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Article Title

Phytochemical screening, phenolic and flavonoid content, and antioxidant methanol extracts of Rhizophoraceae grown in Langsa, Aceh, Indonesia

Abstract

Bruguiera cylindrica, Bruguiera gymnorrhiza, *Ceriops decandra*, *Rhizophora apiculata*, and *Rhizophora mucronata* are mangrove species belonging to the Rhizophoraceae family which has been used as medicinal plant in various parts of the world. In this study, 20 plant parts, including roots, bark, leaves, hypocotyl/fruit, are extracted from five species of Rhizophoraceae mangroves using the maceration method with 80% methanol solvent. The total phenolic content (TPC) was calculated as gallic acid equivalent (GAE) as well as the total flavonoid content (TFC) and quercetin equivalent (QE). Free radical scavenging activity was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH), and GCMS confirmed the phytoconstituents. The phytochemical screening results showed the presence of alkaloids, flavonoids, phenolics (tannins), terpenoids, steroids, and saponins. The highest TPC content was found in the bark, namely *R. mucronata, R. apiculata,* and *C. decandra,* with a composition of 484.39 mg EAG/g, 456.96 mg EAG/g, and 455.38 mg EAG/g, respectively. Furthermore, the highest TFC content was found in the leaves

Antioxidant activity was extremely high in 95% of the studied extracts, with the greatest levels seen in C. decandra species, especially in its bark and roots, with IC_{50} of 2.35 ± 0.01 µg/mL, and 3.23 ± 0 .01 µg/mL. GCMS results detected potential compounds such as pyrocatechol antioxidants, antiarols, and hexadecanoic acid methyl esters.

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Phytochemical screening, phenolic and flavonoid content, and antioxidant methanol extracts of Rhizophoraceae grown in Langsa, Aceh, Indonesia

Abstract. *Bruguiera cylindrica*, *Bruguiera gymnorrhiza*, *Ceriops decandra*, *Rhizophora apiculata*, and *Rhizophora mucronata* are mangrove species belonging to the Rhizophoraceae family which has been used as medicinal plant in various parts of the world. In this study, 20 plant parts, including roots, bark, leaves, hypocotyl/fruit, are extracted from five species of Rhizophoraceae mangroves using the maceration method with 80% methanol solvent. The total phenolic content (TPC) was calculated as gallic acid equivalent (GAE) as well as the total flavonoid content (TFC) and quercetin equivalent (QE). Free radical scavenging activity was tested against 2,2-diphenyl-1-picrylhydrazyl (DPPH), and GCMS confirmed the phytoconstituents. The phytochemical screening results showed the presence of alkaloids, flavonoids, phenolics (tannins), terpenoids, steroids, and saponins. The highest TPC content was found in the bark, namely *R. mucronata*, *R. apiculata*, and *C. decandra*, with a composition of 484.39 mg EAG/g, 456.96 mg EAG/g, and 455.38 mg EAG/g, respectively. Furthermore, the highest TFC content was found in the leaves of *R. apiculata*, *B. cylindrica*, and *R. mucronata* leaves at 15.23 mg QE/g, 12.47 mg QE/g, 12.39 mg QE/g, respectively. Antioxidant activity was extremely high in 95% of the studied extracts, with the greatest levels seen in C. decandra species, especially in its bark and roots, with IC_{50} of 2.35 ± 0.01 µg/mL, and 3.23 ± 0 .01 µg/mL. GCMS results detected potential compounds such as pyrocatechol antioxidants, antiarols, and hexadecanoic acid methyl esters.

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

INTRODUCTION

Indonesia is an archipelagic country where the coastline is 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 a forest area and a diversity of true mangrove species of approximately 8000 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 difficult environmental conditions, including at high salinity (Lopes et al., 2021). They also thrive under natural conditions with high and low temperatures, drought, high luminosity, tides, and waves, where other conventional plants cannot grow (Mishra and Tanna, 2017). These plants develop several 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 (Meot-duros & Magné, 2008).

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). They are produced as by-products of the adenosine triphosphate (ATP) generation in mitochondria when the body's cells use oxygen to make energy. ROS and RNS exert beneficial effects on cellular response and immune function at low or moderate concentrations. 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 (Flieger et al., 2021; Gašparović, 2020). Oxidative stress plays a major role in the development of chronic and degenerative diseases, such as cancer (Gašparović, 2020), cardiovascular disease (Panth et al., 2016), nervous breakdown (Fang et al., 2017), Alzheimer (Bhatt et al., 2020), Parkinson (Dias et al., 2014), aging (Russo et al., 2018), atherosclerosis (Nowak et al., 2017) autoimmune disorders, and arthritis (Flieger et al., 2021). The protection against free radicals can be enhanced by the activity of antioxidants (Lobo et al., 2010). Endogenous antioxidants are metabolites produced by the body's metabolism, while exogenously are produced outside the body. These antioxidants can act as "free radical scavengers" by preventing and repairing damage caused by ROS and RNS (Pham-Huy et al., 2008; Valko et al., 2006). In light of the vast potential of secondary metabolites from halophyte plants, it is important to conduct a study to determine the natural exogenous antioxidant activity derived from mangrove halophyte plants.

