Phytochemical profile

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Phytochemical profile and toxicity of extracts from the leaf of *Avicennia marina* (Forssk.) Vierh. collected in mangrove areas affected by port activities

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ABSTRACT

Mangrove leaves are natural materials with various sources of toxic bioactive compounds that can be developed for the pharmaceutical field. *Avicennia marina* mangrove vegetation on the coast of South Sumatra is dense, and its location is unique because it is close to port activity waste. Environmental influences at the site are thought to have an impact on the diversity of bioactive compounds. The study was conducted to analyze the phytochemical profile of *A. marina* leaf extract using GC–MS analysis and its potential toxicity through Brine Shrimp Lethality Assay. Samples were taken from mangrove vegetation near Tanjung Api-Api Port, South Sumatra. Furthermore, the sample was conducted toxicity test on brine shrimp, total phenol analysis, preliminary phytochemical test, and GC–MS analysis. Based on the results of toxicity tests, the value of LC$_{50}$ ethyl acetate extract amounted to 454 μg/mL, and methanol extract amounted to 740 μg/mL. Furthermore, ethyl acetate extract contained 1.3205 mg GAE/g total phenol, and phytochemical test results contained saponins, flavonoids and steroids. GC–MS analysis detected major compounds, including groups of fatty acids, phenols, terpenes, alkaloids, alcohols, hydrocarbons and minor compounds of cannabinoids and amines. Ethyl acetate extract of *A. marina* leaves produced a structure of bioactive compounds that had been reported to have potential as anticancer, antimicrobial, anti-inflammatory, anti-insecticide and antioxidant. The results of this study were expected to provide important information in finding sources of bioactive compounds, taking into account the more real influence of extrinsic environments.

1. Introduction

Bioactive compounds from mangrove plants may have other benefits as toxic compounds with various new chemical mechanisms. It has great potential in the treatment and cure of cancer (Fabbri and Franzellitti, 2016). Bioactive compounds have been widely used in medicine. The toxicity effect of bioactive compounds extracted from mangrove plants is helpful for the raw materials of anticancer drugs (Bibi et al., 2020). Many plants and marine animals, but mangrove plants have a wealth of bioactive compounds with various biological activity capabilities (Sopalun et al., 2021). New, rapidly growing information states that researchers are revealing the bioactivity capabilities of mangrove plants to produce valuable products in medicine (Glasenapp et al., 2019; Parthiban et al., 2021; Sadeer et al., 2022).

Toxicity is a reaction caused by extracts of natural materials and causes biological disturbances to death in the test organism. The high mortality rate of test organisms indicates that the natural material is worthy of processing to the clinical stage to find anticancer drugs (Hisem et al., 2011). Toxicity with the Brine Shrimp Lethality Assay (BSLA) test is widely chosen because it has several advantages: easy, fast, cheap, and entirely accurate. Some of these factors are needed in the initial screening process for the potential toxicity of a natural material (Hamidi et al., 2014). Studies of the relationship between the toxicity effects of BSLA and the *in vivo* anticancer method have been conducted by several scientists. Both have a straightly proportional relationship. The intense level of toxicity in BSLA is also proven in a test of *in vivo* anticancer methods (Osamudiamen et al., 2020). Many experts consider that screening potential toxicity effects is enough with *in vitro* toxicity methods through the BSLA. The discovery of good toxic activity is more commonly found from plant extracts that live in the tropics because it has more environmental limiting factors (Pan et al., 2012).

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Tropical coastal and marine are exciting areas to explore because of the diversity of chemicals offered by marine and coastal life (Dehm et al., 2021; Rozirwan et al., 2014). Several studies have explored the potential of bioactive compounds derived from tropical marine and coastal by taking place in Asia (Acharya et al., 2020; Moon et al., 2020), South America focuses on the potential of derivative microorganisms (Shishido et al., 2020; Suárez and Chávez, 2018), and Africa tends to study the bioactivity of bioactive compounds from isolated bacteria and fungi (Seukep et al., 2015; Tawfike et al., 2019). Environmental limiting factors change the evolution of species chemically and form bioactive compounds and produce chemical groups such as triterpenoids, alkaloids, flavonoids, steroids, and many more (Nichols et al., 2015). One species that offers a diversity of bioactive compounds is mangroves.

Environmental conditions influence the accumulation of phytochemicals in natural materials. Gololo et al. (2018) stated that the diversity of phytochemical profiles in S. italica leaves could be influenced by geographical location. Mangroves grow in coastal wetlands, so they have unique adaptations to changes in salinity, temperature, nutrients, and excessive radiation (Moteriya et al., 2015; Rozirwan et al., 2021a). In addition to adapting to intrinsic environmental changes, mangroves also adapt to environmental changes caused by extrinsic factors. As a fact, the mangrove species A. marina grows well near Tanjung Api-Api Port activity area in South Sumatra, Indonesia (Almaniar et al., 2021; Saputra et al., 2021). However, the Tanjung Api-Api Port area has experienced organic pollution of polycyclic aromatic hydrocarbons (PAHs). Some types of PAHs are detected above the threshold, as in water containing Naphthalene 15.848 ppb and Benzo[a]pyrene 76.493 ppb, while in sediments, it contains Benzo[a] anthracene 3.16 ppb (Putri et al., 2019). In addition, the content of heavy metals in waters contains Cd 0.02 mg/L and Hg 0.012 mg/L, while in sediments, it contains Cd 0.03 and Hg 0.01 mg/L (Barus, 2017). Of course, the environmental influence of port activity is quite a lot and varied. Port activities significantly impact the surrounding ecosystem, such as accelerating siltation that causes a decrease in water levels that enter mangrove forests (Raharjo, 2016), and polluting materials increased, including heavy metal waste, garbage and smoke pollution (Chakraborty et al., 2021).

