the plant in different parts of the world.

Keywords*: phytochemicals: vitamins; bioactive; drug; minerals*

1. Introduction

Spondias mombin Linn is a fructiferous tree that belongs to the family Anacardiacae. It grows in the coastal areas and in the rain forest into a big tree of up to 15–22mm in height. It is readily common in Nigeria, Brazil and several other tropical forests of the world with high genetic variability among populations (1,2,3,4,5,6). It is called Hog plum in English, *akika* in Yoruba, *ijikara* in Igbo, *tsader maser* in Hausa, *chabbuli* in Fulani and *nsukakara* in Efik (7,8).

The leaves bark and fruit juices of the plant have been widely used for both medicinal and non-medicinal purposes. The tree is commonly used for living fences, in farmlands and shelter by artisans. The fruits are edible. The extracted juice is used to prepare ice cream, cool beverages and jelly in Costa Rica and Brazil. In Amazon, the fruit is used mainly to produce wine sold as 'Vinho de Taperiba', while in Guatemala; it is made into a cider-like drink. It is used in Panama, Peru and Mexico in fairly large quantities as jams (9,10,11). Thus, it has been evaluated as an unconventional source of vitamins A and C (12,13).

All parts of the tree are ethnopharmacologically important. A tea of the flowers and leaves is taken to relieve various inflammatory conditions, stomachache and has wound healing potential (14,15,16). In a recent review, (17,18,19) reported several activities that have been associated with the plant extracts. Some reported pharmacological activities include antibacterial (20,21), antiviral (22), anti-microbial (23), anti-malarial (24), anti-helmintic (25), molluscicidal (26), anti-diarrhoea (27), antiinflammation , haemostatic (28), abortifacient (29), purgative (30), hypnotic (31), wound-healing , enzyme inhibition (32), increases capillary permeability (33), and anti-free radical, anti-aging and reduces glutathione synthesis (34). It has also been reported to have blood lipid-lowering activity (35).

The chemistry of this plant has been reported, although in piece-meals (36). Thus, the reported effects observed with the plant's extracts has been attributed to its constituent compounds of phenols, tannins, anthraquinones and flavonoids (37), as well as caffeoyl ester , 6-alkenylsalcyclic acids (38), and alkaloids, proanthocyanins and saponins (39). The associated link between the compositions of this world-wide cultivated plant and the reported medicinal and non-medicinal uses prompted this study.This work was aimed to evaluate the phytochemical, proximate and mineral contents of the leaves of *S. mombin* with a view to either support or debunk the traditional claims of using this plant in the management of illness.

2. Materials and Methods

2.1. Sample Collection and Preparation:

Fresh leaves and fruits of *S. mombin* were collected from their natural habitat in Okposi Oburu, Ebonyi State of Nigeria, in the month of September, 2012. They were authenticated by Dr. S.E. Okeke, a plant taxonomist of the Department of Plant Sciences and Biotechnology, Imo State University, Owerri, Nigeria and air-dried to a constant weight. The dried leaves and fruits were ground into a fine powder using a mechanical grinder, packaged in glass jars and stored at 4^0 C until analysis (40).

2.2. Extraction Procedure

The leaves and fruits were rinsed in clean water and were air dried to constant weight at 60^0C in a laboratory oven. They were later ground into fine powder with the aid of a clean dry electric grinder (ED-5 Arthur Thomas, USA). A 100g portion of the ground leaves was soaked in 100 ml of water for 12 hours, filtered and then exhaustively extracted with the aid of soxhlet extractor (Gallenkamp, England). The solvent in the extract was then distilled off in a distillatory and evaporated to dryness at 40^0C. The solid extract was placed in a sterile container, labeled and stored at 4^0C in a refrigerator from where portions were taken for the different studies (41).

2.3. Phytochemical Analysis

Prior to quantitative phytochemical analyses, phytochemical screening was carried out on the plant's leaf and extract using standard methods as described by (42) and (43). The percentage compositions of saponins, tannins, alkaloids, flavonoids and cyanogenic glycosides were determined.