This study is interested in the mangrove halophyte plant Rhizophoraceae as a potential source of natural antioxidants and bioactive chemicals that are safe, sustainable, and environmentally friendly. Rhizophraceae, as one of the local natural

resources of Langsa City Mangrove Forest, has been used traditionally to treat various diseases in the world, such as diarrhea, hepatitis, hypertension, diabetes, and childbirth (Bibi et al., 2019; Loo et al., 2008). The study aims to screen and reveal the bioactivity of four plant parts from five selected species, namely *Bruguiera cylindrica, Bruguiera gymnorrhiza, Ceriops decandra, Rhizophora apiculata,* and *Rhizophora mucronata* belonging to the Rhizophoraceae family from Langsa city. Previous studies on cytotoxic bioactivity using the Brine Shrimp Lethality Test (BSLT) method and *Artemia salina L* as animal models have shown that Rhizophoraceae has great potential as a cancer-fighting agent (Indriaty et al., 2022). However, there are no reports of the phytochemical characteristics, phenolic and flavonoid content, antioxidant bioactivity, and phytoconstituents of Rhizophoraceae from Langsa Aceh City. This study is anticipated to contribute to the body of knowledge regarding the bioactivity of medicinal plants from the coast of Langsa City. Thus, their application can be further developed in the drug and pharmaceutical industry.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used include 80% methanol, Mg powder (E. merck), concentrated HCl (E. 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 ($C_7H_6O_5$) (E. merck), Na_2CO_3 (E. merck), $AlCl_3$ (E. merck), CH_3CO_2K (E. merck), and quercetin ($C_{15}H_{10}O_7$) (E.merck).

Plant collection and sample preparation

Plant samples consisting of roots, bark, leaves, and fruit of five Rhizophoraceae species were collected from the Langsa Mangrove Forest Area, Aceh, Indonesia, in January 2021, between 08.00 a.m. and 12.00 p.m., as shown in Figure 1. The plant species include *B. cylindrica, B. gymnorrhiza, C. decandra, R. apiculata,* and *R. Mucronata,* as shown in Figure 2. The diameter of the sampled trees ranged from 10 to 30 cm, and they were washed under running water and cut into small pieces. Samples were dried under shade for 20 days until there was no change in plant weight, then stored in plastic and labeled for further treatment. The sample 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

A total of 20 dry samples were weighed up to 100 g, and were immersed in 1000 ml of methanol for 24 hours at room temperature. The extract was filtered using a glass funnel and Whatman filter paper number 1. Each filtrate was concentrated until thick using a rotating rotary evaporator, namely 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 extracted sample is given the following codes: RBc (*root Bruguiera cylindrica*), RBg (*root Bruguiera gymnorrhiza*), RCd (*root Ceriops decandra*), RRa (*root Rhizophora apiculata*), RRm (r*oot Rhizophora mucronata*), BBc (*bark Bruguiera cylindrica*), BBg (*bark Bruguiera gymnorrhiza*), BCd (*bark Ceriops decandra*), BRa (*bark Rhizophora apiculata*), BRm (*bark Rhizophora mucronata*), LBc (l*eave Bruguiera cylindrica*), LBg (*leave Bruguiera gymnorrhiza*), LCd (*leave Ceriops decandra*), LRa (l*eave Rhizophora apiculata*), LRm (*leave Rhizophora mucronata*); HBc (*hypocotyl Bruguiera cylindrica*), HBg (*hypocotyl Bruguiera gymnorrhiza*), FCt (*fruit Ceriops decandra*), FRa (*fruit Rhizophora apiculata*), and FRm (*fruit Rhizophora mucronata*).

