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Phytochemical screening, phenolic and flavonoid content, and antioxidant activity of Rhizophoraceae methanol extract from Langsa, Aceh, Indonesia

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Abstract. *Indriaty, Djufri, Ginting B, Hasballah K. 2023. Phytochemical screening, phenolic and flavonoid content, and antioxidant activity of Rhizophoraceae methanol extract from Langsa, Aceh, Indonesia. Biodiversitas 24: 2865-2876.* *Bruguiera cylindrica* (L.) Blume, *Bruguiera gymnorrhiza* (L.) Lam., *Ceriops decandra* (Griff.) Ding Hou, *Rhizophora apiculate* Blume, and *Rhizophora mucronata* Lam. are mangrove plant species belonging to Rhizophoraceae that have been used as medicinal plants. Studies on phytochemical screening and bioactivity of Rhizophoraceae mangrove plants from the Aceh region are still limited. This study aimed to analyze the chemical compounds in Rhizophoraceae from Aceh and determine total phenolic content (TPC), flavonoid content (TFC), and antioxidant activity. The chemical compounds were determined using qualitative assay, TLC, and Gas Chromatography-Mass Spectrophotometry (GC-MS). The antioxidant activity was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Twenty extracts from various plant parts of Rhizophoraceae (roots, bark, leaves, and fruit/hypocotyl) were used in this study. The phytochemical screening of Rhizophoraceae plants revealed the presence of alkaloids, flavonoids, phenolics (tannins), terpenoids, steroids, and saponins. The highest TPC content was obtained from the bark of *R. mucronata* (484.39 mg GAE/g extract). Furthermore, the highest TFC was found in the leaves of *R. apiculata* (15.23 mg QE/g extract). Nineteen extracts had very high antioxidant activity (IC50: 2.35±0.01 - 29.84±0.19 µg/mL). The bark and roots of *C. decandra* had the most potent antioxidant activity (IC50: 2.35 0.01 and 3.23 0.01 µg/mL, respectively). The GC-MS revealed the presence of pyrocatechol (15.85%), antiarol (1.44%), and hexadecanoic acid (1.69%), that act as antioxidants. Therefore, it can be concluded that the Rhizophoraceae methanol extract contains phenols in the stem bark and flavonoids in the leaves with very high antioxidant activity as a good source of natural ingredients for future pharmaceutical product development. It is highly recommended to do further research to obtain pure compounds from these plants.

Keywords: Antioxidant, *Bruguiera*, *Ceriops*, *Rhizophora*, phytoconstituents

INTRODUCTION

Indonesia is an archipelagic country with a coastline covered by the most extensive mangrove forest in the world (Hamilton and Casey 2016). The total area of its mangrove forests is approximately 3.2 million ha (Kusmana and Hikmat 2015), with a diversity representing 43 out of 81 true mangrove species worldwide (Ragavan et al. 2016). As part of Indonesia's territory, Aceh province has forest areas and diverse true mangrove species of approximately 8,000 ha and 38 species, respectively (Zurba et al. 2019). The mangrove forest of Langsa City is geographically located in the northern part of Sumatra Island, directly adjacent to the Malacca Strait, and is dominated by Rhizophoraceae, Avicenniaceae, and Sonneratiaceae (Iswahyudi et al. 2020).

Mangroves are halophyte plants that thrive in complex environmental conditions, including high salinity (Lopes et al. 2021). They also thrive under high and low temperatures, drought, high luminosity, tides, and waves, where other conventional plants cannot grow (Rahman et al. 2021). In addition, these plants develop specific adaptive responses, such as synthesizing and accumulating endogenous metabolite compounds to protect cellular structures from stressful environmental conditions (Medini et al. 2014; Rahman et al. 2021). The most important secondary metabolites are found in three structural classes, namely nitrogen-containing compounds (alkaloids and amines), terpenoids, and phenolics (flavonoids, phenolic acids, tannins, and quinones), which serve as new sources of natural antioxidants (Twaij and Hasan 2022).

ROS (reactive oxygen species) and RNS (reactive nitrogen species) play an important role in the pathogenesis of various diseases in humans (Prasad et al. 2017). However, they produce free radicals at high concentrations, which cause oxidative damage to the body's important biomolecules such as lipids, carbohydrates, proteins, enzymes, DNA, and RNA, thereby damaging all cell

structures (Gašparović 2020; Flieger et al. 2021). Oxidative stress plays a significant role in the development of chronic and degenerative diseases, such as cancer (Gašparović 2020), cardiovascular disease (Dubois-deruy et al. 2020), Alzheimer (Bhatt et al. 2020), aging (Russo et al. 2018), atherosclerosis (Batty et al. 2022), autoimmune disorders, and arthritis (Flieger et al. 2021). The protection against free radicals can be enhanced by the activity of antioxidants (Atasoy and Yücel 2021). The use of natural antioxidants derived from plants has received much attention recently because plants are one of the best sources of natural antioxidants such as flavonoids, phenolic, and alkaloid (Tungmunnithum et al. 2018; Macáková et al. 2019). These antioxidant compounds scavenge radicals by preventing and repairing cell damage caused by ROS and RNS.

This study observed the mangrove halophyte plant Rhizophoraceae's bioactive compounds and antioxidant activity as safe, sustainable, and environmentally friendly sources. Several studies on the Rhizophoraceae family have revealed secondary metabolite content and antioxidant bioactivity from plant parts, including *Bruguiera gymnorrhiza* (L.) Blume, *Ceriops decandra* (Griff.) Ding Hou, *Rhizophora apiculate* Blume, and *Rhizophora mucronate* Lam. leaf extracts (Banerjee et al. 2008; Sadeer et al. 2019; Karim et al. 2020; Saragih et al. 2020; Ahad et al. 2021). Furthermore, stem bark extracts of *B. cylindrica*, *B. gymnorrhiza*, *C. decandra*, *R. apiculata*, and *R. mucronata* were used (Banerjee et al. 2008; Gao and Xiao 2012; Simlai and Roy 2012; Gnanadesigan et al. 2017). Those compounds are also found in *C. decandra*, *R. apiculata*, and *R. mucronata* root extracts (Banerjee et al. 2008; Bibi et al. 2019). In addition, studies on the fruit/hypocotyl extracts of *B. gymnorrhiza* and *C. decandra* have been conducted (Hosen et al. 2020). However, there were several parts, including five species of Rhizophoracea, which their phytochemicals and antioxidant activity had not been studied yet, particularly in root and fruit extracts. In addition, the fruit and hypocotyl of *C. decandra* and *B. cylindrica* are hard to obtain in nature due to their small size. They are only found in specific seasons; thus, collecting this sample is difficult.

