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Phytochemical profiling of red mangrove plant (*Rhizophora racemosa*) bark: A factor to its applications

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Abstract
The application of extracts from red mangrove plant (*Rhizophora racemosa*) bark has awakened the need to profile the phytochemicals of the plant. The study characterized the extracts of the red mangrove plant (*Rhizophora racemosa*) to investigate the profile of its phytochemicals. The extraction was conducted using the conventional technique, utilizing water and ethanol as solvents. Classical phytochemical screening techniques and a Gas Chromatography – Flame Ionization Detector (GC-FID) were used to characterize the extracts. Preliminary phytochemical screening of the extracts revealed the presence of tannins, alkaloids, steroids, cardiac glycosides, flavonoids, anthraquinone, saponins, and terpenoids. Terpenoids and alkaloids were absent in the water extract. The GC-FID indicated the concentrations; alkaloids 2.5171 µg/ml, tannins 4.7026 µg/ml, flavonoids 26.084 µg/ml, saponins 4.2997 µg/ml, phenol 11.8429 µg/ml, oxalate 2.3746 µg/ml, phytate 1.9860 µg/ml and steroids 11.9544 µg/ml. The ethanol extract showed the following concentrations; alkaloids 1.6344 µg/ml, tannins 4.6642 ug/ml, flavonoids 28.859 ug/ml, saponins 3.9587 µg/ml, phenol 6.9698 µg/ml, oxalate 2.5587 µg/ml, phytate 0.6926 µg/ml and steroids 12.4892 µg/ml. The high phytochemical content of these extracts implies that they might be used as a therapeutic plant as well as for other industrial purposes.

Keywords: Red mangrove plant, phytochemical, profiling, screening, buan, rivers state

1. Introduction
Mangroves are notably special plants that have developed and are surviving in the region between land and ocean in many humid climates of the tropics and subtropics. However, various descriptions and definitions of their compositions portray them as coastal woodland, tidal forests, and mangrove forests tide. Consequently, a mangrove plant can reach up to 80 feet (24 m) in height in ideal conditions. It is commonly found at a more modest 20 feet (6.1 m). Its bark is thick and a grey-brown colour. Mangrove leaves are 1–2 inches (2.5–5.1 cm) wide and 3–5 inches (7.6-12.7 cm) long, with smooth margins and ellipse shape. They are a darker shade of green on the tops than on the bottoms. The tree produces pale pink flowers [1]. The major family commonly found in Nigeria is *Rhizophoraceae*, commonly called the red mangrove. Its species are *Rhizophora racemos*a, *R. harrisonii*, and *R. mangle.* Of this, *Rhizophora racemosa* is the most abundant, taking about 90% of the mangrove forest [2, 3]. Red mangroves are distinguished by their distinct prop roots system and viviparous seeds. Nigeria has large mangrove forests along the Niger Delta's shore. In terms of area covered, Nigeria's mangrove is the largest in Africa and the fourth largest in the world, with Indonesia being the largest. Brazil>Australia>Nigeria [4]. The mangrove forest extends from Badagry in the West to Calabar in the East, covering a total area of 10,000km^2 along the coast [2], and Rivers State is located in Nigeria's tropical mangrove swamp and rain forest zone. The mangrove swamp belt thins out from the coast northward, giving way to a dense rain forest that has become a secondary forest in some areas [5]. *Rhizophora racemosa* is a species of a mangrove tree in the family Rhizophoraceae. It has a patchy distribution on the Pacific coast of Central and South America, occurs in places on the Atlantic coast of that continent, and has a more widespread range on the Atlantic coast of West Africa [6]. In West Africa, estuaries, bays, and lagoons are fringed by tidal mangrove forests, dominated by *Rhizophora* and *Avicennia*. However, when new mudflats are formed, seagrasses are the first plants that grow on the mud, with *Rhizophora racemosa*, a pioneering species, being the first mangrove to appear. With time, the mud solidifies, and more tree and plant species arrive. On the seaward side, the trees are short but get steadily taller further inland [7]. 2007).

