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Phytochemical Screening and Antimicrobial Activity of *Nypa fruticans* Harvested from Oporo River in the Niger Delta Region of Nigeria

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ABSTRACT: First introduced to check coastal erosion, *Nypa fruticans* has proven to be much more useful. Given the variety of potentials it possess, we decided to analyse the leaves, husks and midveins of this plant for phytochemical bases and also test the antimicrobial property of various extracts against *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa*. Phytochemical screening revealed the presence of alkaloids and polyphenols, and absence of tannins and anthraquinones. Aqueous and ethanolic extracts of the midveins, leaves and husks showed good antimicrobial against all the test organisms. Varying concentrations of the ethanolic extract of the leaves revealed that at concentration of 5% and above gave absolute inhibition of *E. coli*. There is need to reconsider the re-utilization of Nypa Palm in Nigeria.

KEYWORDS: Nypa Palm, Polyphenols, Tannins, phytochemicals, Oporo River, Niger Delta.

INTRODUCTION

Nypa palm (*Nypa fruticans*) is a prostrate-stemmed gregarious palm growing in estuarine conditions akin to mangroves and is sometimes referred to as a mangrove plant as it flourishes in mangrove environment ranging from Queensland to India. It also colonises the upper tidal reaches of rivers. Its habitat is unique in that it does not compete with food crop and its growth is sustainable. It was first introduced from Singapore Botanic Gardens to Calabar in 1908, Oron in 1912 and eventually the rest of the Niger Delta from 1946 to help check coastal erosion [1], [2], [3], [4], [5]. This important and abundant tree which is largely underutilized in Nigeria has been exploited in different parts of the world in different areas. It provides oil, starch and sugar. Its large amount of sap and potential for large amount of sugar production has given it the name sugar alternative with minerals and low glycaemia index. It has potentials for the production of more biofuels such as ethanol and butanol than sugarcane per hectare per year [2]. It has also been used to produce local sweet alcoholic drink called tuba and converts to vinegar on storage. Other regions use the leaflet when burnt as salt and the leaf's epidermis as cigarette paper. Here in the tropics, its leaves is used as roofing material. Despite the numerous potential uses it can be put into, Nypa palm has been tagged an invasive plant following environmental impact assessment conducted recently for oil companies in Nigeria. Its rapid and invasive growth where there is little or no vegetation has seen it almost completely remove the mangroves. Proximate analysis conducted recently on Nigeria's Nypa Palm indicates that the plant is very rich in ash, lignin, cellulose, hemicelluloses, moisture and nitrogen [2], [6]. Another study also established that the fronds and petiole usually treated as waste can be exploited for commercial and industrial uses [1], [2]. A recent study has shown that it is rich in phytochemicals such as phenolics and flavonoids, and even antioxidant properties [7]. However, there is a shortage of information on the antimicrobial properties of this plant. Hence we decided to screen the husks, leaves and midveins of this wonderful mangrove plant for phytochemicals bases and also check the effect of the its extracts on some microorganisms.

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MATERIALS AND METHODS

SOURCE OF SAMPLES

The test organisms were obtained from the University of Calabar Teaching Hospital and University of Calabar Medical Centre. The organisms collected are *Escherichia coli, Klebsiella Pneumonia Staphylococcus aureus, Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* These organisms were cultured and preserved on appropriate media as previously described [8]. The study plant was obtained from Oporo Rivers bank in Akwa Ibom State and identified at the University of Calabar Botanical Garden.

PREPARATION OF AQUEOUS AND ETHANOLIC EXTRACTS

The leaves, husk and tissues of *Nypa fruticians* were dried in the hot air oven at 50^0C and ground into powder in a small mortar. The leaves, husk and tissues were cut into tiny bits before drying. 200g of the powder plant part was weighed into 500ml of distilled water in Erlemeyer flask and allowed to stand for a day. It was filtered through a glass funnel plugged with cotton wool into a sterile reagent bottles and kept in the freezer until ready for use.

About 80g of the dried well ground plant parts was weighed into 200ml of 97% ethanol in a sterile reagent bottles and allowed to stand for two weeks. Aluminium foil was wrapped around each reagent bottle to prevent light and microbial degradation. It was filtered and evaporation of the ethanol carried out using a glass beaker in a water bath leaving behind an oily remnant.

