https://doi.org/10.31925/farmacia.2024.5.25 **ORIGINAL ARTICLE**

INVESTIGATING THE ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS AND PHYTOCHEMICAL PROFILE IN *AVICENNIA ALBA* AND *EXCOECARIA AGALLOCHA* ROOT EXTRACTS AS A DEFENCE MECHANISM AGAINST POLLUTANTS

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Abstract

Plants have various self-defence mechanisms to protect themselves from adverse environmental factors. This study aimed to investigate the antioxidant activity, total phenolics and phytochemical profile of mangrove roots as a defence mechanism against pollutants. The roots of *Avicennia alba* and *Excoecaria agallocha* species were collected from mangrove areas affected by industrial activities and conservation mangrove areas in South Sumatra, Indonesia. Maceration and extraction of all samples were carried out using ethanol as a solvent. Samples were tested for antioxidants against DPPH free radicals, total phenolics by the Folin-Ciocâlteu method, and phytochemical profile screening by GC-MS. Based on the results of the IC_{50} antioxidant of *A. alba* root extract, both regions were classified as very low (344.8 µg/mL and 1062.58 µg/mL) and *E. agallocha* root extract was classified as moderate (109.9 µg/mL and 116.9 µg/mL). The phenolic content of *A. alba* was 20.38 - 55.21 mg GAE/g and *E. agallocha* was 56.70 - 107.18 mg GAE/g. Based on the peaks that were found, the main groups of compounds were, terpenoids, esters, alcohols, fatty acids, aldehydes and steroids. Differences in the ability to produce antioxidant activity in each mangrove species indicate variations in self-defence against oxidative stress due to differences in morphology, habitat and environmental conditions.

Rezumat

Plantele au diverse mecanisme de autoapărare pentru a se proteja de factorii de mediu negativi. Acest studiu și-a propus să investigheze activitatea antioxidantă, prin determinarea conținutului total fenolic, și profilul fitochimic al rădăcinilor de mangrove ca mecanism de apărare împotriva poluanților. Rădăcinile speciilor *Avicennia alba* și *Excoecaria agallocha* au fost colectate din zonele de mangrove afectate de activități industriale și zone de conservare a mangrovelor din Sumatra de Sud, Indonezia. Macerarea și extracția tuturor probelor au fost efectuate folosind etanol ca solvent. Probele au fost testate pentru activitatea antioxidantă prin determinarea radicalilor liberi DPPH, a conținutului total fenolic prin metoda Folin-Ciocâlteu și screeningul profilului fitochimic prin GC-MS. Pe baza rezultatelor IC_{50} obținute pentru extractul de rădăcină de *A. alba*, ambele regiuni au fost clasificate ca foarte scăzute (344.8 µg/mL și 1062.58 µg/mL), iar extractul de rădăcină de *E. agallocha* a fost clasificat ca moderat (109.9 µg/mL și 116.9). µg/mL). Conținutul fenolic al *A. alba* a fost de 20.38 - 55.21 mg GAE/g și in cazul *E. agallocha* de 56.70 - 107.18 mg GAE/g. Pe baza picurilor găsite, principalele grupe de compuși identificate au fost: terpenoide, esteri, alcooli, acizi grași, aldehide și steroizi. Diferențele în activitatea antioxidantă a fiecărei specii de mangrove indică variații în autoapărarea împotriva stresului oxidativ din cauza diferențelor de morfologie, habitat și condiții de mediu.

Keywords: antioxidant, mangrove root, pollutant, phytochemical profile

Introduction

The coast is a potential area because it provides optimal ecological services such as carbon sequestration, biodiversity, pollution reduction and habitat conservation [1-3]. Mangroves are a key ecosystem for coastal areas that can thrive in tropical and subtropical intertidal zones [4]. These plants at the land-sea interface ecologically provide food, breeding and nursery grounds for a wide range of terrestrial and marine organisms [5]. However, as the largest community in coastal areas, mangroves are more vulnerable to pollutants from anthropogenic activities, which can threaten their survival [6, 7]. The declining quality of mangrove forests is a serious threat to the ecosystems of various species of flora and fauna that affect the balance of mangrove and coastal ecosystems [8].