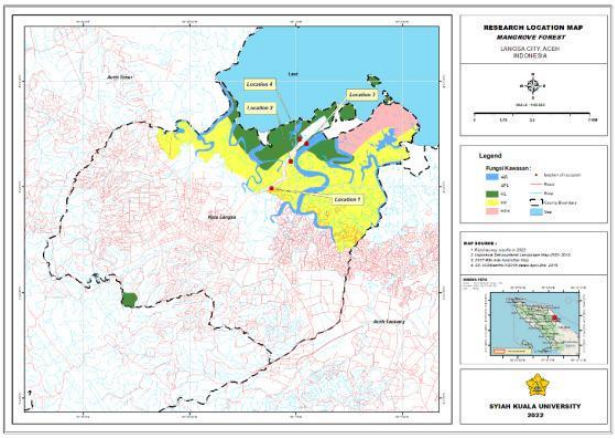

Figure 1. Sampling location in Kuala Langsa Mangrove Forest Area, Aceh Indonesia

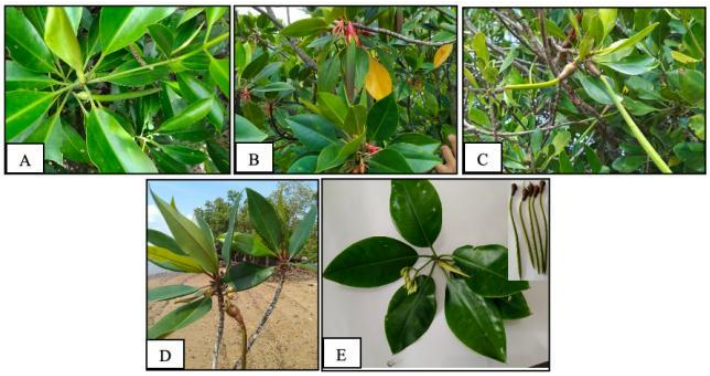

Figure 2. Rhizophoraceae plants. (A) *B. cylindrica*, (B) *B. gymnorrhiza*, (C) *C. decandra*, (D) *R. apiculata*, and (E) *R. mucronata*

Screening qualitative phytochemical compound

The methanol extract was examined qualitatively to detect the presence of phytochemical compounds, including alkaloids, terpenoids, steroids, saponins, flavonoids, and phenolics (Nuraskin et al., 2020). Alkaloids were detected by testing 100 mg of extract dripped with NH_3 (3 ml) and left for two hours until two layers were formed, then 5ml 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. A positive result for alkaloids shows white, yellow, and reddish-brown precipitates. In the terpenoid and steroid tests, 100 mg of extract was taken and dissolved in methanol. The Liebermann-Burchard reagent was added, and the reaction changed to purple or red as an indication of terpenoids. The presence of steroids is indicated by green or blue color. An indication of the saponins' presence was carried out by dissolving 100 mg of methanol extract, then heating and shaking vigorously to show foam which lasted 30 minutes. Afterward, flavonoids were detected by dissolving 100 mg of extract in methanol, as well as adding 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 extract to 5% $FeCl_3$ (5 drops). A resulting dark blue or black sample color indicates the presence of tannins.

Determination of Total Phenolic Contents (TPC)

The total phenolic content of the extract was determined according to the Folin-Ciocalteu method, which is based on Mwamatope's suggestion with slight modifications (Mwamatope et al., 2020). A total of 5 mg extract was dissolved in 0.5 mL methanol pa and added to deionized water until exactly 5 mL. Then, 0.2 mL was taken from the solution and added to

15.8 mL of deionized water and 1 mL of Folin-Ciocalteu reagent. After 5 minutes of incubation, the solution was mixed with 3 mL of 10% (w/v) Na_2CO_3, followed by another incubation for 120 minutes at room temperature. As a standard curve, 5 mg of gallic acid was dissolved in 1 mL of methanol pa and added with deionized water to 10 mL. Furthermore, concentrations of 100 µg/mL, 125 µg/mL, 150 µg/mL, 175 µg/mL, and 200 µg/mL were made from the mother liquor. Each concentration of the dilution results was taken at 0.2 mL, then 15.8 mL of deionized water and 1 mL of Folin Ciocalteu were added. The solution was then mixed with 3 mL of 10% (w/v) Na_2CO_3 after 5 minutes and incubated for 120 minutes at room temperature. The solution absorbance 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 of extract (mg EAG/g). Moreover, all samples were tested in three replicates.