Mangrove bioactivity levels will differ due to the strong influence of environmental limiting factors or habitats in nature. The structure of bioactive compounds in each part of mangroves is also different. For example, in the leaves of A. marina, dominant contains alkaloids, terpenoids, and flavonoids (Jairaman et al., 2019). The stem contains tannins (Osman and Abkar, 2016), and the root contain flavonoids (Al-Mur, 2021). Therefore, the difference in the content of bioactive compounds shows that each part of mangrove has its benefits for human life.

Mangrove A. marina is one type of mangrove that has various bioactive compounds. The bioactive compounds in A. marina include flavonoids, tannins, steroids, saponins, alkaloids, glucosides, and triterpenoids. A. marina is also an anti-cancer, antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory (Lalitha et al., 2021; Tian et al., 2020). Rozirwan et al. (2020) reported that mangrove vegetation on the coast of Tanjung Api-Api is essential for the sustainability of the ecosystem there, and the dominant mangrove species is A. marina, followed by Sonneratia caseolaris, Rhizophora mucronata and Nypa fruticans. As far as we know, this study is the first to reveal the phytochemical profile of mangroves near port activity. The study will analyze phytochemical profiles to determine groups of bioactive compounds and toxicity levels as discover potential new drugs from A. marina leaf extracts collected from mangrove areas that are environmentally affected by port activity.

2. Materials and methods

2.1. Environment characteristics of mangrove sampling area

Environmental influences on mangrove vegetation habitats have been able to affect the presence of bioactive compounds. That was related to environmental limiting factors that triggered the rate of production of bioactive compounds. An environmental condition that experienced fluctuating changes was in the estuary area. Some of the environmental quality values of the waters taken.

Environmental parameters were measured around the sampling location using portable gauges including DO/Temp Meter Gondo, pH Meter HI 83,141 and Handrefractometer. The probe sensor tool was immersed in water directly, then the measurement results were read directly on the tool, except for salinity measurements that did not use a probe (Rozirwan et al., 2022). It included dissolved oxygen concentration, salinity, acidity level and temperature. Some of the measurable environmental characteristics were temperature of 25.2 °C, Dissolved Oxygen 1.98 mg/L, acidity 7, and salinity 15 psu. Water conditions were low tide at the time of sampling. Salinity was not too high because the influence of river water was greater than the

Fig. 1. Map of sample collected location in Tanjung Api-Api Port (Google Earth, 2021).

influence of seawater. In addition, low dissolved oxygen indicated that low tide tended to be calmer and no more turbulence due to the semi-open estuary area.

Samples of *A. marina* leaves were taken from the Banyuasin Estuary area in August 2020. The water conditions at that time were low tide so that it was visible that mangrove ecosystems became the suitable habitat of several species from class crustacea and gastropod. In addition, near the sampling site was also found many anthropogenic pollutants. At the same time, Tanjung Api-Api Port was busy carrying out transportation services. Leaf samples were taken at coordinate points 2.3719750 °S and 104.8046833 °E. The sampling map is presented in Fig. 1.

Identifying the species at the sampling site was using the mangrove guidebooks (Giesen et al., 2007; Noor et al., 2006). The *A. marina* sample taken was the part of the fresh leaves. The samples were taken by random sampling in the intertidal area, a site that could trigger a diversity of phytochemical profiles due to environmental intrinsic factors (Edu et al., 2015; Khalil et al., 2021). Leaf samples were taken in moderation in this study. During the trip, a sample of leaves was put in a box.

2.2. Plant maceration and extraction

Mangrove leaves of 100 g (dry weight) were mashed using a blender and macerated with ethyl acetate 1: 10 (w / v) for 24 h. Maceration was repeated four times until it was not concentrated. Furthermore, in the same sample, followed by maceration using methanol with the exact provisions as before. Maceration was chosen as an extraction method because it is relatively more straightforward, effective and economical (Jones and Kinghorn, 2012). Then, the sample was filtered and evaluated at a temperature of 60 °C. The rough extract was stored at a temperature of 4 °C.

2.3. Brine Shrimp Lethality Assay

2.3.1. Hatching process of Artemia cysts

Brine Shrimp Lethality Assay has been referred to research (Sarah et al., 2017). Hatching was using artificial seawater 30 psu as much as 1 L. The cyst of 5 g was prepared. Lamps and air aeration were given for the hatching process of *A. salina*. This process was left for two days. *A. salina* in the toxicity test contributed to the validation of a more generalized toxicity capability as required in this study.

2.3.2. Preparation of test solution

Toxicity test used a stock solution of 10,000 μg/mL. This solution was produced from 1 g of extracts rushed in 1 mL DMSO and added with aquadest up to 100 mL. The test concentration consists of 2000, 1000, 500, 250, 100, and 50 μg/mL. Five millilitres of each test concentration would be inserted into a vial and filled with ten individuals of *A. salina*. Detailed information on preparing the test solution was presented in Table 1.

Each test concentration was carried out three repetitions. *A. salina* mortality rates were observed after 24 h. These results were to

Table 1
Preparation of test solution.

Concentration (μg/mL)	Extract Solution	Saline Water	Final Volume
2000	10 mL (10,000 μg/mL)	40 mL	50 mL
1000	5 mL (10,000 μg/mL)	45 mL	50 mL
500	2.5 mL (10,000 μg/mL)	47.5 mL	50 mL
250	1.25 mL (10,000 μg/mL)	48.75 mL	50 mL
100	0.5 mL (10,000 μg/mL)	49.5 mL	50 mL
50	0.25 mL (10,000 μg/mL)	49.75 mL	50 mL
0 as control (-)	–	5 mL	50 mL

determine the percentage of mortality and LC_{50}. LC_{50} values were determined by linear regression analysis and probit scale (Wardlaw, 1985).

