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Quantification of Phytochemical Constituents of Ethanol Yellow *Spondias mombin* Leaf Extract in Ogba/Ebgema/Ndoni Local Government Area of Rivers State, Nigeria

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ABSTRACT: Phytochemicals are naturally occurring compounds in plants that have been shown to possess various medicinal activities, making them of great interest for their potential health benefits. Hence, the objective of this paper was to investigate by quantifying the phytochemical constituents of ethanol leaf extract of yellow mombin fruit (Spondias Mombin) collected from Omoku, in Ogba/Ebgema/Ndoni Local Government Area of Rivers State, Nigeria using appropriated standard methods. The data obtained show that the concentration flavonoid in the ethanol leaf extract of yellow mombin fruit (Spondias Mombin) was 144.34 mg/100g, while Alkaloid, Cyanogenic glycosides and Phenolic content were 169.42 mg/100g, 123.74 mg/100g and 218.06 mg/100g respectively. These results indicate that Spondias mombin leaves are rich in bioactive compounds, particularly phenols, which are known for their potent antioxidant properties. The significant presence of alkaloids suggests potential antimicrobial activity, while the flavonoids contribute to anti-inflammatory benefits. Cyanogenic glycosides, although present in lower concentration, necessitate careful consideration due to their potential toxicity. Overall, this quantitative analysis supports the traditional use of Spondias mombin in herbal medicine and underscores the need for further research to explore its full pharmacological potential and ensure safe usage.

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Keywords*:* medicinal properties, bioactive compounds, antioxidant, antimicrobial, anti-inflammatory

Phytochemicals are naturally occurring compounds in plants that have been recognized for their significant therapeutic and medicinal properties. These phytochemicals include flavonoids, alkaloids, phenols, tannins, saponins, cyanogenic glycosides, etc, each contributing uniquely to the health benefits offered by plants (Abbiw *et al*., 2018). The exploration and quantification of these compounds in various plants are critical for understanding their potential in traditional and modern medicine (Balick *et al*., 1996). The hog plum or yellow mombin botanically referred to as *Spondias mombin*, is a tropical tree belonging to the Anacardiaceae family. This plant is widely distributed across tropical regions, particularly in Africa, South America, and parts of Asia. *Spondias mombin* has a rich history in traditional medicine, where various parts of the plant, including the leaves, bark, and fruits, have been used to treat a myriad of ailments such as gastrointestinal disorders, infections, inflammation, and fever. Despite its extensive use in folk medicine, there remains a need for scientific validation of its phytochemical constituents and their associated benefits (Middleton *et al*., 2000). The leaf extract of *Spondias mombin* has garnered particular interest due to its reported medicinal properties. Previous studies have indicated the presence of several bioactive compounds in the leaves, suggesting a correlation between these

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compounds and the plant's therapeutic effects. However, quantitative data on the concentration of key phytochemicals within *Spondias mombin* leaves is limited (Elujoba *et al*., 2005; Ayomide *et al*., 2022). Flavonoids are a diverse group of plant metabolites that are well-known for their antioxidant, anti-inflammatory, and antimicrobial activities (Singleton *et al*., 1965). They play an important role in the plant's defense mechanisms and contribute to the prevention of various human diseases, including cardiovascular diseases and cancers. Alkaloids, another significant class of phytochemicals, are nitrogen-containing compounds that display a wide range of pharmacological activities such as analgesic, antimalarial, and anticancer properties. Phenols are aromatic compounds with potent antioxidant properties, protecting cells from oxidative stress and reducing the risk of chronic diseases. Cyanogenic glycosides, though toxic in large amounts, have been used in controlled quantities for their potential therapeutic effects, including muscle relaxant and anti-hypertensive properties (Ross *et al*., 2002). By quantitatively analyzing these phytochemicals, this study aims to provide a comprehensive profile of the bioactive molecules present in *Spondias mombin* leaves. The findings are expected to contribute to the understanding of the plant's medicinal properties and support its use in traditional medicine. Furthermore, this study seeks to lay the groundwork for future research into the therapeutic potential of *Spondias mombin* and its phytochemical constituents (Rice-Evans *et al*., 1995). Through rigorous scientific analysis, we can bridge the gap between traditional knowledge and modern medicine, ensuring the safe and effective use of plant-based treatments (Chang *et al*., 2002). Hence, the objective of this paper was to investigate by quantifying the phytochemical constituents of ethanol leaf extract of yellow *Spondias Mombin* collected from Omoku, In Ogba/Ebgema/Ndoni Local Government Area of Rivers State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Identification: Fresh leaves of *Spondias mombin* (Ichikara) used in this study included were collected from Omoku, in Ogba/Ebgema/Ndoni Local Government Area of Rivers state, Nigeria. Taxonomical identification and authentication were carried out at the Department of Plant Science, University of Port Harcourt, Rivers State, Nigeria. Analytical grade chemicals were used in this study.

