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Studies on the Antimicrobial Effects of *Spondias mombin* and *Baphia nittida* on Dental Caries Organism

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Abstract: The antimicrobial effect of cold water, hot water and ethanolic extracts of *Spondias mombin* and *Baphia nittida* on cariogenic streptococci isolated from dental caries patients attending the Ebonyi State University Teaching hospital dental clinic Abakaliki was investigated using the agar well diffusion technique. The cold water and ethanolic extracts of *Baphia nittida* showed inhibition zone diameter (IZD) of 10 and 12 mm respectively at 400 mg mL $^{-1}$, while the hot water showed no inhibitory effect. All extracts of *Spondias mombin* did not inhibit the test organism. The cold water and ethanolic extracts of *Baphia nittida* showed Minimum Inhibitory Concentration (MIC) of 100 and 50 mg mL $^{-1}$ respectively. The combination of the cold water extracts of the two herbs showed enhanced activity of 13 mm. Phytochemical analysis of *Baphia nittida* revealed the presence of flavonoids, glycosides, proteins saponins, tannins, carbohydrate and steroidal aglycone. Acute toxicity testing of *Baphia nittida* at a range of 250-5000 mg kg $^{-1}$ bw using mice showed no clinical signs of acute toxicity. No chemical toxicity was observed amongst rats given *Baphia nittida* extracts 500 and 1000 mg kg $^{-1}$ bw after 30 days. *Baphia nittida* may be a potential source of an antimicrobial agent for the treatment and management of dental caries.

Key words: Antimicrobial, *Spondias mombin*, *Baphia nittida*, dental caries, *Streptococcus mutans*, toxicity

INTRODUCTION

Numerous plants and herbs are used all over Nigeria by traditional medicine practitioners. Roots, barks, leaves and stems of various plants are employed in ethnomedicine. Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants (Akobundu and Agyakara 1987; Redo *et al*., 1989; Almagboul *et al*., 1988; Onyeagba *et al*., 2004).

Dental caries is a common disease world wide (Silverstone *et al*., 1981; Rowe *et al*., 1989; Prabhu *et al*., 1992). High prevalence of dental caries has been reported in Nigeria (Odusanya, 1989; Adegbembo *et al*., 1995). This high prevalence is associated with increased access to processed food and sucrose. Dental caries has been reported as a major cause of tooth loss in Nigeria (Dosumu and Denloye 1999). The anti caries properties of some medicinal plants has been widely reported (Okeke, 2003; Linke and LeGeros, 2003; Leitao *et al*., 2004; Uzel *et al*., 2005). In Nigeria Local Medicinal plants are commonly used as chewing sticks and for the treatment of dental disease (Okafor, 2001). There seem to be paucity of information on the scientific evaluation of the effect of these local plants on prevention of dental caries. This paper reports the *in vitro* activities of *Spondias mombin* and *Baphia nittida* tender stems commonly used as chewing stick in the South eastern Nigeria on dental caries organism.

MATERIALS AND METHODS

Collection and preparation of plant leave extracts: Fresh leaves of *Spondias mombin* and *Baphia nittida* were obtained from Enugu, Enugu State and authenticated by a taxonomist in the department of Applied Biology, Ebonyi State University, Abakaliki.

The plant materials were washed, dried at room temperature and pulverized using mechanical grinder. Exactly 50 g of each ground plant preparation was introduced into 250 m L of 95% ethanol and cold water.

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Exactly 50 g of each ground plant material was also introduced into 250 mL of water and boiled for 10 min. The preparations were allowed to stand for 24 h with intermittent shaking. They were then filtered using a Whatman filter paper. The filtrate was evaporated to dryness in a water bath at 40°C (Ibrahim *et al*., 1997). The extracts were stored in the refrigerator at 4°C and used when required.

Source of bacterial isolates: Forty carious lesions were obtained from patients attending Ebonyi State University Teaching Hospital Dental Clinic, Abakaliki, cultured on crystal violet blood agar (Collins *et al*., 1995) and incubated under anaerobic condition at 37°C for 24 h. The isolates were purified by subculturing by the streak plate method (Isu and Onyeagba, 2002) and identified based on colonial morphology, grams reaction and biochemical tests (Cheesbrough, 2002; Collins *et al*., 1999).

Antimicrobial susceptibility screening: The antimicrobial effect of the various extracts on the bacterial isolates was determined using the agar well diffusion technique (Perez *et al*., 1990). A 0.06 mL overnight culture of test organism (Macfarland Standard 0.5) was seeded on the blood agar medium. A sterile cork borer was used to make 4 wells on the media and then 0.06 mL of each extract reconstituted to 400 mg mL^{-1} concentration was introduced into two wells. The same of quantity of erythromycin (2.5 mg mL^{-1}) and distilled water were introduced into the other wells to serve as positive and negative controls. This was done in duplicate. The plates were then incubated anaerobically at 37°C for 24 h and the Inhibition Zone Diameter (IZD) measured in millimeters.