This study conducted the phytochemical content and antioxidant activity of five selected Rhizophoraceae species from various plant parts (roots, bark, leaves, and fruit/hypocotyl). Rhizophraceae, from one of Langsa City Mangrove Forest's local natural resources, has traditionally been used to treat various diseases, such as diarrhea, hepatitis, hypertension, diabetes, and childbirth (Bibi et al. 2019). Further research on Rhizophoraceae's phytochemical screening and antioxidant activity on all plant parts is required to determine which plants and species have the most potent antioxidant potential. In addition, a previous study on cytotoxic bioactivity using the Brine Shrimp Lethality Test (BSLT) method revealed that Rhizophoraceae has excellent potential as a cancer-fighting agent (Indriaty et al. 2022). This study is expected to contribute knowledge regarding medicinal plants' bioactivity from the Langsa City coast. Furthermore, it can be further developed for the pharmaceutical industry.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used in this study were 80% methanol, Mg powder (Merck), concentrated HCl (Merck), 0.5 M HCl, and Mg metal. Qualitative observation of phytochemical tests was carried out according to analytical standards using Liebermann-Burchard, Dragendorff, Mayer, and Wagner reagents from Merck (Selangor, Malaysia). The antioxidant test materials include 1.1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, St Louis, USA), ascorbic acid (Vitamin C) (Sigma-Aldrich, St Louis, USA), 70% and 96% methanol, as well as distilled water. TPC and TFC test materials were Folin-Ciocalteu reagent (Sigma-Aldrich, St Louis, USA), gallic acid ($C_7H_6O_5$) (Merck), Na_2CO_3 (Merck), $AlCl_3$ (Merck), CH_3CO_2K (Merck), and quercetin ($C_{15}H_{10}O_7$) (Merck).

Plant collection and sample preparation

Plant samples (roots, bark, leaves, and fruit/hypocotyl) of five Rhizophoraceae species were collected from the Langsa Mangrove Forest Area, Aceh, Indonesia, in January 2021, between 08.00 AM and 12.00 PM, as shown in Figure 1. The plant species include *B. cylindrica*, *B. gymnorrhiza*, *C. decandra*, *R. apiculata*, and *R. mucronata* (Figure 2). The diameter of the sampled trees ranged from 10 to 30 cm. Plant samples were washed under running water and cut into small pieces. Samples were dried under shade for 20 days to obtain the stable plant weight, then stored in plastic and labeled for further treatment. Plant identification was carried out at the Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia, with identification number B/398-402/UN 11.1.8.4/TA.00.01/2021.

Plant extraction

Furthermore, 100 g of dried samples were macerated in 1,000 mL of methanol for 24 hours at room temperature. Methanol is used as a solvent because it is a polar solvent for extracting polyphenols, besides having a higher solubility with antioxidant compounds than ethanol and water (Truong et al. 2019). The extract was filtered using a glass funnel and Whatman filter paper number 1. Each filtrate was concentrated using a rotary evaporator (Büchi Labortechnik, Germany) at low pressure and controlled temperature ranging from 40 to 50°C. They were then dried in a water bath at 40ºC and left at room temperature until completely dry. The dry extract was stored in airtight vials at room temperature until further use. The maceration activities are consistent with previous study procedures (Ginting et al. 2021). Afterward, each extract had the following codes: RBc (*B. cylindrica* root), RBg (*B. gymnorrhiza* root), RCd (*C. decandra* root), RRa (*R. apiculata*), RRm (*R. mucronata* root), BBc (*B. cylindrica* bark), BBg (*B. gymnorrhiza* bark), BCd (*C. decandra* bark), BRa (*R. apiculata* bark), BRm (*R. mucronata* bark), LBc (*B. cylindrica* leaf), LBg (*B. gymnorrhiza* leaf), LCd (*C. decandra* leaf), LRa (*R. apiculata* leaf), LRm (*R. mucronata* leaf); HBc (*B. cylindrica* hypocotyl), HBg (*B. gymnorrhiza* hypocotyl), FCd (*C. decandra* fruit), FRa (*R. apiculata* fruit), and FRm (*R. mucronata* fruit).

