Scientific African 15 (2022) e01091

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

In vitro antioxidant and antifungal activities of *Rhizophora racemosa* G.F.W. Mey. stem bark extracts

Basil N. Ita[a,*], Samuel I. Eduok[b]

[a] *Department of Chemistry, University of Uyo, Uyo, Akwa Ibom, Nigeria*
[b] *Department of Microbiology, University of Uyo, Uyo, Akwa Ibom, Nigeria*

ARTICLE INFO

Article history:
Received 17 March 2020
Revised 9 December 2021
Accepted 23 December 2021

Editor: DR B Gyampoh

Keywords:
Rhizophora racemosa
Antioxidant activity
Antifungal activity
Trichophyton mentagrophyte
Microsporum canis
Trichophyton rubrum
Epidermophyton floccosum

ABSTRACT

Rhizophora racemosa is used traditionally in Nigeria to treat human microbial infections. In this study, the dichloromethane (DCM), ethylacetate (EAC) and ethanol (ETH) stem bark extracts of *Rhizophora racemosa* were evaluated for their antioxidant and antifungal activities. Antioxidant activity was evaluated by measuring their DPPH and ABTS radical scavenging activities, H_2O_2 scavenging activity, reducing power and metal chelating activity. The antifungal activity against *Trichophyton mentagrophyte, Microsporum canis, Trichophyton rubrum* and *Epidermophyton floccosum* was evaluated by the agar well diffusion and microdilution assays. In the antioxidant assay, ETH extract exhibited a higher radical scavenging activity (DPPH and ABTS), H_2O_2 scavenging activity and reducing power than EAC extract, while the EAC extract demonstrated a greater metal chelating ability than the other extracts. In the antifungal test, EAC and ETH extracts demonstrated inhibitory effects (MIC = 250–440 µg/mL and 180–300 µg/mL, respectively) with the ETH extract being the most effective against the test etiological agents. *Rhizophora racemosa* stem bark may serve as potential sources of new antioxidant and antifungal compounds.

Introduction

Rhizophora racemosa G. F. W. Mey. (Family Rhizophoraceae) is a dominant mangrove species in the Niger Delta Region of Nigeria and the West Coastlines. Traditionally, *R. racemosa* bark is used to treat boils and fungal infections. An infusion of its leaf and bark is used to treat diarrhoea, dysentery, fever, malaria and leprosy [1]. The bark of the plant is rich in tannins. The plant is a good source of pole, timber and fuel because of the large size of the stem. Literature reports on this medicinal plant is scanty, however the lethal dosage of its methanolic leaf extract as well as the antimicrobial potential of its fungal endophytes have been reported [2–3]. Biological activities such as antiviral, antibacterial, antiulcer, anti-inflammatory, anti-hyperglycemia, antiplasmodial, gastroprotective, antiseptic, antidiabetic, antioxidative, anti-HIV, anticancer, and antihelmintic activity of other *Rhizophora* species have been documented [2,4–8,36]. These activities are due to the fact that mangrove plants contain pharmacologically active metabolites with therapeutic properties [7].

* Corresponding author.
E-mail address: basilita@uniuyo.edu.ng (B.N. Ita).

https://doi.org/10.1016/j.sciaf.2021.e01091

Oxidative stress, arising from increased levels of reactive oxygen species (ROS) in cells and tissues is considered a contributing factor in many diseases including diabetes, HIV, cardiovascular diseases, inflammation, gastrointestinal and digestive disorders. These ROS damage biomolecules like proteins, DNA, and lipids, resulting in oxidative damage, lipid peroxidation and DNA strand breaking [9]. Antioxidants scavenge these ROS and relieve oxidative stress [9–10]. However, their usage is hampered by cost, toxicity and efficacy. Plants are considered as promising sources of non-toxic antioxidants which can be exploited in the drug, food and associated industry [8].

Dermatophytes are responsible for superficial mycotic infections. These infections are common in the Niger Delta region of Nigeria, particularly amongst children and rural dwellers. The etiological agents belong to the genera *Epidermophyton, Microsporum* and *Trichophyton*. These infections have high morbidity and, in many cases, are refractive to available antifungal drugs, resulting in increased incidences of dermatophytic infections. Therefore, the need to search for natural products with promising antidermatophytic properties as therapeutic alternatives becomes imperative.

From the review of available literature, there are no research outcomes on the antioxidant and antifungal potential of *R. racemosa*. Thus, the present study was undertaken to investigate the *in -vitro* antioxidant and antifungal activity of *R. racemosa* stem bark extracts on common dermatophytes.

Materials and methods

Chemicals and reagents

1,1- diphenyl-2-picryl hydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), disodium salt of 3-(2-pyridil)-5,6-bis(4- phenylsulfonic acid)-1,2,4-triazine (ferrozine), Folin Ciocalteu reagent, gallic acid, quercetin, butylated hydroxyanisole (BHA), trichloroacetic acid (TCA) were purchased from Sigma (Germany). All other reagents were of analytical grade.

