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Evaluation of Bioactive Compounds in Mangroves: A Panacea towards Exploiting and Optimizing Mangrove Resources

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Abstract
The tissues (leaves, barks and roots) of mangrove species (*Nypa fruticans, Rhizophora racemosa* and *Avicennia africana*) were screened qualitatively and quantitatively for phytochemicals (metabolites). Phytochemical analysis indicated presence of highly polar bioactive compounds (alkaloids, saponins tannins flavonoids and reducing sugar) in their tissues. The concentration of these compounds varied significantly ($P<0.001$). The highest concentrations of alkaloids and saponins were in *A. africana,* flavonoids and tannins in *R. racemosa* and reducing sugars in *N. fruticans*. The existence of these metabolites suggests the possible contributions and potentials of the mangroves to medicine and the environment.
Keywords: Mangrove species, Metabolites, Polar bioactive compounds, Medicine.

Introduction

It has been observed that one of the biggest challenges of Africa as a continent is the obvious under exploitation and utilization of our forest resources (Ikpeme *et al.,* 2012), including mangrove trees. According to FAO (2003), approximately 4 percent of Nigeria's rain forest disappear everyday, which could have served as reservoirs for pharmaceutical/therapeutic precursors and industrial raw materials. Latif *et al.* (2002) affirmed that forest resources directly contribute up to 80 percent of the livelihood of the people in countries living in extreme poverty. There is a current global trend in the utilization of natural plant remedies thus creating enormous need for database on the properties and uses of medicinal plants. This becomes expedient owing to the need to broaden raw material base of agro-allied and pharmaceutical industries coupled with the paradigm shift from the use of synthetic chemicals in food processing and their likes. Mangroves which are halophytic trees dominate the intertidal zone along coastlines, estuaries and islands in tropical regions of the world (Hogmath, 1999; Middleton and Mckee, 2001; Karynak and Tentranlt, 2003). Mangrove plants are rich sources of saponins, alkaloids, and flavoniods (Sharaf *et al.,* 2000; Itoigawa *et al.,* 2001; Khafage *et al.,* 2003; Mfilinge *et al.,* 2005; Basyuni *et al.,* 2007), glycosides and saponins are rich in sterols, terpenes (Akinyama *et al.,* 2001; Lu *et al.,* 2004; Sparg *et al.,* 2004), polyphenols (Agoramoorthy *et al.,*2008), where they have either acted as antiviral, antibacterial, antifungal (Bandaranayake, 1998), antimalarial, anticancer (Sharaf *et al.,* 2000) or combination of activities.

From the foregoing, it becomes imperative that if mangroves are evaluated phytochemically, they could add value to the discovery of new natural chemotherapeutic which will be a sure one way to optimizing mangrove resources. The therapeutic potential of mangroves notwithstanding, also provide other raw materials such as tannin for leather industries, food, fodder (Bandaranayake, 2002). This study therefore, aims at identifying and quantifying the amounts of crude phytochemical constituents present in the mangroves of the Cross River estuary.

Materials and Methods

The study area covered the mangrove forests of the Great Kwa River, east of the Cross River estuary which flows into the Gulf of Guinea. This area lies within latitudes 04° 45′ and 04° 15′ North of the Equator and longitudes 008° 15′ and 008° 30′ East of Greenwich Meridian along the eastern border of the University of Calabar, Calabar, Nigeria.

Five trees of each mangrove *species* (*Nypa fruticans, Rhizophora racemosa* and *Avicennia africana*) were randomly selected from the study area and tagged. Senescent leaves, barks and roots were obtained from the tagged trees and used for phytochemical screening. The samples collected were stored and transported to the laboratory in black polyethylene bags to prevent contamination. The samples were rinsed with deionized water in the laboratory and then used for preparation of aqueous and ethanol crude extracts. Ethanol crude extracts of the plant parts were prepared by homogenizing with ethanol (10ml/g fresh weight) for 5 mins in electric blender (National Food Grinder, Model MK 308, Japan). The homogenate was left to stand overnight in the dark and then filtered (Harbone, 1984). The filtrate was evaporated in a rotary evaporator under reduced pressure at 40°C.