Spondias mombin Leaf Extract: Fresh leaves were plucked from *Spondias mombin* tree plants and washed 4 times with tap water and finally three times with distilled water. The washed leaves were dried for 48 hr to remove residual moisture. Dried leaves were ground to powdery form and kept for further use. *Spondias mombin* (50 g) was soaked in 50 ml of absolute ethanol and extracted using cold maceration method. It was then was percolated in 500 ml of 99% ethanol in 1-L conical flask and stored for 3 days with constant shaking. The mixture was then filtered using a funnel and Whatman filter paper No. 1. The mixture was evaporated using rotary evaporator and the extracts recovered were stored at 4 ^{o}C in a refrigerator (Sofowora, 1993).

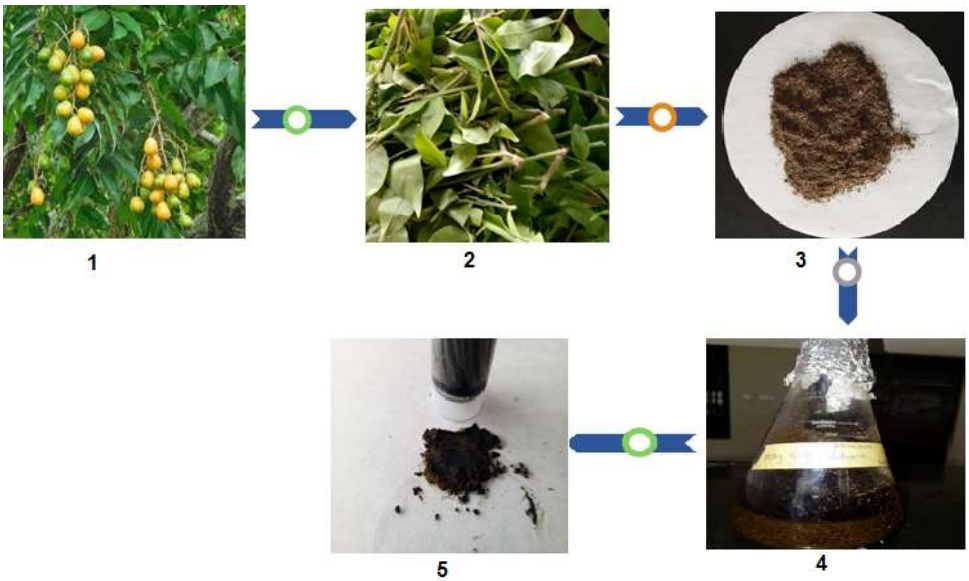

Fig 1: Stages of *Spondias mombin* (*Sm*) leaf processing in this study
*Key: **1** = Spondias mombin Tree; **2** = Fresh Sm-Leaf; **3** = Ground Sm-Leaf; **4.** Soaked Sm-Leaf; **5** =Sm extract*

Percentage Moisture Content of Spondias mombin Leaf: The percentage moisture content for *Spondias mombin* leaf extract was determined by weighing the sample when wet. The weight recorded was 145.8 g. The sample was dried to ensure that there is no moisture content and 32.18 g was obtained. The moisture content was determined using the formula in equation 1.

$$\%\ \text{miosture content} = \frac{\text{weight of dry sample}}{\text{weight of the wet sample}} \times 100 \quad (1)$$