Plant material and extraction

Stem bark of *R. racemosa*, collected in March 2019 from a mangrove forest in Ifiayong (5º 2.59′N, 8º 2.42′E) within Akwa Ibom State, Nigeria was identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo, Nigeria by Prof. M. Bassey, with voucher number UUH 4062. The plant material was air dried for 9 days and powdered using a laboratory mill. The powdered material (100 g) was successively macerated with 700 mL of dichloromethane (DCM), ethyl acetate (EAC) and ethanol (ETH) respectively for 48h. The extracts were filtered using a filter paper, evaporated to dryness *in - vacuo* and stored in an air tight container at 4 °C until further use. Extraction yields of 4.88%, 8.43% and 14.65% were obtained for the DCM, EAC and ETH extracts respectively.

Phytochemical analysis

The DCM, EAC and ETH were screened qualitatively for phytochemical constituents using standard procedures [11–13].

Evaluation of antioxidant activity

The antioxidant activity of the extracts was determined by evaluating its DPPH and ABTS radical scavenging activities, hydrogen peroxide scavenging activity, reducing power and metal chelating potentials.

Evaluation of DPPH activity

The solvent extracts were prepared by dissolving 0.01 g of the dried extract in 10 mL of the appropriate solvent (DCM, EAC or ETH) and the concentration adjusted to 20–100 µg/mL. Each extract (1 mL, at these concentrations) was mixed with 1 mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm with a UV-Vis spectrophotometer (Specord S600. Analytik Jena AG). The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

$$\mathrm{DPPHscavengingeffect}(\%) = \left[\left(\mathrm{A_{blank}} - \mathrm{A_{sample}}\right)/\mathrm{A_{blank}}\right] \times 100$$

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Butylated hydroxy anisole (BHA) was used as positive control [14].

Evaluation of ABTS activity

ABTS radical was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in water (1:1). This mixture was allowed to stand for 12 h in the dark at room temperature. Thereafter, the mixture was diluted with methanol to an absorbance of 0.7 at 734 nm. Then, 2.94 mL of this ABTS solution was mixed with 60 μL of each extract and incubated

at 37 °C for 20 min in the dark. After incubation, the absorbance was read at 734 nm. The percentage inhibition was calculated using the equation:

$$\%\text{inhibition} = \left[A_{blank} - A_{sample}\right)/A_{blank}\right] \times 100$$

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Vitamin C was used as positive control [15].

Evaluation of hydrogen peroxide scavenging activity

Briefly, 0.01 g of the dried extract was dissolved in 10 mL of the appropriate solvent and the concentration adjusted to 20–100 µg/mL. Extracts at these concentrations (1 mL) were mixed with 400 µL of H_2O_2 (5 mM) in phosphate buffer (pH 7.4; 100 mM) and incubated for 20 min, after which the absorbance was read at 230 nm against a blank. The H_2O_2 inhibition (%) was determined using the equation:

$$H_2O_2 \text{scavengingactivity}(\%) = \left[\left(A_{blank} - A_{sample}\right)/A_{blank}\right] \times 100.$$

Vitamin C was used as positive control [16]

Evaluation of reducing power

The reducing power was determined according to the method of Oyiazu [17]. Each sample (10–100 µg/mL) in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.60), 2.5 mL of 1% potassium ferricyanide and the mixture incubated at 50 °C for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200 g for 19 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (µg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from the regression equation. Vitamin C was used as positive control.

Evaluation of metal chelating activity

Metal chelating activity was determined according to the method of Decker and Welch [18] with some modifications. Briefly, 0.01 g of the dried extract was dissolved in 10 mL of the appropriate solvent and the concentration adjusted to 20–100 µg/mL. 0.5 mL of each extract was mixed with 0.05 mL of 2 mM $FeCl_2$ and 0.1 mL of 5 mM ferrozine. The total volume was diluted with 2 mL methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

$$\text{Scavengingactivity}(\%) = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \times 100$$

where $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as a positive control.

Determination of total phenolics

Concentration of total phenolics in the extracts was determined using the Folin- Ciocalteu reagent [19]. Briefly, 0.01 g of the dried extract was dissolved in 10 mL of the appropriate solvent and the concentration adjusted to 100 µg/mL. Each extract (0.1 mL) was dissolved in 0.5 mL (1/10 dilution) of the Folin-Ciocalteu reagent and 1mL of water/methanol (1:2) was added. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1.5 mL of 20% Na_2CO_3 solution was added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of the fraction was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was employed as the standard and the results expressed in mg gallic acid equivalent per gram (mg GAE/g).