The resultant residue was re-dissolved in 3ml ethanol to yield the crude ethanol extract for use in other assays. The aqueous extract was prepared by homogenizing with distilled water (10ml/2g fresh weight) for 5 mins in a homogenizer. The homogenate was left to stand for 24 h at room temperature. The aqueous mixture was then filtered using Whatman No.1 filter paper. The filtrate was used for the bioactive assays.

Qualitative analysis of the phytochemical components in plant extracts

For Alkaloids, Meyers and Dragendoof's tests was adopted according to the method of Sofowora (1993). 2ml of each extract was added to 5ml of 1% aqueous HCl and stirred in a water bath. A part (1ml) of the filtrate of each extract was titrated against Dragendoof's reagent and a second part (1ml) against Meyer's reagent drop wise. Turbidity and precipitation with either of the reagents indicated the presence of alkaloids.

Salkovski test was adopted in test for glycosides according to the method of Trease and Evans, (1989); Sofowora, (1993). 2ml of each extract was dissolved in 2ml of chloroform and 2ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a lower layer. A reddish-brown colour at the interface indicates the presence of glycosides.

Frothing test was adopted in test for saponins according to the method of Harbone, (1984) and Sofowora, (1993). 2ml of each extract was mixed with 20ml of distilled water and boiled in a water bath and then filtered. Then 10ml of the filtrate was added to 5ml of distilled water and shaken vigorously for a stable persistent froth. The froth was then treated with 3 drops of olive oil and shaken vigorously. The formation of emulsion indicated the presence of saponins.

Ferric chloride test was adopted in test for tannins according to the method of Trease & Evans, (1989) and Sofowora, (1993). 2ml of each extract was stirred with 10ml of distilled water in a test tube and heated in a water bath and then filtered. Ferric chloride (0.1%) was added drop wise and observed for brownish green or blue-black colouration, indicating presence of tannins.

Aluminium chloride test was adopted in test for flavonoids according to the method of Harbone, (1984) and Sofowora, (1993). 5ml of dilute ammonia solution was added to 2ml of each plant extract. Then concentrated sulphuric acid was added. A yellow coloration disappearing on standing indicated the presence of flavonoids.

Fehling's solution test was adopted in test for reducing sugars according to the method of Harbone, (1984). 2ml of each plant extract was added to 5ml of Fehling's solution in a test tube and heated in the water bath for 5 mins. The formation of brick-red coloration of precipitates indicated the presence of reducing sugars.

Potassium ferrocynide test was adopted in test for polyphenols according to the method of Harbone, (1984). 2ml of each plant extract was added to 5ml of distilled water in a test tube and heated for 30 mins in a water bath. Then 1ml of 1% ferric chloride was added to the mixture and followed by the addition of 1ml of 1% potassium ferrocyanide solution. The formation of green-blue colouration indicated the presence of polyphenols.

Aqueous hydrochloric acid test was adopted in test for phlobatannins according to the method of Trease and Evans, (1989). 2ml of each plant extract was boiled with 5ml of 1% aqueous solution of hydrochloric acid (HCl). Deposition of red precipitate was evidence for the presence of phlobatannins.

Borntrager's test was adopted in test for anthraquinones according to the method of Trease and Evans, (1989). 2ml of each extract was shaken with 10ml of benzene and filtered. Then 5ml of 10% nitric acid was added to the filtrate and shaken. The presence of pink red or violet colouration in ammonical (lower) phase indicated the presence of free anhraquinones

Ammonium test was adopted in test for hydroxymethyl anthraquinones according to the method of Sofowora, (1993). 2ml of each extract was treated with 5ml of 5% ammonia solution. The formation of a red colour or precipitate indicated the presence of hydroxymthyl anthraquinones.

Quantitative determination of the phytochemical components

Alkaloid determination

5g of each dried ground sample was placed in a 250ml beaker. 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4hours. This was filtered and the extract concentrated on a water bath to ¼ of original volume. Concentrated ammonium hydroxide was added drop wise to the content until precipitation was complete. The resulting solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed (Harbone, 1984).