Percentage Yield of Spondias mombin Leaf: The percentage yield of *Spondias mombin* extract was determined using equation 2.

$$\%\ \text{yield of the extract} = \frac{\text{weight of extract}}{\text{weight of the sample}} \times 100 \quad (2)$$

Quantitative Phytochemical Test

Estimation of Alkaloids: Five milliliters of phosphate buffer at pH 4.7 was added 1 ml of the test extract, followed by 5 ml of Bromocresol Green (BCG) solution. The mixture was shaken with 4 ml of chloroform. The resulting extracts were collected in a 10 ml volumetric flask and then diluted to the mark with chloroform (Zhu *et al*., 2021). The absorbance of the complex in chloroform was measured at 470 nm against a blank prepared in the same manner but without the test extract. Atropine was used as a standard material, and the assay results were compared with Atropine equivalents (Yadav *et al*., 2022).

Estimation of Flavonoids: The total flavonoid content was determined using the Aluminum chloride method with catechin as a standard. One milliliter of the test sample and 4 ml of water were added to a volumetric flask with a total volume of 10 ml. After 5 min, 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminum chloride were added (Magwaza *et al*., 2020). Following a 6-min incubation at room temperature, 2 ml of 1 M sodium hydroxide was added to the reaction mixture. The final volume was then immediately adjusted to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank using a spectrophotometer. The results were expressed as catechin equivalents, calculated in mg catechin per g of dried extract (Kim *et al*., 2022).

Estimation of Phenols: The total phenolics content in various solvent extracts was determined using the Folin-Ciocalteus reagent (FCR). Two milliliters of filtrate were combined with 0.4 ml of FCR, which had been diluted to 1:10 (v/v). After a waiting period of 5 min, 4 ml of sodium carbonate solution was added. The final volume of the tubes was adjusted to 10 ml with distilled water, and the mixture was left to stand for 90 min at room temperature (Wabaidur *et al*., 2020). Absorbance of the samples was measured against a blank at 750 nm using a spectrophotometer, specifically the model (Apel 3000UV). A calibration curve was constructed using catechol solutions as standards, and the total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight based on the standard plot (Podolina *et al*., 2019).

Estimation of cyanogenic glycosides: One milliliter extract from each plant part was mixed with 10 ml of freshly prepared Baljit's reagent, which consists of 95 ml of 1% picric acid and 5 ml of 10% NaOH (Goldstein *et al*., 1985). After 1 hr, the mixture was diluted with 20 ml of distilled water, and the absorbance was measured at 495 nm using a Shimadzu UV-VIS spectrophotometer model 160A (Kyoto, Japan) (Adsersen *et al*., 1988).

RESULTS AND DISCUSSION

Recent scientific studies have established (Kumar *et al.*, 2023) that a relationship between the consumption of phytochemicals such as carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, polysaccharides, etc., with health benefits such as prevention of diabetes, obesity, cancer, cardiovascular diseases, etc. This has led to the popularization of phytochemicals. Nowadays, foods containing phytochemicals as a constituent (functional foods) and the concentrated form of phytochemicals (nutraceuticals) are used as a preventive measure or cure for many diseases. The health benefits of these phytochemicals depend on their purity and structural stability. The yield, purity, and structural stability of extracted phytochemicals depend on the matrix in which the phytochemical is present, the method of extraction, the solvent used, the temperature, and the time of extraction. Hence, the phytochemical constituents of ethanol leaf extract of yellow mombin fruit (*Spondias Mombin*) collected from Omoku, in Ogba/Ebgema/Ndoni Local Government Area of Rivers State, Nigeria was carried out. The quantitative phytochemical and UV analyses for *Spondias mombin* leaf extract were summarized in Table 1 and Figure 2.

