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Anti-Microbial Evaluation of a Herbal Dental Remedy: Stem Bark of *Nuclea latifolia*-Family Rubiaceae

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Abstract: The aim of the study was to evaluate the antimicrobial activity of the stem bark of *Nuclea latifolia* used as a dentrifice by the local populace. The crude powdered sample was evaluated for the chemical and antimicrobial effects. The methanolic and chloroform extracts were subjected to different organisms of clinical isolates *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Streptococcus varidans*, *Staphylococcus aerues*, *Penicillium nonatum*, *Candida albicans* and *Aspergillus niger*. The Minimum Inhibitory Concentrations (MIC) were also obtained. The results of the study revealed significant antibacterial effect of the extracts. The study thus justifies the ethno medicinal use of the plant as a dental remedy.

Key words: Stem bark, *Nuclea latifolia*, methanolic, aqueous extract, antimicrobial organisms

INTRODUCTION

Ethno medicine has played an important role in Africa and Western societies and in Nigeria particularly. Herbal medicine is an integral part of Traditional medicine (WHO, 2003; Annk and Stephen, 1997).

Herbal medicine is the major component of the traditional medicine that can be subjected to scientific investigations and adaptations (Hoareau, 1999). It is through the scientific investigations that many of the first generation plant drugs came into existence (Murray, 1995). These were usually simple botanicals employed in their more or less crude form and sometimes as food supplements (Shaw *et al.*, 1997). Several effective medicines used in their natural state such as cinchona, opium, belladonna and aloe were selected as therapeutic agents based on empirical evidence of their clinical application by traditional societies. The industrial revolution gave rise to a second generation of plant based drugs. These are products of scientific processing of the plant extracts to isolate their active constituents. These include quinine from *cinchona*, reserpine from *Rauwolfia vomitoria* and cocaine from erythrozyllum *coca* leaves, Vincristine and Vinblastine from periwinkle (*Vinca roseus*, *Catharanthus roseus* syni) (Iwu *et al.*, 1999).

Herbal dentrifices are (for this purpose) gaining popularity and high patronage in the western countries (Hammmer-Beem *et al.*, 2006). Many studies have shown

that the herbal dentrifices are as good as the conventional ones (in controlling the oral bacteria reservoir. (Van der weijden *et al.*, 1998; Mullally *et al.*, 1995; Tanner and Still Man, 1993; Velden, 1998).

Nauclea latifolia (Rubiaceae) is one of such herbal preparations that have been used traditionally for treating different disease conditions. *Nauclea latifolia* is a shrub or small spreading tree that is a widely distributed savannah plant. It is found in the forest and fringe tropical forest. Medicinal uses vary from one traditional setting to another; its traditional uses include fever medicine, chewing stick, toothaches, dental caries, septic mouth and malaria, diarrhea and dysentery (Lamidi *et al.*, 1995). It is commonly known as pincushion tree. The Hausa call it Tafashiya (Gbile, 1984). In Igbo it is known as Ogwu-iba and Egbeyes in Yoruba. It is native to tropical Africa and Asia. The plant has sweet scented flower heads. It produces red fruits that resemble a large, rather hard strawbery, with many seeds which have a pleasant taste but act as emetic if eaten in excess (Oliver, 1960). The plant is found in many parts of Nigeria, for example Sokoto, Zaria, Jos and Benin. The aqueous extract of leaves of the plant has been used as a remedy for diabetes mellitus in Northern Nigeria (Gidado *et al.*, 2005). It has been reported that it is one of the sixth most prescribed medicinal plants among the Igede people of Benue State (Igoli *et al.*, 2005). The plant also has been reported to have antihypertensive and laxative activities (Aknanabiatu *et al.*, 2005).

The present study was undertaken to establish the scientific bases for the traditional use of the plant *Nauclea latifolia* as a dental remedy and to identify the possible active principles and to appraise its clinical potential.

MATERIALS AND METHODS

Collection and identification of plant material: A large quantity of the stem bark of the plant, *Nauclea latifolia*, was collected from the forests of Igbodo, a south eastern part of Delta state, Nigeria between January and February, 2007. The plant was identified and authenticated by Dr. B A Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City. The material was sun dried for one week. This was further subjected to another one week of air-drying.

Extraction and preparation of extract: The dried stem bark material was then reduced to very fine powder using a mechanical grinder. The pulverized material (300 g) was transferred into the soxhlet extractor and subjected to gradient extraction using different solvents of increasing polarity. n-hexane 600 mL was first used, followed by chloroform 600 mL and lastly 600 mL of methanol. The extracts were concentrated to dryness using rotary evaporator at reduced pressure. The extracts were further dried to semi-solid with the aid of an oven at 20°C. The dried extract was stored in the refrigerator at a temperature of -4°C until use.