2.4. Total phenol contents and preliminary phytochemical screening

Analysis of total phenol extract of *A. marina* leaves was using the Folin-Ciocalteau method seen in detail in previous studies (Suh et al., 2014). Preliminary phytochemical screening was used to detect the groups of phytochemical compounds of *A. marina* leaf extract described by Harborne (1984), Tiwari et al. (2011). Some of the phytochemicals tested in this study included alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. This preliminary phytochemical test relied on color and physical changes, so it was classified as a conventional qualitative test (Shaikh and Patil, 2020).

2.5. Gas chromatography-mass spectroscopy analysis

GC−MS analysis was performed to find out the components of bioactive compounds contained in *A. marina* leaf extract. The extract was injected as much as 1 L into the rt x 5 ms column and helium as the carrier gas with a split ratio of 1: 50. The oven temperature was set at 50 °C for ±5 min, slowly increasing the temperature by 5 °C/ min to a maximum temperature of 280 °C and maintained at that temperature for 5 min. Samples were injected at a temperature of 280 °C. Spectrum graphs of analysis results were compared to the data on Library Wiley 7 (Rahim et al., 2018).

2.6. Data analysis

2.6.1. Percentage of depreciation and extract weight
The calculation of percentage of depreciation was used to calculate the rate of depreciation of the leaves and extract samples with formula (1). The weight percentage calculation was calculated using formula (2).

$$\% \text{ Depreciation} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \qquad (1)$$

$$\% \text{ Extract weight} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100 \qquad (2)$$

Based on the formulas, the final weight was used for the dry weight of the sample and the weight of the crude extract while the initial weight was used for the wet weight of the sample and the weight of the dry powder.

2.6.2. Percentage of mortality and LC_{50}
The calculation of% mortality was analyzed based on the data of *A. salina* that died after 24 h of observation using formula (3). LC_{50} was analyzed using the standard linear regression curve formula (4).

$$\% \text{ Mortality} = \frac{\text{Number of dead individuals}}{\text{Number of test individuals}} \times 100 \qquad (3)$$

$$y = a + \text{bx} \qquad (4)$$

De Alencar et al. (2014) distinguished mortality into three categories: nontoxic (< 50%), moderate toxic (50%−75%), very toxic (75%−100%). The LC_{50} value was obtained from the data analysis of the mortality percentage, total individual test and test concentration (μg/mL). Before the LC_{50} value was determined, there were several values that needed to be known, such as the mortality probit value and the Log 10 concentration test. Based on (Nguta et al., 2012), LC_{50} value categories included nontoxic (> 1000), weak toxic (500−1000), moderate toxic (100−500), and very toxic (< 100). These values were analyzed through regression using Microsoft Excel 2019.

Table 2
Depreciation percentage of weight and percentage of extract.

Sample	Sample weight (gr)		Depreciation percentage (%)	Weight percentage (%)
	Wet	Dry		
A. marina leaves	1000	320	68	32
Solution	**Extract weight (gr)**		**Depreciation percentage (%)**	**Extract percentage (%)**
	Dry powder	**Crude extract**		
Ethyl acetate	100	15.98	84.02	15.98
Methanol	100	6.49	93.51	6.49

3. Results

3.1. Description of mangrove A. marina

Mangrove ecosystems found in Banyuasin Estuary were quite dense. Samples of *A. marina* leaves taken as samples of this study were obtained in the first row of zones. Several types of mangroves were found in this location, including *S. caseolaris, R. mucronata,* and *N. fruticans*.

Leaves *A. marina* was green with leaf bones that cross from base to tip of the leaf. The leaves were elliptical and extended round, and the ends tapered until slightly round. The leaves had stalks that were at the base. The back of the leaves was gray-white, so it was different from the color of the front of the leaves. *A. marina* is found in the shape of a large and tall tree. The skin of the stem peeled off in a small part on almost the entire skin surface. The peeling skin was delicate textured and gray-white. The root of *A. marina* had a characteristic as breathing root type. The roots were like small woods or pencils with an upright position that appeared from the ground. However, the roots were not hard and not stiff.

3.2. Extract characteristics of A. marina mangrove leaves

Depreciation of *A. marina* leaves from wet to dry was 68% (Table 2). Depreciation was able to be interpreted as the moisture content lost during the drying process of the leaves. In addition, dry leaves prevented spoilage by bacteria and extended the shelf life of the sample.

The percentage of extract weight resulted was 15.98% or weight of 15.98 g in ethyl acetate extract and 6.49% or weight 6.49 g in methanol extract. Each solvent experienced depreciation of 84.02% and 93.51% for ethyl acetate and methanol.

3.3. Determination of brine shrimp mortality

Mortality obtained based on the results of ethyl acetate extract was presented in Table 3. Mortality was classified as strong from the results of each concentration including 2000 μg/mL (90%), 1000 μg/mL (70%), 500 μg/mL (47%), 250 μg/mL (30%), 100 μg/mL (20%), 50 μg/mL (3%), and negative control (0%). This mortality result was higher than methanol extract.

Mortality obtained based on the results of methanol extract was presented in Table 4. Mortality was classified as strong too from the results of each concentration including 2000 μg/mL (73%), 1000 μg/mL (57%), 500 μg/mL (43%), 250 μg/mL (27%), 100 μg/mL (7%), 50 μg/mL (3%), and negative control (0%). This mortality result was lower compared to ethyl acetate extract.

Mortality percentage at concentration 1000 μg/mL was 57%. This value was enough to indicate that the polar extract of *A. marina* leaves also had toxic activity. However, the toxic level of methanol extract was still less strong when compared to its semi-polar extract because, at a concentration of 1000 μg/mL, there was a difference of 13%.