2.4 Qualitative Identification of Tannins

This was carried out as described by (44). An aqueous extract of the sample and the ethanol extract was obtained dispersing 2g of the sample in 10ml of distilled water and ethanol the mixture was shaken for 30mins in a mechanical shaken and filtered through Whatman No 42 filter paper. The filtrate was used as the aqueous and ethanol extract. An aliquot (2ml) of filtrate was measured into a test tube and 3ml of distilled water the tannin content was and ethanol were added to it. This was shaken gently to mix well and 2 drops of kinetic chloride solution was added. A dark precipitate was indicative of the presence of tannins in the test sample. The test was conducted against a blank control consisting of water and ferric chloride. The result is presented in Table 1. Calculated as shown below % Tannin =

$$\frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{Vf}{Va} \times D$$

- W = Weighing of sample analysed
- AU = Absorbance of the test sample,
- AS = Absorbance of the Standard Tannin Solution
- C = Concentration of the standard in mg / ml
- Vf = Total filtrate volume
- Va = Volume of filtrate analysed
- D = Dilution factor where applicable

2.5. Test for Saponins

Using (45) method, the sample was subjected to the froth test. A quantity of the aqueous and ethanol extract (2ml) was mixed with 5ml of water and ethanol in a test tube. The mixtures were shaken and the presence of a steady froth observed was taken as indicative of the presence of saponins.

$$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times \frac{100}{1}$$

- W1 = Weight of Empty Evaporation Dish,
- W2 = Weight of dish + Saponin Extract

2.6. Test for Flavoniods

The aqueous and ethanol extracts (2ml) was dispersed into a test tubes and a few drop of bench sodium hydroxide solution was added to it. The formation of a yellow colouration indicative the presence of flavonoids. The presence of flavonoids was confirmed by adding magnesium turnings and a few drops or concentration HCL to each extract both the aqueous and ethanol extract. The appearance of magenta red colour showed the presence of flavonoids. The presence of flavonoid was further confirmed by adding a few drops conc. H_2SO_4 to the yellow solution obtained above the decolourisation of the solution confirmed the presences of flavonoids in each case of blank was carried out as a control(46)

$$\% \text{ Flavonoids} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times \frac{100}{1}$$

- W1 = Weight of Empty Evaporation Dish,
- W2 = Weight of dish + flavonoid extract.

2.7. Test for Alkaloids

The presence of alkaloids in the test sample was investigated using the Mayers and Wagners reagents separately. The processed sample (2g) was weighed into a small boiling tube and 20ml of 5% ethanolic sulphuric acid solution was added to it. The mixture was boiled in a water bath for 5minutes after cooling it was filtered through Whatman No 42 filter paper. A 2ml aliquot of the filtered was dispersed into a test tubes in duplicates for each sample both ethanol and aqueous extract while 2ml of distilled water and ethanolic H_2SO_4 reagent blank were placed in a third and fourth test tubes respectively as controls. To the first tube, 3 drops of Wagners reagent were added. Also 2 drops of Wagners reagents were added to one of the control test tubes. To the second portion in the test tube, 2 drops of Mayers reagents were added the same was also done for the second control test tube. The formation of an orange precipitate in the test sample showed the presence of alkaloids.

$$\% \text{ Alk.} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times \frac{100}{1}$$

- W1 = weight of empty filter paper
- W2 = weight of paper and alkaloids precipitate

2.8. Test for Cyanogenic Glycoside (HCN)

The sample was subjected to HCN using the alkaline picrate colourimeter method described by (47). Stripes of filter paper were cut from Whatman No 1 filter paper. The alkaline picrate solution was prepared by dissoluting 1g of small volume of minimally warm water the volume was made up to 200ml with distill water and ethanol for the ethanol extract and dissolution water for aqueous extract the picrate paper was prepared by dipping rectangular pieces of filter paper in a saturated (0.05ml) aqueous picric acid previously neutralized and filtered. The stripes were kept in an oven to dry. Meanwhile, 2g of each sample of ethanol and aqueous extract was mixed with 5ml of distilled water and ethanol in a test tube. A stripe of alkaline picrate paper was carefully lung in the test tubes, taking care not to let the paper touch the simple mixture.