Determination of flavonoids

A slightly modified version of the method of Sabhasree et al. [14] was used to determine the flavonoid contents of the fractions. Each extract (0.1 g) was mixed with 20 mL of 80% aqueous methanol, then filtered to obtain a clear filtrate. A 0.5 mL aliquot of this filtrate was taken in a test tube and 3 mL of distilled water and 0.3 mL of 0.5% sodium nitrite were added. The solution was mixed and allowed to stand at room temperature for 5 min. To this solution, 0.6 mL of 10% aluminium chloride was added. After 6 min, 2 mL of 1 M sodium hydroxide was also added. The solution was then diluted with distilled water to make the final volume up to 10 mL. The absorbance was read at 510 nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin and results expressed in mg quercetin equivalent per gram (mg QE/g).

Table 1
Phytochemical analysis of *R. racemosa* stem bark extracts

Constituent	Extract		
	DCM	EAC	ETH
Alkaloids	-	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Coumarins	+	+	+
Saponins	_	+	+
Cardiac glycosides	-	+	+

+ = present, - = absent.

Fungal strains

The dermatophytes used in this study (*Trichophyton mentagrophyte, Microsporum canis, Trichophyton rubrum* and *Epidermophyton floccosum*) were isolated from clinical specimens of infected patients (children) attending a Hospital in Uyo, Akwa Ibom State, Nigeria and identified by microscopy and culture in accordance with the method of Rebell and Taplin [20]. The fungi were maintained on Sabouraud dextrose agar (SDA) slants at 4 °C.

Preparation of spore suspension

The fungi were cultured on SDA and incubated at 32 ± 2 °C for 7–9 days, after which the dermatophytes were suspended in normal saline (0.9%) and the turbidity adjusted to 0.5 McFarland standard to give a final suspension of 1.5×10^6 CFU/mL.

Antifungal activity

Antifungal activity of the extracts was determined by the agar well diffusion method in 10 cm petri dishes using SDA. Six wells were punched using a sterile cork borer (4 mm diameter) and 20 µL of the different extract concentration (500, 1000 and 2000 µg/mL) was introduced into the well and allowed for 10 min at room temperature for diffusion. Ketoconazole (10 µg/mL) was used as positive control, while DMSO was used as negative control. The agar plates were incubated at 32 ± 2°C for 7–9 days [21]. Determinations were carried out in triplicates and the inhibition zone diameter (mm) noted.

Minimum inhibitory concentration (MIC) was determined by the broth microdilution technique based on the Clinical and Laboratory Standards Institute (CLSI) document M38-A2 [22] with some modifications. A 96 - well microtiter plate was used. Briefly, two - fold dilutions of the extract (6400 - 25 µg/mL) and ketoconazole (16 - 0.03 µg/mL) were performed in SDA diluted to a final inoculum concentration of 5.0×10^4 CFU/mL with RPMI-1640, buffered to pH 7 with morpholine propane sulfonic acid (MOPS). Each well contained 100 μL of the test substance and 100 μL of the fungal spore suspension. The inoculated plates were incubated at 28 °C for 7 days. Fungal growth in each well was evaluated by observing and comparing the turbidity of the test wells to that of the control wells. The MIC was defined as the lowest concentration of extract/control showing no visible growth. For minimum fungicidal concentration (MFC), 10 μL of suspension from the MIC was re-inoculated on SDA and incubated at 28 °C for 7 days. MFC was defined as the lowest concentration that yielded negative subcultures.

Statistical analysis

All experiments were performed in triplicates. The data were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS version 20.0, IBM Corp, USA). Levels of significance was maintained at 95% for each test.

Results and discussion

Phytochemical analysis of *R. racemosa* stem bark extracts revealed the presence of alkaloids, tannins, tepenoids, coumarins, saponins and cardiac glycosides in the EAC and ETH extracts (Table 1). However, a negative test was obtained for alkaloids, saponins and cardiac glycosides in the DCM extract.

These secondary metabolites are known from published reports to exhibit a wide range of biological activities including antimicrobial, anti-inflammatory, hypotensive, antiprotozoal, antioxidant, anticancer and antidiabetic activities [23–27].

Increasing number of studies have shown that oxidative stress plays an important role in the pathological processes of diseases such as cancer, diabetes, digestive, gastrointestinal, cardiovascular and neurodegenerative diseases. Approaches aimed at reducing oxidative damage such as the use of plant - derived antioxidants may be good therapeutic alternatives because they are less toxic than their synthetic counterparts [4]. In this study, the antioxidant activity of extracts of *R.*