3.4. Determination of LC$_{50}$

Based on this study, ethyl acetate extract resulted in a 454 μg/mL concentration with a value of R^2 (0.96815). In comparison, methanol extract resulted in a 740 μg/mL concentration with a value of R^2 (0.990497) (Table 5). This value depended on the mortality rate of 50% on each extract, as presented in Tables 3 and 4.

In BSLA, the toxicity classification of ethyl acetate extract was moderate toxic, and methanol extract was weak toxic (Table 5). These results were in line with the mortality ability shown by both types of extracts. Compared to methanol extract, ethyl acetate extract of *A. marina* leaves may have more potential bioactive compounds.

3.5. Total phenol determination and preliminary phytochemical of ethyl acetate extract

This study obtained a quantitative phenol value of 1.3205 mg GAE/g from the ethyl acetate extract (Table 6). The total phenol of phenolic compounds was related to the equivalent value of gallic acid.

Based on the results of this study, the extract of ethyl acetate leaves *A. marina* contains a group of bioactive compounds that included saponins, flavonoids and steroids (Table 7). This preliminary phytochemical test could not detect the presence of a group of tannins, alkaloids and triterpenoids compounds (Fig. 2).

3.6. GC−MS analysis of ethyl acetate extract

GC−MS chromatogram for retention time difference was presented in (Fig. 3). The characteristics of chemical compounds

Table 3
Mortality percentage of ethyl acetate extract.

Concentration (μg/mL)	Initial number of *A. salina*	*A. salina*		% Mortality
		Dead	Alive	
2000	30	27	3	90
1000	30	21	9	70
500	30	14	16	47
250	30	9	21	30
100	30	6	24	20
50	30	1	29	3
0	30	0	30	0

Table 4
Mortality percentage of ethyl acetate extract.

Concentration (μg/mL)	Initial number of *A. salina*	*A. salina*		% Mortality
		Dead	Alive	
2000	30	22	8	73
1000	30	17	13	57
500	30	13	17	43
250	30	8	22	27
100	30	2	28	7
50	30	1	29	3
0	30	0	30	0

Table 5
Determination and classification of LC$_{50}$.

Sample extract	Linear regression			LC$_{50}$ (μg/mL)	Category
	a	b	R^2		
Ethyl acetate extract	1.786805	0.250794	0.96815	454	Moderate
Methanol extract	1.586076	0.449192	0.990497	740	Weak

Table 6
Determination of total phenol of ethyl acetate extract.

Sample	Sample condition	Unit	Phenol
Ethyl acetate extract	Green crude extract	mg GAE/g	1.3205

Table 7
Bioactive compound groups in the phytochemical test of ethyl acetate extract.

Phytochemical	Analysis results	Analysis type
Tannins	−	Qualitative
Saponins	+	Qualitative
Flavonoids	+	Qualitative
Alkaloids	−	Qualitative
Steroids	+	Qualitative
Triterpenoids	−	Qualitative

interpreted by peaks were identified and characterized by comparing the mass spectrum with the Wiley 7 library. Analysis of compounds with GC−MS analysis revealed the presence of compounds as many as fifty peaks (Table 8). Compounds that had a total area large enough or referred to as major compounds as many as nineteen compounds include 2-Cyclopentene-1-undecanoic acid ($C_{16}H_{28}O_2$), Triethylene glycol monohexyl ether ($C_{12}H_{26}O_4$), 2-Undecenyl acetate ($C_{13}H_{24}O_2$), 2,6-Octadiene 4,5-dimethyl ($C_{10}H_{18}$), Decanoic acid, 1-ethylethyl ester ($C_{13}H_{26}O_2$), 11-Tetradecen-1-ol, acetate ($C_{16}H_{30}O_2$), Estran-3-one, 17-(acetyloxy)-2-methyl-, (2.alpha.,5.alpha.,17.beta.) ($C_{21}H_{32}O_3$), 1,5,9-Cyclododecatriene, 1,5,9-trimethyl ($C_{15}H_{24}$), Urs-12-en-28-ol ($C_{30}H_{50}O$), Neomenthyl acetate ($C_{12}H_{22}O_2$), Limonene Dioxide 2 ($C_{10}H_{16}O_2$), Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.) ($C_{27}H_{46}O$), Veridiflorol ($C_{15}H_{26}O$), 1-(Methylencyclopropyl)-ethanol ($C_6H_{10}O$), 4-Cyclopentene-2,2-D2-1,3-diol trans ($C_5H_6D_2O_2$), Thiocytosine ($C_4H_5N_3S$), 2-Benzyloxyethylamine ($C_9H_{13}NO$), Heptanoic acid ($C_7H_{14}O_2$), Cyclopentaneundecanoic acid, methyl ester ($C_{17}H_{32}O_2$), while the minor compounds as many as twenty two compounds included Tetradecane ($C_{14}H_{30}$), Hexane 1-bromo ($C_6H_{13}BR$), Kauren-19-yl-acetate ($C_{22}H_{34}O_2$), l-Limonene ($C_{10}H_{16}$), Butane 2-iodo-3-methyl ($C_5H_{11}I$), 2,7,13-Tetradecatriene ($C_{14}H_{24}$), 2-Cyclopentene-1-undecanoic acid ($C_{16}H_{28}O_2$), Linalyl acetate ($C_{12}H_{20}O_2$), 3-n-Hexyl-.delta.9-tetrahydrocannabinol ($C_{22}H_{32}O_2$), Cyclonon-4-ynone ($C_9H_{12}O$), Heptanoic acid ($C_7H_{14}O_2$), Nerol ($C_{15}H_{26}O$), Butane 1-chloro-3-methyl ($C_5H_{11}CL$), O-Decylhydroxylamine ($C_{10}H_{23}NO$), Decane 2-methyl ($C_{11}H_{24}$), 5-Methyloctene-1 (C_9H_{18}), 6,9,12-Octadecatrien-1-ol ($C_{18}H_{32}O$), 2-Butenethioic acid, s-[2-(acetylamino)ethyl] ester ($C_8H_{13}NO_2S$), 2-Decenoic acid methyl ester ($C_{11}H_{20}O_2$), Methyl dodecadienoate ($C_{13}H_{22}O_2$), 3,4-Hexanediol 2,5-dimethyl, ($C_8H_{18}O_2$), Hydroperoxide heptyl ($C_7H_{16}O_2$).