The paper was held in a position with aid of a cork which was used to close the mouth of the test tube. A blank control consisting of the picrate paper hanging over 5ml of distilled water and ethanol was set up. All the tubes were incubated for 18hrs (overnight) and observed for colour change the formation (or development) of an orange colour indicated the presence of HCN.

$$\% \text{ HCN} \quad \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{100} \times D$$

While W = weight of sample analyzed,

- Au = absorbance of the test sample,
- As = absorbance of the test standard HCN solution,
- C = concentration of the standard in mg/ml,
- D = dilute factor where applicable.

2.9. Test for Phenols

2mls of the extracts was mixed with 5mls of water. This mixture was soaked and filtered. Neutral ferric chloride solution was added to the sample and observed to fro green, blue and or black coloration by (48). According to methods described by (49). Oxalate content was determined by the spectrophotometric methods of (50).

3. Results

3.1. Quantitative analysis of fruits and leaves of Spondias mombin

Quantitative phytochemical screening in revealed the presence of saponins, alkaloids, flavonoids, tannins and cyanogenic glycosides, while their quantitative estimations in the leaf gave saponins (11.96%), alkaloids (18.32%), flavonoids (12.84%), tannins (1.24mg/100ml) and phenol (0.53mg/100ml) whereas in the fruit the following phytochemicals were recorded: saponins (13.41%), alkaloids (9.0%), flavonoids (2.84%), tannins (1.24mg/100ml) and phenol (0.08mg/100ml) as shown in Figure 1 below.

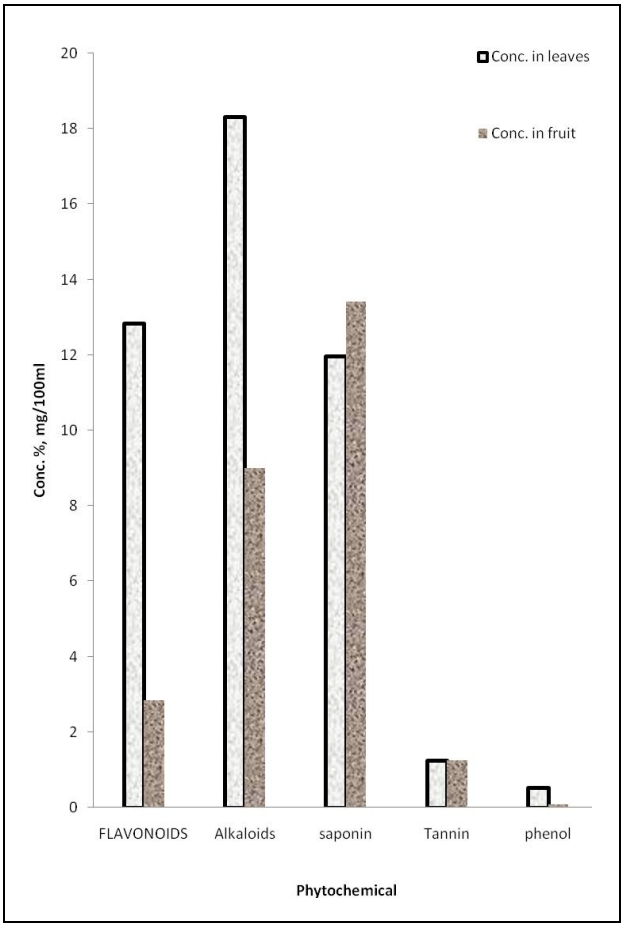

Figure 1: Phytochemical quantitative analysis of fruits and leaves of Spondias mombin

