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Antimicrobial properties of alcoholic extract of African peach (*Nauclea latifolia*) leaves

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Abstract
This study presents results of the antimicrobial properties of *Nauclea latifolia* leaves commonly called African Peach. The ethanolic extract of the leaves at different concentrations was tested on some seven pathogenic bacteria; *Escherichia coli, Staphylococcus aureus, Streptococcus iniae, Bacillus subtilis, Pseudomonas aeruginosa, Samonella typhi and Aeromonas hydrophila* using agar well diffusion assay. Results reveal that ethanolic extracts of *N. latifolia* had better antibacterial potentials than the methanolic extracts. *Nauclea latifolia* leaves showed the highest antibacterial activity in the ethanolic extracts on *Bacillus subtilis* with a zone of inhibition of 27.5 ± 0.25 mm while the lowest antibacterial activity was recorded on *Escherichia coli* with a zone of inhibition of 9.18 ± 0.04 mm. No antibacterial activity was recorded in the control (distilled water). The results signify that the alcoholic extracts of *N. latifolia* leaves can serve as a cheap source of raw material in the production biomedical products.

Keywords: antimicrobial properties, African peach (*Nauclea latifolia*)

Introduction

The use of medicinal plants for the treatment of many diseases is associated with folk medicine from different parts of the world. Naturally occurring compounds from plants, fungi and microbes are still used in pharmaceutical preparations in pure or crude forms. Medicinal plants are increasingly being used in most parts of the world as: analgesic and anti-inflammatory, anticonvulsant (Oyewole and Amabeoku, 2006) [1], hypoglycemic and hypocholesterolemic (Brai *et al*., 2007) [2], wound healing and reducing cancer risk (Nayak *et al.*, 2008) [3]. Herbal remedies have increasingly become attractive alternatives to prevent or treat hypercholesterolemia, especially for those with cholesterol at the borderline levels. Excellent safety profile, cost effectiveness and multiple beneficial effects on improving wellbeing, have collectively contributed to the emerging trend of increasingly usage of herbal supplements. A number of studies have shown that the reduction of LDL-cholesterol with medicinal plants will reduce the incidence of cardiovascular diseases and overall death rate (Nayak *et al.*, 2008) [3]. Cardiovascular disease is the leading cause of death accounting for nearly 50% of all deaths in the western world (Thomas and Rich, 2007) [4]. Due to wide spread of western diet to the developing world there has been explosive increase in the rates of obesity and hypercholesterolemia in the developing world. Controls of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing cardiovascular diseases (Stacy and Egger, 2006) [5]. However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages have been reported by Neuvonen *et al* (2006) [6]. Therefore, development of additional therapies for controlling cholesterol levels is important, especially for those with better safety profile and lower cost.

Nauclea latifolia (Smith) belongs to the family Rubiaceae, Genus *Nauclea* and Species *latifolia*. It is a straggling shrub or small tree, native to tropical Africa and Asia. It bears an interesting flower, large red ball fruit with long projecting stamens. The red fruit is edible but not appealing. *Nauclea latifolia* is an evergreen multi-stemmed shrub or tree. It is widespread in the humid tropical rain forest zones or in the savanna wood lands of west and central Africa. The common name of the plant is African peach .The commonly used parts of the plant include bark, fruit, roots, stem and sap. In Nigeria, *Nauclea latifolia* is called Egbesi [Yoruba], uburuinu/nbitinu [Igbo] and tafashiya or tuwonbiri [Hausa], Mbom- mbog (Efik) in the local languages. The plant fruit contains vitamins A, B1, B2, C and E (Nkafamiya *et al*., 2006) [7]. The wood of *Nauclea latifolia* is termite resistant and is used as live stakes in farms. All parts of the plant species are rich source of mono-terpene indole alkaloid. The fruit is a major source of food for the baboons, livestock, reptiles, birds and man. It is used for the treatment of stomach ache when the decoction of the bark and leaves are infused. It is also used in the treatment of fever, diarrhea and even as anti-parasitic drug. The sticks are used as chewing stick and a remedy against tuberculosis (Esimore *et al*., 2003) [8].