Tab. 1: Quantitative phytochemical analysis

Phytochemicals	Result
Flavonoid (mg/100g)	144.34
Alkaloids (mg/100g)	169.42
Cyanogenic Glycosides (mg/100g)	123.74
Phenols (mg/100g)	218.06

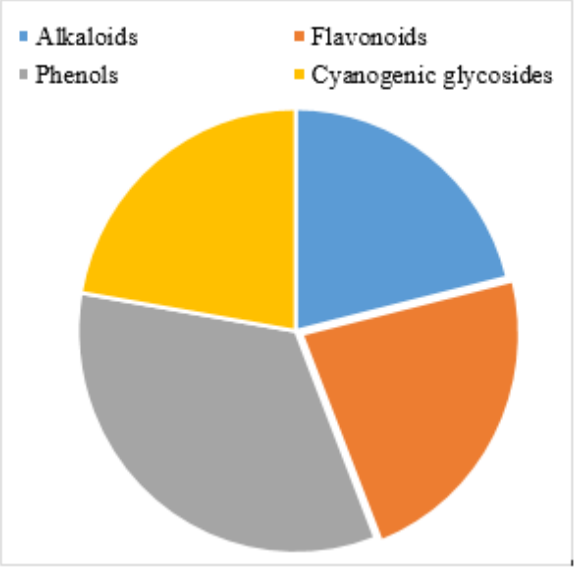

Fig 2: Ultra-Violet visible spectrum composition of Sm-extract

The study provides a comprehensive analysis of the phytochemical content in the leaf of *Spondias mombin*, highlighting significant concentrations of flavonoids, alkaloids, phenols, and cyanogenic glycosides. The percentage moisture content of *Spondias mombin* leaves at 22.50% reveals that a notable portion of the leaf mass was water, which was important for understanding the drying process and the potential for spoilage if not properly dried and stored.

The percentage yield of the extract at 78.90% demonstrates a highly effective extraction process, yielding a significant amount of material from the original leaf sample. This efficiency is beneficial for applications requiring concentrated extracts, such as in medicinal or nutritional uses. The high yield also suggests that *Spondias mombin* leaves are rich in extractable compounds, which might have significant practical applications.

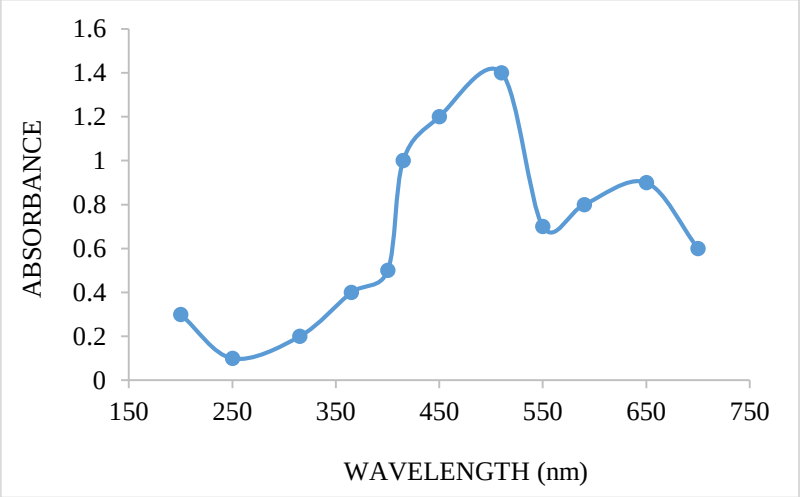

Fig 3: Ultra-violet visible spectrum of flavonoids composition of Sm-extract

The presence of 144.34 mg/100g of flavonoids in the leaf extract of *Spondias mombin* in Figure 3, indicates that this plant could be a significant source of natural antioxidants. This level of flavonoid content supports its traditional use in treating inflammation and suggests potential for preventing oxidative stress-related diseases like cardiovascular diseases and cancers. The use of the Aluminum chloride method for quantification reinforces the accuracy of this measurement, highlighting the plant's substantial levels of beneficial compounds.

Comparatively, recent studies have shown varying flavonoid contents in different plant species. For example, microwave-assisted extraction from *Alpinia oxyphylla* leaves resulted in a total flavonoid content of 28.24% under optimal conditions (Liga *et al*., 2023). Another study on *Populus* species revealed different flavonoid contents, with red poplar varieties having higher levels of quercetin, rhamnetin, isorhamnetin, and kaempferol compared to their green counterparts (Yang *et al*., 2023). These comparisons indicate that *Spondias mombin* has a relatively high flavonoid content, making it a competitive source of natural antioxidants in comparison to other plants.

