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Research Article

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Phytochemical and antimicrobial evaluations of the methanol stem bark extract of N*eocarya macropylla*

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ABSTRACT

An assessment of phytochemical composition and antimicrobial studies of the methanol stem bark extract of Neocarya macrophylla was carried out. Phytochemical screening was conducted using standard procedures and the antimicrobial property of the extract was evaluated against clinical isolates including Streptococcus pyogenes, Streptococus feacalis, Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas fluorescence, Candida albicans and Candida krusei using agar diffusion and broth dilution methods. The result of the preliminary phytochemical analysis of the extract revealed the presence of carbohydrates, alkaloids, flavonoids, anthraquinones, tannins, saponins, glycosides, steroids and triterpenes. Susceptibility test (at 2mg) showed inhibition range of 22-34mm against S. pyogenes, B. subtilis, B. cereus, E. coli, C. albicans. No activity was observed against S. feacalis, C. krusei and P. fluorescence. The Minimum Inhibitory Concentrations (MIC) range was 2.5-5.0mg/ml and the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) range was 5-20mg/ml against the sensitive organisms. The findings of this research indicate that the crude methanol stem bark extract of N. macrophylla contains bioactive components that have antifungal and broad spectrum antibacterial properties.

Keywords*: Neocarya macrophylla*, stem bark, methanol extract, Phytochemical screening, Antimicrobial evaluation

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INTRODUCTION

Multiple resistance to antimicrobial agents has become a global problem in recent years largely due to indiscriminate use of antimicrobial drugs commonly employed for the treatment of infectious diseases. Some statistics indicate that about 14 to 17 million people die each year from infectious diseases [1, 2]. This high mortality could be due to extreme poverty of people in the developing countries compared to developed countries, lack of suitable vaccine, lack of access to antibiotics and the emergence of antibiotic-resistant strains [3]. All these drawbacks compel the search for effective alternative antimicrobial substances from various sources including medicinal plants.

Many plants employed in ethno-medicine for the management of microbial infections have been identified and studied using modern scientific approaches and the results revealed their potential in the field of pharmacology [4]. *Neocarya macrophylla* (Sabine) Prance (formerly) *Parinari macrophylla* Sabine is commonly known as Gingerbread plum or Neou oil trees. It belongs to the chrysobalanaceae family which is composed of 17 genera and

525 species widely distributed along coastal savannahs from Senegal to Liberia, woody savannahs of Southern Mali, Niger and Northern Nigeria (Personal Communication). In Nigerian traditional medicine, it is used to treat diarrhoea, asthma, dysentery, cancer, tooth decay, snakebite, pain, inflammation and skin infections [6]. Some preliminary phytochemical screening and physico-chemical studies of the seed oil [6] as well as the antimicrobial studies on the fruit and root bark of the plant [7, 8] were previously undertaken.

This study was aimed at phytochemical screening and the evaluation of the antimicrobial potential of the methanolic stem bark extract of *Neocarya macropylla*.

EXPERIMENTAL SECTION

Collection and Identification of Plant material

The plant sample of *Neocarya macrophylla* was collected in November 2012 at Jega, Jega Local Government Area of Kebbi State. It was identified by U.S Gallah of the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University by comparing with herbarium reference voucher specimen (No. 3197).

Preparation of the extract

The stem bark of the plant was shade dried, pulverized, labelled and stored at room temperature for use. The powdered stem bark (3000g) was extracted with methanol using maceration method for 10 days with occasional shaking. The extract was evaporated in-vacuo using rotary evaporator at 40^{o}C to afford a reddish-brown residue (396g) subsequently referred to as the crude methanol extract (ME).

Preliminary Phytochemical Investigation

Portion (1g) of the methanol extract was subjected to preliminary phytochemical screening for the presence of secondary metabolites using standard procedures [9, 10].

Test Organisms

Clinical isolates of *Streptococcus pyogenes*, *Streptococus feacalis*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas fluorescence*, *Candida albicans* and *Candida krusei* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. All the isolates were checked for purity and maintained in slants of nutrient agar (for bacteria) and in slants of sabouraud dextrose agar for fungi.

Antimicrobial Screening

The antimicrobial activity of the methanolic stem bark extract of *Neocarya macrophylla* was first determined through susceptibility test using agar diffusion. 0.2g of the extract was weighed and dissolved in 10ml DMSO to obtain a concentration of 20mg/ml. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions and sterilized at 121^{o}C for 15mins. It was poured into sterile petri dishes, allowed to cool and solidify. The sterilized medium was seeded with 0.1ml of standard inoculum of the test microbe; the inoculum was spread evenly over the surface of the medium by the use of a sterile swab. Standard sterile cork borer of 6mm in diameter was used to bore a well at the centre of each inoculated medium. The wells were filled with 0.1ml of the solution of the extract (2mg) and allowed to diffuse for I hour. Incubation of the inoculated medium was made at 37^{o}C for 24hrs, after which the medium was observed for the zone of inhibition of growth; the tests were done in duplicates and the zone of inhibition was measured with a transparent ruler. The mean of the results was recorded in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the broth dilution method. Mueller Hinton broth was prepared according to manufacturer's instructions; 2mls of the medium was dispensed in screw-capped test tubes and sterilized at 121°C for 15 min. and allowed to cool; MC-Farlands standard turbidity scale number 0.5 was prepared. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison. At that point, the concentration of the test microbe was about 1.5 x 10^{8}cfu/ml. Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml.. 0.1ml of the standard inoculum of the test microbe was then inoculated into the different concentrations of the extract in the broth. The tubes were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi respectively after which the plates were

observed for turbidity (growth). The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each micro-organism.