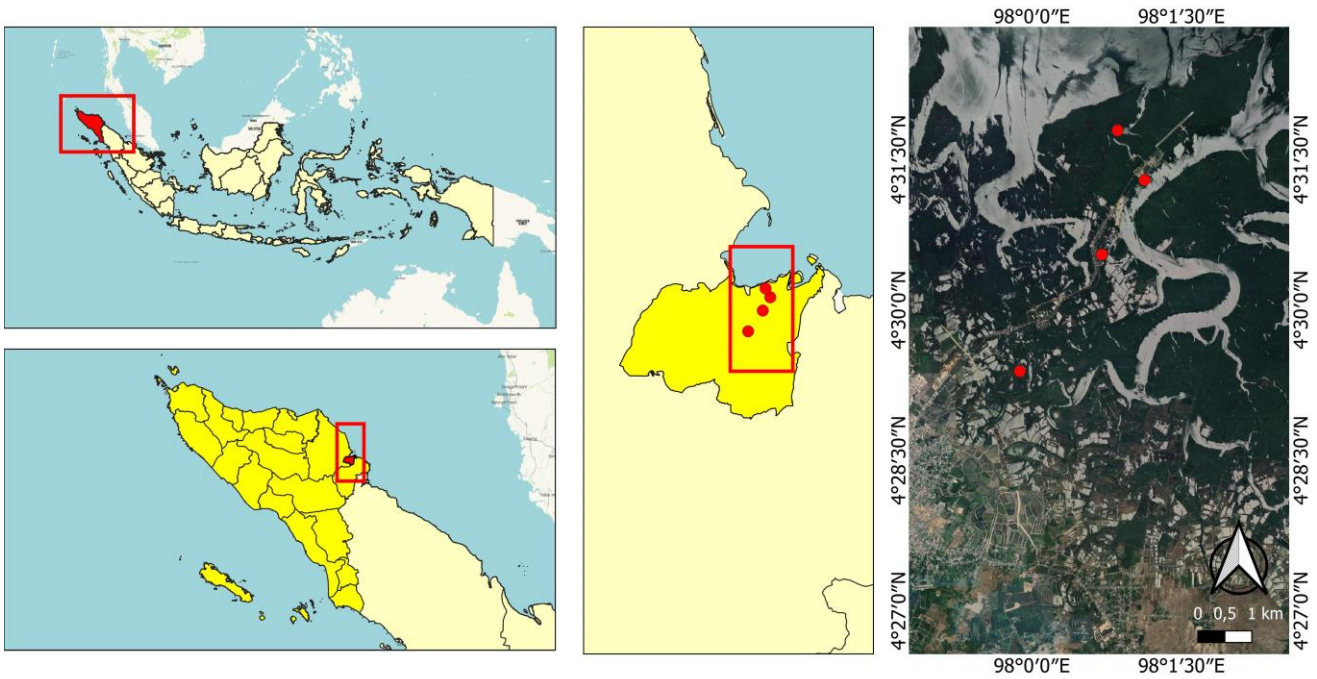

Figure 1. Sampling location in Kuala Langsa Mangrove Forest Area, Aceh Indonesia

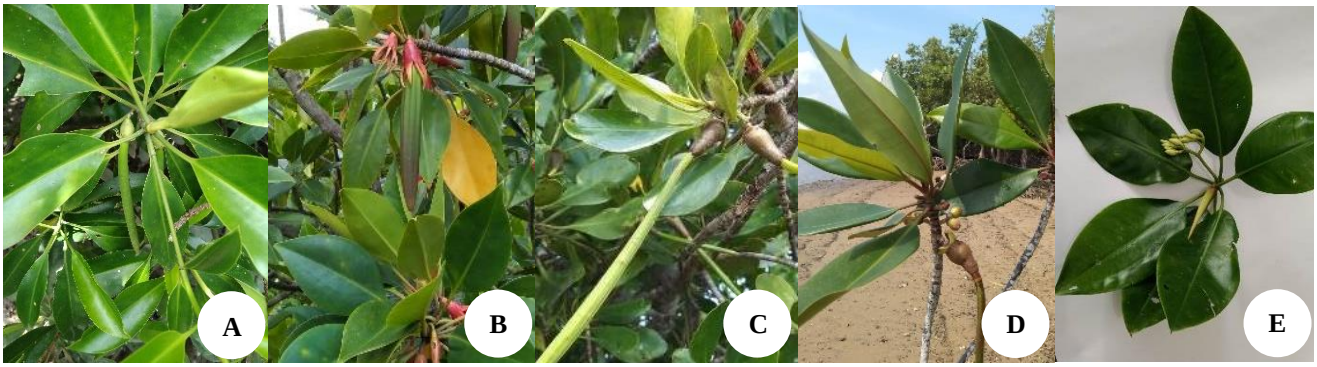

Figure 2. Rhizophoraceae plants. A. *B. cylindrica*; B. *B. gymnorrhiza*; C. *C. decandra;* D. *R. apiculate*; and E. *R. mucronata*

Qualitative phytochemical screening

Phytochemical compounds of methanol extracts were determined qualitatively, including alkaloids, terpenoids, steroids, saponins, flavonoids, and phenolics (Nuraskin et al. 2020).

Alkaloids

100 mg of extract was added with NH_3 (3 mL) and left for two hours until two layers were formed, then 5 mL chloroform was added. The dissolved layer was separated into three test tubes. Then, Mayer's, Wagner's, and Dragendrof's reagents were added to the first, second, and third tubes, respectively. White, yellow, and reddish-brown precipitates indicated a positive result for alkaloids.

Terpenoid and steroid

The Liebermann-Burchard reagent was added to 100 mg of extract dissolved in methanol. The presence of purple or red indicates the presence of terpenoids, while steroids are indicated by green or blue.

Saponin

100 mg of extract was dissolved in methanol, then heated and shaken vigorously. The formation of foam which lasted 30 minutes, indicated the presence of saponin.

Flavonoids

100 mg of extract was dissolved in methanol and added with Mg^{2+} powder and HCl solution in methanol (1:1). A red or purple color indicates the presence of flavonoids. Phenolics (tannins) were detected by adding 100 mg of the extract with 5% $FeCl_3$ (5 drops). The presence of dark blue or black color indicates the presence of tannins.

Profiles of phytochemicals using Thin Layer Chromatography (TLC)

Silica gel G_{60} F_{254} thin Layer Chromatography **(**TLC) plates were activated in a 50°C oven for 30 minutes. A capillary tube was used to apply the 50 µL sample to the TLC plate (Kafelau et al. 2022). The mobile phase is chloroform: methanol (6:4). Each 25 µL extract was dissolved in methanol and transferred onto the TLC plate using a capillary tube. After developing the TLC in the mobile phase, the TLC plate was sprayed with vanillin sulfate reagent (Ambarwati et al. 2015). Vanillin sulfate reagent: 1 g vanillin was dissolved in 20 mL of 70% ethanol and then added with 20 mL of 5% sulfuric acid.

Determination of Total Phenolic Contents (TPC)

The total phenolic content of the extract was determined according to the Folin-Ciocalteu method with slight modifications (Mwamatope et al. 2020). First, 5 mg extract was dissolved in 0.5 mL methanol p.a and added with deionized water to precisely 5 mL. Then, 0.2 mL of the mixture was added with 15.8 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. Next, the solution was mixed with 3 mL of 10% (w/v) Na_2CO_3 after 5 minutes and incubated for 120 minutes at room temperature. In addition, as the standard curve, 5 mg of gallic acid was dissolved in 1 mL of methanol p.a and added with deionized water to 10 mL. Various concentrations of gallic acid (100 µg/mL, 125 µg/mL, 150 µg/mL, 175 µg/mL, and 200 µg/mL) were made from the stock solution to obtain the standard curve. Next, as much as 0.2 mL was taken from each concentration and added with 15.8 mL of deionized water and 1 mL of Folin Ciocalteu reagent. After 5 minutes of incubation, the solution was added with 3 mL of 10% (w/v) Na_2CO_3 and incubated again for 120 minutes at room temperature. After incubation, the absorbance of the mixture was measured with a UV Vis Spectrometer (Shimadzu UVmini-1240, Kyoto, Japan) at a wavelength of 765 nm. The total phenolic content was expressed as mg gallic acid equivalent per g extract (mg EAG/g extract). All samples were tested in three replicates.

Determination of Total Flavonoid Contents (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Phuyal et al. 2020), and quercetin was used as the standard. First, 5 mg extract was dissolved in methanol p.a to obtain precisely 5 mL. Next, 1 mL of the solution was added with 3 mL methanol, 0.2 mL $AlCl_3$, 0.2 mL potassium acetate, and 5.6 mL deionized water. Next, a standard solution was prepared by dissolving 5 mg of quercetin in methanol p.a to a volume of 5 mL and diluted to a series of concentrations of 20, 40, 60, 80, and 100 µg/mL. Afterward, 1 mL of each concentration was added with 3 mL methanol p.a, $AlCl_3$ (0.2 mL), potassium acetate (0.2 mL), and distilled water (5.6 mL). The samples were incubated for 30 minutes at room temperature (25°C). After incubation, the absorbance of the solution was measured with UV-Vis spectrophotometer (λ 440 nm). The total flavonoid content was expressed as mg quercetin equivalent per g of extract (mg QE/g extract). All samples were tested in three replicates.

Antioxidant DPPH 2,2-Diphenyl-1-picrylhydrazyl Assay

Antioxidant activity was determined using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method developed by Yahya et al. (2021). 2.5 mg of the extract was mixed with two drops of 2% dimethyl sulfoxide (DMSO, Merck-Germany) to increase solubility and left for 24 hours. Extracts were prepared at various concentrations (1.56 µg/mL, 3.125 µg/mL, and 6.25 µg/mL) in methanol p.a and homogenized using a sonicator. Then, 4 mL of this solution was added to 1 mL of DPPH solution (dissolve 7.9 mg of DPPH powder in 50 mL of methanol p.a). The solution was homogenized and incubated in the dark incubator for 30 minutes at 37°C. The absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer, and methanol p.a was used as a blank. Ascorbic acid was used as a positive control, and a similar procedure was carried out at concentrations of 1, 3, 6, 9, 12, and 15 g/mL. Furthermore, the inhibition percentage of DPPH radicals was calculated to obtain the IC_{50} value. IC_{50} is the extract's concentration causing 50% inhibition of DPPH radicals. The extract samples with the highest antioxidant activity were further analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu QP2000A, Kyoto, Japan) to determine the phytoconstituents.