Phytochemical screening: The crude powdered sample was subjected to phytochemical screening testing for the presence of alkaloids, Tannins, flavonoids and saponins using the method of Trease and Evans (1989).

The powdered stem bark (5 g) was boiled with water on a steam bath for 30 min. After filtration, the filtrate obtained was tested for the presence of alkaloids using alkaloidal reagents like Dragendorff's, Mayer's, Wagner's and Hager's reagents.

This procedure was repeated using 10% H₂SO₄ as extracting solvent. Methanol and chloroform were separately used as extracting solvent. The filtrates obtained from each was evaporated to dryness on a water bath after which the residues were dissolved in 1% H₂SO₄, filtered and the filtrate tested for the presence of alkaloids using alkaloid reagents.

For the saponins, about 0.1 g of the plant material was extracted with 5 mL of water for 2 min and filtered. About 0.1 mL of the filtrate was diluted to 1 mL with water. The mixture was shaken vigorously for 2 min and observed for frothing.

In order to test for the Tannins, portion of the extract was dissolved in chloroform, shaken vigorously with 50 mL distilled water, boiled for 5 min and filtered. Volume was made up to 50 mL. To a few drops of filtrate was added an equal volume of 15% ferric chloride and observed for colour change.

The test for the presence of flavonoid involves the addition of 2 mL of the extract to a solution of dilute NaOH and concentrated HCl. The colour change was also observed.

Antimicrobial activity: Microbial strains-clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus viridans*, *Escherichia coli*, *Aspergillus niger*, *Penicillium notatum* and *candida albicans* were all obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, university of Benin, Benin City, Nigeria.

Antimicrobial screening-Ager-well diffusion method was used (NCCLS, 1999; Bailey and Elvyn, 1970; Barry and Thornsberry, 1985; Cruickshank *et al.*, 1975; Silva *et al.*, 1996). Inocula of test organisms obtained from source were obtained by growing each pure isolate in nutrient broth (oxid cm 31) for 18 h at 37°C. 0.2 mL was then used to seed a molten nutrient agar medium cooled to 45°C. This was poured unto sterile Petri dishes and used for analysis.

The fungal isolates were treated in a slightly different way. They were first grown in sabourand dextrose broth (oxid cm 41) and assayed using sabourand agar. The fungal isolates were incubated at 25°C for 72 h.

Extracts were tested at 100 mg concentration. This was prepared by dissolving 1 g of crude extract in 2.5 mL of sterile distilled water to pure 400 mg mL⁻¹. 0.25 mL was then delivered into wells (8 mm in diameter) bored unto the surface of the already seeded nutrient agar plates.

Commercial antibiotics prepared with ciprofloxacin 25 µg (SIGMA) and amoxicillin, 25 µg (Silva Hill) were used in parallel in the agar-well diffusion method. The plates were incubated at 37°C for 24 h and the zones of inhibition were measured in millimeter diameter and recorded.

Determination of Minimum Inhibitory Concentration (MIC): The agar-well diffusion method was also used. The extracts were incorporated into molten nutrient agar at concentrations of 2.5, 2.5, 5.0, 7.5, 10, 15 and 20 mg mL⁻¹. A loopful of the test isolates diluted 10⁶ cfu mL⁻¹ was used to streak the plates and incubated. The minimum inhibitory concentration of the extract was regarded as the lowest concentration that did not permit growth of the test organism.

RESULTS AND DISCUSSION

The phytochemical test results are confirmatory of the published report of the key constituents of *Nauclea latifolia* (Iwu *et al.*, 1999) (Table 1). The chloroform fraction contains more of the alkaloid than the methanolic fraction. This is an indication of the nature of the alkaloids present. They are most likely to be of the intermediate polarity. The roots have been reported to contain indole-quinolizidine alkaloids, as well as glyco-alkaloids and saponins (Iwu, 1999). The stem bark is known to contain monoterpene indole alkaloids (Shigemori *et al.*, 2003).

The antimicrobial results of present research equally confirmed the reported antimicrobial properties of the root (Deeni and Hussain, 1991).

Antibacterial activities of the different fractions are indications of either difference in nature of the constituents or in concentration. The chloroform extract showed higher antimicrobial activity both in spectrum and potency (MIC). The methanolic extraction is active only against two organisms (one Gram positive and one Gram negative) while the chloroform extract showed activity against all the six tested bacteriae. Their non-activity against fungi however is not in conformity with the reported antimicrobial activity of the root of *Nauclea latifolia*. The root has been reported to show activity against fungi (Iwu, 1993).

The different anti-bacterial activities of the two fractions (methanol and chloroform) are probably due to the amount and types of alkaloids present in the fraction. The methanolic extract showed slight presence of alkaloid (++) while the chloroform extract showed higher presence of alkaloid (+++). The significant antibacterial activity could be due to presence of alkaloids.