Tannin determination

500mg of each sample was placed in 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted into a test tube and mixed with 2ml of 0.1M ferric chloride in 0.1N hydrochloric acid and 0.008M potassium ferrocyanide. The absorbance was measured at 280mm within 10 mins. Tannin contents were expressed as a percentage of the dried faction (Van-Burden and Robinson, 1981).

Saponin determination

50mg of each ground sample was added to 100ml of 20% aqueous ethanol in a conical flask. This was heated in

a hot water bath at 55°C for 4hrs with continuous stirring. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90°C. The concentrate was transferred into a 250ml separating funnel and 20ml diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added to the purified aqueous extract. The extract was washed twice with 10ml of 5% aqueous sodium chloride and then heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as a percentage of the dried faction **(**Obadoni and Ochuko, 2001).

Flavoniod determination

1g of each ground sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness (constant weight) over a water bath. The flavonoid content was calculated as a percentage of the dried faction (Boham and Kocipal-Abyazan, 1994).

Reducing sugars determination

1g of each ground sample was diluted with water (10ml) and titrated with standard Benedict reagent. The sample was hydrolyzed with standard acid (0.5NHCl). The hydrolyzed fraction gave the total reducing sugars. Results obtained were calibrated using standard curve of glucose (Harbone, 1984).

RESULTS

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 are presented in Table 1. The analysis showed that the phytochemicals, alkaloids, glycosides, tannins, saponins, flavonoids, polyphenols and reducing sugars were present in all the plants. Phlobatanins and anthroquinones were only present in the leaves of *N. fruticans* bark and bark and roots of *R. racemosa.* Quantitative estimation of the percentage crude chemical constituents in the mangrove plants studied is summarized in Table 2.

The average concentrations (mg/100g dry weight; mean ± standard error) of major phytochemicals in the tissues of the mangrove species show that the plants accumulated in bioactive compounds such as alkaloids, saponins, tannins, flavonoids and reducing sugars (Fig. 1). Analysis of variance (ANOVA) of concentrations of the major phytochemicals in tissues of the mangrove species show significant variations in the different species ($P < 0.001$) with *A. africana* having the highest and *N. fruticans* the lowest concentrations of accumulated phytochemicals (Table 3 and 4). There was also significant difference in phytochemicals present in the plant tissues with bark tissues having the highest and the root tissues the lowest concentrations.

From the analysis, all mangrove species had the highest mean concentrations of reducing sugars and high concentrations of flavonoids with varying alkaloids, saponins and tannins concentrations (Table 4 and Fig. 2).

There were variations in phytochemicals present in the tissues of mangrove plant species. The leaves and bark of *A. africana* respectively had the highest concentration of alkaloids; for saponins, the barks of *A. Africana* and *N. fruticans* had higher concentrations. In all bioactive compound analysis, the highest concentration of tannins (1.40) was evident in the leaf and bark of *R. racemosa.* For flavonoids, the leaf and bark of *R. racemosa* had highest concentrations. Reducing sugars analysis produced highest concentrations in bioactive compounds present in all mangrove species tested with the highest being 54.73 in roots of *N. fruticans* & lowest (12.28) in leaves of same species.

The LSD (5%) as summarized in Table 2 unravels reducing sugars as bioactive compounds with highest concentrations. This is accompanied by flavonoids, alkaloids, saponins and tannins respectively.

Discussion

The investigated mangrove plants (*N. fruticans, R. racemosa* and *A. africana*) contained secondary metabolites such as alkaloids, saponins, tannins and flavonoids. Phytochemical screening revealed strong presence of most of these bioactive compounds in aqueous than in ethanolic extracts (Table 1), suggesting that the bioactive compounds may be highly polar. The variance might probably be due to differential classes of metabolites inherent in mangrove. This is in line with the findings of Ijeh *et al.* (2005) and Junaid *et al*. (2006), but at variance with the findings of Obi and Onuoha (2000) who reported alcohol was the best solvent for extraction of most secondary plant metabolites. The presence of these metabolites in mangroves may account for their potential usefulness as medicinal plants. Marine plants have been recommended as important sources of potentially useful secondary metabolites with natural resources of complex molecules valued and exploited for novel drugs as well as in food supplements for their pharmacological properties (Riguera, 1997).