Anthropogenic activities have resulted in the degradation of nearly one billion hectares of land globally due to both agricultural and industrial activities [9]. Pollutants released by anthropogenic activities, such as heavy metals, nutrients, organic pollutants and microplastics, can be harmful to the growth and development of plants and animals if the concentration exceeds the threshold [10]. These pollutants will be carried by river currents and experience a build-up until they can accumulate in the estuary area [11]. Pollutants that are initially present in the water column will settle to the bottom of the water and accumulate in aquatic biota [12]. The process of accumulating pollutants can cause stress for mangroves and cause an increase in reactive oxygen species (ROS) that trigger oxidative stress [13].

Plants have specific self-defence mechanisms to detoxify ROS, which include antioxidant enzyme activity as well as non-enzymatic antioxidants. Increased enzymatic activity takes the form of the formation of superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen [6, 14]. Uncontrolled ROS can cause serious disruption to the normal metabolism of plant cells through oxidative damage to lipids, proteins and nucleic acids [15]. Plants also have the ability to produce non-enzymatic antioxidant activity as a form of self-defence mechanism in the form of increased activity of antioxidant compounds such as phenolics and flavonoid groups that function as pro-factors against various environmental stresses [16, 17].

Some information about how the activity of antioxidant enzymes changes in mangrove species when they are exposed to environmental stress has been obtained. However, most studies have not explored the role and contribution of non-enzymatic antioxidant activity in two types of species with different forms, habitats and regions at the same time. Therefore, these two species are good materials to clarify the self-defence mechanism in mangrove. In addition to having differences in terms of zoning or habitat, *Avicennia alba* and *Excoecaria agallocha* species also have different root systems. Roots are the part of the plant that is directly related to the accumulation process of moisture, nutrients and pollutants through sediment as a growth medium [18-20]. Plant roots release root exudates such as organic and inorganic compounds, which in turn increase or decrease the availability of contaminants in the root zone [21, 22]. In addition, mangrove ecosystems affected by industrial activities will be compared with conservation areas to comparatively look at changes in non-enzymatic antioxidant activity.

Materials and Methods

Root collection

This research was conducted in August 2023. Root samples were *Avicennia alba* and *Excoecaria agallocha* species taken from industrial and conservation areas on the East Coast of Banyuasin, South Sumatra. The first area was chosen with consideration of industrial activities along the Musi River that empties into the Payung Island mangrove forest, such as docks, aquaculture, oil exploitation and oil processing that produce pollutants [23, 24]. Reported pollutants are nutrients, heavy metals, organic pollutants and microplastics [25, 26]. The comparison area is the Barong River area and also includes the Sembilang National Park conservation area, which represents an area away from industrial activities [27, 28].

Identification, preparation, maceration, deconstruction and antioxidant activity tests on samples were carried out at the Marine Bioecology Laboratory and Marine Instrumentation Oceanography Laboratory, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indonesia.

Plant maceration and extraction

The mangrove roots of *A. alba* and *E. agallocha* from two areas, each 200 g (dry weight), that had been mashed and macerated with ethanol solvent as much as 1 L (1:5 b/v) for 2 x 24 h. According to [29, 30], polar ethanol solvents is more effective to extract secondary metabolites. The result of maceration in the form of a solution is then filtered using Whatman 40 filter paper. The macerate is evaporated at a water bath temperature of 60°C until the solvent evaporates completely to produce a paste-like formation (crude extract) of mangrove roots. The crude extract was then stored at room temperature.

Antioxidant activity evaluated by DPPH assay

Antioxidant activity analysis using ethanol solvent refers to making a 0.1 μM DPPH solution as much as 50 mL. The parent solution of ascorbate (2000 ppm) was prepared by homogenizing 10 mL of the solution. A series of dilutions was then made to obtain concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 62.5 ppm. In each concentration, 1 mL of a 0.1 μM DPPH solution was added, homogenised and incubated for 30 minutes in a dark place. Next, the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 517 nm [31]. The antioxidant activity of the extract is expressed as IC_{50}, which has characteristic criteria to determine the strength of its antioxidant content (Table I) using the following formula:

$$\%\, inhibition = \frac{blank\ abs - sample\ abs.}{blank\ abs} \text{ x } 100\%.$$

The results for the IC_{50} were entered into a linear regression equation, with the sample concentration as the abscissa (X-axis) and the percentage of antioxidant inhibition as the ordinate (Y-axis) using the y = ax + b [32].