GC−MS chromatograms were peaks with different time retention ranges that translated as bioactive compound properties. The total area revealed the quantitative number of bioactive compounds detected and interpreted into percent units. The information of compound names became important data for identified groups of bioactive compounds. Based on the detected peak that the major group of compounds included groups of fatty acids, phenols, terpenoids, alkaloids, alcohols, hydrocarbons and minor groups of cannabinoids and amines (Table 9).

4. Discussion

The Tanjung Api-Api area is in the eastern coastal region of South Sumatra, known as the largest area of mangrove vegetation in western Indonesia. In this region grew several mangrove genera such as

Fig. 2. Mangrove *A. marina* at the sampling site, (A) leaf; (B) stem and root.
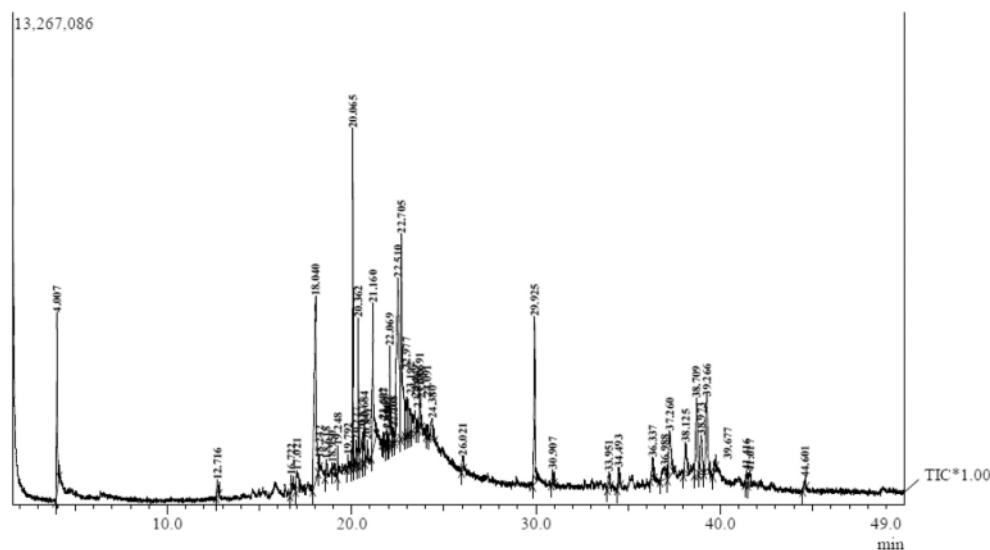
Fig. 3. GC–MS chromatogram of ethyl acetate extract.

Avicennia, Rhizophora, Sonneratia, Bruguiera and *Nypa* based on direct observations and previous research (Verheugt et al., 1991). Muddy and tidal-influenced environmental conditions are perfect for mangrove habitats. In addition, the surface temperature of its waters includes optimal conditions for biota life (Saputra et al., 2021). Rozirwan et al. (2021b) stated that the source of nutrients and anthropogenic affect the environmental quality of estuary waters. Mangrove *A. marina* was found in the intertidal zone near the port which is a source of waste from anthropogenic activities. The uniqueness of this habitat has created a new study on the characteristics of the phytochemical profile of *A. marina* in various habitats. Previous studies have shown that *Avicennia* mangroves have better phytochemical characteristics in relation to pollutant-induced sites. Based on studies at different sites with the characteristics of heavy metal induction, there was a significant correlation between the increase in the content of sediment pollutants (potentially toxic metals) of *A. marina* and *Avicennia sp.* with its biochemical ability as a plant detoxification mechanism (Aljahdali and Alhassan, 2020; Bakshi et al., 2018; Ghosh et al., 2021).

Extraction of bioactive compounds *A. marina* in this study used multilevel extraction to improve the quality of the extracted compounds based on their polarity group (Akbar et al., 2021). Multilevel maceration uses solvents from low polarity values to high polarity values. Based on this, multilevel maceration is very effective and accurate for exploring its compounds' bioactivity (Gori et al., 2021).

According to Table 3, *A. marina* leaves experienced 68% depreciation after being dried by the indirect sunlight method. Another study showed a depreciation value of almost the same at 70.59% (Puspitasari et al., 2018). Table 3 showed that the weight of the extract obtained was 15.98% in ethyl acetate solvents and 6.49% in methanol solvents. The value was generated from the initial powder weight of 100 g during the maceration process. Other studies also showed methanol solvent extracts to be 3.8% lower than semi-polar solvents (Manilal et al., 2009). Ethyl acetate extract is more than methanol extract based on multilevel extraction methods. That suggests that ethyl acetate solvents can dissolve more bioactive compounds in polar and non-polar properties (Hamzah et al., 2021).

Mortality rates of ethyl acetate extract and methanol leaves *A. marina* can be seen in Tables 4 and 5. Based on these results, it was found that ethyl acetate extract has a higher mortality rate than methanol extract. Based on the classification by De Alencar et al.