Determination of minimum inhibitory concentration: The Minimum Inhibitory Concentration (MIC) was determined for the cold water and ethanolic extracts of *Baphia nattida*. The extracts were reconstituted to concentrations of 400, 200, 100, 50, 25 and 12.5 mg mL^{-1} and used for antimicrobial sensitivity screening as described earlier. The lowest concentration of each extract that showed noticeable inhibition was regarded as the MIC.

Antimicrobial susceptibility test for herbal combinations: The reconstituted 400 mg mL^{-1} cold water, hot water and ethanolic extracts of the herbs were mixed in equal proportions and used for antimicrobial susceptibility as described above.

Phytochemical analysis: The Phytochemical analysis of the *Baphia nittida* extracts were done using the methods described by Iwu and Chiori (1984).

Acute and chronic toxicity testing: The acute toxicity of the extracts were done using laboratory mice. Seventy mice (8-10 weeks) old weighing 18-22 g were divided randomly into seven equal groups (A-G). While Groups A-E were given a single dose of 250, 500, 750, 1500, 3000 and 5000 mg kg^{-1} bw of *Baphia nttida* via the intraperitoneal route, group G mice were given 2 mL of distilled water. The mice groups were observed for clinical signs of acute toxicity.

Before commencement of chronic toxicity testing, 1.0 mL of blood was collected from the experimental animals and analyzed for Packed Cell Volume (PCV), Haemoglobin Concentration(HB), Red Blood Cell Count (RBC), Erythrocyte Sedimentation Rate (ESR) and Total Luekocyte Count (TLC).

Chronic toxicity testing was done with fifteen 8-10 weeks old male albino rats weighing 62-120g. The rats were divided into three groups (A-C). Group A rats were fed diets containing 500 mgkgbw of *Baphia nttida,* Group B rats were fed with diet containing 1000 mg kg^{-1} bw of the same extract while Group C rats were fed with normal diet without herbal preparation. The body weights of the rats and signs of toxicity were monitored for 30 days. Then 1.0 mL of blood was collected from the rats for haematotogical analysis namely PCV, RBC count, HB concentration, ESR and TLC (Brouder and Pugh, 1977; Berkowitz and Katzung, 2001). The mean values of the parameters and standard deviations were determined. The rats were then sacrificed and their liver and kidney excised and fixed in 10% formalin. They were stained and observed with the light microscope.

RESULTS

Bacterial isolates: The isolates were identified as *Streptococcus mutans*, *S. salivarius* , *S. sobrinus* and *S. mitior*. The frequency of isolation was *Streptococcus mutans* (78%), *S. salivarius* (12%), *S. sobrinus* (6%) and *S. mitior* (4%), respectively.

Susceptibility test and MIC: The cold water and ethanolic extracts of *Baphia nittida* showed inhibitory activity with Inhibition Zone Diameter (IZD) of 10 and 12 mm at 400 mg mL^{-1}, respectively. All the extracts of *Spondias Membin* and the hot water extract of *Baphia nittida* produced no inhibitory activity against the test organism-*Streotococcus mutans*. The combinations of the cold water extracts of the two herbs produced an inhibition zone diameter of 13 mm while that of the ethanolic extracts showed inhibition of 9 mm on *S. mutans*. The combinations of the hot water extracts of two herbs showed no inhibitory effect against the test organism.

Table 1: Inhibition zone diameter and Minimum Inhibitory Concentration (MIC) of *Baphia nittida* extracts on *Streptococcus mutans*

Concentrations (mg mL^{-1})	Zones of inhibition (mm)		
	Cold water	Hot water	Ethanol
400	10	N1	12
200	9	N1	9
100	6	N1	6
50	N1	N1	2
25	N1	N1	N1
12.5	N1	N1	N1
Erythromycin (control) 2.5 mg mL^{-2}	20	20	20

N1-No visible inhibition

Table 2: Phytochemical analysis of *Baphia nittida*

Phytochemical constituents tested	Present or absent
Alkaloids	-
Flavonoids	++
Glycosides	Tr
Protein	++
Saponins	++
Tannins	+++
Carbohydrate	+
Cardiac glycoside	-
o- and c- glycosides	-
Cyanogenic glycoside	-
Anthracene glycoside	-
Reducing sugar	-
Steroidal aglycone	++

- = Absent, + = Present in varying degrees, Tr = Trace

Table 3: Changes in body weight, packed cell volume and red blood cell count of rat groups before and after administration of *Baphia nittida* extracts