GC-MS analysis

Methanol extract of *C. decandra*'s bark and root had the most potent antioxidant activity; therefore, they were analyzed using GC-MS. The procedure of GC-MS analysis follows Masyudi et al. (2022). GC-MS analysis was carried out on the GC-MS Gas Chromatograph with Auto Sampler 5975A (Agilent Technologies 7890A), Mass Selective Detector, and data system in Chemstation. First, the sample was dissolved in methanol p.a., then 5 µL was injected into the GC-MS using helium (He) gas through a capillary column with a 1.2 mL/minute rate and a split ratio 8:1 psi. The injector and detector were programmed at 250ºC and 230ºC, with operating temperatures of 280ºC and 140ºC, respectively. The National Institute of Standards and Mass Spectral Technology (NIST-MS) spectrometer database interpreted the mass spectrum fragmentation pattern.

Data analysis

The data for each sample was obtained from three replications, and the values are expressed as the average (± standard error). The data included phenolic and flavonoid content, and the IC_{50} was statistically analyzed. The data were tested for normal distribution using the Shapiro-Wilk test, and statistical significance was obtained through a one-way analysis of variance (ANOVA). Furthermore, a comparison of individual averages was generated from Duncan's test using the computer program SPSS for Windows, version 21.

RESULTS AND DISCUSSION

Extraction and qualitative phytochemicals of Rhizophoraceae

The yield percentage and phytochemical compounds of roots, bark, leaves, and hypocotyl/fruit of Rhizophoraceae species are presented in Table 1, while the appearance of plant extracts is in Figure 3. The highest yield was in *C decandra* leaves extract (25.462%), followed by *B. gymnorrhiza* leaves extract (22.87%). A previous study by Malik et al. (2017) showed that the yield of methanol extract of Rhizophoraceae, i,e., *B. cylindrica*, and *R. apiculata* leaves extract without grinding were 7% and 3.5%, lower than those in this study, namely by 21.96% and 15.27%. This study uses finely ground samples in the extraction process, which results in the broader sample surface interacting with the solvent. Therefore, the compound diffused out of the cell optimally. Methanol provides a higher solubility, so the compound diffuses out of the cell optimally (Borges et al. 2020). Methanol molecule has a polar arrangement of oxygen and hydrogen atoms. One side (hydrogen) is positively charged, and the other (oxygen) is negatively charged; therefore, it can extract polar and non-polar compounds simultaneously (Nawaz et al. 2020). The ability of the solvent to extract the material depends primarily on the compound's solubility, the product's mass transfer kinetics, the solute's interaction strength with the appropriate solvent, the heat and mass of the solvent and dissolved compound (Dhanani et al. 2017).

Figure 3 shows that the mangrove plant extracts of Rhizophoraceae generally have a solid form and are reddish brown. The leaf and fruit liquid extracts have a sticky and oily texture. Previous studies showed that the bark extracts of *R. mucronata* and *C. decandra* had a reddish-brown color (Hendrawan 2021; Rumengan et al. 2021). The reddish-brown color of the extract indicated the presence of tannin, that have chromophore groups in conjugated C=C and C=O bonds, which absorb and produce color to a compound (Rumengan et al. 2021). In addition, there are also plant pigments that affect other colors in Rhizophoraceae extracts derived from chlorophyll a, chlorophyll b, lutein, beta-carotene, and violaxanthin (Pringgenies et al. 2017).

The phytochemical analysis showed the presence of tannins in all extracts. Tannins are classified as natural polyphenolic compounds. Condensed tannins are composed of flavonoids that were detected in all Rhizophoraceae extracts. Another phytochemical compound revealed was alkaloid, except for the root extract of *R. apiculata*. Alkaloids play a significant role in plants by protecting them from predators and regulating their growth (Heinrich et al. 2021). Alkaloids are known as anesthetic, cardioprotective and anti-inflammatory agents (Heinrich et al. 2021). In addition, steroids were only detected in *B. cylindrica*, *R. apiculata*, and *R. mucronata* leaves, while terpenoids were detected in almost all extracts. Steroids are categorized as growth hormones, while terpenoids protect plants from abiotic and biotic pressures, growth hormones, anti-inflammatory, antioxidants, anticancer, antiseptic, antiplasmodial, astringent, digestive, and diuretic in therapeutic elements (Andreu et al. 2018).

Table 1. The yield of methanol extract and the phytochemical compounds of Rhizophoraceae methanol extract

Extracts	Extract features	Yield (%)	Alkaloids	Steroids	Terpenoids	Saponins	Flavonoids	Phenolic (tannin)
RBc	Dense, reddish-brown, oily	12.383	+	-	+	-	+	+
RBg	Dense, reddish brown	5.849	+	-	+	-	+	+
RCd	Dense, reddish brown	7.046	+	-	+	-	+	+
RRa	Dense, reddish brown	7.653	-	-	+	-	+	+
RRm	Solid, blackish brown, accompanied by white crystal grains	9.883	+	-	+	-	+	+
BBc	Dense, sticky, blackish brown	8.850	+	-	+	-	+	+
BBg	Solid, dark red	11.094	+	-	+	-	+	+
BCd	Solid, dark red	20.388	+	-	+	-	+	+
BRa	Solid, brittle, blackish red	14.172	+	-	+	-	+	+
BRm	Dense, brittle, hard, blackish brown	12.583	+	-	+	-	+	+
LBc	Liquid, sticky, blackish green, slightly oily	21.962	+	+	-	+	+	+
LBg	Liquid, sticky, blackish green, slightly oily	22.857	+	-	+	+	+	+
LCd	Solid, blackish red	25.462	+	-	+	-	+	+
LRa	Liquid, sticky, blackish green, red oily	15.267	+	+	-	+	+	+
LRm	Dense, soft, green, blackish brown	13.667	+	+	-	-	+	+
HBc	Liquid, sticky, greenish-brown, oily	16.464	+	-	+	+	+	+
HBg	Dense, reddish brown	11.576	+	-	+	+	+	+
FCd	Liquid, sticky, blackish red	17.165	+	-	+	-	+	+
FRa	Solid, like jelly, blackish-red brown, accompanied by white crystal grains	12.416	+	-	+	-	+	+
FRm	Solid, reddish brown, with white crystal grains	9.259	+	-	+	-	+	+

Note: RBc: Methanol extract of *B. cylindrica* root; RBg: *B. gymnorrhiza* root; RCd: *C. decandra* root; RRa: *R. apiculata* root; RRm: *R. mucronata* root; BBc: *B. cylindrica* bark; BBg: *B. gymnorrhiza* bark; BCd: *C. decandra* bark; BRa: *R. apiculata* bark; BRm: *R. mucronata* bark; LBc: *B. cylindrica* leaf; LBg: *B. gymnorrhiza* leaf; LCd: *C. decandra* leaf; LRa: *R. apiculata* leaf; LRm: *R. mucronata* leaf; HBc: *B. cylindrica* hypocotyl; HBg: *B. gymnorrhiza* hypocotyl; FCd: *C. decandra* fruit; FRa: *R. apiculata* fruit; FRm: *R. mucronata* fruit