*Avicennia africa*na was found to have the highest mean concentrations of alkaloids and saponins. The highest concentration of alkaloids was found in the leaves while the bark had the highest concentration of saponins (Fig. 1, Table 2&3). Alkaloids and saponins are a group of important secondary plant metabolites which are structurally diverse with diverse pharmacological properties (Buckingham, 2001). They include

steroidal alkaloids and triterpenoids which are regarded as important potential natural sources for medicinal compounds because of their wide range of biological activities (Sparg *et al*., 2004). These activities include anti-inflammatory (Kim *et al*., 2005), anti-carcinogenic and cardio protective effect in hyper-cholesterocemic condition (Jang *et al*., 2004). Triterpenoids also comprise the major proportions of non-saponifiable lipids identified in cuticular waxes of mangroves which constitute the primary source of lipid input from mangrove into the environment and act as biomarkers for organic matter because of their stability during sedimentation and diagenesis (Koch *et al*., 2003, 2005). Terpenoids also have physiological significances. Alkaloids and saponins are also known to play some metabolic roles in animal systems. They act as surfactants that can be used to enhance the penetration of large molecules such as proteins through cell membranes (Francis *et al*., 2002). Saponins have been used in the manufacture of shampoos and insecticides due to their foaming ability (Okwu, 2003). Steroidal alkaloids are known to play important role in hormonal balance and as such are used in the synthesis of steroid hormones (Edeoga *et al*., 2005). Saponins have also been used as adjuvants in vaccines (Skene and Sutton, 2006). They are promoted as dietary supplements and nutriceuticals because of their metabolic role in animal systems (Asl and Hosseinzalleh, 2008). However alkaloids and saponins are often bitter to taste and as such can reduce palatability (Francis *et al*., 2002). They have also been shown to exhibit anti-microbial activities (Singh *et al*., 2002; Gilbert *et al*., 2002; Opitz *et al*., 2008).

The mangrove *R. racemosa* had the highest mean concentrations of flavonoids and tannins, leaves had the highest concentration of flavonoids and the stem, the highest concentration of tannins (Fig. 2, Table 4). Flavonoids are polyphenols which are only synthesized in plants. They have anti-oxidative and anti-inflammatory actions that work in human body to enhance health and possibly minimize certain effects of ageing. It has been discovered that they can block the action of enzymes and deactivate substances that promote the growth of cancers (Sharaf *et al*., 2000; Itoigawa *et al*., 2001; Sellappan and Akoh, 2002; Khafagi *et al*., 2003; Verena *et al*., 2006). They have been found to also have the ability to inhibit low density lipoprotein oxidation by free radicals (Rufer and Kulling, 2006). They have been found to have negative correlation with incidence of coronary heart disease and inhibit platelet aggregation. The bark of *Rhizophora* species has traditionally been used as powerful astringent in diabetics, hemorrhages and angina. They are also used for traditional fishnet dyeing (Agoramoorthy *et al*., 2008). These uses can be attributed to the tannin contents. Tannins have been extensively studied for their potential anti-bacterial (Akiyama *et al*., 2001; Lu *et al*., 2004), anti-viral (Cheng *et al*., 2002; Quideau *et al*., 2004; Lu *et al*., 2004), anti-parasitic (Kolodziej and Kiderlin, 2005), anti-carcinogenic (Yang *et al*., 2000; Tanimura *et al*., 2005) and anti-AIDS (Nonaka *et al*., 1990) effects. Apart from their medical uses and potentials, tannins are important in leather tannery because of their ability to produce different colours with ferric chloride or sulphate. They are also responsible for colour changes in food (Edeoga *et al*., 2006). They have also been used as component in industrial particle board adhesive (Bisanda *et al*., 2003). Recent studies have shown that products containing tannins (in low dosages) in diets can be beneficial (Schiavone *et al*., 2008). The incorporation of *R. racemosa* leaves as feed additives for broiler chicks led to a lineal body weight gain and enhanced performance of breeding cocks (Wekhe *et al*., 2007).