TEST FOR PHYTOCHEMICAL COMPONENTS

Phytochemical screening of the plants extracts was carried out using methods previously reported [9],[10], [11], [12].. These are briefly described below.

ALKALOIDS AND CARDIAC GLYCOSIDE

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of Alkaloids. About 0.5g of the extract was dissolved in 2ml of chloroform. After which H_2SO_4 was carefully added to form a lower layer. A reddish brown colour at the inter-phase indicated the presence of steroidal ring which is the aglycone portion of the cardiac glycosides.

ANTHRAQUINONES AND PHLOBATANNINS

5g of each plant extract was shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonia cal (lower) phase indicated the presence of free anthraquinones. For phlobatannis, an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate was taken as evidence for the presence of phlobatannis.

ANTHRANOIDS AND POLYPHENOLS

Exactly 2g of grounded pant material was boiled with 5ml of potassium hydroxide. The solution was filtered through glass tube. The filtrate was treated with acetic acid and the resulting solution was mixed Toluene. The upper layer was transferred to another test tube and potassium hydroxide added. The presence of red colour indicates the presence of anthranoids. For polyphenols, 2g of well-grounded plant material was heated with 10ml of distilled water for 30 minutes. Then a mixture of 10% ferric chloride 1ml and 1% potassium ferricyanide 1ml was added to the solution. This was then filtered; the formation of a green blue colour indicates the presence of polyphenols.

TANNINS, SAPONINS AND FLAVONOIDS

About 5g of each portion of plant extract was shared with 10ml of distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black green or blue green precipitate was taken as evidence for the presence of tannins. For saponins, about 0.5g of each plant extract was shaken with water in a test tube. Frothing which persists on warming was

taken as evidence for the presence of saponins. About 2mls of the alcoholic extract of the plant part was added to a few pieces of magnesium metal followed by the addition of a few drops of concentrated hydrochloric acid. The formation of orange, red, crimson or magenta was taken as an evidence for the presence of flavonoid.

ANTIMICROBIAL TEST OF EXTRACTS

Briefly, a cork borer was used in cutting filter papers into 9mm in diameter. The filter papers were wrapped with aluminium foil and sterilized in the hot air oven. A colony of each test organisms was sub-cultured on nutrient broth and incubated at 37^0C for 6 hours, to ensure that the bacteria were in logarithmic phase of growth. They were then inoculated on Mueller-Hinton agar plates that have solidified. The sterilized filter paper disc were soaked in the respective test extracts (aqueous and ethanolic) and placed on the agar plates using sterile forceps. The plates were incubated at 37^0C for 24 hours. After incubation the zones of inhibition were observed. The diameter of the zones of inhibition was measured in quadruplicates and the mean was determined.

MINIMUM INHIBITORY CONCENTRATION (MIC) OF ETHANOLIC EXTRACT OF *NYPA FRUTICANS* LEAVE ON *E. COLI*

The largest zone of inhibition observed in this experiment was given by the ethanolic extracts *Nypa fruticans* leaves on E coli. The test was performed by soaking 200g of well ground dried leaves of *Nypa fruticans* in 500ml of 97% ethanol in a sterile reagent bottle and allowed to stand for two weeks. It was then filtered and ethanol was evaporated in a beaker on a water bath leaving an oily remnant. The percentage by volume (1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25% and 30%) of the oily remnant was prepared by dissolving 0.1g, 0.2g, 0.3g, 0.4g, 0.5g, 1g, 1.5g, 2g, 2.5g and 3g in 10ml of distilled water respectively. This was incorporated into sterile nutrient agar aseptically poured into sterilized petridishes appropriately labelled, swirled to mix and allowed to solidify. A culture of *Escherichia coli* grown for 6 hours was then streaked on the nutrient agar plates and observed for growth after incubation at 37^0C for 24 hours.