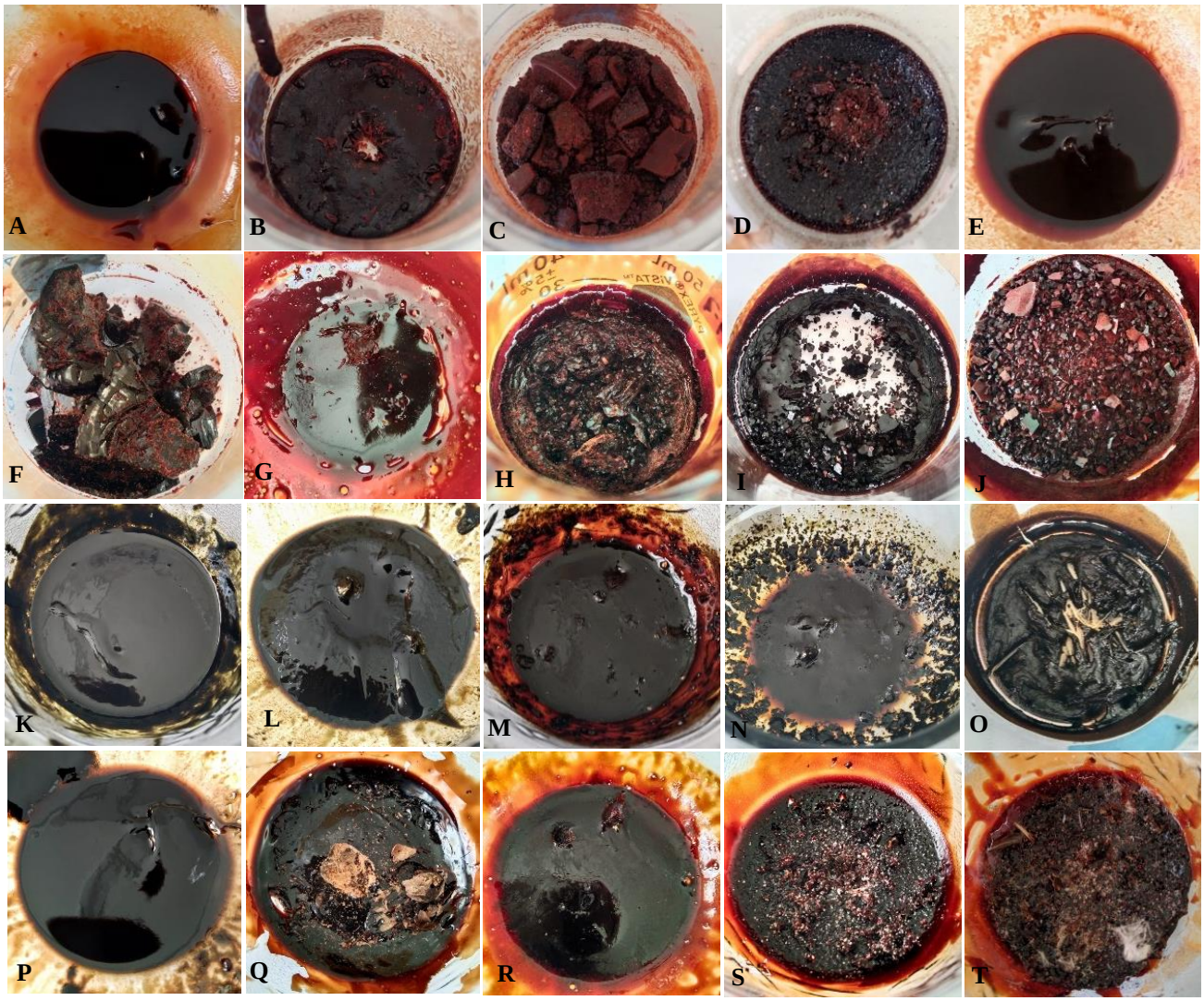

Figure 3. Methanol extract of A. *B. cylindrica* root; B. *B. gymnorrhiza* root; C. *C. decandra* root; D. *R. apiculata* root; E. *R. mucronata* root; F. *B. cylindrica* bark; G. *B. gymnorrhiza* bark; H. *C. decandra* bark; I. *R. apiculata* bark; J. *R. mucronata* bark; K. *B. cylindrica* leaf; L. *B. gymnorrhiza* leaf; M. *C. decandra* leaf; N. *R. apiculata* leaf; O. *R. mucronata* leaf; P. *B. cylindrica* hypocotyl; Q. *B. gymnorrhiza* hypocotyl; R. *C. decandra* fruit; S. *R. apiculata* fruit; T. *R. mucronata* fruit

The presence of consistent foam indicates that the sample contains saponins. Saponins were found in the leaf extracts of *B. cylindrica*, *B. gymnorrhiza*, *R. apiculata*, and hypocotyl extracts of *B. cylindrica* and *B. gymnorrhiza*. These natural glycosides have pharmacological properties, such as cytotoxic activity and antitumor (Juszczak et al. 2021). Phenolics and flavonoids were found in all parts of the plant samples, and these components are produced by plants to protect or promote growth under unfavorable conditions (Andreu et al. 2018). Moreover, phenolic compounds and flavonoids are generally known for their antioxidant properties (Andreu et al. 2018; de la Rosa et al. 2018; Juszczak et al. 2021). Different bioactive compounds, such as alkaloids, steroids, terpenoids, saponins, flavonoids, and phenolics, show that these plants may have the potential as medicinal plants.

Phytochemical analysis was also performed by thin-layer chromatography (TLC) analysis (Figure 4). The TLC plates sprayed with vanillin sulfate showed that each extract from E1 to E20 had a reddish-brown stain, indicating polyphenols' positive presence (Figure 4B), i.e., phenolic acids, tannins, and flavonoids (Cutrim and Cortez 2018). The presence of black spots or bands under 256 nm indicates polyphenols (Figure 4C) (La et al. 2020). After spraying with vanillin sulfate (at E11-E15), the yellow and orange color showed the presence of flavonoids (La et al. 2020). Furthermore, purplish-blue spots in each extract (E1-E20) (Figure 4B) indicate the presence of terpenoids, which is confirmed by blue fluorescence in UV 365 light (Figure 4C). The bright blue fluorescence color under 365 nm UV light indicates the presence of alkaloids in the extract. According to Hanani (2014), some alkaloids give blue or yellow fluorescence, for example, strychnine, purine, and brucine.