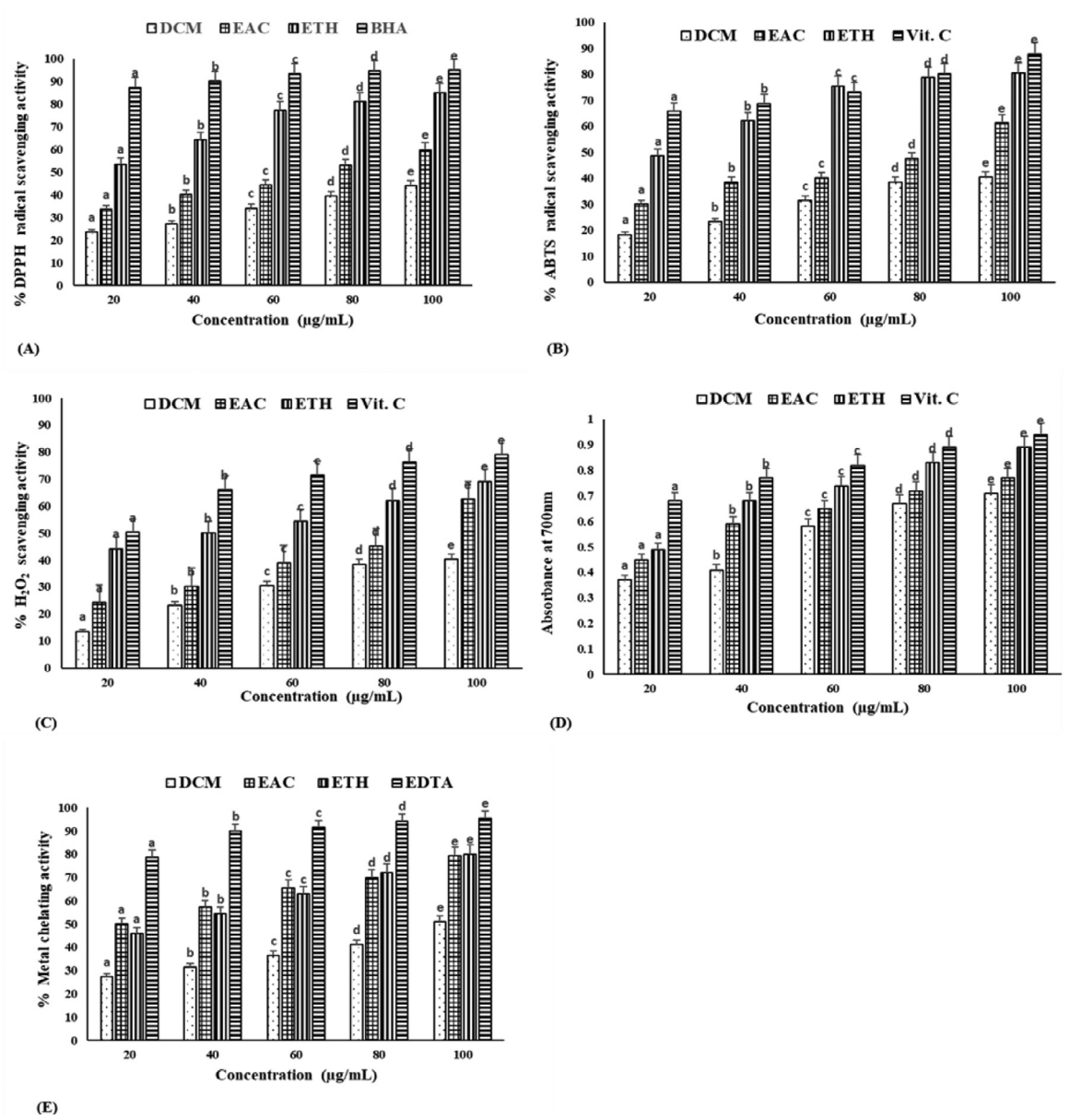

Fig. 1. (A)-DPPH radical scavenging activity of *R.racemosa* extracts; (B)-ABTS radical scavenging activity of *R.racemosa* extracts.(c)-H_2O scavenging activity of *R.racemosa* extracts; (D)-Reducing power of *R.racemosa* extracts. (E)-Mental chelating activity of R.racemosa extracts. Values are mean of triplicate determinations±SD.Bars having different letters are significantly different ($p \leq 0.05$).

racemosa was evaluated using various *in vitro* models (DPPH, ABTS, hydrogen peroxide, reducing power and metal chelating activity) since the antioxidant activity of a plant extracts cannot be accessed by a single method because it involves a number of mechanisms and synergies between them [9]. In the DPPH assay, the scavenging activity of the extracts increased significantly ($p \leq 0.05$) in a dose - dependent manner (Fig. 1A), with the dichloromethane (DCM), ethylacetate (EAC) and ethanol (ETH) extracts scavenging 23.57, 33.61 and 53.57% of the DPPH radicals at 20.μg/mL respectively. This increased to 44.16, 60.02 and 85.16%, respectively at 100 μg/mL. Generally, ETH extract scavenged higher amounts of the DPPH radical than the other extracts (IC_{50} = 36.18 μg/mL) as indicated in Table 2. Lower scavenging activity have been reported for *Schima wallichii* stem bark, while a higher activity has been reported for *R. mucronata* dichloromethane and methanol leaf extracts [4,28]. The variations of DPPH activity observed may be attributed to the content of phytocompounds, climate, soil composition and specie.

