Evaluating Extracts of *Spondias mombin* for Antimicrobial Activities

[1]Umeh, E. U., [2]Igoli, J. O., [1]Agada, E. O. and [1]Usman, S. S.
[1]Department of Biological Sciences, University of Agriculture, Makurdi, Benue State, Nigeria.
[2]Chemistry Department, University of Agriculture, Makurdi, Benue State, Nigeria.

Corresponding author: Umeh, E. U. Department of Biological Sciences, University of Agriculture, Makurdi, Benue State, Nigeria. **Email:** jceu1@yahoo.com

Abstract

The plant Spondias mombin Linn. also called yellow mombin in English, Igongo/Ichankla in Idoma, and Uchakuru in Igbo, is common in the forest and savanna regions of Nigeria. It is used in several countries of the world to treat various ailments including infectious diseases. Water, chloroform, methanol, and petroleum ether extracts of leaf, root, and bark of the mature plant were screened for antimicrobial activity using an indicator-based microdilution technique. Inhibition was measured as minimal inhibitory concentration (MIC). The growth of Streptococcus pyogenes (mean MIC = 0.139 mg), Candida albicans (mean MIC = 0.148 mg), Salmonella typhi (mean MIC = 0.226 mg), Escherichia coli (mean MIC = 0.265 mg), and Staphylococcus aureus (mean MIC = 0.289 mg) in broth cultures were inhibited. Inhibition was significantly correlated with plant parts (r = -0.435; p < 0.05), leaf extracts having the greatest inhibitory effect (mean MIC = 0.060 mg), and the bark extracts the least (mean MIC = 0.389 mg). Statistical tests show that the mean MICs of leaf and bark extracts differ significantly (p <0.05), whereas those of leaf and root do not. The plant part by solvent interactive effect was significant (p < 0.05), suggesting that the MICs of the various plants differ significantly according to extraction solvent. Preliminary phytochemical test on aqueous extracts of the plant revealed the presence of saponins and tannins as the main phyto-constituents of the plant. These findings demonstrate the possible effectiveness of the plant, especially its leaf extracts, in treating microbial infections.

Keywords: Indicator-based microdilution technique, Antimicrobial activity, *Spondias mombin*

Introduction

The age old fight against human diseases coupled with the emergence of drug resistant microorganisms have given rise to the need to explore the environment for bioactive compounds with antimicrobial effects. Being new, such compounds may not have the problem of microbial resistance, and with some structural modification their activity could be diversified. The plant *Spondias mombin* Linn. (Family Anacardiaceae) commonly called yellow mombin (English), Igongo/Ichankla (Idoma) or Uchakuru (Igbo), is common in the forest and savanna regions of Nigeria. It is used in several countries (e.g. South America) to treat various ailments.

The plant exhibits antibacterial, antifungal and antiviral activities (Ajao *et al*., 1985; Caceres *et al*., 1995; Corthout *et al*., 1987; Gill, 1992; Abo *et al*., 1999; Calderon *et al*., 2000; and Taylor, 2006). Hot water and alcohol extracts of fruits, leaves, root and stem bark of this plant are used in form of infusions, decoctions, or concoctions. In Nigerian folk medicines, the plant is usually mixed with other plant materials and is used to treat diseases such as dysentery, sore-throats, cough, leprosy and stomach aches (Ajao *et al*., 1985). In addition, the leaves may be used as a direct squeeze or they may be soaked in alcohol, or boiled in water to extract the active components.

Even though the antimicrobial activities of a number of local herbs found within Middle Belt of Nigeria have been investigated (Lis-Balchin *et al*., 1996; Sanusi, 1999; Oluma *et al.,* 2004; Umeh *et al*., 2005), little information is available on the antimicrobial property of *Spondias mombin L*. growing in Benue State. The purpose of this study, therefore, was to substantiate the antimicrobial activity of *S. mombin*, and compare the minimal inhibitory concentrations of the aqueous and organic solvent extracts of the various plant parts.

Since herbal medical practice is common in developing countries (Ellof, 1998), it is necessary that the antimicrobial activities of herbal plants against aetiological agents of diseases be investigated. The results of this study may validate the use of the plant in herbal medical practice, and may provide cheaper, readily available and alternative cure for infectious diseases.

Materials and Methods

Test plants: The leaves, root and bark of *Spondias mombin* were obtained from a hog plum tree in North Bank area of Makurdi town, Benue State, Nigeria. The plant was identified by botanists: Dr. S.S. Usman and Prof. H.O.A. Oluma, Department of Biological Sciences, University of Agriculture, Makurdi, according to the Floral of West Tropical Africa.