Rat group treatments	Mean body weight (g)		Mean packed cell volume (%)		Main red blood cell count (10^6 cells µL^{-1} of blood)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
500 mg kg^{-1} bw	80.50±9.60	141.98±11.54	41.80±1.79	41.60±1.14	5.24±0.24	5.13± 0.23
1000 mg kg^{-1} bw	81.00±12.79	157.50±10.49	41.40±1.90	42.00±2.00	5.25±0.22	5.06±0.09
Untreated control	78.60±15.31	155.40±9.89	41.80±1.30	41.20±1.10	5.21±0.36	5.06 ±0.10

Values are means±standard deviation

Table 4: Changes in haemoglobin concentration, erythrocyte sedimentation rates and total leukocyte counts of rat groups before and after administration of *Baphia nittida* extracts

Rat group treatment	Mean haemoglobin concentration (g dL^{-1})		Mean erythrocyte sedimentation rates (mm h^{-1})		Mean total leukocyte counts (10^3 cells µL^{-1} of blood)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
500 mg kg^{-1} bw	13.04±0.38	13.26± 0.36	0.66± 0.17	0.42±0.13	9.59± 0.70	15.38±1.15
1000 mg kg^{-1} bw	13.02±0.46	13.12±0.23	0.56± 0.11	0.34±0.11	9.27±1.22	15.44±1.06
Untreated control	13.04± 0.38	13.14±0.37	0.62±0.18	0.38±0.18	9.61±0.64	15.62±1.39

Values are means±standard deviation

The MIC of the cold water extract and ethanolic extracts of *Baphia nittida* on *Streptococcus mutans* was 100 and 50 mg mL^{-1}, respectively (Table 1).

Phytochemical analysis: Phytochemical analysis of *Baphia nittida* shows that it contains varying degrees of flavonoids, glycosides, protein, saponins tannins, carbohydrate and steroidal aglycone (Table 2).

Toxicity testing: The acute toxicity showed that all the mice groups administered 250-5000 mg kg^{-1} bw of *Baphia nittida* extracts did not exhibit any clinical signs of acute toxicity 24 h post treatment. The chronic toxicity test show that there was no significant difference ($p>0.05$) between the rat groups before and after herbal treatment in all the parameters studied (Table 3 and 4). Histopathological examination showed congestion of the liver of rats given 500 and 1000 mg kg^{-1} bw of *Baphia nittida* extracts although no degenerative changes were observed. No necrotic or degenerative changes in the kidney tissues of all the rats given 500 and 1000 mg kg^{-1} bw of *Baphia nittida* extracts were observed.

DISCUSSION

The isolation of *Streptococcus mutans* in 78% of the caries samples emphasizes the fact that *S. mutans* is the aetiologic factor in dental caries. This agrees with previous reports on the aetiologic agent of dental caries (Silverstone *et al*., 1981; Loesche, 1986; Samaranayake, 1996; Brooks *et al*., 2001).

That *Baphia nittida* extracts produced visible inhibitory activity against *S. mutans* is remarkable. This plant is commonly used an chewing stick in Eastern Nigeria. It is rather surprising that the hot water extract of *Baphia nittida* had no noticeable inhibitory effect on the test organism since the cold water extract had effect. The inhibitory effect observed with the cold water extract of *Baphia nittida* confirms the efficacy of this herb as used by the local inhabitants in Eastern Nigeria for the management of dental caries. The anticaries activity of some medicinal plants had been previously reported. Okeke (2003) has reported the effect of *Newbouldia Leavis*; Brazilian green propolis and *Braccharis drancunculifolia* has also been reported to have anticaries effect (Leitao *at al*., 2004).

Spondias mombin did not produce any visible inhibitory activity against the test organism. It is however surprising that when the cold water extract of *Spondias* mombin was combined in equal proportion with that of *Baphia nittida*, the combination produced an Inhibition Zone Diameter (IZD) of 13 mm which is slightly higher than the inhibition by *Baphia nittida* alone. Although this enhanced activity cannot be absolutely attributed to synergy, it is not unlikely that the combination could produce better result when the concentration of the active ingredients from both plants are isolated and used. Earlier studies had reported an interaction effects of medicinal herbal extracts on micro organisms (Adetunji, 1999; Amadi *et al*., 2004).

The Phytochemical studies of *Baphia nittida* revealed the presence of flavonoids, glycosides, proteins, saponins, tannins, carbohydrate and steroidal aglycone in varying degrees. Some of these compounds have been reported to have a range of antimicrobial activity (Trease and Evans, 1983; Sato *et al*., 2003). In recent times there has been an increase in the use of natural products from plants for therapy and the interest in their biological activity have also widened (Valerio and Gonzales, 2005). The usefulness of these preparations is often marred by their high toxicity. It is interesting that the acute and chronic toxicity tests conducted in this study using mice and albino rats respectively indicated no obvious signs of both type of toxicity. *Baphia nittida* has remained a chewing stick of choice in the south eastern Nigeria since ancient times. Results of this study suggest further investigation of *Baphia nittida* as a potential source of drug for caries treatment and management.

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