Table 2
Antioxidant activity of *R. racemosa* stem bark extracts

Parameter	Extracts			Controls		
	DCM	EAC	ETH	BHA	EDTA	Vit. C
DPPH scavenging activity*	105.52	72.06	36.18	12.45	-	-
ABTS scavenging activity*	113.48	96.63	39.42	-	-	31.83
H_2O_2 scavenging activity*	113.29	79.98	55.77	-	-	39.41
Reducing power*	56.56	45.84	36.71	-	-	26.10
Metal chelating activity*	93.35	44.42	46.16	-	16.69	-
Total Phenolics (mg GAE/g)	11.34	34.52	53.39	-	-	-
Flavonoid (mg QE /g)	-	19.21	27.18	-	-	-

* IC_{50} (μg/mL) is the effective concentration where ABTS, DPPH and H_2O_2 radical is scavenged by 50%, ferrous ion is chelatedby 50% and the absorbance is 0.5 for reducing power. IC_{50} was obtained using the regression equation.

Table 3
Correlation between antioxidant assays, total phenolic and flavonoids*

	DPPH	ABTS	H_2O_2	RP	MC	TP	FL
DPPH	1						
ABTS	0.92	1					
H_2O_2	0.99	0.85	1				
RP	0.99	0.88	0.99	1			
MC	0.70	0.42[c]	0.79	0.76	1		
TP	0.99	0.87	0.99	0.99	0.77	1	
FL	0.94	0.74	0.98	0.96	0.89	0.97	1

* high positive correlation between the parameters, except c = low positive correlation.

Like the DPPH assay, the ABTS scavenging activity also increased significantly with increasing concentrations of the extracts. (Fig. 1B). The ETH extract showed a more powerful ABTS radical scavenging activity than the EAC and DCM extract. The observed trend was ETH (IC_{50} = 39.42 μg/mL) > EAC (IC_{50} = 96.63 μg/mL) > DCM (IC_{50} = 113.48 μg/mL).

A similar result was obtained for the H_2O_2 scavenging activity (Fig. 1C). ETH extract showed stronger H_2O_2 scavenging activity than EAC and DCM extracts in a dose- dependent pattern. However, the observed activity was lower than Vitamin C. Ramalingam and Rajaram [29] reported similar H_2O_2 scavenging activities for various leaf extracts of *R. apiculata*.

Fig. 1D shows the reducing power of *R. racemosa* extracts. Reducing power of an extract is regarded as an indicator of antioxidant activity as it measures the ability of the extract to break free radical chain by hydrogen donation [9]. Amongst the extracts, ETH extract exhibited the highest reducing power in a dose-dependent manner. Lower reducing power was exhibited by EAC and DCM extracts.

The ability of the extract to chelate transition metals such as iron was also evaluated. This is significant because iron may accelerate lipid oxidation by the lysis of hydrogen and lipid peroxides to form free radicals by the Fenton process [30]. In this study, ferrous-ion chelating assay increased in a dose - dependent manner (Fig. 1E), with EAC extract demonstrating outstanding chelating ability than ETH extract at all concentrations except at 100 μg/mL. Chelating ability increased in the order: EAC (IC_{50} = 44.42 μg/mL) > ETH (IC_{50} = 46.16 μg/mL) > DCM (IC_{50} = 93.35 μg/mL).

Generally, results obtained from the antioxidant study demonstrate that ETH and EAC extracts exhibited outstanding antioxidant activity in the various *in-vitro* models and could donate hydrogen to free radicals, scavenge organic radicals, and chelate metal ions that may be involved in lipid peroxidation processes [10].

Correlation between the different antioxidant assays and phenolics were evaluated (Table 3). Total phenolics showed good correlation with the antioxidant assays, such as DPPH (R^2 = 0.99), ABTS (R^2 = 0.89), H_2O_2 (R^2 = 0.99), reducing power (RP) (R^2 = 0.99) and metal chelating activity (MC) (R^2 = 0.77). A similar correlation was obtained for the flavonoid content. This is in agreement with reports that antioxidant activity is positively corelated with contents of phenolics. Also, there was positive correlation between flavonoid contents (FL) and total phenolics (TP), suggesting that phenolic compounds are important contributors to the observed antioxidant activity of the extracts and played important role in the beneficial effect of this plant [31–32].

The antifungal activity of DCM, EAC and ETH stem bark extracts of *R. racemosa* against *T. mentagrophyte, M. canis, T. rubrum* and *E. floccosum* was evaluated by the agar well diffusion method and their inhibition zone diameter (IZD) measured. The extracts showed significant inhibitory effects against the dermatophytes in a dose-dependent manner (Fig. 2). The DCM extracts exhibited the lowest activity against the dermatophytes at all concentrations (IZD < 14 mm). At a concentration of 2000 µg/mL, ETH extract was most active against *T. mentagrophyte* (IZD = 26.13 mm) and *E. floccosum* (IZD = 29.28 mm), while EAC extract was most active against *M. canis* (IZD = 28.21 mm) and *T. rubrum* (IZD = 33.14 mm). This result suggests a higher sensitivity of the dermatophytes to EAC and ETH extracts.