OPTICAL DENSITY OF *ESCHERICHIA COLI* AT DIFFERENT CONCENTRATIONS OF ETHANOLIC EXTRACTS OF *NYPA FRUTICANS* LEAVES

Precisely 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25% and 30% of the oily remnant of *Nypa fruticans* leaves was incorporated into sterile nutrient broth culture medium in test tubes. The test tubes were appropriately labelled and then one drop of a culture of *Escherichia coli* grown for 6 hours was then added to each of the labelled test tubes. The test tubes were then incubated at 37^0C for 24 hours. They were observed for growth as shown by turbidity of the medium. After incubation, the optical density (O.D) of the contents of the test tubes was measured using the colorimeter with a wave length of 540nm. A graph of optical density against percentage concentration of extract was plotted.

RESULTS

The results of the study are as presented in the tables and figure below. Table 1 indicates the phytochemical bases that we screened for in the samples. Polyphenol and alkaloids were present in the leaves, husk and mid-vein tissues of the plant. However, anthraquinones and tannins were not detected in all three samples. As shown in table two, the extracts from the Nypa plant showed excellent antimicrobial activity against the tested organisms. None of the tested organisms showed resistance.

Table 1: Phytochemical Screening of Nypa fruticans Parts

Phytochemical Bases	Leaves	Husk	Tissues
Alkaloids	+	+	+
Cardiac glycosides	+	-	-
Anthraquinones	-	-	-
Phlobotannins	-	+	-
Anthranoids	+	+	-
Polyphenols	+	+	+
Tannins	-	-	-
Saponins	-	+	+
Flavonoid	+	-	+

Table 2: Antimicrobial Activity (mm) of the Aqueous and Ethanolic Extracts of the Leaves, Midvein Tissues and Husk of Nypa fruticans on Selected Microorganisms

Test Bacteria	*Leaves*		*Husk*		*Midvein Tissues*	
	Ethanolic	*Aqueous*	*Ethanolic*	*Aqueous*	*Ethanolic*	*Aqueous*
Staphylococcus aureus	25.00±0.15	26.00±0.18	25.40±1.00	24.10±0.31	24.00±0.37	24.20±0.30
Esherichia coli	33.00±1.16	28.50±0.58	25.10±0.42	22.40±0.62	25.20±0.42	22.00±0.15
Klebsiella pneumonia	20.00±0.00	23.30±0.32	25.30±0.31	15.00±0.00	23.60±0.18	25.10±0.13
Staphylococcus epidermidis	26.00±0.19	24.00±0.37	20.00±0.37	16.00±0.10	25.10±0.32	26.20±0.32
Pseudomonas aeruginosa	24.20±0.32	25.20±0.31	24.00±0.12	23.20±0.33	15.00±0.10	14.00±0.10