The IC_{50} characteristic values are categorised based on concentration as follows: concentrations below 50 μg/mL are considered "very strong", between 50 - 100 μg/mL as "strong", between 100 - 150 μg/mL as "moderate" and between 150 - 200 μg/mL as "low".

Determination of phenol content

Analysis of total phenol content in a sample was carried out by the Folin-Ciocâlteu method [33, 34]. A 1000 ppm gallic acid standard solution was prepared (50 mL total), followed by serial dilutions to create concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm, each in 5 mL volumes. From these, aliquots of 1 mL, 2 mL, 3 mL, 4 mL and 5 mL were pipetted into 10 mL volumetric flasks to create a 100 ppm gallic acid standard solution. Separately, 50 mg of the sample was weighed, mixed with 2 mL of methanol and 5 mL of distilled water and homogenised in a 10 mL volumetric flask.

In the standard series of dilutions, 0.5 mL of 50% Folin-Ciocâlteu reagent was added, distilled water was added until the limit was reached for 5 minutes, and 5% Na_2CO_3 (1 mL) was added to incubate in a dark place without light for 1 hour. After incubation, the sample was measured using a UV-VIS spectrophotometer with a wavelength of 750 nm.

Gas chromatography-mass spectroscopy (GC-MS) analysis

Analysis was conducted to determine the components of bioactive compounds contained in root extract. The spectrum graph of the analysis results was compared with the data bank in Wiley Library 7 [35, 36].

Results and Discussion

Description of mangrove roots

The mangrove species found in the field were *Avicennia alba* in the coastal zone and *Excoecaria agallocha* in the inland zone (Figure 1). In general, these two species have different root characteristics.

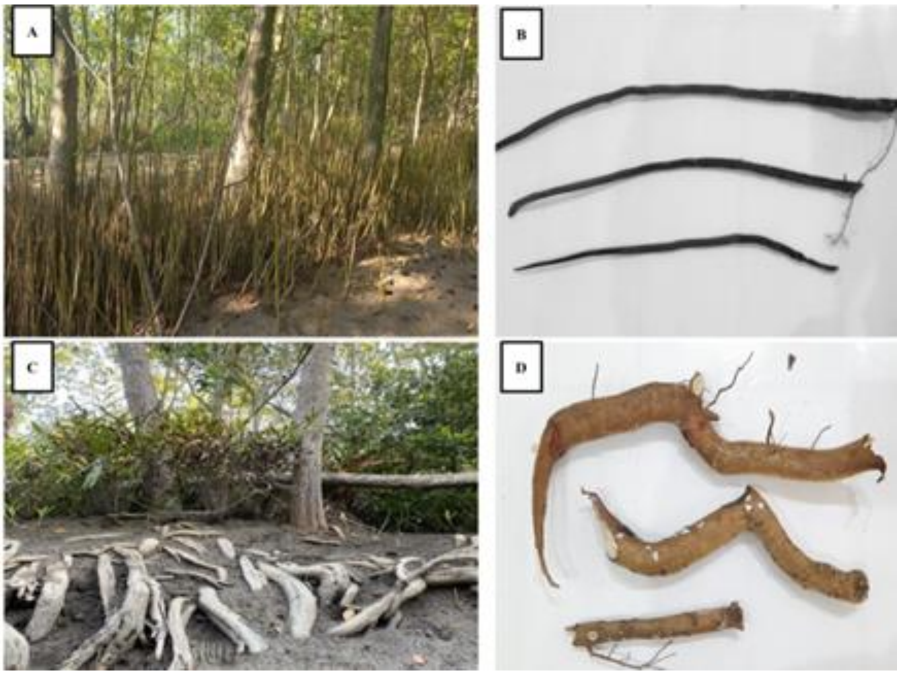

Figure 1.
Description of mangrove roots: (A-B) *A. alba*, (C-D) *E. agallocha*

The genus *Avicennia* includes a type of pneumatophore root that arises vertically from the cord root, having aerenchyma that can amount to up to 70% of the root volume. Pneumatophores aid in the exchange of gases, especially oxygen and carbon dioxide, between the roots and the atmosphere [37, 38]. This can affect sediment oxygenation and redox. The roots are characterised by being dense, can penetrate deeper soil layers, and can help in binding and retaining surrounding sediments, resisting erosion and maintaining soil structure [39]. While *E. agallocha* has a type of lateral roots spreading and mixing with each other; the supraterranean band produces elbow-shaped pegs of pneumatophores [40].