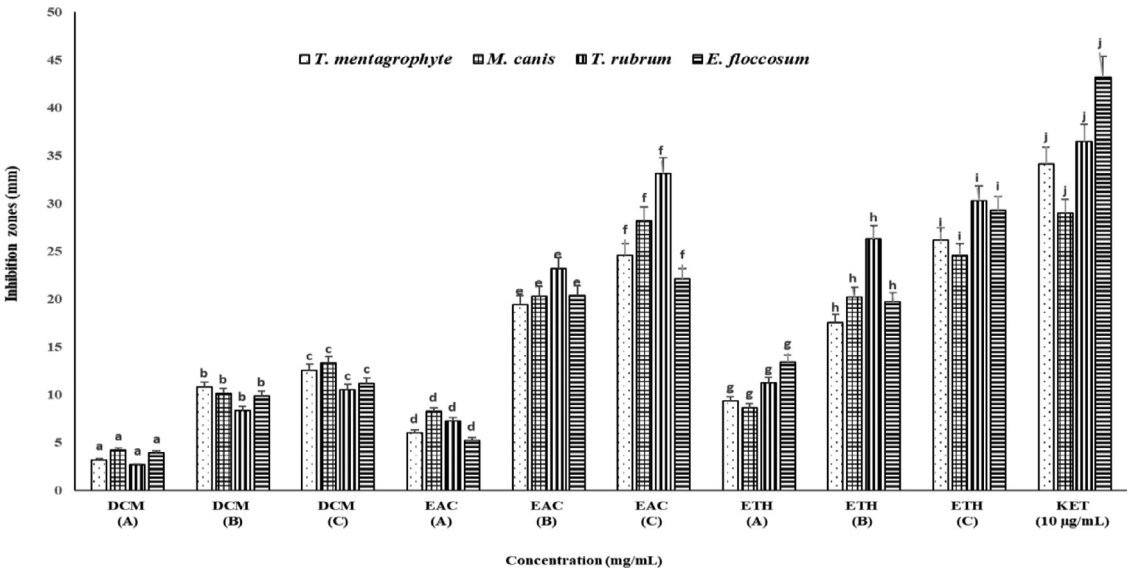

Fig. 2. Antifungal activity of *R. racemosa* extract. (A)-0.5 mg/mL;(c)-2.0 mg/mL. Values are mean of triplicate determinations±SD.Bars having different letters are significantly different ($p \leq 0.05$).

Table 4
Minimum inhibitory concentrations and minimum fungicidal concentrations of *R. racemosa* extracts against test organisms.

Microorganism	Extracts (µg/mL)						Control (µg/mL)	
	DCM		EAC		ETH		KET	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
T. mentagrophyte	1890	3780	320	640	270	270	2	4
M. canis	1630	3360	270	270	300	600	3	3
T. rubrum	2340	5480	250	500	180	360	2	4
E. floccosum	2550	5100	440	880	260	520	4	4

DCM = dichloromethane; EAC = ethylacetate; ETH = ethanol; KET = ketoconazole.

Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were also determined by the broth microdilution method (Table 4). EAC and ETH extracts showed considerable inhibitory effects against the tested dermatophytes (MIC = 250–440 µg/mL and 180–300 µg/mL respectively) than the DCM extract. With regards to the EAC extract, observed MIC trend was *T. rubrum* > *M. canis* >*T. mentagrophyte* > *E. floccosum*. For ETH extract, highest MIC was against *T. rubrum* (180 µg/mL), followed by *E. floccosum* and *T. mentagrophyte* (260 µg/mL and 270 µg/mL respectively), then *M. canis* (300 µg/mL). Ketoconazole was effective at concentrations of 2 µg/mL against *T. mentagrophyte*, 3 µg/mL against *M. canis*, 2 µg/mL against *T. rubrum* and 4 µg/mL against *E. floccosum*. Minimum fungicidal concentrations (MFC) were the same or only one two-fold dilution above the MIC.

Numerous studies have demonstrated that medicinal plants possess antidermatophytic activity. Mahmoudvand et al. [33] reported lower antidermatophytic activity from extracts of *Berberis vulgaris* against *T. mentagrophytes, T. rubrum, M. canis* and *M. gypseum*. Similar result exists for extracts of *P. tomentosa* and *M. scaber* against *T. rubrum, T. mentagrophytes* and *M. gypseum* as well as *E. umbelliflora* against *E. floccosum, M. canis, M. gypseum, T. rubrum* and *T. mentagrophytes* [34]. Higher activity has been reported for the dichloromethane and methanol crude extracts of *Coula edulis* with MIC of 1.25–5 µg/mL [35], while similar activity has been reported for various extracts of *R. racemosa* against non-dermatophytic fungi [36]. Solubility and diffusability of the extract may be responsible for the observed variation. Overall, the increased activity observed in ETH than EAC extract suggests a higher concentration of phyto - compounds in the former which was able to either supress the synthesis of ergosterol and other macromolecules or react with membrane sterols in dermatophytes [37].