Consequently, *Rhizophora racemosa* is largely wind-pollinated, and the fruit produces propagules that may fall into the water and be dispersed by wind and currents [8].

Plant phytochemicals can be produced from a variety of plant components, including the bark, leaves, flowers, roots, fruits, and seeds. As a result, the therapeutic effects of this plant have been related to a variety of phytochemicals such as alkaloids, flavonoids, saponins, phenols, steroids, and so on [9]. Phytochemicals, which are also referred to as secondary metabolites, are found in plant foods, and other plant extracts are numerous and exhibit various physiological actions. Apart from the nutritional values of different plants foods, and extracts, they have equally proved to be strong agents of disease prevention and treatment [10] and have also been applied as indicators in titration [11]. These phytochemicals that have found wide therapeutic applications include alkaloids, flavonoids, tannins, saponins, carotenoids, etc. For instance, many alkaloids are extremely toxic and may act as neuromuscular poisons, enzyme inhibitors, or membrane transport inhibitors. Alkaloids (morphine, codeine, and cocaine) are used as pain killers, anaesthetics, antimalarial, stimulants, and insecticides [12, 10]. Flavonoids also play vital roles as anti-inflammatory, anti-allergic, and anti-cancer roles [13]. Flavonoids have been shown to possess anti-hypertensive properties [14, 15]. Tannins cause protein inactivation, hence used as insecticides. They also possess astringent properties. Tannins have been shown to inactivate Polio Virus, Herpes simplex, and other enteric viruses [16]. Saponins also serve as natural antibiotics, reducing cardiovascular diseases and reduction in cholesterol levels [10]. The study characterized the extracts from the red mangrove plant (*Rhizophora racemosa*) bark to investigate the phytochemical profile of the plant.

2. Materials and Methods

2.1 Collection of the Plant Sample

The plant sample was collected from Buan Mangrove Forest in Khana Local Government Area, Rivers State, Nigeria. Buan lies between 4º 36' 43.668" N and 7º 29' 27.78" E. The sample was collected using a matchet to remove the bark from the red mangrove trunk.

2.2 Preparation and Extraction of the Plant Sample

The plant's bark was stripped, rinsed with distilled water to remove sand and debris, and then air-dried for seven days. The dried sample was crushed and kept in airtight cellophane until it was time to utilize it. To obtain a noticeable comprehensive extraction of the active components in the plant samples, about 10 g of the red mangrove plant was soaked in 100 mL of various solvents: ethanol and distilled water and was permitted to stand for 2 days. The sample solutions were filtered, and the filtrates were pre-concentrated and stored for future use [17-20].

2.3 Qualitative Phytochemical Screening

Qualitative phytochemical screening of secondary metabolites was carried out using the methods described by Onwuka [21].

2.4 GC-FID Analysis

2.4.1 Extraction of Phytochemicals

About 1g of the sample was weighed and transferred in a test tube, and 15ml ethanol and 10ml of 50%m/v potassium hydroxide were added. The test tube was allowed to react in a water bath at 60^0C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water, and 3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate, and the solvent was evaporated. The sample was solubilized in 1000µl of pyridine of which 200µl was transferred to a vial for analysis.

2.4.2 Quantification by GC-FID

The analysis was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280ºC with the spitless injection of 2ul of sample and a linear velocity of 30cms^{-1}. Helium 5. 0pa.s was the carrier gas with a flow rate of 40 ml min^{-1}. The oven operated initially at 200^0C. It was heated to 330^0C at a rate of 3^0C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320^0C. Phytochemical was determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in µg/ml.

3. Results and Discussion

3.1 Classical Phytochemical Screening

Preliminary phytochemical screening revealed the presence of tannins, alkaloids, steroids, cardiac glycosides, flavonoids, anthraquinones, saponins, and terpenoids in the extracts. Terpenoids and alkaloids were absent in the water extract, as presented in Table 1. Table 1 shows the results obtained from classical phytochemical screening conducted.