Fig 1: African Peach (*Nauclea Latifolia*) Leaves

Biochemical evaluation of the plant (*Nauclea latifolia*) indicates that the plant has high anti- diabetic, high anti-hypertensive and anti-abortifacient properties (Nworgu *et al.*, 2010) [9]. In African traditional medicine, *Nauclea latifolia* has neuropharmacological effects and it is also being used as herbal recipe for its cardiovascular activities. The crude extract of the roots have been shown to have anti-hypertensive effect (Nworgu *et al.*, 2010) [9]. The purpose of this study is to evaluate phytochemical screening of methanolic extract of *nauclea latifolia* leaves. The present study was prompted by the claim of some traditional health practitioners in parts of Nigeria that the fruits, leaves and roots of *Nauclea latifolia* are effective remedies for the management and/or control of hypertension and certain cardiac disorders. With the increasing incidence of cardiovascular diseases in the urban and rural population throughout the world, there is clear need for development of indigenous, inexpensive botanical sources of anticholesterolemic crude or purified drugs. This, therefore, necessitates the need for research into alternative medicine or therapeutic agents that retain therapeutic efficacy, and can be taken for long durations, devoid of, or with minimal side effects and with low prescription cost. This work will provide pharmacological justification (or otherwise) for the ethno medical uses of the *latifolia* roots in the management, control and/or treatment of certain cardiovascular disorders.

Materials and Methods

Sample collection and preparation

Nauclae latifolia leaves were collected from the botanical garden of University of Cross River. The leaves were washed in distilled water, air – dried at room temperature for 31 days and grounded with a hammer mill into powder. About 180g of the fine powder of the plant leaves was aseptically soaked in 1000 ml of ethanol and stirred at regular interval (every 3 hours), filtered using a sterile muslin cloth after which the extraction was obtained, air-dried and store at 25^0C until used. This procedure was repeated for methanol and water.

Media Preparation

Media such as nutrient broth (Oxoid), nutrient agar (Biolife) and Mueller-Hinton agar (Hi Media) used were prepared according to manufacturer's instruction. All these media were allowed to cool after sterilization to about 45^0C before pouring into petri dishes.

Test Microorganisms/Counts

The microorganisms isolated were; *Escherichia coli, Staphylococcus aureus, Streptococcus iniae, Bacillus subtilis, Pseudomonas aeruginosa, Samonella typhi and Aeromonas hydrophila.* The pure cultures were labeled, sub-cultured on nutrient agar slants and nutrient broth(s) and were preserved in the refrigerator at 40ºC until it is required for study. The gills, skin, intestine and liver sample of *Clarias gariepinus* (cat fish) were separately macerated and put into sterile clapped test tube containing sterilized distilled water and homogenized. Serial dilution was carried out and 1ml each from 10-3 and 10-5 dilution factors were dispersed into petri dishes that were appropriately labeled and molten sterilized medium was poured aseptically into petri dish.

The plates were swirled gently for even distribution of inoculated and allowed to set/gel and then incubated at 37ºC for 24 hours. The organisms grew into visible different colonies after 24 hours. Total viable counts and enterobacteriacea counts were determined, the result were expressed in Log_{10}CFU/g.

Antibacterial Activity Assay

A well diffusion assay was used. Pre-poured indicator pathogen (4 mm depth) was overlaid with a 10 ml soft agar (0.7%) lawn of indicator culture (thus generating a potential mat for the indicating of bacteria). Wells of 10 mm diameter were cut into these agar plates using cork borer and 0.2ml of these plants extract was placed into each well. Distilled water was used as control. The plates were examined for zones of inhibition which was scored positive, if the width of the clear zone was 10 mm or longer. The diameter of the inhibition zones were taken to be proportional to the logarithm of the antimicrobial compounds in *N. latifolia* leaves.

Minimum Inhibitory Concentration

Minimum inhibitory concentration of *N. latifolia* leaves was assessed based on a micro broth dilution method in a test tube. 180 mg/ ml of the plant extract were made in 2 ml volume of broth to 1.76 mg / ml. One row of the test was inoculated with 0.02 ml of 1 in 10 dilution of the overnight broth culture of the organism. The test was incubated at 37ºC for 24 hours aerobically. The minimum inhibitory concentration was the lowest concentration that prevented the growth of bacterial after 24-hour incubation.

Results and Discussion

The microbial load of cat fish tissues (skin, gills, intestine and liver) is presented in Table 1; the results obtained revealed that the highest enterobacteriacea counts was recorded in the gills and least in liver and intestine while no enterobacteriacea counts was recorded in control. Also, the highest total viable count was recorded in gills and least in liver. The result of this study shows that the microbial load of the gill, skin liver and intestine of *C. gariepinus* varies, the gill having the highest value of total viable counts and enterobacteriacea counts. This is in accord with the report of Shalaby *et al.*, (2006) [11] and Olusola *et al*., (2017) that the bacteria load is greater on the gills and skin than any part of the fish as these parts are ones constantly exposed to challenges.