Test organisms: *Candida albicans*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus pyogenes* isolated from clinical specimens (vaginal swab, stool, throat swab, and wound swab) and identified at the TOSEMA Diagnostic Laboratories, Makurdi, and as described by Benson (1990) were used for the antimicrobial activity assays.

Extraction procedure: A slight modification of the method described by Umeh et al. (2005) was used.

Thirty centiliters (30 cL) of solvent (water, chloroform, methanol, or petroleum ether) were added to dry pulverized leaf, stem bark, or root (100 g) of *S. mombin*. The resulting suspension was allowed to stand in a tightly covered bottle for 48 h at room temperature (30^{o}C) after which it was filtered using Whatman's filter paper No. 1. The filtrate was collected as plant extract in sterile test tube and was concentrated by evaporation using the exposure method. Extract was reconstituted in 10% dimethylsulphoxide (DMS) for antimicrobial assay.

Antimicrobial activity assay: The antimicrobial activity of the various plant extracts was assayed using a slight modification of the microdilution technique described by Drummond and Waigh (2000). An indicator solution was prepared by dissolving one tablet of resazurin dye in 40 ml of sterile water. An overnight broth culture of a test microorganism in nutrient broth was diluted serially in 0.1% peptone water to obtain 10^6 colony forming units mL^{-1} of broth culture. Prior to serial dilution, the viable count of the overnight culture was estimated using the standard plate count. Each extract was serially diluted two-folds (to give concentrations of 1.00 – 0.0078 mg) in an appropriate solvent (dimethylsulphoxide) and placed in microtitre wells so that each well contained one hundred microliters (100 µL) of a dilution. The wells were labeled 1 – 10. An equal amount of broth culture (100 µL) and indicator solution (100 µL) were placed one after the other in each labeled well. Growth control solution comprised indicator solution and broth culture, while the sterile control consisted of indicator solution and sterile broth. The microtitre tray was incubated at 37^{o}C for 6 hours. Blue coloured solution meant growth inhibition in test wells, while pink coloured solution indicated growth or absence of inhibition. The highest dilution showing growth inhibition was taken as the minimal inhibitory concentration (MIC).

Phytochemical tests: Preliminary phytochemical screening of the aqueous and organic solvent extracts of the bark, leaves and roots of *S. mombin* were carried out as described by Sofowora (1982), Abo *et al.* (1999) and Calderon *et al.* (2000). The phytochemical tests were the frothing test for saponins, ferric test for tannins, Salkowski's test for alkaloids and steroids, and Fehling's test for glycosides.

Results

Extracts of pulverized bark, leaf, and root of *S. mombin* in organic solvents and water were assayed for antimicrobial activity. Preliminary phytochemical test of aqueous extracts of the plant revealed the presence of saponins and tannins as the main phyto-constituents of the plant. Table 1 shows the extent of inhibitory activity. Most of the extracts demonstrated a high degree of antimicrobial activity. For instance, water and organic solvent extracts of the leaf inhibited growth of all the test microorganisms giving 100% inhibition rate. On the contrary, not all the extracts of stem bark inhibited microbial growth; for instance, chloroform extract of bark did not show antimicrobial activity against *E. coli, S. aureus*, or *S. pyogenes* (Table 2).

Table 1: Frequency of microbial inhibition (per cent) by extracts of *Spondias mombin* according to structural parts

Extracts	Rate of microbial growth inhibition (%)	Total number of tests (%)
leaf	20 (100)	20 (100)
root	19 (95)	20 (100)
bark	14 (70)	20 (100)

**Using One-Way ANOVA test, the F-test (= 2.470) has a significant level less than 0.05 and indicates that the antimicrobial activity of leaf, root, and stem of the S. mombin differed significantly from each other. Similarly, the correlation coefficient (r) was 0.435 ($p < 0.05$) and suggests a relationship between antimicrobial effect and plant tissue.*

Petroleum ether extract also did not have any effect on *E. coli* or *S. typhi*. Percentage inhibition of stem bark therefore was 70%. The clinical isolates of *Candida albicans*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, used as test organisms, were susceptible to the inhibitory effect of the extracts although to varying degrees.

Antimicrobial activity, measured by minimal inhibitory concentration (MIC), was also significantly associated with plant structure (r_{60} = 0.430; p = 0.001): leaf extracts exhibited more antimicrobial activity than bark extracts (Table 1).

The MICs of the various plant parts are presented in Table 2. The leaf extracts exhibited the greatest antimicrobial activity (mean MIC = 0.060 mg), while bark extracts exhibited the least (0.389 mg). Comparison of mean differences using post hoc test (statistical analytical technique) showed that the mean MIC of leaf extracts differed significantly from that of bark extracts, but not from that of root extracts.