Species of *A. alba* and *E. agallocha* were found in the characteristics of clay substrate. Based on the results of research [41], clay substrate is strong at absorbing organic matter, so many groups of macrozoobenthos animals were found. Characteristics of mangrove substrates are often found in mud, loam and sandy [42, 43]. Mangrove substrates have some special characteristics that are different from sediments in ordinary land environments. This is due to the high-water availability, changing salinity levels and tidal movements along the coastal zone [44, 45]. Accumulation of organic and inorganic material often occurs in the mangrove substrate to provide nutrients for growth and development [46, 47]. According to [48], organic material can come from fallen mangrove leaves and the decomposition of organisms in the environment. While inorganic materials such as heavy metals can come from anthropogenic activities around mangrove ecosystems, they tend to stay in the sediment for a long time [49]. Plant roots have a colloidal surface and can attract and retain heavy metal ions from sediment solutions through the process of adsorption

[50]. However, the presence of excessive roots will produce antioxidant enzyme and non-enzyme activities as a form of self-defence from environmental stress [51, 52].

Characterictics of mangrove roots extract

The percentage of weight shrinkage of wet and dry leaf samples was 58% for *A. alba* roots and 30.8% of *E. agallocha* roots for industrial areas. While in the conservation area, the percentage of shrinkage of *A. alba* roots were 56% and *E. agallocha* roots were 27%. These results show that the water content contained in the leaves of *A. alba* is higher than in the leaves of *E. agallocha* in both areas (Table I).

Table I

Depreciation percentage of weight

Area	**Sample roots**	**Sample weight (g)**		**Depreciation percentage (%)**	**Weight percentage (%)**
		Wet	**Dry**		
Industry	*A. alba*	500	210	58	42
	E. agallocha	500	346	30.8	69.2
Conservation	*A. alba*	500	220	56	44
	E. agallocha	500	365	27	73

Removal of moisture content in the sample can be done by drying until the moisture content is completely lost, because the content of the compounds contained in the sample will be better if the sample is in a dry condition. Moisture content can affect the stability of bioactive compounds during the extraction process. Some compounds may be more stable or less susceptible to chemical degradation or oxidation in the presence of water. Meanwhile, the extraction process for *A. alba* and *E. agallocha* root samples were carried out using ethanol solvent. The results show the weight of the extract produced by the leaves of *A. alba* and *E. agallocha* which is the highest in *E. agallocha* found in conservation areas at 1.96% (Table II).

Table II

Percentage of etanol extract

Area	**Sample roots**	**Extract weight (g)**		**Depreciation percentage (%)**	**Extract percentage (%)**
		Dry powder	**Crude extract**		
Industry	*A. alba*	200	3.86	98.07	1.93
	E. agallocha	200	4.3	98.28	1.72
Conservation	*A. alba*	200	2.63	98.69	1.32
	E. agallocha	200	4.9	98.04	1.96

Maceration and extraction are part of the process of testing bioactive compounds. The solvent used in the extraction process aims to separate the substance of bioactive compounds in mangrove root extract [53]. Ethanol is an amphipathic solvent that can dissolve compounds that are both polar and nonpolar [54]. Mangroves often contain various types of compounds with polar and nonpolar properties and ethanol can effectively take on a large number of diverse bioactive compounds [55, 56]. The highest extraction weight percentage results can indicate that the extraction method used is efficient in removing compounds from mangrove samples [57]. The high extraction yield may indicate that the sample is rich in compounds that are thought to have biological activity or other potential uses [58].

DPPH radical scavenging activity

The results of antioxidant tests on two types of mangrove roots from two different areas using the DPPH radical reduction method using an ethanol solvent (Table III). The content of IC_{50} on mangrove root samples in industrial areas for *A. alba* amounting to 344.8 μg/mL is classified as very low, and *E. agallocha* of 109.9 μg/mL is classified as moderate. While in the conservation area for *A. alba* amounting to 1062.58 μg/mL is classified as very low, and *E. agallocha* of 116.9 μg/mL is classified as moderate.