Conclusion

This work indicates that *R. racemosa* stem bark extracts exhibited varying degrees of antioxidant activity in the *in-vitro* models, with EAC and ETH extracts demonstrating excellent ability to effectively scavenge free radicals, chelate metal ions and donate hydrogen. In addition, the extracts showed promising activity against the tested dermatophytes, with EAC and ETH extracts being the most effective. This study suggests that EAC and ETH extracts of *R. racemosa* stem bark can serve

as leads for alternative and renewable source of phyto - based antioxidants with antifungal potential for the treatment of dermatophytic infections in humans.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Basil N. Ita: Visualization, Investigation, Writing – original draft. **Samuel I. Eduok:** Formal analysis, Writing – review & editing, Investigation.

Funding

Nil

References

[1] C.N. Duke, J.A. Allen, (2006) Rhizophora mangle, R. samoensis, R. racemosa, R. harrisonii (Atlantic-East Pacific Red Mangrove). Species Profiles for Pacific Island Agroforestry, 10, 1-18
[2] C.N. Ariole, A.E. Akinduyite, Antibacterial potential of indigenous red mangrove (*Rhizophora recemosa*) fungal endophytes and bioactive compounds identification, Int. J. Microbiol. Mycol. 4 (4) (2016) 14–24.
[3] B.E. Angalabiri-Owei, J.C. Isirima, Evaluation of the lethal dosage of the methanol extract of *Rhizophora racemosa* leaf using Karber's Method, Afr. J. Cell. Path 2 (2014) 65–68.
[4] N. Suganthy, K.P. Devi, *In vitro* antioxidant and anticholinesterase activities of *Rhizophora mucronata*, Pharm. Biol. 54 (1) (2016) 118–129.
[5] J.C. Sanchez, R.F. Garcia, T.M. Cors, 1,1-diphenyl-2-picrylhydrazyl radical and superoxide anion scavenging activity of *Rhizophora mangle* (L) bark, Pharmacog. Res. 2 (5) (2010) 279–284.
[6] E. Rocca, J. Steinmetz, M. Kassim, M.S. Ibrahim, H. Osman, Antioxidant activity of mangrove *Rhizophora apiculata* bark extracts, Food Chem. 107 (2008) 200–207.
[7] R. Sundaram, S.J. Inbaneson, S. Palavesam, M. Gnanadesigan, *In vitro* antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine – sensitive *Plasmodium falciparum*, Parasitol. Res. 108 (4) (2011) 873–878.
[8] J.N. Mishra, N.K. Verma, *In vitro* antihelmintic activity of *Rhizophora mucronata* leaves against *Pheritima posthuma*, Int. J. Chem. Sci. 2 (1) (2018) 5–7.
[9] V. Kumar, M. Lemos, M. Sharma, V. Shiram, Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl, Free Radic. Antioxid. 3 (2013) 55–60.
[10] K. Sowndhararajan, S.C. Kang, Free radical scavenging activity from different extract of leaves of *Bauhinia vahliii* Wight and Arn, Saudi J. Biol. Sci. 20 (2013) 319–325.
[11] G.O Ajayi, J.A. Olagunju, O. Ademuyiwa, O.C. Martins, Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica* Linn, J. Med. Plants Res. 9 (2011) 1756–1761.
[12] G.E. Trease, W.C. Evans, Pharmacognosy. W.B. Saunders, 15th edn., W. B. Saunders Company, 2002, p. 406.
[13] A. Sofowora, Screening plants for bioactive agents, in: Medicinal Plants and Traditional Medicine in Africa, 2nd edn., Spectrum Books Ltd, Ibadan, Nigeria, 1993, pp. 134–156.
[14] B. Subhasree, R. Baskar, R.L. Keerthana, R.L. Susan, P. Rajasekran, Evaluation of antioxidant potential in selected green leafy vegetables, Food Chem. 115 (2011) 1213–1220.
[15] C.S. Obiang, J. Ondo, G.N. Atome, L.O. Engonga, J.D. Siawaya, E.N. Emvo, Phytochemical screening, antioxidant and antimicrobial potential of stem barks of *Coula edulis* Baill, *Psedospondias longifolia* Engl. and *Carapa klaineana* Pierre from Gabon, Asian Pac. J. Trop. Dis. 6 (7) (2016) 557–563.
[16] I. Gulcin, M. Oktay, E. Kirecci, O. Kufreviolu, Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts, Food Chem. 83 (2003) 371–382.
[17] M. Oyiazu, Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutri. 44 (1986) 307–315.
[18] E.A. Decker, B. Welch, Role of ferritin as lipid oxidation catalyst in muscle food, J. Agr. Food Chem. 38 (1990) 674–678.
[19] H. Gul, I. Bhakshu, F. Ahmed, I. Qureshi, I. Ghazi, Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays, BMC Compl. and Altern. Med. 11 (2011) 64–71.
[20] G. Rebell, D. Taplin, Dermatophytes, Their Recognition and Identification, University of Miami Press, Coral Gables, Florida, 1978.
[21] W.R. Devi, B. Singh, C.B. Singh, Antioxidant and anti-dermatophytic properties of leaf and stem bark of *Xylosma longifolium* Clos, BMC Compl. Altern. Med. 13 (2013) (2013) 155–164.
[22] Clinical and Laboratory Standards Institute (CLSI), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentuous Fungi; Approved Standard- Second Edition CLSI document M38-A2 (ISBN 1-56238-668-9). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2008.
[23] I.J. Sagbo, A.E. Crock, W. Otang-Mbeng, Phytochemical screening and gas chromatography – mass spectrometry analysis of ethanol extract of *Scambiosa columbabria* L, Pharmacogn. Res. 12 (2020) 35–39.
[24] N. Akhtar, B.M. Ihsan-ul-Haq, Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plants, Arabian J. Chem. 11 (2018) 1223–1235.
[25] M.B. Patel, S. Mishra, Hypoglycemic activity of alkaloidal fraction of *Tinospora cordifolia*, Phytomedicine 18 (2011) 98–106.
[26] G. Devendran, U. Balasubramanian, Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L leaves, Asian J. Plant Sci. Res. 1 (2011) 44–48.
[27] O.O. Owolabi, D.B. James, I. Sani, B.T. Andongma, O.O. Fasanya, B. Kure, Phytochemical analysis, antioxidant and anti-inflammatory potential of *Feretia apodanthera* root bark extracts, BMC Compl. Altern. Med. 18 (2018) 12–20.
[28] K. Lalhminghlui, G.C. Jagetia, Evaluation of the free - radical scavenging and antioxidant activities of Chilauni, *Schima wallichii* Korth *in vitro*, Future Sci. 4 (2) (2018) 1–12.
[29] V. Ramalingham, R. Rajaram, Enhanced antimicrobial, antioxidant and anticancer activity of *Rhizophora apiculata*: an experimental report, 3 Biotech 8 (200) (2018) 1–13.
[30] M. Ozturk, F. Aydogmus- Ozturk, M.E. Duru, Antioxidant activity of stem and root extracts of Rhuba (*Rheum ribes*): An edible medicinal plant, Food Chem 103 (2007) 623–630.