Chloroform extract of leaf was however the most active (mean MIC = 0.0437 mg) of leaf extracts, whereas aqueous extract was the least active (mean MIC = 0.0713 mg). The organisms most susceptible to antimicrobial activities of leaf extracts were *E. coli* (mean MIC = 0.0468 mg) and *S. pyogenes* (mean MIC = 0.0486 mg).

The most active of root extracts was the chloroform extract (0.0246 mg), and the most susceptible organism to inhibitory effects of root extracts was *S. typhi* (mean MIC = 0.0544 mg). Aqueous extract of bark (0.0750 mg) was the most inhibitory to microbial growth, and *C. albicans* was the most susceptible organism (mean MIC = 0.1602 mg). Overall, S. pyogenes was most susceptible to antimicrobial activities of all extracts of *S. mombin* (mean MIC = 0.1391 mg).

Discussion

Extracts of *Spondias mombin* were evaluated for antimicrobial activity against some clinical isolates of bacteria and yeast.

Table 2: Minimal inhibitory concentrations (MIC) of aqueous and organic solvent extracts of *S. mombin* parts

Part of plant used	Extraction solvent	Minimal Inhibitory Concentrations (mg)					Mean MICs	Number of tests
		C. albicans	*E. coli*	*S. typhi*	*S. aureus*	*S. pyogenes*		
Leaf	**Aqueous**	.0156	.0310	.1250	.0600	.1250	.0713	5
	Chloroform	.0312	.0156	.0156	.1250	.0312	.0437	5
	Methanol	.2500	.0156	.0620	.0070	.0070	.0683	5
	Petroleum Ether	.0620	.1250	.0156	.0625	.0310	.0592	5
	Mean	.0897	.0468	.0546	.0636	.0486	.0606	20
Root	**Aqueous**	.2500	.5000	.0312	-	.0625	.3687	5
	Chloroform	.0156	.0312	.0620	.0070	.0070	.0246	5
	Methanol	.5000	.1250	.0625	.5000	.0310	.2437	5
	Petroleum Ether	.0150	.2500	.0620	.0600	.2500	.1274	5
	Mean	.1952	.2266	.0544	.3918	.0876	.1911	20
Bark	**Aqueous**	.2500	.0312	.0312	.0312	.0312	.0750	5
	Chloroform	.0156	-	.2500	-	-	.6531	5
	Methanol	.1250	.0620	-	.1250	.0310	.2686	5
	Petroleum Ether	.2500	-	-	.5000	.0620	.5624	5
	Mean	.1602	.5233	.5703	.4141	.2810	.3898	20
Total	**Aqueous**	.1719	.1874	.0625	.3637	.0729	.1717	15
	Chloroform	.0208	.3489	.1092	.3773	.3461	.2405	15
	Methanol	.2917	.0675	.3748	.2107	.0230	.1935	15
	Petroleum Ether	.1090	.4583	.3592	.2075	.1143	.2497	15
	Mean	.1483	.2655	.2264	.2898	.1391	.2138	60

The susceptibility of all the clinical isolates to inhibitory effect of the plant extracts implies broad spectrum activity. All the extracts possessed inhibitory effect, although at varying degrees. This result suggests that the 'active compound(s)' although probably present in all parts of the plant extracts investigated, may have been extracted in varying amounts.

Plants are known to produce chemicals as protection against herbivores and for their well being or survival. Competition and disease could provoke the production of compounds toxic to other species. Generally, fatty acids, oils and lower molecular weight terpenes are extracted in petroleum ether, while chloroform extracts these same less or nonpolar compounds to medium polar compounds. In addition to all these, methanol extracts the most polar compounds including tannins, waxes, sugars, anthrocyanidines, plant pigments, and others.

Inhibition was, however, significantly associated with plant part, leaf extracts having the greatest activity. Maiga *et al.* (2005) had reported the leaf to be more biologically active than bark. Chemicals as protectants are produced more in the leaves than bark as herbivores are more likely to eat leaves than bark. Hence they may have the higher concentrations of the 'active compou6nds' than the bark. The activity of leaf did not however, differ from that of the root. Some chemical substances that act as protection against microorganisms may be present in the root.

The inhibitory effect of leaf and bark differed and supports the report of Taylor (2006) that leaves and bark have different properties, actions, and traditional uses. Chloroform fraction of leaf extract had the greatest activity – probably flavonoids, polyphenolics, and alcohols are responsible for the inhibitory effect of the leaf. Chloroform fraction of root was also the most active fraction of the plant. The result could be attributed to a class of compounds of such polarity that are more soluble in chloroform than in the other solvents used. Chloroform also extracts some medium polar to polar compounds.

The findings of this study not only support the use of *S. mombin* in trado-medical practice, but underscore its importance in treating microbial infections.

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