Total Phenolic Content (TPC)

The total phenolic content (TPC) was expressed as mg gallic acid equivalent per g of extract (mg EAG/g) (Figure 5.) The highest TPC compound was found in the stem bark of *R. mucronata* (484.39 mg EAG/g), which was significantly

higher than that of *R. apiculata* bark (456.96 mg EAG/g), and *C. decandra* bark (455.38 mg EAG/g). The results showed that the phenolic content was higher in the bark than in the roots, leaves, and fruit. A previous study of the three mangrove species in the Rhizophoraceae family, namely *B. gymnorrhiza, C. decandra,* and *R. mucronata*, shows have high phenolics in their bark than in roots and leaves (Banerjee et al. 2008). The TPC content of *C. decandra*, *R. mucronata, and B. gymnorrhiza* bark was 94.41±9.63, 40.47±3.18, and 35.86±2.04 mg GAE/g, respectively (Banerjee et al. 2008). These results were lower than that revealed in this study. A study by Haq et al. (2011) showed that the TPC of the bark and leaves of B. *gymnorrhiza* were 268.47±0.12 and 178.73±0.23 mg GAE/g, respectively that, were similar to the results of this study, which are 267.09 and 115.19 mg GAE/g (Haq et al. 2011). The bark accumulates phenolic compounds more than in the leaves. As in *Salix alba* (L.), there are 29 phenolic compounds in the leaves and 34 in the bark (Piątczak et al. 2020). The bark of mangrove plants is a rich source of tannins, used mainly for the traditional painting of nets and boats (Bandaranayake 2002). A previous study also reported that TPC was present in the methanol extract of *R. apiculata* twigs (220.50±3.33 mg GAE/g) (Sadeer et al. 2019). Meanwhile, in this research, *R. apiculata* bark has twice the TPC as the twig extract in the study of Sader et al. (2019). Differences between the TPC in this study and previous studies due to several factors, namely geographical origin, plant maturity, environmental factors (temperature, ultraviolet light, CO_2 levels in the atmosphere), and solvents used in the extraction process (Sukweenadhi et al. 2020). The phenolic content of plants is directly related to their antioxidant activity (Phuyal et al. 2020). Phenolic compounds can reduce free radicals by donating hydrogen (Chen et al. 2020). According to Zhang et al. (2022), antioxidant activity is related to high phenol content, and phenolic compounds contribute to most plants' antioxidant activity. Phenols have an aromatic ring containing one or more hydroxyl groups capable of scavenging free radicals, donating hydrogen atoms or electrons, or chelating metal cations (Costa et al. 2021).

Total Flavonoid Content (TFC)

The presence of flavonoid compounds was found in all extracts of Rhizophoraceae) (Figure 6). *R. apiculata* leaves had the highest flavonoid content (15.23 mg QE/g), which was significantly higher than *B. cylindrica* leaves (12.47 mg QE/g) and *R. mucronata* leaves (12.39 mg QE/g). The higher TFC was in the leaves compared to the roots, bark, and fruit of the five species of Rhizophoraceae mangroves. The results are similar to Agati et al. (2020) that flavonoids in most plants are produced in the leaf mesophyll cells in the chloroplast, which acts as an antioxidant against endogenous ROS and stabilizer of the chloroplast outer sheath membrane. Flavonoids in mangroves also significantly protect plants from exposure to intense UV radiation (Ferreyra and Casati 2021).

The ethanol extract of *R. mucronata* leaves in a study by Adhikari et al. (2017) had twice the content of flavonoid (24.42±0.32 mg QE/g) than that in this study. Another report by Krishnamoorthy et al. (2011) revealed that methanol bark extract of *B. cylindrica* and *C. decandra* contained TFC of 11.6 and 15 mg QE/g respectively, higher than those produced in this study. It might be caused by different extraction techniques (Sadeer et al. 2019). In this study, plant nutrient uptake may also be a significant factor. The results also showed that the extracts with a high TPC had a low TFC value, indicating no relationship between the amount of TPC and TFC because the phenolic compounds may not be from the flavonoid class (Yahya et al. 2021). In addition, it can also be associated with the different wavelength standards (gallic acid and quercetin) used. The total phenolic content was detected using the wavelength on the gallic acid standard (λ 765 nm), while the total flavonoids were detected using the quercetin standard (λ 440 nm).

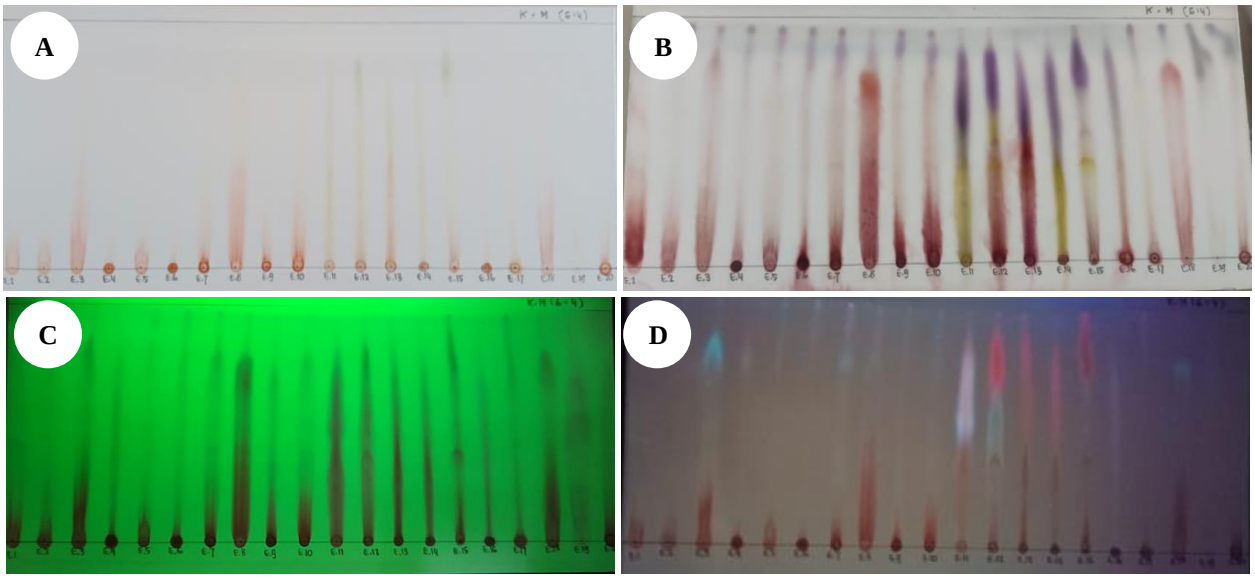

Figure 4. Chromatograms profile of Rhizophoraceae extract (E1-E20) developed using chloroform: methanol 6:4; A. Before spraying with vanillin sulfate; B. After spraying with vanillin sulfate; C. Observed under UV light 254 nm; D. Observed under UV light 365 nm

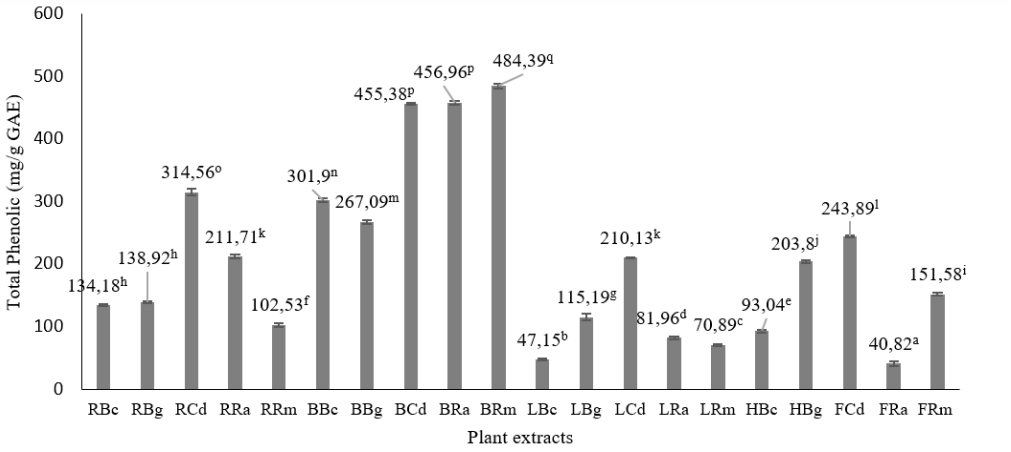

Figure 5. Total phenolic content of Rhizophoraceae extracts (the same notation shows no significant difference (P>0.05))