[31] T. Islam, Y. Xiaoming, B. Xu, Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China, LWT Food Sci. Technol. 72 (2016) 423–431.
[32] M.E. Jemli, R. Kamal, I. Marmouzi, A. Zerrouki, Y. Cherrah, K. Alaoui, Radical Scavenging activity and ferric reducing ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.), Adv. Pharmacol. Sci. 6392656 (2016) 1–6.
[33] H. Mahmoudvand, S.A.A. Mousavi, A. Sepahvand, F. Sharififar, B. Ezatpour, F. Gorohi, E.S. Dezaki, S. Jahanbakhsh, Antifungal, antileishmanial and cytotoxicity activities of various extracts of *Berberis vulgaris* (Berberidaceae) and its active principle berberine, ISRN Pharmacol. 602436 (2014) 1–6.
[34] K.E. Machado, V.C. Filho, R.C.B. Cruz, C. Meyre-Silva, A.B. Cruz, Antifungal activity of *Eugenia umbrelliflora* against dermatophytes, Nat. Prod. Commun. 4 (9) (2009) 1181–1184.
[35] J.D.D. Tamokou, J.R. Kulate, D. Gasting, P.N. Efouet, A.J. Njouendou, Antidermatophytic and toxicological evaluation of dichloromethane-methanol extract, fractions and compounds isolated from *Coula edulis*. Iran, J. Med. Sci. 2 (2011) 111–121.
[36] A. Chiavaroli, K.I. Sinan, G. Zengin, M.F. Mahomoodally, N.B. Sadeer, O.K. Etienne, Z. Cziáky, J. Jek, J. Glamo - Clija, M. Sokovi, L. Recinella, L. Brunetti, S. Leone, H.H. Abdullah, P. Angelini, G.A. Flores, Identification of chemical profiles and biological properties of Rhizophora racemosa G. Mey. extracts obtained by different methods and solvents, Antioxidants 9 (533) (2020) 1–30.
[37] M.A. Ghannoum, L.B. Rice, Antifungal agents: mode of action, mechanism of resistance and correlation of these mechanisms with bacterial resistance, Clin. Microbiol. Rev. 12 (4) (1999) 501–517.