Table 1: Classical Phytochemical Screening

Sample	Tannin	Saponin	Cardiac Glycoside	Steroids	Terpenoids	Alkaloids	Anthraquinone	Flavonoids
Water Extract	+	+	+	+	-	-	+	+
Ethanol Extract	+	+	+	+	+	+	+	+

+ = Present, - = Absent

3.2 GC-FID Results of the Red Mangrove Plant (*Rhizophora racemosa*)

Gas Chromatography-Flame Ionization Detector (GC-FID) is a recent technology that gives reliable evidence on the types and amounts of phytochemicals present in a given plant sample Azubuike *et al*. [30]. The GC-FID analysis results of the red mangrove plant showed that it contains varying amounts of flavonoids, alkaloids, tannins, saponins, phenols, oxalate, phytate, and steroids. The types and amounts of the different phytochemicals present are listed in Table 2. The analysis showed that the phytochemicals were more dissolved in water than in ethanol. Except for flavonoids, oxalate, and steroids were more dissolved in ethanol than in water. The water extract showed the

following concentrations for alkaloids 2.5171 µg/ml, tannins 4.7026 µg/ml, flavonoids 26.084 µg/ml, saponins 4.2997 µg/ml, phenol 11.8429 µg/ml, oxalate 2.3746 µg/ml, phytate 1.9860 µg/ml and steroids 11.9544 µg/ml. The ethanol extract showed the following concentrations for alkaloids 1.6344 µg/ml, tannins 4.6642 µg/ml, flavonoids 28.859 µg/ml, saponins 3.9587 µg/ml, phenol 6.9698 µg/ml, oxalate 2.5587 µg/ml, phytate 0.6926 µg/ml and steroids 12.4892 µg/ml.

Table 2: GC-FID Results of Red Mangrove Plant (*Rhizophora racemosa*) extracts

Phytochemicals	Water Extract µg/ml	Ethanol Extract µg/ml
Alkaloids		
Ribalinidine	2.1051	1.2586
Lunamarin	0.4120	0.3758
Total	2.5171	1.6344
Tannins		
Tannin	4.6449	4.6509
Proanthocyanin	0.0577	0.0133
Total	4.7026	4.6642
Flavonoids		
Naringin	1.3379	1.3002
Anthocyanin	11.7721	1.8913
Flavanones	5.1779	5.0215
Epicatechin	0.0070	0.00631
Kaempferol	1.0170	
Flavones	1.7047	8.2617
Naringenin	5.7874	2.3658
Flavan 3 ol		10.0123
Total	26.084	28.859
Saponins		
Sapogenin	4.2997	3.9587
Total	4.2997	3.9587
Phenol		
Phenol	10.1873	4.9880
Resveratrol	1.6556	1.9818
Total	11.8429	6.9698
Oxalate		
Oxalate	2.3746	2.5587
Total	2.3746	2.5587
Phytate		
Phytate	1.9860	0.6926
Total	1.9860	0.6926
Steroids		
Steroids	11.9544	12.4892
Total	11.9544	12.4892
Grand Total	65.7613	61.8366