Antioxidant activities

DPPH is a stable free radical; if it receives hydrogen atoms, the color of the DPPH solution changes from purple to yellow due to an increase in free radical scavenging, thereby decreasing the absorbance in spectrophotometer measurements (Shamsuzzaman et al. 2021). The antioxidant activity of the methanol extract of Rhizophoraceae is shown in Table 2. The results showed that 95% of the 20 Rhizophoraceae extracts showed very high antioxidant activity, as indicated by their IC_{50} values ranging from 2.35±0.01 µg/mL to 29.84±0.19 µg/mL. According to Molyneux, antioxidant activity is classified as a very high, high, moderate, and weak antioxidant activity with the IC_{50} value of <50 µg/mL, 50-100 µg/mL, 101-150 µg/mL, and >150 µg/mL, respectively (Molyneux 2004). The stem bark of *C. decandra* was the most potent antioxidant with the IC_{50} value of 2.35±0.01 µg/mL), followed by its roots of *C. decandra* (3.23±0.01 µg/mL) and *R. apiculata* barks (3.30±0.01 µg/mL). A previous study by Krishnamoorthy et al. (2011) showed that methanol extract of *C. decandra* barks had the IC_{50} value of 2.1±0.28 µg/mL, and *B. cylindrica* barks of 5.5±0.58 µg/mL that were not significantly different from the results of this study.

A study by Banerjee et al. (2008) showed that the IC_{50} of *C. decandra* stem bark (65.5±1.35 µg/mL), *R. mucronata* stem bark (193.82±11.14 µg/mL), and stem bark of *B. gymnorrhiza* (254.69±21.26 µg/mL) that were lower than the result of this study. Meanwhile, a study by Hossain et al. (2011) showed that ethanol extract of *C. decandra* bark has lower antioxidant activity than this study, as indicated by IC_{50} of 12.90 ± 0.97 µg/mL (Hossain et al. 2011). The solvent used in extraction affects the antioxidant activity of the sample because it is related to the polarity and solubility of the active compounds, especially phenolic compounds which play a significant role in scavenging free radicals (Malik et al. 2017). The potent antioxidant activity of the mangrove halophytes is related to the environmental conditions of growth that are usually highly saline environments (de Silva and Amarasinghe 2021). ROS production increases under these conditions, necessitating the role of an efficient antioxidant system (Qasim et al. 2017). Consequently, tolerant plants synthesize bioactive compounds, including polyphenolic antioxidants, to protect metabolic functions from oxidative damage (Falleh et al. 2012; Santander et al. 2022).

Generally, it showed the potent antioxidant activity of five Rhizophoraceae mangrove species in the bark. It was consistent with the reports of Banerjee et al. (2008) that the bark of the three species of Rhizophoraceae mangroves (*B. gymnorrhiza*, *C. decandra*, and *R. mucronata*) had the most potent antioxidant activity. The bark comprises up to 20% of the dry weight of woody plants and contains polysaccharides, lignin, suberin, tannins, or phenolic acids (Zhang 2010). Furthermore, mangrove plant species are good sources of polyphenols such as tannins (Neimsuwan et al. 2017). Previous results showed that the tannin content of mangroves in the bark and stems was twice higher than the leaves, accounting for 66.6% and 33.4%, respectively (Hilmi et al. 2021). In this study, we suspect that tannins are the active compounds responsible for antioxidant activity. Structurally, they are polyphenols that contain more hydroxyl substituents that can donate hydrogen atoms to scavenge free radicals.

This study also showed that the IC_{50} value of the extract was in line with the TPC value. The previous studies showed that TPC has a high correlation in predicting the antioxidant activity of DPPH compared to TFC (Aryal et al. 2019; Mwamatope et al. 2020; Yahya et al. 2021). The results showed that *C. decandra* had the highest antioxidant activity compared to the other four species. So, *C. decandra* is the most active mangrove species in the family Rhizophoraceae, indicating its potential as a source of natural antioxidants for therapeutic ingredients. Therefore, further analysis of *C. decandra* bark and root extract was performed using GC-MS to determine their phytoconstituent.

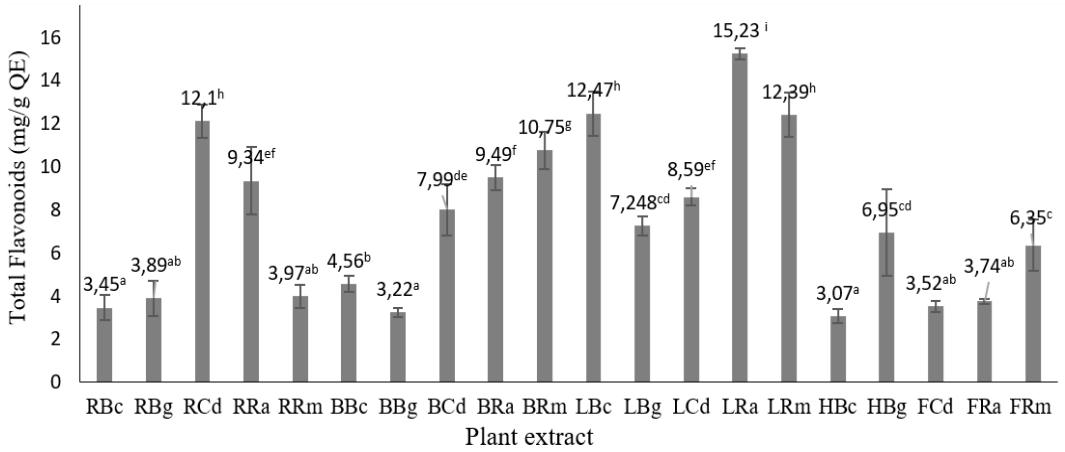

Figure 6. Total Flavonoid content of Rhizophoraceae extracts (the same notation shows no significant difference (P>0.05)

Table 2. Antioxidant activity of methanol extract of 20 extracts of 5 Rhizophoraceae species