(2014), the mortality group is divided into three groups: more than 50% categorized as nontoxic, between 50 to 75% categorized as weak toxic, and between 75 to 100% categorized as highly toxic. The LC_{50} value of both extracts indicated a value of below 1000 μg/mL. LC_{50} of ethyl acetate extract results were more potent than methanol extract with moderate toxic category based on toxicity category (Nguta et al., 2012). This toxicity test showed that there are bioactive compounds that are suspected to be more toxic in ethyl acetate solvents. The structure of bioactive compounds in ethyl acetate extract contains significant compounds such as 2-Cyclopentene-1-undecanoic acid, 2-Undecenyl acetate, 1-Methylethyl ester, 11-Tetradecen-1-ol acetate, 1,5,9-Cyclododecatriene 1,5,9-trimethyl that had been reported to have toxic activity against living cells (Jia et al., 2019; Sandhya et al., 2016).

The study of the toxicity activity of bioactive compounds is important as data on the exploration of the potential of natural material drugs (Singh et al., 2021). In traditional and modern science, *A. marina* leaves have not been directly utilized in new products for human needs. The content of bioactive compounds in mangrove species is very diverse, as evidenced by the peak detected on chromatograms (Fig. 3). Based on Gajula et al. (2020), mangrove leaf parts have the most bioactive compounds among other parts of plants. In addition to the group of bioactive compounds, the leaves have various other chemicals such as amino acids, vitamins and minerals that are useful for the growth of small organisms that live in mangrove environments (Vinoth et al., 2019). Bioactive compounds can be found in phenolic groups of compounds (Andreu et al., 2018). The phenol compound found in the study was 2,6-Octadiene 4,5-dimethyl (5.26%). Other bioactive compounds found in the alkaloid group were Thiocytosine (1.8%), 2-Benzyloxyethylamine (1.46%), and O-Decylhydroxylamine (0.43%). Terpenes group compounds are found quite a lot in some derivatives. Monoterpenes included Neomenthyl acetate (2.99%), Limonene dioxide 2 (2.7%), l-Limonene (0.68%), and Linalyl acetate (0.54%). Sesquiterpenes included 1,5,9-Cyclododecatriene 1,5,9-trimethyl (4.37%), Veridiflorol (2.11%) and Nerol (0.46%). Diterpenes, triterpenes and steroids found only one compound included Kauren-19-yl-acetate (0.73%), Urs-12-en-28-ol (3.14%), and Cholestane 4,5-epoxy-(4.alpha.,5.alpha.) (2.43%). Derived compounds from these groups have been identified in thousands of compounds, and new bioactive compounds are made (Pereira et al., 2016). Derivatives of phenol compounds with characteristics of one or two hydroxy

Table 8
Proposed peak order, retention time, area, concentration, compound name and molecular formula.

Peak#	R. time	Area	Conc.%	Name of compounds	Molecular formula
1	4.007	13,462,905	3.93	Carbon dioxide	CO_2
2	12.716	1,583,492	0.46	Nerol	$C_{15}H_{26}O$
3	16.722	1,812,107	0.53	Cyclonon-4-Ynone	$C_9H_{12}O$
4	17.021	5,009,458	1.46	2-Benzyloxyethylamine	$C_9H_{13}NO$
5	18.040	37,031,626	10.81	Triethylene glycol monohexyl ether	$C_{12}H_{26}O_4$
6	18.242	1,389,158	0.41		
7	18.618	1,998,600	0.58	2-Cyclopentene-1-undecanoic acid	$C_{16}H_{28}O_2$
8	18.950	1,838,059	0.54	Linalyl acetate	$C_{12}H_{20}O_2$
9	19.248	1,289,280	0.38	5-methyloctene-1	C_9H_{18}
10	19.792	1,612,959	0.47	Heptanoic acid	$C_7H_{14}O_2$
11	20.065	16,005,354	4.67	11-Tetradecen-1-ol, acetate, (Z)	$C_{16}H_{30}O_2$
12	20.227	2,747,488	0.80	11-Tetradecen-1-ol, acetate, (Z)	$C_{16}H_{30}O_2$
13	20.362	10,255,358	2.99	Neomenthyl acetate	$C_{12}H_{22}O_2$
14	20.577	6,365,770	1.86	1-(Methylencyclopropyl)-ethanol	$C_6H_{10}O$
15	20.684	3,678,361	1.07	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$
16	20.851	2,096,771	0.61	Butan, 2-iodo-3-methyl-	C_5H_{11}
17	21.160	17,547,012	5.12	Decanoic acid, 1-methylethyl ester	$C_{13}H_{26}O_2$
18	21.692	617,857	0.18	Hydroperoxide, heptyl	$C_7H_{16}O_2$
19	21.767	642,751	0.19	3,4-Hexanediol, 2,5-dimethyl	$C_8H_{18}O_2$
20	21.909	942,573	0.28	2-Decenoic acid, methyl ester	$C_{11}H_{20}O_2$
21	21.980	845,060	0.25	Methyl dodecadienoate	$C_{13}H_{24}O_2$
22	22.069	9,245,099	2.70	Limonene dioxide 2	$C_{10}H_{16}O_2$
23	22.200	282,797	0.08		
24	22.268	1,528,231	0.45		
25	22.510	45,661,450	13.32	2-Cyclopentene-1-undecanoic acid	$C_{16}H_{28}O_2$
26	22.705	25,337,975	7.39	2-Undecenyl acetate	$C_{13}H_{26}O_2$
27	22.977	6,200,635	1.81	4-Cyclopentene-2,2-D2—1,3-diol, trans-	$C_5H_6D_2O_2$
28	23.058	4,104,455	1.20	Hexane	C_6H_{14}
29	23.192	4,958,943	1.45		
30	23.449	6,161,600	1.80	Thiocytosine	$C_4H_5N_3S$
31	23.575	1,040,455	0.30	2-Butenethioic acid, s-[2-(acetylamino)ethyl] ester	$C_8H_{13}NO_2S$
32	23.691	4,921,174	1.44	Heptanoic acid	$C_7H_{14}O_2$
33	24.091	1,192,786	0.35	6,9,12-Octadecatrien-1-ol	$C_{18}H_{32}O$
34	24.380	4,452,957	1.30		
35	26.021	1,851,758	0.54	3-n-Hexyl-.delta.9-tetrahydrocannabinol	$C_{22}H_{32}O_2$
36	29.925	18,041,587	5.26	2,6-Octadiene, 4,5-dimethyl	$C_{10}H_{18}$
37	30.907	1,342,743	0.39	Decane, 2-methyl	$C_{11}H_{24}$
38	33.951	2,320,675	0.68	l-Limonene	$C_{10}H_{16}$
39	34.493	2,821,804	0.82	1-Bromohexane	$C_6H_{13}Br$
40	36.337	3,214,441	0.94	Tetradecane	$C_{14}H_{30}$
41	36.988	4,778,893	1.39		
42	37.260	10,756,069	3.14	Urs-12-en-28-ol	$C_{30}H_{50}O$
43	38.125	7,245,643	2.11	Veridiflorol	$C_{15}H_{26}O$
44	38.709	14,988,168	4.37	1,5,9-Cyclododecatriene, 1,5,9-trimethyl	$C_{15}H_{24}$
45	38.973	8,335,532	2.43	Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.)	$C_{27}H_{46}O$
46	39.266	15,490,576	4.52	Estran-3-one, 17-(acetyloxy)—2-methyl-, (2.alpha.,5.alpha.,17.beta.)	$C_{21}H_{32}O_3$
47	39.677	1,540,005	0.45	1-Chloro-3-methylbutane	$C_5H_{11}CL$
48	41.416	2,103,040	0.61	2,7,12-tetradecatriene	$C_{14}H_{24}$
49	41.617	2,506,908	0.73	Kauren-19-yl-acetate	$C_{22}H_{34}O_2$
50	44.601	1,476,784	0.43	O-Decylhydroxylamine	$C_{10}H_{23}NO$
Total		342,675,182	100		