Table III

Classification of IC_{50}

Area	**Sample roots**	**Linear regression**			**IC_{50} (μg/mL)**	**Category**
		a	**b**	**R^2**		
Industry	*A. alba*	15.551	40.868	0.9223	344.8	Very low
	E. agallocha	33.569	107.76	0.9791	109.9	Moderate
Conservation	*A. alba*	30.12	159.89	0.9878	1062.58	Very low
	E. agallocha	35.707	120.02	0.9628	116.9	Moderate

Based on the IC_{50} classification results for *A. alba* in both areas is included in the very low. Previous research explains that the *Avicennia* genus is a mangrove found in the foremost zone and directly facing the waters [59]. *Avicennia spp*. has strong and dense aerial roots that are very effective in capturing and holding mud and various pollutants that drift in the waters [37, 60]. As a plant species that is periodically submerged in water, the stilt roots owned by mangroves are able to take, absorb, or reduce contaminants through the dilution process [61, 62]. Therefore, it is suspected that the absorbed contaminants do not cause excessive oxidative stress on the roots and do not increase the production of secondary metabolites. Another study in the Island of Weno area, Chuuk State of Micronesia found that the antioxidant activity of *Rhizophora stylosa* roots amounted to 41.3% and *Sonneratia alba* 40.7% [63]. While the IC_{50} value in *E. agallocha* species in both regions is included in the medium category. *E. agallocha* in this study was found in the inland zone. This zone is rarely submerged by sea water and is more often exposed to the influence of lower tides. This is thought to be the cause of the low water content in *E. agallocha* roots, as presented in Table II. The findings suggest that most of the anti-oxidant enzymes in roots are synchronised to reduce stress efficiently [35]. The differences that occur in the ability to produce antioxidant enzyme and non-enzyme activities in each mangrove as a form of self-defence against oxidative stress are due to differences in terms of morphology, habitat, tides, sediment substrate and environmental conditions [57, 64, 65]. Various environmental conditions caused by pollutants can pose a serious threat to the health and sustainability of mangrove ecosystems. Some information on the impact of pollutants on plant biochemical processes is summarised in Table IV.

Table IV

Influence of pollutants on plant biochemical processes throughout in the world

Location	Source of pollutant	Impact	Ref.
Hinchinbrook Channel and Port Douglas	Heavy metals, oil residues, herbicides and raw wastewater	Photosynthesis, growth and biomass are reduced due to its influence and ultimately increase mortality.	[67]
The Can Gio Mangrove Forest (Southern Vietnam)	Heavy metals	Decrease in mangrove forest biomass.	[68]
The Fundão dam disruption in Brazil	Heavy metals	The occurrence of oxidative stress symptoms may be caused by the reduced efficiency of antioxidant defence by Cu^{2+} and Zn^{2+}.	[69]
The Rabigh lagoon, Red Sea	Heavy metals	Sediment damage due to heavy metals in a gradual pattern has the potential to have negative impacts on biogeochemical cycles, with potentially lethal consequences for the survival of mangroves.	[70]
Industrial areas and a control area	Heavy metals	Increases hydrogen peroxide (H_2O_2) activity, malondialdehyde content and enzymatic and non-enzymatic antioxidant activity. However, it reduces total carbohydrates and protein, secondary metabolite content (phenols and flavonoids) and free radical scavenging activity (DPPH).	[71]
A systematic review	Microplastics and nano-plastics	Stronger inhibition of most physiological pigments, photosynthesis and biochemical indicators in plants.	[72]
A systematic review	Polystyrene micro-plastics (PS-MPs)	PS-MPs damage leaf photosynthetic pathways and inhibit protein synthesis. SOD activity decreased and CAT decreased.	[73]
A systematic review	Nano- and micro-plastics (NMPs)	Inhibits the growth of biomass and plant length in plant species.	[74]

Phenol content of mangrove roots

Phenol content of mangrove roots was measured by adding Folin-Ciocâlteu reagent to the sample solution tested (Table V).

Table V

Total phenolic content of the obtained mangrove root extracts

Area	Sample roots	Phenol (mg GAE/g)
Industry	*A. alba*	55.21
	E. agallocha	107.18
Conservation	*A. alba*	20.38
	E. agallocha	56.70