3.2. Phytochemical constituents of leave and fruits of Spondias mombin

Carbohydrates were found to be present in very high concentration in the leaves extract while the fruit contains a trace concentration. Reducing sugar is present in the leaf in trace concentration while absent in the fruit. Protein is absent in both the leaf and fruit of *Spondias mombin.*

The following phytochemicals tannin, flavonoids, glycosides, alkaloids, steroids, terpenoids, resins and oil were found to be present in the leaves and fruits of *Spondias mombin* in different concentration as shown in Table 1 below.

s/n	Constituent	Relative presence in leave extract	Relative presence in fruits extract
1.	**Carbohydrates**		
a.	Carbohydrates	++	+
b.	Polysaccharide	-	-
c.	Reducing sugar	+	-
2.	**protein**		
		-	-
a.	Protein	-	-
b.	α-amino acid	-	-
C	Aromatic amino acid	-	-
D	Tyrosine **Phytochemical**	-	-
3.	Tannin		
4.	Flavonoids	+++	+++
5.	Glycosides	+++ -	- -
6.	Alkaloids	+	-
7.	Steroids	++ ++	- -
8.	Terpenoids	+++	-
9.	Resins	-	+++
10.	Oil	+++ +++	+++ +++

Table 1: Phytochemical constituents of leave and fruits of Spondias mombin

Key

- +++ Present in very high concentration
- ++ Present in moderate concentration
- + Present in trace concentration
- \- Absent

4. Discussion and Conclusion

4.1. Discussion

S. mombin leaves and fruits are among the forages given to domestic animals in SouthEastern Nigeria. The young leaves are also cooked as green vegetables (51). The present study shows that high contents of saponins (4.80%), alkaloids (18.32%) and flavonoids (12.84%) were observed in the leaves while the fruits contains saponins (4.80%), alkaloids (9.0%) and flavonoids (2.84%) of the plant. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Saponin is a known anti-nutritional factor that can reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intra lumenal physicochemical interaction or other yet unidentified activity (52). This may account for the non-significant serum lipid-lowering effect observed with ingestion of *S. mombin* leave and fruit extract in our previous studies (53). Alkaloids are beneficial chemicals to plants. They help in repelling predators and parasites. However, when ingested by animals, they affect glucagon, thyroid stimulating hormone and inhibit certain mammalian enzymic activities (54). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity (55). The content of these phytochemicals may be associated with the reported oxytocic and abortifacient activity of the plant's leaf extract (56). The plant leaves and fruits also contain flavonoids, which are phenolic compounds that serve as flavouring ingredients of spices and vegetables (57). Flavonoids and other phenolic derivatives have been identified in *S. mombin* leaves with anti-herpes, antioxidant and anti-aging properties (58). Furthermore, flavonoids, alkaloids and tannins observed in the plant had lower concentrations and have been associated with the observed antimicrobial effects in various studies involving plant extracts (59). Their presence in *S. mombin* may account for the plant's reported anti-microbial, anti-bacterial and molluscicidal , anti-viral, anti-malarial and anti-helminthic (60) activities.

The major nutritional compositions of *S. mombin* leaves and fruits were found to be carbohydrates and proteins was absent. The good distribution of nutrients in the leaves and fruits may explain its use as one of the forage feed given to domestic animals. When compared with some other common vegetables domestic animals graze on, *S. mombin* leaves and fruits contain fairly good quantities of carbohydrates as in table 1. When compared to the common vegetables cited above as well as *Vernonia amygdalina* (61). This may justify the earlier effort at evaluating the plant's use as an unconventional source of vitamins A and C in the production of juices, ice

creams and jellies and help to ward off infections, while vitamin A helps to maintain good sight and prevents certain diseases of the eye. Both vitamins also have antioxidant properties and may protect against some forms of cancer (62).

4.2. Conclusion

In conclusion, the study has revealed that leaves of *S. mombin* are potential source of pharmacologically active phytochemicals. It has also shown the leaves and fruits as an important source of nutrients for domestic animals.

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