

Phytochemical, antioxidant and microbial inhibitory effects of *Spondias mombin* leaf and stem bark extracts.

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Abstract

AIM: The study evaluated the phytochemical, antioxidant and microbial inhibitory properties of the leaf and stem bark extracts of *Spondias mombin*.

METHODOLOGY: Ten grams of the leaves and stem bark were each extracted with 100ml of 80% ethanol, hot and cold water respectively and allowed to stand for 24 hrs. The phytoconstituents, antioxidant vitamins C and E and antimicrobial testing were carried by standard methods.

RESULTS: There were the presence of the tested phytoconstituents in both the leaves and stem bark extracts using different extraction media. The leaves contain saponins, alkaloids and tannins in all the extraction media while flavonoids, alkaloids and tannins were detected in all the extraction media of the stem bark. The leaf extract contained more vitamin C and E than the stem bark extracts. The ethanolic extract showed increased activity against *Staphylococcus aureus* when compared to the inhibitions observed with the other tested microorganisms.

CONCLUSION: The results demonstrate that the plant has both medicinal and antioxidant properties.

Key words: *Spondias mombin*, phytochemical, antioxidant, microbial.

I. Introduction.

Medicinal plants have been recognized to be of great importance to the health of individuals and communities. Among these medicinal plants, *Spondias mombin* has been reported to have significant medicinal and economic values (1). The anti bacterial, antimicrobial, antiviral and anti fungal potentials of the plant have been reported (2,3,4,5). The plant was recommended for use by pregnant women after five months of pregnancy (6) while the mineral analysis of the plant has also been documented (7,8). The abortifacient activity of an aqueous extract of *Spondias mombin* was reported by Offiah and Anyanwu (9). The plant has been shown to have a wide range of phytoconstituents such as tannins, saponins and anthraquinone glycosides (3). The plant has been reported to be used in the treatment of many disease conditions in the Eastern part of Nigeria by the natives. The associated link between the composition of this world-wide cultivated plant and the reported medicinal and economic values prompted this study aimed at evaluating the phytochemical and antioxidant properties as well as the microbial inhibitory effects of the leaf and stem bark extracts of the plant.

II. Materials and Methods.

Collection of plant material and preparation of extracts.

The leaf and stem bark of the plant were freshly collected from Obokwu Obinze in Owerri-West Local Government Area of Imo state, Nigeria. They were identified by Dr. S.C. Okeke of the Department of Plant Science and Biotechnology, Imo state University, Owerri, Nigeria. The leaves and stem bark were properly washed with distilled water and dried at room temperature. The samples were each ground into fine powder with a mechanical blender and stored in air tight bottle glass jar at 4 °C prior to use. Ten grams of the leaves and stem bark were each extracted with 100ml of 80% ethanol, hot and cold water, respectively and allowed to stand for 24 hours.

Phytochemical screening.

The methods described by Trease and Evans (10) were used to determine alkaloids and saponins while tannin and flavonoids were determined as described by Bohm and Kolipai-Abyazan (11).

Microbial testing.

Indicator organisms: the microorganisms used were local isolates from the Microbiology Department of the Federal University of Technology, Owerri. The cultures were maintained throughout the duration of the research on agar slant.

Preparation of medium: the nutrient agar (IABM) used for the test was prepared as directed by the manufacturer and sterilized to 121 °C for 15 min.

Antibacterial activity test: the diluted extracts were tested for their antimicrobial potency using the agar-well technique as described (12, 13). The test for antimicrobial activity was carried out with *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Pneumonia* and *Staphylococcus aureus*.

With a sterile 1ml pipette, 0.2ml of the broth culture of the test organism was put in a sterile petri dish and 18ml of the sterile molten diagnostic agar was added. The plates were mixed by swirling and then allowed to sit. A sterile cork borer was used to bore wells into the medium. The wells were then filled with 0.1ml of the extracts in triplicates. The culture plates were kept in sterile inoculation chambers for 2hrs to facilitate diffusion of the solutions. The plates were then incubated at 37 °C for 24 hrs. The zones of inhibition of microorganism for each extract was measured using a calibrated ruler.

Antioxidant vitamins.

Vitamins C and E were assayed on the extracts as described by Ojiako and Akubugwo (14).

Statistical analysis. All data were expressed as mean ± standard deviation. Statistical significance of the difference was analyzed using one way analysis of variance (ANOVA) by SPSS version 16 for windows.

III. Results.

Table 1. Phytochemical screening of the leaf and stem bark extracts of *Spondias mombin*.

	Saponin	Flavonoids	Alkaloids	Tannins
Leaf extract				
Ethanol extract	+	-	+	+
Hot water extract	+	+	+	+
cold water extract	+	+	+	+
Stem bark extract				
Ethanol extract	+	+	+	+
Hot water extract	+	+	+	+
Cold water extract	-	+	+	+

+ = present; - = not present.

Table 2. Antioxidant vitamin contents of the leaf and stem extracts *Spondias mombin*.