groups such as flavonoids, tannins, phenolic acids, and simple phenols are important as anticancer, antiviral, anti-inflammatory (Nour et al., 2016), antioxidant, antimicrobial (Eswaraiah et al., 2020) and antidiabetic (Rasouli et al., 2020). Alkaloids have nitrogen atoms in their chemical structure, giving toxic biological effects to some living organisms (Erharuyi et al., 2014) and small insects (Ji et al., 2018). Terpenes have different uniqueness. This compound has a carbon atomic structure with a considerable amount of five, so that it is divided into hemiterpenes C_5, monoterpenes C_{10}, sesquiterpenes C_{15}, diterpenes C_{20}, sesterterpenes C_{25}, triterpenes C_{30}, tetraterpenes C_{40}, and polyterpenes C_n (n more than forty) (Böttger et al., 2018).

The toxicity activity of the bioactive group of compounds has been studied gradually to find superior bioactivity potential. Several groups of pure compounds that were successfully identified based on GC—MS chromatography in leaf *A. marina* of this study came from major groups of fatty acids, phenols, terpenoids, alkaloids, alcohols,

hydrocarbons and minor groups of cannabinoids and amines. The group of fatty acids consists of cyclopentenyl fatty acids, ester fatty acids, lauric acids, monounsaturated fatty acids, heptanoic acids, and simple fatty acids. Cyclopentenil fatty acid compound is 2-Cyclopentene-1-undecanoic acid that is reported as antibiotic (Almahli, 2017). Ester fatty acids are 2-Undecenyl acetate reported as antimicrobial (Naulidia et al., 2020) and 2-Decenoic acid methyl ester as flavouring agent (Mohansrinivasan et al., 2015). Lauric acid was 1-Methylethyl ester reported as antibacterial, antifungal, antiviral and antioxidant agent (Sandhya et al., 2016), and Methyl dodecadienoate had not been reported the bioactivity. The unsaturated fatty acid compound is 11-Tetradecen-1-ol acetate, an antimicrobial and antioxidant (Ge et al., 2019). Heptanoic acid is hydroperoxide heptyl that is reported to be antimicrobial (Mohini et al., 2013). Simple fatty acids are Heptanoic acid reported as antimicrobial (Mohini et al., 2013) and Cyclopentaneundecanoic acid methyl ester as antimicrobial (Kumar et al.,

Table 9
Information of compound names, chemical structures, mass spectral and compound bioactivities.