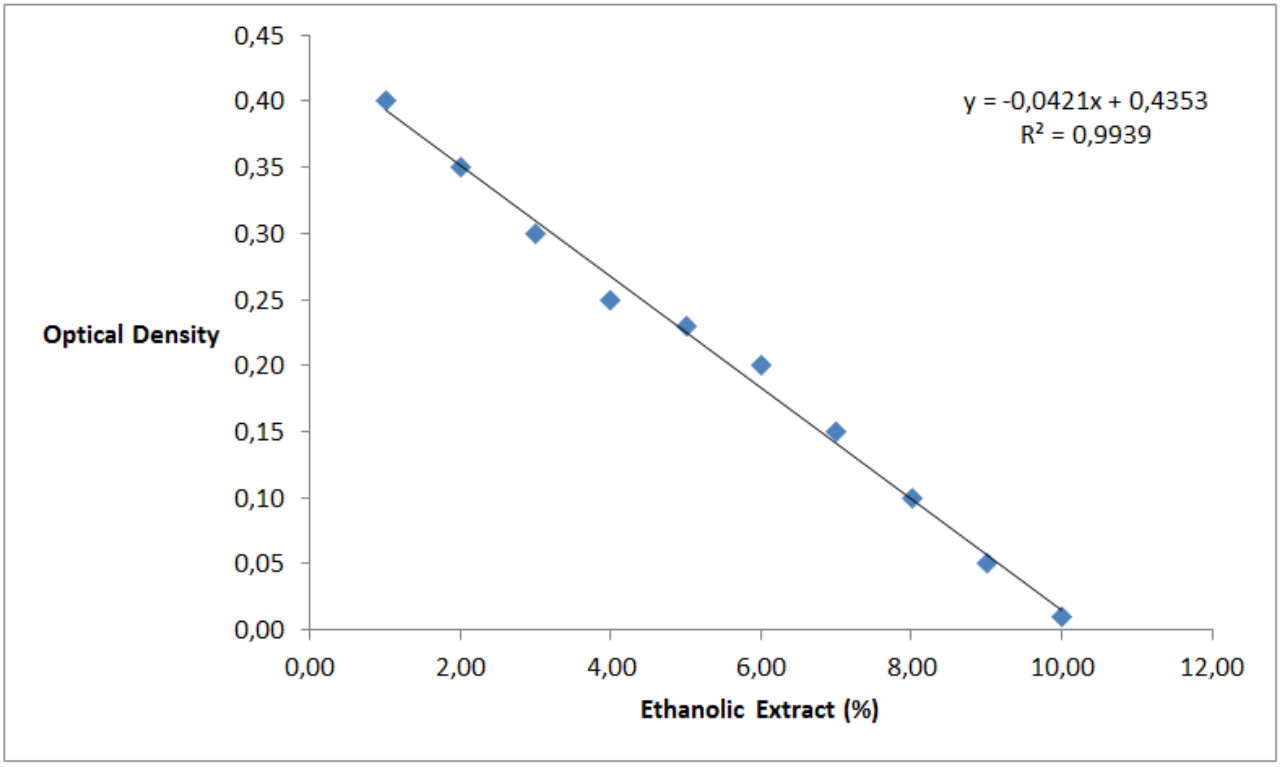

Figure 1: Growth rate of E. coli. at different concentrations of ethanolic extract of Nypa fruticans leaves. The graph shown above shows the decreasing optical density of oily remnant of the ethanolic extract on E. coli. The higher the concentration, the lower the optical density or the lesser the growth observed. The high R^2 valve shows a strong correlation between the variables.

DISCUSSION

Nigeria abounds with a wide variety of plants which are both medicinal and nutritional in value [13]. One of such plant is the Nypa fruticans. As already mentioned, these plants have good potential for the production of sugar, ethanol (biofuels), pulp for paper making and vinegar production in addition to its use as housing material [2],[6]. The nutritional properties of these mangrove invasive plants and its possible microbial properties is only begining to emerge [14], [15], [16], 17, [18]. Our study has shown that Nypa fruticans is a rich source of various phytochemical bases such as alkaloids, cardiac glycosides, polyphenols, phlobotannins, saponins and anthranoids. The presence of polyphenol in the husk of Nypa fruticans was also confirmed in another study and even in larger quantity. The same study also showed the presence of moisture, crude protein, fat, ash, fibre, carbohydrate and even toxicants. The seeds and Husk have also been shown to contain important nutrients such as sodium potassium and Iron [15]. The excellent antimicrobial activity exhibited by our extracts can be attributed to the presence of polyphenols. Both aqueous and ethanolic extract of the plants showed good antimicrobial properties on routine microorganisms such as *Esherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Klebsiella pneumonia.* Studies showed that the plant had excellent anti-bacteria activity against Vibrio species [14] and also on *Fusarium oxysporum* [16]. The aqueous and ethanolic extract of the leaves did show

good inhibition on *E. coli* and its MIC was seen at 5% of the ethanolic extract. The growth survival curve of the ethanolic extract on E coli, shows that the higher the concentration, the lower the optical density or growth of *E. coli.* Generally, at 5% and above, very scanty or no growth was observed.

CONCLUSION

Although, Nypa palm has become very invasive in its growth in the mangroves of the Niger Delta region, there is need to rediscover the plant altogether. Studies have shown that it can be put to a variety of uses such as biofuels, as livestock feed and paper manufacturing in addition to its other conventional uses. Our study have also been able to confirm that the plant is indeed rich is important phytochemical bases. However, there is need to establish the main bioactive agent and also rethink the applications of this wonderful palm away from checking coastal erosion.

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