Phenol has antioxidant properties and can help protect plant tissues from damage caused by free radicals. Therefore, the total phenol test can provide information regarding the potential antioxidant activity of mangrove root extract. In this study, the highest quantitative phenol value was found in *E. agallocha* at 107.18 mg GAE/g from the industrial area and the smallest in *A. alba* at 20.38 mg GAE/g from the conservation area. The result of total phenol in this study was directly proportional of the antioxidant activity IC_{50} value in Table V. The ability of mangroves to have antioxidant activity is inseparable from their total phenol content. Total phenol content is directly proportional to the antioxidant activity of a material; the greater the total phenol value, the greater the antioxidant activity in a sample [29]. Based on the results of this study for *A. alba* has a low total phenol content compared to *E. agallocha*. This is strongly suspected due to the environmental factors that make the leading zone mangroves experience more environmental pressure from pollutants and physical and chemical habitat factors In line with previous research reporting total phenol in the roots of *A. marina*, which is in the coastal zone of 26.11 mg GAE/g is smaller than *B. gymnorrizha* amounting to 344.02 mg GAE/g is located in the inland zone [75]. Mangrove ecosystems located in the leading zone tend to have special adaptations to survive in coastal environments that are often inundated by seawater due to tides [76]. Their ability to cope with the impact of pollutants by reducing their concentration and toxic effects through their water content so that the pollutants absorbed are not excessive [77]. Meanwhile, according to [78], nonenzymatic antioxidant activity cannot be generated exclusively because there is a certain threshold for excess free radicals. Instead, the nonenzymatic antioxidant system is usually regulated and activated when free radicals or oxidative stress exceed the capacity of the normal defence system [79].

GC-MS analysis of E. agallocha root extract

The GC-MS analysis conducted using *Excoecaria agallocha* mangrove root samples from industrial areas is particularly significant due to its classification in the medium IC_{50} category, indicating moderate potency in biological activity inhibition. Notably, the IC_{50} values for *E. agallocha* are higher compared to those obtained from *Avicennia marina* samples. This suggests that *E. agallocha* may possess a more favourable profile for extracting bioactive compounds, which is crucial for evaluating its potential applications in environmental remediation and pharmacological research. The graph obtained presented 30 peak area. The compounds detected were terpenoids, esters, alcohols, fatty acids, aldehydes and steroids The compounds identified based on the peak height of the chromatogram and mass spectrum on the chromatogram graph of the analysis results that have been matched are identical to the mass spectrum in the data base library: WILEY 7 (Table VI).

Table VI

Retention time, peak area, compound name, formula and compound group

Ret. time	***Peak Area %***	**Compound name**	**Formula**	**Compound group**
10.87	2.38	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1 -(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	Terpenoids
12.31	3.73	Cubenol	$C_{15}H_{26}O$	Terpenoids
14.64	0.93	Benzyl Benzoate	$C_{14}H_{12}O_2$	Ester
17.75	1.02	Bicyclo[9.3.1]-pentadeca-3,7-dien-12-o l, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-	$C_{20}H_{34}O$	Terpenoids
18.35	2.20	1-Heptatriacotanol	$C_{37}H_{76}O$	Alcohol
18.40	2.20	Naphthalene, decahydro-1,1,4a-trimethyl-6-methyle ne-5-(3-methyl-2,4-pentadienyl)-, [4aS-(4aà,5à,8aá)]-	$C_{21}H_{34}$	Terpenoids
18.57	5.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acids
18.93	0.57	1H-Naphtho[2,1-b]-pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pen tamethyl-, [3R-(3à,4aá,6aà,10aá,10bà)]-	$C_{26}H_{42}O$	Terpenoids
19.80	1.87	Kaur-16-ene	$C_{20}H_{32}$	Terpenoids
21.36	8.29	Bicyclo[9.3.1]-pentadeca-3,7-dien-12-o l, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-		Terpenoids
21.65	6.70	Thunbergol	$C_{20}H_{34}O$	Terpenoids
21.73	1.84	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{18}H_{30}O_2$	Fatty acids
22.05	6.35	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	$C_{20}H_{30}O$	Terpenoids
23.09	0.71	Butyl 6,9,12,15-octadecatetraenoate	$C_{22}H_{36}O_2$	Ester
23.36	7.16	1H-Naphtho[2,1-b]-pyran-8(4aH)-one, 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-	$C_{21}H_{34}O_2$	Terpenoids
23.81	2.65	Ethyl 5,8,11,14,17-icosapentaenoate	$C_{22}H_{34}O_2$	Ester
23.94	1.97	1-Heptatriacotanol	$C_{37}H_{76}O$	Alcohol
24.80	1.53	9H-Naphtho[2,1-b]-pyran-9-one, 3-ethenyldodecahydro-7-(hydroxymeth yl)-3,4a,7,10a-tetramethyl-, [3R-(3à, 4aá, 6aà, 7à, 10aá, 10bà)]-	$C_{22}H_{34}O_3$	Terpenoids
25.54	4.11	i-Propyl 5,8,11,14,17-eicosapentaenoate	$C_{23}H_{36}O_2$	Ester
26.29	0.81	Preg-4-en-3-one, 12,17-dihydroxy-20-nitrilo-	$C_{20}H_{27}NO_3$	Steroids
27.41	0.89	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	Terpenoids
27.81	2.27	2-[4-methyl-6-(2,6,6-trimethylcyclohex -1-enyl)-hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	Aldehyde
31.84	2.74	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	Ester
35.49	0.52	Stigmasterol	$C_{29}H_{48}O$	Steroids
36.33	2.21	ç-Sitosterol	$C_{29}H_{50}O$	Steroids
36.53	11.69	á-Amyrin	$C_{32}H_{52}O_2$	Terpenoids
37.56	6.08	Lupeol	$C_{30}H_{50}O$	Terpenoids
37.63	3.23	9,19-Cyclo-9á-lanostane-3á,25-diol	$C_{30}H_{52}O_3$	Terpenoids
39.20	1.29	Lup-20(29)-en-3-ol, acetate, (3á)-	$C_{32}H_{52}O_2$	Terpenoids
39.42	1.60	Octadecanoic acid, 2,3-bis[(1-oxotetradecyl)-oxy]-propyl	$C_{49}H_{94}O_6$	Ester