Sample	leaf		Stem bark		
	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Vitamin E (mg/dl)
Ethanol extract	0.26±0.02	1.00±2.90	0.25±0.09	0.28±0.02	
Hot water extract	0.27±2.06	1.03±4.09	0.23±0.18	0.20±4.71	
Cold water extract	0.28±4.21	1.01±0.90	0.30±2.07	0.25±0.21	

Results are mean ± SD.

Table 4. Microbial inhibitory effects of the *Spondias mombin* leaf and stem bark extracts.

Sample	Leaf extracts				Stem bark extract.			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. Pneumonia</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. Pneumonia</i>	<i>S. aureus</i>
Ethanol extract	+(20%)	++(40%)	++(40%)	+++ (60%)	+(20%)	++(40%)	+(20%)	+++ (60%)
Water extract	-	-	-	+(20%)	-	-	-	+(20%)
Cold water extract	-	-	-	+(20%)	-	-	-	+(20%)

+ = level of inhibition.

RESULTS.

The preliminary phytochemical screening of the leaves and stem bark ethanol, hot and cold water extracts of *Spondias mombin* are shown in Table 1. The results show the presence of the tested phytoconstituents in both the leaves and stem bark extracts using different extraction mediums. The leaves contain saponins, alkaloids and tannins in all the extraction mediums while flavonoids, alkaloids and tannins were detected in the extraction medium of the stem bark. The results of the antioxidant vitamins C and E are presented in Table 2. The results show the presence of the vitamins in the leaf and stem bark extracts. As

indicated, the leaf extract contains more vitamin C and E compared to the stem bark extracts. The results of the antimicrobial activity of the ethanol, aqueous hot and cold extracts of *Spondias mombin* leaf and stem bark extracts are shown in Table 3. The ethanol extract inhibited all the tested organisms in both the leaf and stem bark. The ethanol extract exhibited increased activity against *Staphylococcus aureus* as compared to the inhibitions seen with the other tested microorganisms. The results, therefore, showed that the anti microbial principle in the plant were mostly extracted with ethanol.

IV. Discussion.

The study had used phytochemical, antioxidant and microbial indices to evaluate the biochemical potentials of *Spondias mombin*, a local botanical plant used in herbal medicinal practice in some states in Eastern Nigeria. The plant stem bark and leaf extracts showed copious amounts of saponin, alkaloids, flavonoids and tannins. The above constituents are bioactive compounds with different effects (13,15).

The flavonoids are known to protect against allergies and inflammation, and represent the most common and widely distributed groups of plant phenolics that serve as flavoring ingredients of spices and vegetables (16,17). Flavonoids and other derivatives have been identified in *Spondias mombin* plant with anti-herpes, antioxidant and anti-aging properties (18). While flavonoids have been reported to be free radical scavengers, super antioxidants and with strong anticancer activity (19), they also provide anti-inflammatory activity as antioxidants (7, 20). This could be the reason for the use of the plant in the treatment of intestinal troubles in herbal medicine (17).

Alkaloids are of therapeutic significance. The pure substance is used to repel parasites and predators (21). When ingested by animals, they affect glucagon, thyroid stimulatory hormone and inhibit some mammalian enzymatic activities (22). Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agents due to their analgesic antispasmodic and antibacterial potentials (7, 23).

Tannins are known to improve wound healing and inflamed mucus membrane (7). The study showed high presence of tannins in both leaf and stem bark extracts supporting the strong use of *Spondias mombin* in healing wounds, various ulcers, frost-bite and burns in traditional herbal medicine (1). The tannins, flavonoids and alkaloids as seen in the results give credence to the reported anti-microbial (24) anti-viral (18), and anti-helminthic (25) properties of *Spondias mombin*.

The stem bark and leaf extracts also contained the antioxidant vitamins C and E. Antioxidants repair free radical damages to the cells (29). The presence of antioxidant molecules suggests that *Spondias mombin* can be used as vitamin supplement probably during oxidative stressed conditions. The results of the study showed that the plant has high nutritive value which could attenuate physiological oxidative stress due to its high concentration of vitamin E and C as well as flavonoids contents (27). The presence of vitamin C in the extracts may confirm the reported wound healing property of the plant (28). The presence of vitamin C in *Spondias mombin* leaves and stem bark, implies that it can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer (17, 29).

The microbial investigations showed that the ethanolic stem bark and leaf extracts of *Spondias mombin* inhibited *E. coli*, *Pseudomonas*, *Klebsiella* and *Staphylococcus*. The results also indicated that all the extracts inhibited *Staphylococcus aureus*. The inhibition of *Staphylococcus aureus* and *E. coli* is an indication that the plant can be used as remedy for gastro-enteritis and pneumonia (13). This will serve as a scientific basis supporting the local use of *Spondias mombin* in the treatment of diseases like minor urinogenital infections.

Conclusion: The ethanolic extract of *Spondias mombin* leaf and stem has antimicrobial activity against *E. coli*, *Klebsiella* and *Staphylococcus aureus*. The active principles of the extract that showed these effects were not identified but the presence of flavonoids, alkaloids and tannins coupled with the antioxidant vitamins in the plant gives credence on the herbal use of the plant.

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