The mangrove *N. fruticans* had the highest mean concentration of reducing sugars. This may be attributed to its high yield of sweet sap which can be tapped and drunk as is boiled to produce brown sugar or fermented to form alcohol or vinegar (Tan, 2001). It had the least mean concentrations of alkaloids and flavonoids (Fig. 2, Table 4). The low concentrations of these metabolites in *Nypa* may be responsible for its limited adaptiveness to undiluted sea water. *Nypa* is usually found in large river dominated estuaries in wet tropical climates (Duke, 2006; Wightman, 2006). Shoot biomass of *N. fruticans* has been found to be a good source for remediation of metal ion polluted effluents (Wankasi *et al*., 2006).

CONCLUSION

The mangrove plant tissues contained highly polar bioactive compounds (alkaloids, saponins tannins flavonoids and reducing sugar). The concentrations of these compounds varied significantly ($P<0.001$) with the highest concentrations of alkaloids and saponins in *A. africana,* flavonoids and tannins were in *R. racemosa* and reducing sugars in *N. fruticans*. The presence of these metabolites is an indication of their potentials as medicinal plants. The results obtained from this study can be valuable for further studies on their genera (*Rhizophora, Avicennia, Nypa*) and the recognition of their medicinal and bioactive values can enhance conservation plans for mangroves in Nigeria. This study has implications in pharmacology as natural sources for medicinal compounds presently and in the future for some are yet unforeseen need. The adaptability of the mangrove trees to salinity could be optimized genetically-as to isolate alien genes, which will be transgressed into other crops vulnerable to this ecological problem.

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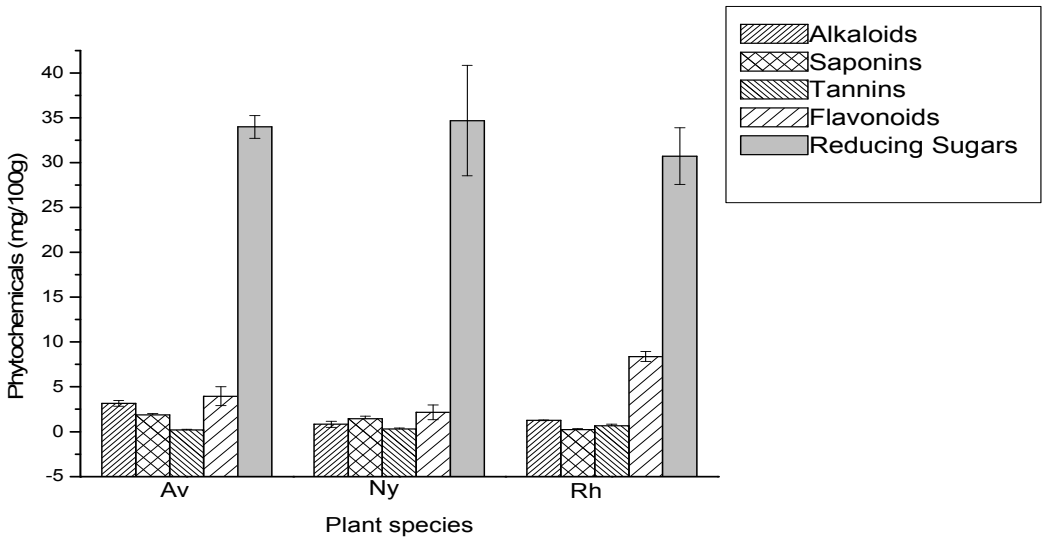

Av = *Avicennia africana*
Rh = *Rhizophora racemosa*
Ny = *Nypa fruticans*

FIG. 1: Concentrations of phytochemcals (dry weight ± SE) in mangrove plants sampled from a mixed mangrove forest at Esuk Mba of the Great Kwa river east of the Cross river estuary, Nigeria.