Overall, the significant flavonoid content in *Spondias mombin* underscores its potential health benefits and aligns with findings from other studies on the antioxidant properties of flavonoid-rich plants (Liga *et al*., 2023; Yang *et al*., 2023).

The study found a substantial amount of alkaloids (169.42 mg/100g) in the leaf extract of *Spondias*

mombin as shown in Figure 4, suggesting potential antimicrobial, analgesic, and antimalarial properties. This aligns with traditional uses of the plant.

Comparatively, a study on *Cassia alata* showed alkaloid content of 137.23 mg/100 g, which was significantly lower than *Spondias mombin* (Aileen *et al*., 2022). Another study on *Lannea egregia* reported notable cardioprotective effects, emphasizing the therapeutic potential of alkaloid-rich extracts (Aileen *et al*., 2022). These comparisons highlight *Spondias mombin* as a particularly potent source of alkaloids with promising medicinal applications.

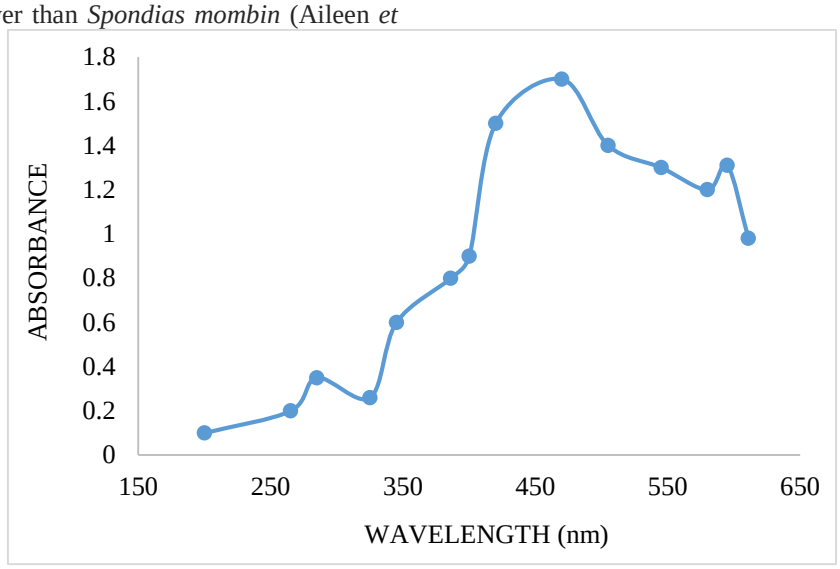

Fig 4: Ultra-violet visible spectrum of alkaloids composition of Sm-extract

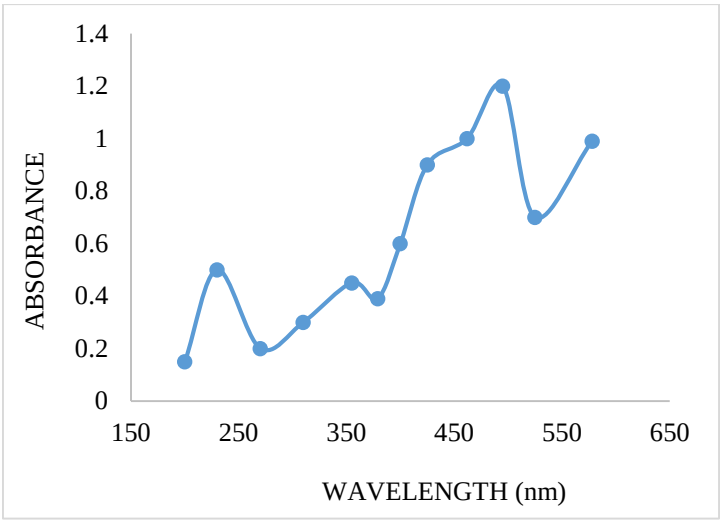

Fig 5: Ultra-violet visible spectrum of cyanogenic glycosides composition of Sm-extract

The study identified a cyanogenic glycoside content of 123.74 mg/100g in *Spondias mombin* leaves as shown in Figure 5, which, though relatively low, was still significant due to the potential release of hydrogen cyanide upon metabolism. This finding aligns with other studies that have explored the presence of cyanogenic glycosides in edible plants and their associated risks and benefits.