Determination of Total Flavonoid Contents (TFC)

The total flavonoid content (TFC) was determined using the appropriate aluminum chloride colorimetric method (Phuyal et al., 2020), and quercetin were used as the standard. A total of 5 mg extract was dissolved with methanol pa to obtain exactly 5 ml. From this solution, 1 ml was taken, then 3 mL methanol, 0.2 mL $AlCl_3$, 0.2 mL potassium acetate, and 5.6 mL deionized water were added. A standard solution was prepared, and 5 mg of quercetin was dissolved in methanol pa to obtain exactly 10 ml. A dilution of 20, 40, 60, 80, and 100 µg/mL was carried out. Afterward, 1 ml of each concentration was added to methanol pa (3 mL), 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 ^oC), and the absorbance of the solution was measured with a UV-Vis spectrophotometer (λ 440 nm). The total flavonoid content was expressed as mg quercetin equivalent per g of extract (mg QE/g). 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. (Yahya et al., 2021). A total of 2.5 mg of the extract was added with two drops of 2% dimethyl sulfoxide (DMSO, Merck-Germany) and left for 24 hours. Extracts were prepared at various concentrations in 1.56 µg/mL, 3.125 µg/mL, and 6.25 µg/mL in methanol pa and sonicated using a sonicator. Then, 4 ml of this solution was taken and added to 1 ml of DPPH solution (which comprises 7.9 mg of DPPH powder with a molecular weight of 394.32 g/mol in methanol pa up to 50 mL). The solution was homogenized and incubated in the dark incubator for 30 minutes at 37^{o}C. The absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer, and methanol pa was used as a blank. A similar procedure was carried out at concentrations of 1, 3, 6, 9, 12, and 15 µg/mL, using ascorbic acid as a positive control. Furthermore, the percentage of DPPH radical inhibition activity was calculated to obtain the IC_{50} value, which is the concentration of the extract 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

GCMS analysis was performed on the methanol extract of *C. decandra's* bark and root, which are the most potent. GC-MS analysis used the GC-MS Gas Chromatograph with Auto Sampler 5975A (Agilent Technologies 7890A), Mass Selective Detector, and data system in Chemstation. The sample was prepared first by dissolving with methanol pa, then 5 µL was injected into the GC-MS using helium (He) gas through a capillary column with a total rate of 1.2 mL/minute and a split ratio of 8:1 psi. The injector and detector were at 250ºC and 230ºC, with operating temperatures of 280ºC and 140ºC, respectively. Furthermore, the components are to be eluted and detected using a mass detector. The mass spectrum fragmentation pattern formed is adjusted to the spectrometer database from the National Institute of Standards and Mass Spectral Technology (NIST-MS).

Data analysis

The test data for each sample was carried out in triplicate, and the values are expressed as the average (± standard error). 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

One of the important steps to obtain plants' bioactive compounds is extraction. Its efficiency refers to the yield produced and obtaining the widest possible range of phytochemical compounds (Gupta, 2012). The results of percent yield and phytochemical tests on the roots, bark, leaves, and hypocotyl/fruit of species of Rhizophoraceae plants are presented in Table 1. Meanwhile, the appearance of plant extracts can be seen in Figure 3. The highest extract yields reached 25.462% in *C decandra* leaves, and 22.857% was found in *B. gymnorrhiza* leaves. In a previous study by Malik et al. (2017) on the

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methanol extract of Rhizophoraceae, the result showed that the yield of *B. cylindrica* and *R. apiculata* leaves extracted without grinding are 7% and 3.5%, which are lower than those produced in this study, namely by 21.96% and 15.27%. Sample preparation of this study was carried out by chopping the sample finely, which gave a wider sample surface to interact with the solvent, this helps the compound diffuse out of the cell until it was saturated. the use of methanol as a solvent in the extraction process provides a higher solubility of compounds because it helps the compound diffuse out of the cell until it is broadly saturated, polar, and non-polar compounds (Tiwari et al., 2011).The 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 and easily evaporate (Bonventre, 2014). The solvent's ability 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).

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Table 1. Results of Phytochemical Tests and 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	+	-	+	-	+	+

Description: extract sample of methanol root *B. cylindrica* (RBc), root *B. gymnorrhiza* (RBg), root *C. decandra* (RCd), root *R. apiculata* (RRa), root *R. mucronata* (RRm), bark *B. cylindrica* (BBc), bark *B. gymnorrhiza* (BBg), bark *C. decandra* (BCd), *bark R. apiculata* (BRa), bark *R. mucronata* (BRm), leave *B. cylindrica* (LBc), leave *B. gymnorrhiza* (LBg), leave *C. decandra* (LCd), leave *R. apiculata* (LRa), leave *R. mucronata* (LRm), hypocotyl *B. cylindrica* (HBc), hypocotyl *B. gymnorrhiza* (HBg), fruit *C. decandra* (FCd), fruit *R. apiculata* (FRa), and fruit *R. mucronata* (FRm).