Determination of Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

MBC/MFC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar broth was prepared, sterilized at 121^{o}C for 15mins, and transferred into sterile petridishes to cool and solidify. The contents of the MIC in the serial dilution were sub-cultured into the prepared medium and incubated at 37^{o}C for 24 hrs; the plates were observed for colony growth; the MBC/MFC was the plate with lowest concentration of the extract in serial dilution without colony growth [11].

RESULTS AND DISCUSSION

The results of preliminary phytochemical screening and the antimicrobial activity of methanol extract of stem bark of *N. macrophylla* are presented in Tables 1- 4.

Table 1: Preliminary Phytochemical Screening of *N. macropylla*

Constituents	Test	Inferences
Carbohydrates	Molisch's test Fehling's test	+ +
Glycosides	Fehling's test	+
Alkaloids	Mayer's test Dragendorf's test	+ +
Flavonoids	Ferric chloride test NaOH test	+ +
Anthraquinones	Borntrager's test	+
Saponins	Frothing test	+
Steroids & Triterpenes	Lieberman-Buchard Salkowski test	+ +
Tannins	Lead Sub-acetate	+

Key: + presence of constituent - absence of constituent

Table 2: Antimicrobial activity of methanol stem bark extract of *N. macrophylla*

Extract ME (mg/ml)	Zone of Inhibition (mm)		
Test Organisms	**ME (2mg/ml)**	**Ciprofloxacin**	**Fluconazole**
Streptococcus pyogenes	28	35	-
Streptococus feacalis	-	37	-
Bacillus subtilis	31	41	-
Bacillus cereus	34	45	-
Escherichia coli	24	35	-
Pseudomonas fluorescence	-	-	-
Candida albicans	22	-	35
Candida krusei	-	-	32

Key: mean zone of inhibition measured in millimeter (mm), - = activity not detected, ME= methanol extract

Table 3: Minimum Inhibitory Concentration of methanol stem bark extract of *N. macrophylla* against the test organisms

Minimum Inhibitory Concentration (mg/ml)					
Test Organisms	**20**	**10**	**5**	**2.5**	**1.25**
Streptococcus pyogenes	-	-	-	OA	+
Streptococus feacalis	-	-	-	-	-
Bacillus subtilis	-	-	-	OA	+
Bacillus cereus	-	-	-	OA	+
Escherichia coli	-	-	OA	+	++
Pseudomonas fluorescence	-	-	-	-	-
Candida albicans	-	-	OA	+	++
Candida krusei	-	-	-	-	-

Key: - = no turbidity (no growth), OA= MIC, + = turbid (light growth), ++ = moderate turbidity

Table 4: Minimum Bactericidal/Fungicidal Concentration of methanol stem bark extract of *N. macrophylla* against the test organisms

Minimum Bactericidal/Fungicidal Concentration (mg/ml)					
Test Organisms	**20**	**10**	**5**	**2.5**	**1.25**
Streptococcus pyogenes	-	OA	+	++	+++
Streptococus feacalis	-	-	-	-	-
Bacillus subtilis	-	-	OA	+	++
Bacillus cereus	-	OA	+	++	+++
Escherichia coli	-	OA	+	++	+++
Pseudomonas fluorescence	-	-	-	-	-
Candida albicans	OA	+	++	+++	+++
Candida krusei	-	-	-	-	-

Key: - = no colony growth, OA= MBC/MFC, + = scanty colonies growth, ++ = moderate colonies growth, +++ = heavy colonies growth

Preliminary phytochemical screening

The result revealed the presence of carbohydrates, alkaloids, flavonoids, anthaquinones, saponins, tannins, glycosides, steroids and triterpenes. These constituents have been reported to be associated with different pharmacological activities of plants [12].

Antimicrobial activity

The extract exhibited varying degree of antimicrobial effect against the test organisms. The susceptibility tests result showed inhibition range of 22-34mm against *S. pyogenes*, *B. subtilis*, *B. cereus*, *E. coli*, *C. albicans*. The most sensitive organism was *B. cereus* (34 mm) and the least was *C. albicans* (22 mm). No activity was observed against *S. feacalis*, *C. krusei* and *P. fluorescence*. The Minimum Inhibitory Concentrations (MIC) range was 2.5-5.0mg/ml; the extract recorded highest activity against *B. cereus*, *B. subtilis* and *S. pyogenes* at 2.5 mg/ml. The low MIC values suggest good activity of the extract against the test organisms. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) range was 5-20mg/ml against the test organisms further established that the extract has antimicrobial activity against the susceptible organisms and the highest activity was recorded on *B. subtilis* at 5 mg/ml while the least bactericidal effect was on *C. albicans* at 20mg/ml.

Bacteria such as *E. coli*, *B. cereus* can cause diarrhea [13] and dysentery [14]. *S. pyogenes* and *C. albicans* are causative agents of skin and mouth infections [15]. Plants-based products have been utilized as a source for antimicrobial compounds [16]. The antimicrobial activity of the extract against the test organisms could be attributed to the presence of different secondary metabolites detected in the plant. Several high-quality investigations have examined the relationship between these constituents and antimcrobial activity [17, 18]. Antimicrobial activity of saponins [19, 20], flavonoids [17, 21], alkaloids [22, 23], glycosides [24, 25], tannins [26, 27, 28], steroids [17, 29, 30] and anthraquinones [31, 32] have been reported. Stigmasterol and a flavonoid glycoside were recently isolated from the plant [33].

CONCLUSION

Conclusively, the results of the study suggest that the stem bark of *N. macrophylla* contains bioactive constituents with antimicrobial effect and lends credence to the ethno medicinal use of the plant in the management of microbial infections such diarrhoea, dysentery, tooth decay and skin infections. Bioactivity-guided isolation and characterization of the active principles is being undertaken.

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