Extracts	Absorbance			IC_{50} (µg/mL)
	1.56(µg/mL)	3.125(µg/mL)	6.25(µg/mL)	
RBc	6.62 ±0.14	11.59±0.07	19.90±0.18	16.94±0.15 ^{j}
RBg	11.67±0.14	18.64±0.07	38.93±0.07	8.20±0.01 ^{e}
RCd	30.32±0.20	47.83±0.12	86.88±0.18	3.23±0.01 ^{ab}
RRa	16.14±0.18	16.61±0.18	51.08±0.07	6.10±0.01 ^{d}
RRm	11.12±0.296	18.33±0.24	30.87±0.07	10.80±0.04 ^{g}
BBc	19.7±0.136	35.53±0.07	68.51±0.00	4.49±0.002 ^{c}
BBg	15.24±0.068	30.79±0.12	58.95±0.07	5.26±0.01 ^{cd}
BCd	36.82±0.068	64.94±0.14	93.38±0.07	2.35±0.01 ^{a}
BRa	27.97±0.235	48.41±0.12	86.60±0.12	3.30±0.01 ^{ab}
BRm	23.82±0.07	46.22±0.07	84.68±0.14	3.52±0.003 ^{b}
LBc	7.38±0.17	8.58±0.13	9.20±0.17	119.15±3.53 ^{n}
LBg	9.38±0.11	17.08±0.17	26.21±0.06	12.93±0.02 ^{h}
LCd	21.01±0.13	31.81±0.06	50.75±0.13	6.10±0.02 ^{d}
LRa	9.20±0.17	12.00±0.11	18.21±0.19	22.73±0.24 ^{l}
LRm	9.92±0.22	13.41±0.22	23.56±0.11	15.28±0.04 ^{i}
HBc	6.72±0.17	11.16±0.17	18.79±0.17	18.44±0.33 ^{k}
HBg	16.94±0.23	32.53±0.06	35.33±0.22	9.87±0.06 ^{f}
FCd	21.48±0.11	35.70±0.17	65.25±0.06	4.63±0.01 ^{c}
FRa	7.38±0.13	10.14±0.22	14.50±0.11	29.84±0.19 ^{m}
FRm	16.61±0.17	28.97±0.17	51.54±0.063	6.02±0.007 ^{d}
AA	8.98±0.2	21.30±0.1	86.26±0.1	5.1±0.02 ^{cd}

Note: AA: Ascorbic acid, the same letter notation indicates no significant difference in treatment (P>0.05)

Phytoconstituents of the extracts

Two potential extracts with the highest antioxidant activity were analyzed further using GC-MS to determine their bioactive compounds. The identified major compounds are presented in Tables 3 and 4. Table 3 showed that the methanol extract of *C. decandra* bark contained 12 identified compounds, with pyrocatechol (15.85%) being the highest. Another compound with 96% similarity was Antiarol (1.44%), hexadecanoic acid methyl ester (1.69%) with 91%, 2-butyne-1,4-dione, 1-(2,3-dihydro-3,3-dimethyl-1H-inden-5-YL)-4phenyl- (2.44 %) with 90%, 8-oxo-beta-erythroidine (1.58%) with 90%, and vitamin E (1.09%) with 90% similarity.

A previous study shows that pyrocatechol compounds have antioxidant activity that can reduce free radicals (Kosobutskii 2014). Antiarol isolated from the stem of *Eucalyptus globulus* Labill. that are included in aromatic phenols had moderate DPPH free radical activity, antibacterial and antifungal (Celeiro et al. 2019). Hexadecanoic acid is a fatty acid found in plants with several biological activities, including antioxidant and hypocholesterolemic properties (Siswadi and Saragih 2021) and antitumor (Yang et al. 2018). 8-oxo-beta-erythroidine belonged to the erythroidine alkaloids and was also isolated from the *Erythrina poeppigiana* (Walp.) O.F.Cook bark methanol extract with great potential as a phytoestrogen. It reduces the risk of breast cancer MCF-7 (Djiogue et al. 2014; Ambardhani et al. 2019). The extract also contained α-tocopherol as vitamin E, a fat-soluble antioxidant that preserves vital fatty acids from oxidation and reduces inflammatory response (Elgendy et al. 2022).

Table 4 contained several of the same compounds between the methanol extract of *C. decandra* bark and roots, i.e., pyrocatechols (3.47%) and antiarol (1.27%). Both compounds have antioxidant properties.

Table 3. Identified phytochemical compounds of the *Ceriops decandra* bark methanol extract by GC-MS

Name of compound	Molecular formula	Retention time	Relative area (%)	Molecular weight (g/mol)	SI
Pyrocatechol	$C_6H_6O_2$	14.24	8.92	110	96
Pyrocatechol	$C_6H_6O_2$	14.58	6.93	110	96
Antiarol	$C_9H_{12}O_4$	27.28	1.44	184	96
1,2-benzenediol	$C_6H_6O_2$	28.91	2.87	110	25
Allomycin	$C_{29}H_{42}N_6O_9$	29.78	1.85	618	30
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.01	1.69	270	91
5-oxo-7,7-dimethyl-5,6,7,8-tetrahydro coumarin	$C_{11}H_{12}O_3$	30.19	2.47	192	43
2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	30.48	6.27	192	55
2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	31.14	18.76	192	55
2-methyl-1-thia-cyclopentane	$C_6H_8O_2$	31.74	34.31	112	52
1H-indene, 1-ethylideneoctahydro-7A-methyl-,cis	$C_{12}H_20$	32.22	2.05	164	64
2-butyne-1,4-dione, 1-(2,3-dihydro-3,3-dimethyl-1H-inden-5-YL)-4phenyl-	$C_{16}H_{10}O_2$	32.53	2.44	234	90
8-oxo-beta-erythroidine	$C_{16}H_{17}NO_4$	32.81	1.58	273	90
vitamin E	$C_{29}H_{50}O_2$	41.54	1.09	430	90

Table 4. Identified phytochemical compounds of *Ceriops decandra* root methanol extract by GC-MS

Name of compound	Molecular formula	Retention time	Relative area (%)	Molecular weight (g/mol)	SI
2,4-hexadiene, 3-fluoro-2,5-dimethyl-	$C_8H_{13}F$	7.58	6.77	128	90
Imidazolidinetrione, methyl-	$C_4H_8N_2S$	8.67	3.51	116	64
Pyrocatechol	$C_6H_6O_2$	14.43	2.44	110	96
Pyrocatechol	$C_6H_6O_2$	20.09	1.03	110	83
2-ethoxyphenol	$C_8H_{10}O_2$	25.50	3.15	138	35
Antiarol	$C_9H_{12}O$	27.26	1.27	184	95
4-(1-acetyl-2,2-dimethylcyclopentyl)-3-buten-2-one	$C_{12}H_{16}O_2$	29.03	1.95	192	52
2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	30.19	15.59	192	45
2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	30.47	17.01	192	50
2-trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	30.76	28.56	192	55
6-Octadecenoic acid	$C_{18}H_{34}O_2$	31.50	4.68	283	46
2-methyl-2,3-divinyl oxirane	$C_7H_{10}O$	32.21	1.99	110	50
Patchoulene	$C_{15}H_{24}$	32.52	2.27	204	60
Norolean-12-ene	$C_{29}H_{48}$	49.36	1.02	397	62

In conclusion, this study shows that Rhizophoraceae extracts have a high potential as antioxidant sources that benefit human health by resisting free radicals. It contains phytochemical compounds such as alkaloids, phenolics, tannins, terpenoids, steroids, and saponins. Rhizophoraceae had a high content of phenolics, flavonoids, and antioxidant activity, particularly in the stem bark. Phytoconstituents in the Rhizophoraceae that act as antioxidants include Pyrocatechols, Antiarols, and Hexadecanoic acid methyl esters. Further research is needed on using ethanol and water solvents and partitioning using semi-polar and non-polar solvents, such as ethyl acetate and n-hexane in extracts, that show high potential for antioxidant bioactivity and purification of Rhizophoraceae active compounds.

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