Based on Figure 3, it can be explained that the mangrove plant extracts of Rhizophoraceae generally have a solid form and are reddish brown. The liquid extracts have a sticky and oily texture found in leaf and fruit extracts. Some previous studies on the bark extracts of *R. mucronata* and *C. decandra* have shown that it produces a reddish-brown color (Hendrawan, 2021; Rumengan et al., 2021). The reddish-brown color in the extract is caused by the presence of tannin compounds. Tannins have chromophore groups in the form of conjugated C=C and C=O bonds, which absorb and impart color to a compound (Rumengan et al., 2021). In addition, there also plant color 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 test showed the presence of tannins in all extracts. Tannins are classified as natural

polyphenolic compounds, the condensed tannins are composed of flavonoids. Furthermore, flavonoid compounds were detected in all Rhizophoraceae extracts. Other phytochemical elements detected were alkaloid compounds, except for the root extract of *R. apiculata*. Alkaloids play a significant role in plants by protecting them from predators and regulating their growth (Chik et al., 2013). The bioactive properties of alkaloids are known as an anesthetic, cardioprotective and anti-inflammatory agents (Heinrich et al., 2021). Steroid phytochemical results were only detected in *B. cylindrica*, *R. apiculata*, and *R. mucronata* leaves, while terpenoids were detected in almost all extracts of. According to Andreu et al, steroids in plant are useful as growth hormones, while terpenoids protect plants from abiotic and biotic pressures as growth hormones, anti-inflammatory, antioxidants, anticancer, antiseptic, antiplasmodial, astringent, digestive, and diuretic in therapeutic elements (Andreu et al., 2018). The presence of consistent foam indicates that the sample contains saponins. Furthermore, saponins were found in the extracts of *B. cylindrica*, *B. gymnorrhiza*, and *R. apiculata* leaves, as well as *B. cylindrica* and *B. gymnorrhiza* hypocotyl. These natural glycosides have several pharmacological properties, such as cytotoxic activity, and act as anti-tumor (Podolak et al., 2010). Phenolic compounds and flavonoids were found in all parts of the plant samples. These components are produced by plants to defend themselves or to promote growth under unfavorable conditions (Andreu et al., 2018). Moreover, phenolic compounds and flavonoids are generally known as the largest phytochemical molecules providing antioxidant properties that are produced by plants (Andreu et al., 2018; de la Rosa et al., 2018; Ota et al., 2017; Panche et al., 2016). The presence of different bioactive compounds in each plant, such as alkaloids, steroids, terpenoids, saponins, flavonoids, and phenolics, shows that these plants have the potential as medicinal plants.

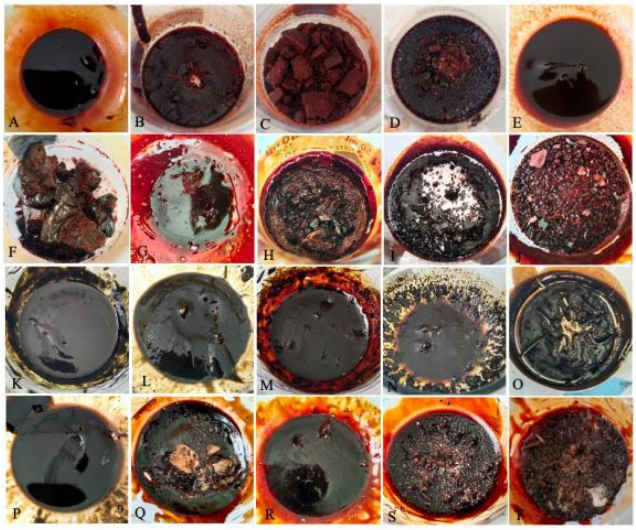

Figure 3. Extract samples of methanol (A) *B. cylindrica root*, (B) *B. gymnorrhiza root*, (C) *C. decandra root*, (D) *R. apiculataroot root*, (E) *R. mucronata root*, (F) *B. cylindrica bark*, (G) *B. gymnorrhiza bark*, (H) *C. decandra bark*, (I) *R. apiculata bark*, (J) *R. mucronata bark*, (K) *B. cylindrica leave*, (L) *B. gymnorrhiza leave*, (M) *C. decandra leave*, (N) *R. apiculata leave*, (O) *R. mucronata leave*, (P) *B. cylindrica hypocotyl*, (Q) *B. gymnorrhiza hypocotyl*, (R) *C. decandra fruit*, (S) *R. apiculata fruit*, and (T) *R. mucronata fruit.*