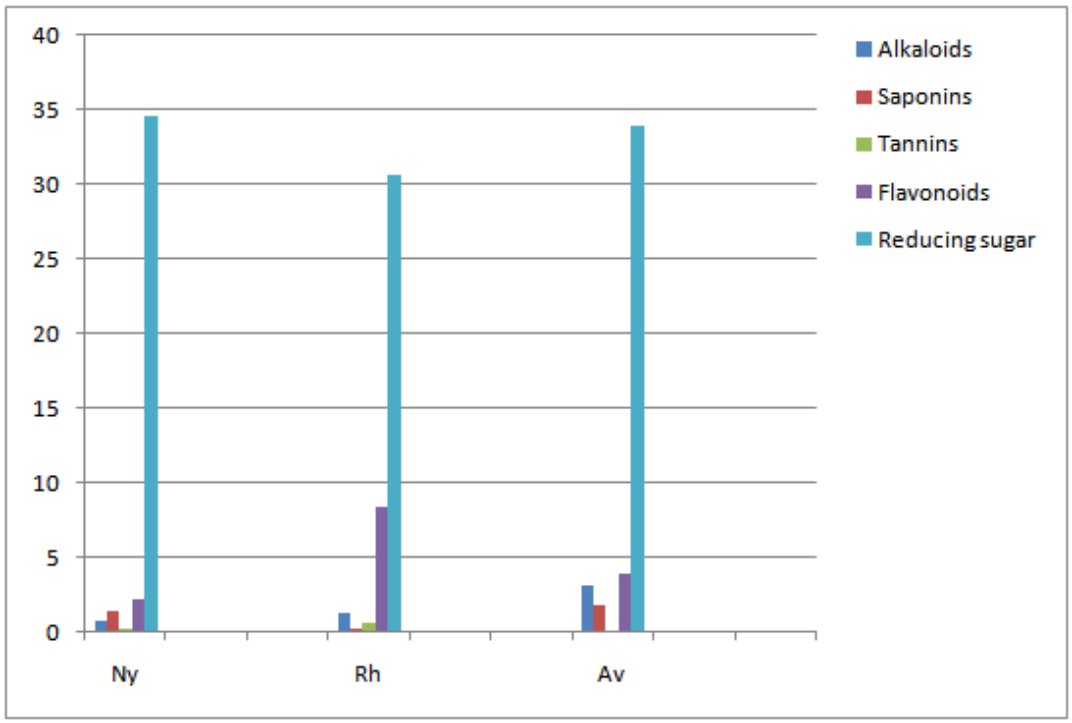

Fig 2: Graph of mean concentration of phytochemicals (mg /100g, dry weight) in mangrove species, sampled from a mixed mangrove forest at Esuk Mba, of Great Kwa River, of the Cross River estuary, Nigeria.

TABLE 1: Phytochemical screening of mangrove plants (*Nypa fruticans, Rhizophora racemosa, Avicennia africana*) from a mixed mangrove forest at Esuk Mba of the Great Kwa River, east of Cross River estuary, Nigeria.

Plant tissues	Alkaloids		Glycosides		Saponins		Tanins		Flavonoids		Reducing sugars		Polyphenols		Phlobatanins		Anthroquinones		Hydroxmethyl anthroquinones	
	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE
NyL	++	++	+	++	-	+	++	-	+++	+++	+++	+++	++	++	++	+	-	++	+	-
NyB	++	++	++	++	++	-	+	+	+	+	++	+	+++	+++	-	-	-	-	-	-
NyR	+	+	+	+	++	+	-	-	-	-	+++	+	++	++	-	-	-	-	-	-
RhL	++	+	++	++	-	+	+	-	++	++	++	++	++	++	-	-	-	-	-	-
RhB	+	++	+	++	+	++	+	-	++	+++	+++	+++	+++	+++	-	+	-	+	-	+
RhR	++	+	++	+	-	-	-	-	++	++	++	++	++	-	+	-	-	+	-	+
AvL	++	+	+	+	+	+	+	-	+	+	++	++	+	++	-	-	-	-	-	-
AvB	++	+	++	++	++	++	-	+	+	+	++	++	+++	+++	-	-	-	-	-	-
AvR	++	+	+	+	+	+	-	-	-	-	++	+	++	-	-	-	-	-	-	-

Legends

+ = Slight presence, ++ = Strong presence, +++ = Very strong presence, - = Absent, EE = Ethanol extract, AE = Aqueous extract, Ny = *Nypa fruticans* Rh = *Rhizophora racemosa,* Av = *Avicennia africana,* L = Leaves, B = Bark, R = Roots.