The screening results of secondary metabolite compounds from mangrove root extracts using GC-MS can provide information on the chemical composition of the extracts and the potential bioactive compounds contained in the mangrove roots. Based on the results of GC-MS screening, the compounds detected were terpenoids, esters, alcohols, fatty acids, aldehydes and steroids. These compounds have the potential to serve as plant defence mechanisms against environmental stress and pathogens. Secondary metabolites in plants are not only important sources of natural compounds, but they also play an important role in plant defence mechanisms against infections and other environmental hazards [79-81]. In line with the opinion of another researchers [83], plant defences are adaptations that reduce the damage and death caused by herbivores and pathogens. Additional plant compounds play a critical role in interactions with pathogens [83, 84]. Secondary metabolite compounds, such as phenolics, alkaloids and essential oils, play an important role in plant metabolism, namely compounds against herbivores, insect and pathogen defence, pigmentation, growth and development and germination regulation [85, 86]. Terpenoids, flavanols, flavonoids and others are some examples of phytochemicals that emerge in response to environmental stress, and they play a key role in regulating immune responses in plants [87, 88]. According to the provided data, terpenoid compounds appear very frequently in the mangrove root extract of *E. agallocha*. Terpenoid compounds have an important role as a self-defence mechanism for plants against environmental stress, which is influenced by surrounding industrial activities [89, 90]. Some terpenoid compounds have antioxidant, antibacterial and anti-inflammatory properties that can help protect plants from pathogenic infections that can damage roots and other tissues [91-93]. Several previous research studies also reported the benefits of terpenoid compounds, especially in mangroves. Terpenoids from the mangrove plant *Xylocarpus moluccensis* may have the potential to inhibit SARS-CoV-2: an *in silico* strategy [94]. Findings research of [95], in the detection that the roots of the Asian mangrove *Rhizophora mucronata* had activity against pro-inflammatory cyclooxygenase and lipoxidase.

Conclusions

In both industrial and conservation areas, *A. alba* roots showed very low antioxidant activity, while *E. agallocha* showed moderate antioxidant activity. The total phenol content in the roots of *A. alba* was lower than that of *E. agallocha*. GC-MS screening results contain various phytochemical compounds such as terpenoids, esters, alcohols, fatty acids, aldehydes and steroids. These compounds have the potential to act as antioxidant agents and able to assist protect mangrove plants from oxidative stress caused by pollutants. The differences in the ability to produce antioxidant activity between the two mangrove species indicate variations in the defence mechanisms against oxidative stress. This variation can be caused by differences in morphology, habitat and environmental conditions where the species grows. Further research on factors influencing antioxidant activity and phytochemical composition in mangrove species could provide deeper insight into plant adaptation to polluted environments.

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Conflict of interest

The authors declare no conflict of interest.

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