The results of the phytochemical test screening were strengthened by thin-layer chromatography (TLC) analysis, which is presented in Figure 4. TLC analysis was performed with a 6:4 ratio of chloroform to methanol as the mobile phase and silica gel 60 F_{254} as the stationary phase. After being sprayed with the vanillin sulfate stain remover, in visible light as in Figure 4-B, showed that each extract from E1 to E20 had a reddish-brown stain, indicating the positive presence of polyphenols. Polyphenols include phenolic acids, tannins, and flavonoids (Cutrim & Cortez, 2018). The presence of black color under UV 256 illumination on the TLC plate is indicative of the presence of polyphenols (Figure 4-C). The positive reaction that forms polyphenols or tannins is the presence of black stains on the TLC plate (Jawala et al., 2020). Other colors seen on the TLC plate under visible light (Figure 4-B) are yellow and orange (at E11-E15), which represent leaf extracts from five Rhizophoraceae species with a high flavonoid concentration. This is in accordance with Jawala's report when he identified the presence of flavonoid compounds (Jawala et al., 2020). Furthermore, purplish-blue spots in each extract (E1-E20) under visible light (Figure 4-B) indicate positive terpenoids, which is confirmed by blue fluorescence in

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UV 365 light (Fig. 4-C). Furthermore, the bright blue fluorescence color at 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 alkaloids (Hanani, 2014)(Hanani, 2014).

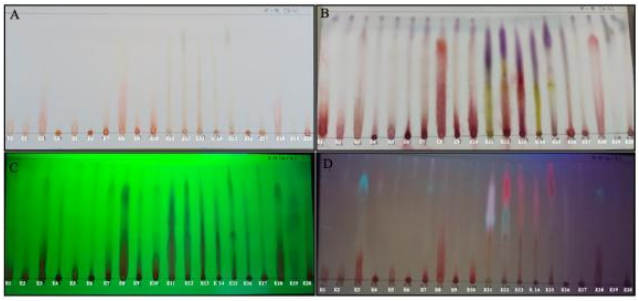

Figure 4. Thin layer chromatography (TLC) of 20 groups of chemical compounds Rhizophoraceae extract E1-E20 (from RBc-FRm extract) using chloroform: methanol 6:4 (A) before spraying with vanillin sulfate, (B) in visible light after spraying vanillin sulfate, (C) exposure to UV light 254 nm and (D) exposure to UV light 365nm

Total Phenolic Content (TPC)

The total phenolic content (TPC) in this study was expressed as mg gallic acid equivalent per g of extract (mg EAG/g), as shown in Figure 5. The highest TPC component of the extract was sequentially found in the stem bark of *R. mucronata* (484.39 mg EAG/g), which differed significantly from the bark of *R. apiculata* (456.96 mg EAG/g), and the bark of *C. decandra* (455.38 mg EAG/g). It is interesting that we found that in the five Rhizophoraceae plants species, the phenolic content was very highly concentrated in the bark compared to the roots, leaves, and fruit. This is consistent with a previous report that three mangrove species analyzed in the Rhizophoraceae family, namely *B. gymnorhizza*, *C. decandra*, and *R. mucronata* have high phenolics in their bark compared to roots and leaves (Banerjee et al., 2008). The TPC content of *C. decandra*, *R. mucronata, and B. gymnorrhiza* bark was 94.41 ± 9.63 mg GAE/g, 40.47± 3.18, and 35.86 ±2.04, respectively (Banerjee et al., 2008). even though it is lower than the results of our study, this is because the extract used is a liquid extract, not a dry extract as used in this study. In addition, the TPC values of the bark and leaves of B. *gymnorrhiza*, which are 268.47 ± 0.12 mg GAE/g and 178.73 ± 0.23 mg GAE/g as reported by Haq et al, were not different from the results of this study, which are 267.09 mg GAE/g and 115.19 mg GAE/g (Haq et al., 2011). Bark as a place for the accumulation of 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). According to Bandaranayake, mangrove bark is a rich source of tannins, used mainly for the traditional painting of nets and boats (Bandaranayake, 2002). Previous studies also reported that the highest methanol extract in *R. apiculata* twigs was 220.50 ± 3.33 mg GAE/g (Sadeer et al., 2019). In the nearest organ, *R. apiculata* bark has twice the TPC compared to twig extract in the study of Sader et al. There are differences, between the TPC in this study and previous studies due to several factors, namely geographical origin, plant maturity, environmental factors (temperature, ultraviolet light, CO2 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 agents, and hydrogen donors can scavenge free radicals (Wojdylo et al., 2007). According to Mansouri et al., antioxidant activity is related to high phenol content, and the majority of plants come from phenolic compounds (Mansouri et al., 2005). Phenols have an aromatic ring containing one or more hydroxyl groups which are 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 during the initial qualitative phytochemical screening of Rhizophoraceae. Furthermore, as shown in Figure 6, the TFC test was performed quantitatively using quercetin as the standard solution and expressed as mg Quercetin Equivalent (QE) per gram dry weight of the extract (mg QE/g). *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). There was an interesting pattern in the TFC test, namely the highest TFC was found in the leaves compared to the roots, bark, and fruit of the five species of Rhizophoraceae mangroves. This is consistent with the report of Agati et al. 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 (Agati et al., 2012). Flavonoids in mangroves also play a significant role in protecting plants from exposure to strong UV radiation (Agati et al., 2007).The ethanol extract of *R. mucronata* leaves in a related