Table 1: Microbial load of gills, skin, intestine and liver of *Clarias gariepinus*

Organ (Log_{10}CFU/g)	Organism microbial load	
	Enterobacteriacea counts	Total viable counts
gills	6.47	6.51
Skin	6.12	6.51
Intestine	6.23	6.29
Liver	6.10	6.43
Control	-	-

Both Ethanolic and methanolic extracts of *N. latifolia*, leaves showed antibacterial activities on pathogens isolated from *Clarias gariepinus* (Table 2) However, *Nauclea latifolia* leaves showed the highest antibacterial activity in the ethanolic extracts on *Bacillus subtilis* with a zone of inhibition of 27.5 ± 0.25 mm while the lowest antibacterial activity was recorded on *Escherichia coli* with a zone of inhibition of 9.18 ± 0.04 mm. Also, *Streptococcus pyrogens* did not show any diameter of zone of inhibition in ethanolic extracts of *N. latifolia* leaves but

recorded a high antibacterial activity in the methanolic extract with a zone diameter of 10.50 ± 0.12 mm. This shows that *Streptococcus pyrogens* are resistant to the antimicrobial agent present in the methanol extract, attributed to the fact that the bacteria is inherently resistant to many antibiotics and non-antibiotic antimicrobial agents due to the permeability barrier afforded by their outer membranes (el-mahmood *et al*, 2008; Nyong *et al*, 2021) [12, 10].

Table 2: Antibacterial activities (diameter of zone of inhibition mm) of methanolic and ethanolic extracts of *N. latifolia* leaves

Pathogens	Ethanol extract	Methanol extract
Bacillus subtilis	27.50 ± 0.21	10.50 ± 005
Pseudomonas aeruginosa	20.00 ± 0.03	17.10 ± 0.29
Streptococcus pyogens	-	10.50 ± 0.12
Escherichria coli	9.30 ± 0.16	9.18 ± 0.10
Salmonella typhi	21.00 ± 0.26	15.02 ± 0.03
Aeromonas hydrophila	9.50 ± 0.08	11.50 ± 0.29
Staphylococcus iniae	21.00 ± 0.45	14.50 ± 0.17

The minimum inhibitory concentration of ethanolic and methanolic extract of *N. latifolia* leaves against seven (7) isolated bacteria from *Clarias gariepinus* revealed 4.5 mg/ml and 9.0 mg/ml for the methanolic and ethanolic extracts respectively (Table 3). *Nauclea latifolia* leaves extracts showed antibacterial activity against the isolated fish pathogens from *Clarias gariepinus* except *Streptococcus pyrogens* that did not show any diameter of zone of inhibition in ethanolic extracts of *N. latifolia* leaves. This results is in agreement with the report of El-Mahmood *et al*., (2008) [12] who reported that ethanol and methanol extracts of *N. latifolia* leaves respectively exhibited antimicrobial activity against the microorganisms tested (*Pseudomonas aeruginosa, Klebsiealla pneumonia, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi).* This result shows antibacterial activity of plant active ingredients that inhibit bacterial growth which was in agreement with the findings of Okwori *et al* (2008). The result also shows that ethanolic extracts had better diameter of zone of inhibition when compared to the methanol extracts, this may be due to the fact that ethanol is the best solvent for the
active compounds extracted from the plant. Also, the difference in antibacterial activity of a plant extracts might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light, water), time of harvesting of plant materials and drying method used before the extraction process.

Table 3: Minimum inhibitory concentration (µg/ml) assay of methanolic and ethanolic extracts of *N. latifolia* leaves

	Ethanol									Methanol								
Isolates	1800	900	450	225	112	56	24	14	7	1800	900	450	225	112	56	24	14	7
B. subtilis	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
S. pyogens	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
E. coli	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
S. typhi	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
A. hydrophila	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
S. iniae	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+

- inhibition, + no inhibition

The minimum inhibitory concentration assay carried out on *N. latifolia* leaves indicates that 450µg/m and 900µg/m for ethanolic and methanolic extracts is required to inhibit the seven isolated fish pathogen (*P. aeruginosa, A. hydrophila, B. substillis, Salmonella typhi, S. iniae, Streptococcus pyogens, and E. coli*). The minimum inhibitory concentration of both the ethanolic and methanolic extracts of the *N. latifolia* leaves showed appreciable inhibitory effect when compared to the control which support the report of Okwori *et al.,* (2008) who used serial doubling dilution method, recorded inhibitory and bactericidal activity on the test organisms (*Pseudomonas aeruginosa, Klebsiealla pneu monia, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi).*

In conclusion, The results of the present study shows that *N. latifolia* leaves exhibit a wide range of antimicrobial activity which can be used for the treatment of fish diseases and improvement of fish yield in aquaculture. Therefore, it can be concluded that the methanolic and ethanolic extracts leaves of *N. latifolia* possesses a broad spectrum of antimicrobial potentials and may be useful in the formulation of antimicrobial agent that could be used for the treatment of microbial infections of different species.

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