The high presence of saponins in the methanolic fraction probably did not influence the antibacterial property except in the case of *S. aureus* and *P. aeruginosa*. These two organisms are the only two out of the six that are susceptible to the antibacterial activity of the methanolic extract (Table 2). They are only sensitive at high concentration (MIC) of 10 and 20 mg mL⁻¹; while the corresponding chloroform extract activity was 2.5 and 15.0 mg mL⁻¹, respectively. This shows that the activity may be due to slight presence of alkaloid in the methanolic extract compared to the higher presence in the chloroform extract. The scientific bases for its traditional use as an anti-dental caries, mouth rinsing antiseptic agent, septic mouth treatment, diarrhea and dysentery treatment, are therefore justifiable. The MIC studies showed that that potency is low. It requires 100 µg of the extract to produce the same result as 25 µg of ciprofloxacin and amoxicillin, respectively (Table 3).

Table 1: Phytochemical constituents of *Nauclea latifolia*

Test	Methanolic extract	Chloroform extract
Alkaloidal tests	Present ++	Present +++
Saponins	Present +++	Present ++
Carbohydrates	Present +++	Present +
Reducing sugar	Absent	Absent -

+: Indicates presence of constituents; ++: Indicates high amount of constituents; +++: copious amount of constituents and -: indicates absence of components

Table 2: Anti-microbial minimum inhibition concentration determination

Organisms	Methanolic extract (mg mL ⁻¹)	Chloroform extract (mg mL ⁻¹)
<i>Staph aureus</i>	10	2.5
<i>Bacillus subtilis</i>	-	10.0
<i>Streptococcus varidas</i>	-	5.0
<i>E. coli</i>	-	5.0
<i>Klebsiella pneumonia</i>	-	5.0
<i>Pseudomonas aeruginosa</i>	20	15.0
<i>Aspergillus niger</i>	-	-
<i>Penicillium notatum</i>	-	-
<i>Candida albicans</i>	-	-

Table 3: Comparative zones of inhibition (mm)

Organisms	Methanolic extract	Chloroform extract	CIPRO	AMX	DMSO
	100 µg mL ⁻¹	100 µg mL ⁻¹			
<i>Staph. aureus</i>	16	26	32	21	-
<i>Bacillus substillis</i>	-	20	30	20	-
<i>Streptococcus varidans</i>	-	18	32	20	-
<i>Pseudomonas auregenosa</i>	4	18	34	20	-
<i>Klebsiella pneumoniae</i>	-	20	28	-	-
<i>Aspergillus niger</i>	-	-	NT	NT	-
<i>Penicillium notatum</i>	-	-	NT	NT	-
<i>Candida albicans</i>	-	-	NT	NT	-

NT: Not Tested; AMX: Amoxicillin; CIPRO: Ciprofloxacin; DMSO: Dimethyl sulphoxide and -: Absence of zone of inhibition

The antibacterial activity of *Nauclea latifolia* is of good clinical significance. It's traditional use as a chewing stick, remedy for dental and oral infections serves two major purposes of maintaining good oral hygiene and preventing reservoir status of the mouth for systemic pathogens (respiratory and cardiovascular infections). Studies have implicated the role of poor oral hygiene in such life threatening diseases like chronic obstructive pulmonary disease and cardiovascular disorders (Scannapieco, 1999).

Herbal dentifrices are (for this purpose) gaining popularity and high patronage in the western countries (Hammmer-Beem, 2006) and many studies have shown that the herbal dentifrices are as good as the conventional ones (in controlling the oral bacteria reservoir (Van der Weijden *et al.*, 1998; Mullally *et al.*, 1995). In the Nigerian context price and availability therefore puts the herbal dentifrices at advantage over the conventional ones (Eisenberg *et al.*, 1993).

The findings from this research revealed that the herbal remedy (*Nuclea latifolia*) under investigation has

shown some good scientific bases for its use as a herbal dental remedy due to its significant antimicrobial activity.

CONCLUSION

The research was able to produce scientific bases for the traditional use of *Nauclea latifolia* stem bark as a dental remedy. The broad spectrum antimicrobial activity is a pointer to this. However, the clinical potential could not be appraised due to absence of toxicological studies. To improve the safety and consistency of this traditional herbal remedy, like other herbs, additional research is needed to define the pharmacology, stability and bioavailability of this product (Bayly *et al.*, 1995; Perharic *et al.*, 1994).

Therefore, further studies are therefore needed for the isolation and characterization of the active constituents and determination of structural activity relationship which will serve as a template for the development of semi-synthetic drugs. This will require the structural elucidation of the isolated active constituents using spectroscopic techniques such as nuclear magnetic resonance NMR, Infra red spectrophotometry IR, mass spectrometry, UV spectroscopy and elemental analysis.

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