study showed a flavonoid content of 24.42 ± 0.32 mg QE/g, which is twice higher as the result of this study (Adhikari et al., 2017). Other reports also revealed that *B. cylindrica* and *C. decandra*'s bark was macerated with methanol for 24 hours using a soxhlet containing TFC of 11.6 mg QE/g and 15 mg QE/g, which were higher than those produced in this study (Krishnamoorthy et al., 2011). Besides the extraction technique, TFC can be identified differently in samples depending on environmental conditions and plant nutrient uptake (Sadeer, 2019). In this study, plant nutrient uptake may also be a significant factor. When viewed further, the number of TFC shows a different pattern from TPC. Extracts with a high TPC show a low TFC value, indicating no relationship between the amount of TPC and TFC because the phenolic compounds detected may not be from the flavonoid class (Yahya et al., 2021).It can also be associated with the standards (gallic acid and quercetin) that have been 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).

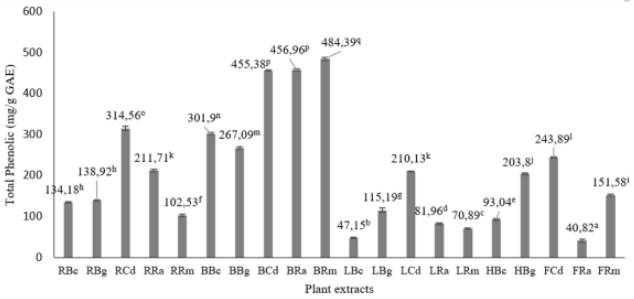

Figure 5. Total phenolic content of Rhizophoraceae extracts (the same notation shows no significant different treatment (P>0.05))

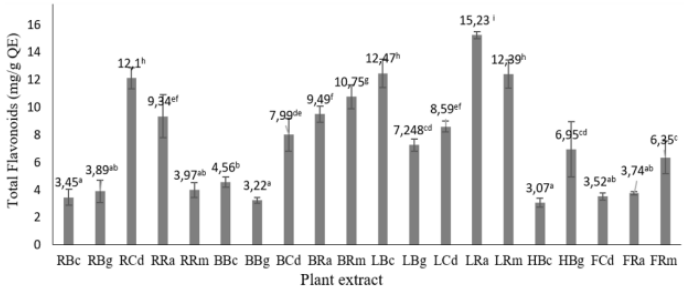

Figure 6. Total Flavonoid content of Rhizophoraceae extracts (the same notation shows no significant different treatment (P>0.05)

Antioxidant activities

Antioxidant testing is a way to identify the bioactivity of secondary metabolites in plants. The DPPH free radical used is a stable compound. When the DPPH accepts hydrogen atoms from plant extracts, the color of the test 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 methanol extract from each part of the mangrove Rhizophoraceae plant is shown in Table 2. Furthermore, several interesting facts can be analyzed from this plant. First, it can be explained that 95% of samples of the 20 Rhizophoraceae mangrove plant extracts showed very high antioxidant activity, as indicated by the range IC_{50} values from 2.35 ± 0.01 µg/mL to 29.84 ± 0.19 µg/mL. According to Molyneux, a compound in a material is classified as having very high, high, moderate, and weak antioxidant activity when the IC_{50} value is <50 µg/mL, 50-100 µg/mL, 101-150 µg/mL, and > 150 µg/mL, respectively (Molyneux, 2004). The strongest antioxidant activity in this study was shown in the stem bark of *C. decandra* (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). Based on data from previous studies using the same solvent, the IC_{50} of *C. decandra* barks (2.1 ± 0.28 µg/mL) and *B. cylindrica* barks (5.5 ± 0.58 µg/mL) were not significantly different from those generated in this study (Krishnamoorthy et al., 2011). However, in another study using liquid methanol extract, the antioxidant activity was lower than the results of our study, with 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) (Banerjee et al. 2008). Meanwhile, Hossain et al. (2011) used different solvents and found that the 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 type of solvent also affects the antioxidant activity of the sample because it is related to the polarity and solubility of the active compounds, especially phenol phenolic compounds which play a significant role in the scavenging of free radicals (Malik et al., 2017). The very high antioxidant activity of the mangrove halophytes is related to the environmental conditions they grow. Naturally, mangrove halophytes are designed to grow and survive in a harsh saline environment (Alhdad et al., 2013). 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 key metabolic functions from oxidative damage (Falleh et al., 2012; Santander et al., 2022).