For instance, cassava, a staple crop in many parts of the world, contains cyanogenic glycosides ranging from 75 to 1000 ppm, depending on various factors such as plant variety and processing methods. Improperly processed cassava can lead to cyanide toxicity, highlighting the importance of adequate preparation to ensure safety (Islamiyat *et al*., 2016). Similarly, studies on bamboo shoots have reported varying levels of cyanogenic glycosides, with concentrations of up to 105 mg/kg in some varieties (Islamiyat *et al*., 2016). The method used in the current study involving Baljit's reagent was a reliable technique for quantifying cyanogenic glycosides,

providing consistent results comparable to those found in other plants known for their cyanogenic content. These findings underline the need for proper processing and handling of *Spondias mombin* leaves to mitigate potential toxicity while harnessing their therapeutic benefits (Jonathan., 2019; Meri and Christian., 2021).

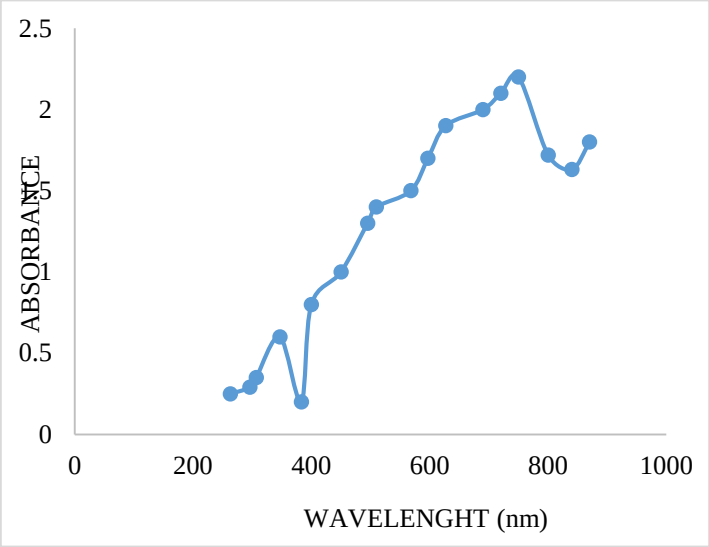

Fig 6: Ultra-violet visible spectrum of phenols composition of Sm-extract

The study found a high phenolic content of 218.06 mg/100g in *Spondias mombin* leaves as represented in Figure 6, suggesting significant antioxidant potential. This level was comparable to or higher than those found in other medicinal plants. For instance, recent research on various medicinal plants have reported total phenolic contents ranging from 113.47 mg GAE/g to over 311.6 mg GAE/g in different species using similar Folin-Ciocalteus methods (Mehmood *et al*., 2022). These comparisons highlight *Spondias mombin* as a particularly potent source of phenolic compounds, which can protect against cellular damage and reduce the risk of diseases like cancer and cardiovascular conditions.

The Folin-Ciocalteus reagent method used for quantification was a standard procedure, ensuring accurate measurement of phenolic content. These findings strongly support the plant's traditional use for its medicinal properties.

Conclusion: The study revealed significant concentrations of various bioactive compounds. The high phenolic content suggests strong antioxidant potential, which can help in protecting cells from oxidative stress and reducing the risk of chronic diseases. The substantial alkaloid concentration indicates potential antimicrobial, analgesic, and antimalarial properties. Flavonoids contribute to antioxidant, anti-inflammatory, and antimicrobial benefits, further validating the medicinal value of *Spondias mombin*. However, the presence of cyanogenic glycosides, although in lower concentration, necessitates careful consideration due to their potential toxicity. Overall, this study provides a scientific basis for the traditional medicinal uses of *Spondias mombin* leaf and highlights its potential for further pharmacological research and development into modern therapeutic agents.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author.

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