Preliminary phytochemical screening revealed the presence of tannins, alkaloids, steroids, cardiac glycosides, flavonoids, anthraquinone, saponins, and terpenoids in the ethanol extract plants. Terpenoids and alkaloids were absent in the water extract, as presented in Table 1. Edu *et al.* [22] reported the qualitative analysis and profile of the plant tissues (leaves, barks, and roots of *N. fruticans, R. racemosa* and *A. africana*) in aqueous and ethanolic extracts and revealed that the phytochemicals, alkaloids, glycosides, tannins, saponins, flavonoids, polyphenols and reducing sugars were present in all the plant tissues. Poompozhil & Kumarasamy [23] and Ukoima *et al.* [24] reported the presence of saponin and terpenoids in plants of the same family. For saponin and terpenoids, the current study's findings coincided with Poompozhil and Kumarasamy [23] and Ukoima *et al.* [24]. Secondary metabolites such as alkaloids, saponins, tannins, and flavonoids were found in the red mangrove plants studied *(R. racemosa*). Phytochemical screening indicated that the majority of these bioactive components were more abundant in ethanolic extracts than in aqueous extracts. However, other researchers have reported these phytochemicals to be more present in aqueous extracts, suggesting that the bioactive compounds may be highly polar. The variance among the different extracts might probably be due to differential classes of metabolites inherent in red mangrove plants. The current study's findings differ from those of Ijeh *et al.* [25] and Junaid *et al.* [26] but coincide with those of Obi & Onuoha [27], who found that alcohol was the best solvent for extracting most secondary plant metabolites. Edu *et al.* [22], Poompozhil & Kumarasamy [23] also reported on the phytochemical constituents of some selected mangroves, whose results agreed with that of the present study. Ganesh & Vennila [28] reported the same phytochemical compounds in their research. In the family Rhizophoraceae, four taxa *Bruguiera cylindrica, Ceriops decandra, Rhizophora apiculata*, and *R. mucranata* are evaluated for phytochemicals. The phytochemicals present in all four taxa were flavonoids, saponins, and terpenoids. Findings from the present study agreed with studies by Udeozo *et al.* [29], which revealed the presence of all the tested secondary metabolites, which include; flavonoids, alkaloids, saponin, protein, tannin, steroids, terpenoids, glycosides, and carbohydrates. The red mangrove plant *R. racemosa* is reported to have the highest mean concentrations of flavonoids and tannins. Flavonoids are polyphenols that are only synthesized in plants, therefore, are present in plants when experimented. According to the current and evaluated studies, red mangrove plant tissues contain highly polar bioactive chemicals (alkaloids, saponins, tannins, flavonoids, and reducing sugar).

Gas Chromatography-Flame Ionization Detector (GC-FID) is a recent technology that gives reliable evidence on the types and amounts of phytochemicals present in a given plant sample Azubuike *et al.* [30]. The results of the GC – FID analysis of the red mangrove plant (*Rhizophora racemosa*) showed that it contains varying amounts of flavonoids, tannins, saponins, alkaloids, phenolic acid, oxalate, phytate, and steroids. Emejulu *et al.* [9] phytochemical analysis reported the presence of saponins, sapogenin, catechin, kaempferol, rutin, anthocyanin, ribalinidine, Luna marine, phenols, tannins, oxalate, phytate and spartein in extracts of Mucuna Pruriens. Findings from the present study agreed with the reports of Emejulu *et al.* [9]. The current investigation, however, did not discover spartein. The current research's findings coincided with Amadi *et al.* [31] in their study on proximate, GC-FID, and micronutrient analyses of *Azadirachta indica* extracts. Njoku and Obi [32] observed a similar finding for *C. papaya* and *Adenia cissampeloides*. The current study also coincided with the findings of Azubuike *et al.* [30] on their research phytochemical comparative screening of aqueous extracts of *Hura crepitans* (L) leaves, stem barks, and roots by GC-FID.

Mangroves are distinctive plants that have evolved and thrived between land and ocean in many humid tropics and subtropics [1]. The Rhizophoraceae family is the most prevalent in Nigeria, and they are collectively known as the red mangrove. *Rhizophora racemosa, R. harrisonii*, and *R. mangle* are its three species. *Rhizophora racemosa* is the most common, accounting for around 90% of the mangrove

forest [33]. Edu *et al*. [22], in their study on qualitative analyses and profiling of the plant tissues (leaves, barks, and roots of *N. fruticans*, *R. racemosa* and *A. africana*) reported that *Rhizophora racemosa* had the highest mean concentrations of flavonoids and tannins [37]. Generally, mangrove plants are rich sources of saponins, alkaloids, flavonoids, and glycosides [35-40]. They are also rich in sterols, terpenes [40-42], and polyphenols [43]. Findings from the present study agree to the presence of these phytochemicals in *Rhizophora racemosa.*

4. Conclusion

Dyes were extracted from the plant based on the findings. The phytochemical screening of the plant extracts revealed that the plant is high in a variety of phytochemicals.

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