It is particularly intriguing that the very strong antioxidant activity of five mangrove Rhizophoraceae species has the same pattern, concentrated in the bark. This was consistent with the reports of Banerjee et al, that of the three species of Rhizophoraceae mangroves (*B. gymnorrhiza, C. decandra*, and *R. mucronata*) the strongest antioxidant activity was found in the bark (Banerjee et al. 2008). 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, mangroves are a good source of polyphenols such as tannins (Neimsuwan et al., 2017). Previous results have shown that the tannin content of mangroves in the bark and stems was two times higher than the leaves, accounting for 66.6% and 33.4%, respectively (Hilmi et al., 2021). In this study, we suspect that the active compounds responsible for the antioxidant activity are tannins. Structurally, they are polyphenols that contain more hydroxyl substituents and donate hydrogen atoms to scavenge free radicals.

The result of this study also showed that the antioxidant IC_{50} profile was similar to the TPC value, which was highest in the bark and lowest in the leaves. This is consistent with the result of a previous study that TPC is more specific and 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). In addition, it was also discovered that although there are different antioxidant activities in each species of Rhizophoraceae mangrove plants, there were similarities. Based on observations of the four parts of the plant, *C. decandra* had the highest antioxidant activity compared to the other 4 species. This illustrates that, *C. decandra* is the most active mangrove species in the family Rhizophoraceae, demonstrating its potential as a source of natural antioxidants for therapeutic ingredients. Therefore, using the GC-MS technique, the sample with the highest antioxidant activity, namely *C. decandra* bark and root, was filtered for phytoconstituent analysis.

Table 2. Antioxidant activity of methanol extract of 20 samples of Rhizophoraceae plant parts

Extracts	Absorbent			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: Ascorbat acid (AA), the same letter notation indicates no significant difference in treatment (P>0.05).

Phytoconstituents of the extracts

GCMS was used to analyze two potential extracts with the highest antioxidant activity for their bioactive compounds. The dominant bioactive compounds using GCMS are shown in Tables 3 and 4. Based on the results of Table 3, the GCMS of the methanol extract of *C. decandra* bark contained 12 compounds. Pyrocatechol has high similarity with the data in GCMS, as indicated by 15.85% content with 96% similarity. Other compounds with similarity include Antiarol (1.44%) with 96% similarity, 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). In addition, the stem of *Eucalyptus globulus* contains antiarol compounds that are included in aromatic phenols and show moderate DPPH free radical activity, and function as antibacterial and antifungal (Celeiro et al., 2019). Furthermore, hexadecanoic acid acts as an anti-tumor detected in the ethanol extract of *Pleurotus ferulae* (Yang et al., 2018). The compound 8-oxo-beta-erythroidine belongs to the erythroidine alkaloids class from the *Erythrina poeppigiana* bark methanol extract. It has great potential as a phytoestrogen that can mimic the effects of estrogen, thereby reducing the risk of breast cancer MCF-7 (Djiogue et al., 2014). There are also α-tocopherol compounds from plant extracts that can meet the needs of vitamin E in humans. Wheat germ oil contains vitamin E, a fat-soluble antioxidant that preserves vital fatty acids from oxidation and lowers inflammatory response (Traber and Atkinson, 2008).

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Table 3. GCMS results of the *Ceriops decandra* bark methanol extract

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-tetrahydrocoumarin	$C_{11}H_{12}O_3$	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

Based on Table 4, the GCMS found the same compounds between the methanol extract of *C. decandra* bark and roots. The same compounds between the two extracts have a similarity index of greater than 80%, including Pyrocatechols (3.47%) and antiarols (1.27%). Both of these compounds, as described above, have activity as antioxidants.

Table 4. GCMS results of *Ceriops decandra* root methanol extract

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-divinyloxirane	$C_7H_{10}O$	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, the mangrove halophyte plants of the Rhizophoraceae family, including *B. cylindrica*, *B. gymnorhizza*, *C. decandra*, *R. apiculata*, and *R. mucronata* species, contain an alkaloid, phenolic, tannin, terpenoid, steroid and saponin phytoconstituents. The TPC content of Rhizophoraceae was very high in the stem bark, the highest TFC content was found in the leaves, and the antioxidant activity was very high specifically for *C. decandra* species. Furthermore, Rhizophoraceae

plants are a good source of natural antioxidants for medicinal use. Further studies are recommended to purify and identify specific antioxidant-active compounds that can be applied as anticancer agents.

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