TABLE 2: Concentration of phytochemicals (mg /100g, dry weight) in tissues of mangrove species, sampled from a mixed mangrove forest at Esuk Mba, of Great Kwa River, of the Cross River estuary, Nigeria.

Species	Tissues	Alkaloids	Saponins	Tannins	Flavonoids	Reducing sugars
Ny	L	2.12 ± 0.04^{e}	0.54 ± 0.03^{c}	0.63 ± 0.01^{d}	5.42 ± 0.08^{d}	12.28 ± 0.02^{a}
	B	0.2 ± 0.02^{a}	2.50 ± 0.03^{h}	0.31 ± 0.011^{b}	1.12 ± 0.02^{b}	37.00 ± 0.41^{e}
	R	0.12 ± 0.03^{a}	1.31 ± 0.03^{e}	01.100^{a}	01.100^{a}	54.73 ± 0.55^{h}
Rh	L	1.30 ± 0.05^{c}	0.17 ± 0.03^{b}	1.40 ± 0.02^{e}	9.72 ± 0.04^{g}	37.54 ± 0.03^{f}
	B	1.13 ± 0.03^{b}	1.55 ± 0.03^{f}	1.40 ± 0.02^{f}	9.72 ± 0.04^{g}	37.92 ± 0.03^{f}
	R	1.36 ± 0.07^{c}	0.1010^{a}	01.100^{a}	6.10 ± 0.06^{e}	36.07 ± 0.59^{d}
Av	L	4.23 ± 0.13^{g}	1.28 ± 0.03^{d}	01.100^{a}	4.71 ± 0.08^{c}	38.61 ± 0.55^{g}
	B	3.22 ± 0.03^{f}	2.54 ± 0.01^{c}	0.56 ± 0.03^{c}	7.18 ± 0.02^{f}	33.35 ± 0.02^{c}
	R	2.00 ± 0.19^{d}	1.74 ± 0.02^{g}	01.100^{a}	01.100^{a}	29.99 ± 0.06^{b}
	LSD (5%)	**0.0828**	**0.023**	**0.0141**	**0.147**	**0.3885**

LSD = Least Significant Difference (5%), Ny = *Nypa fruticana*, Rh = *Rhizophora racemosa*, Av = *Avicennia africana*, L = Leaves, B = Barks, R = Roots

TABLE 3: Mean concentration of phytochemicals (mg /100g, dry weight) in mangrove species, sampled from a mixed mangrove forest at Esuk Mba, of Great Kwa River, of the Cross River estuary, Nigeria.

Species	Al	Sa	Ta	Fl	Rs
Ny	0.81	1.45	0.31	2.18	34.67
Rh	1.26	0.24	0.65	8.39	30.71
Av	3.15	1.86	0.19	3.96	33.98

Ny = *Nypa fruticans*, Rh = *Rhizophora racemosa*, Av =*Avicennia africana*, Al = Alkaloids, Sa = Saponins, Ta = Tannins, Fl = Flavonoids, Rs = Reducing sugars.

TABLE 4: Results of analyses of variance (ANOVA) for concentrations of phytochemicals (mg/100g) dry weight) in mangrove plants tissues, sampled from a mixed mangrove forest at Esuk Mba, east bank of the Great Kwa River, east of the Cross River estuary Nigeria.

		Alkaloids			Saponins			Tannins			Flavonoids			Reducing sugars		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species(s)	2	13.857	1916.4	0*	6.36	12014.57	0*	0.513	2662.327	0*	92.072	41780.46	0*	40.238	261.104	0*
Plant tissue(P)	2	13.657	646.9	0*	3.43	6488.64	0*	1.292	6710.077	0*	53.839	24431.1	0*	731.093	1744.046	0*
S x P	4	1.472	203.2	0*	0.483	911.818	0*	0.408	2116.5	0*	11.263	5110.76	0*	525.183	3407.903	0*
Error	18	0.007			0.001			0			0.002			0.154		

- = Significant difference (P<0.001); Species = *Nypa fruticans*, *Rhizophora racemosa*, *Avicennia africana;*
- Plant tissues =Leaves, barks, roots; df = Degrees of freedom; MS= Mean squares; F = Variance ratio, P = Level of probability.