Bioactive compounds with their structures, mass spectral and bioactivities
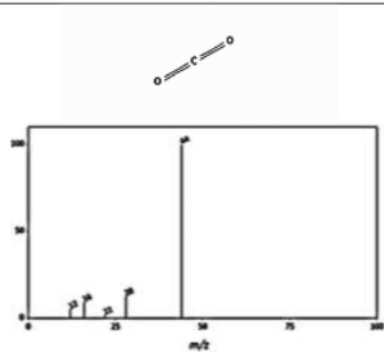
Carbon dioxide
Not reported
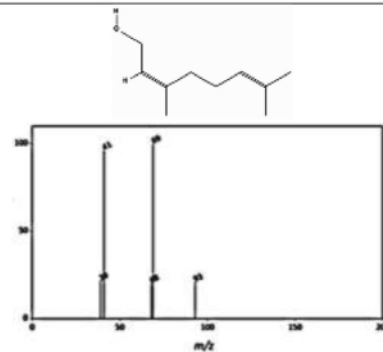
Nerol
Anticancer
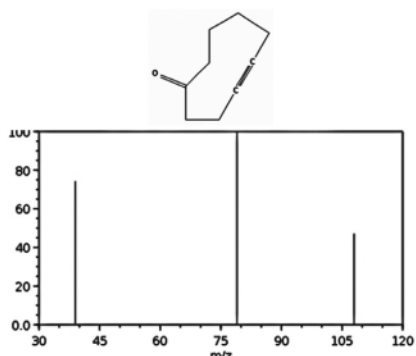
Cyclonon-4-Ynone
Not reported
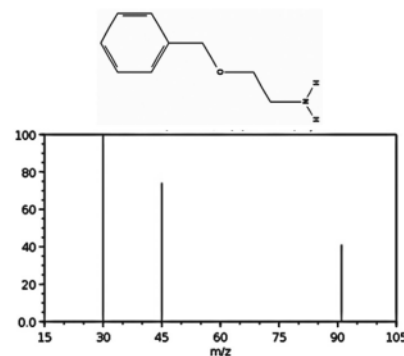
2-Benzyloxyethylamine
Not reported
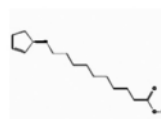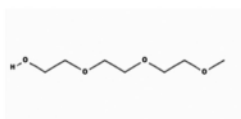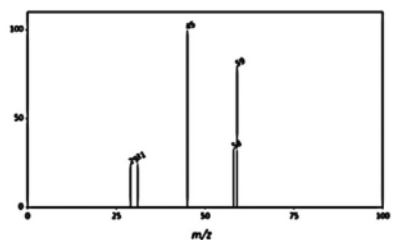
Triethylene glycol monohexyl ether
Not reported
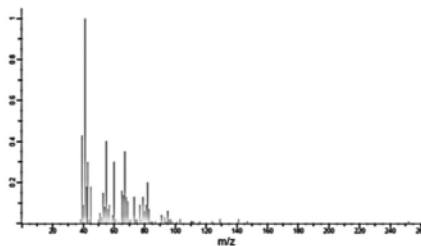
2-Cyclopentene-1-undecanoic acid
Antibiotic
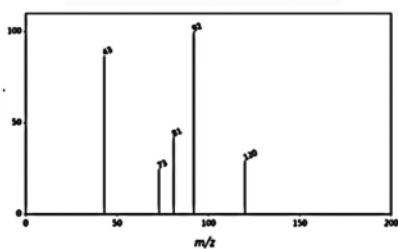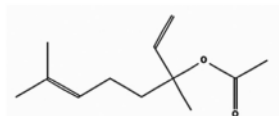
Linalyl acetate
Anticancer
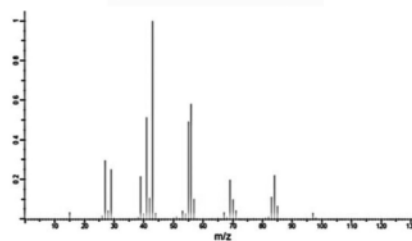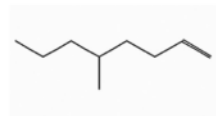
5-Methyloctene-1
Not reported
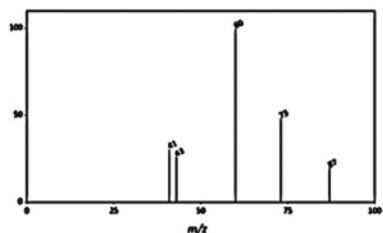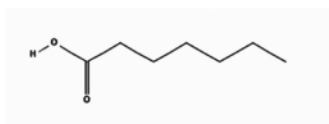
Heptanoic acid
Antimicrobial
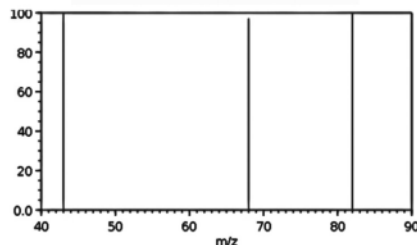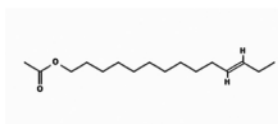
11-Tetradecen-1-ol, acetate, (Z)
Antimicrobial, antioxidant
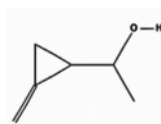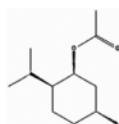
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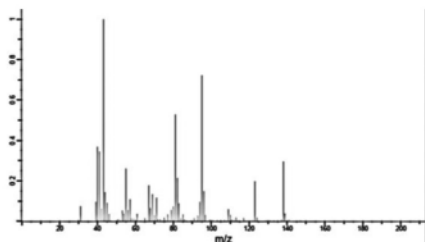
Neomenthyl acetate
Antioxidant
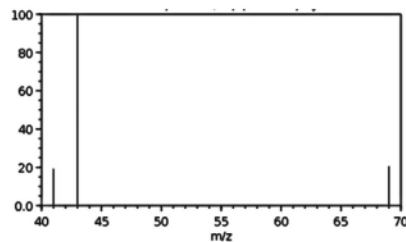
1-(Methylencyclopropyl)-ethanol
Anticancer
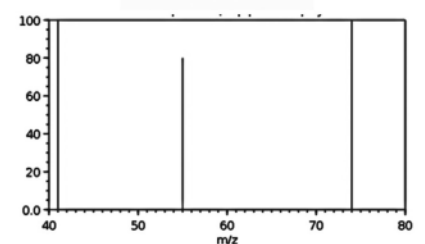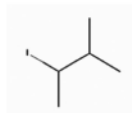
Cyclopentaneundecanoic acid, methyl ester
Antimicrobial
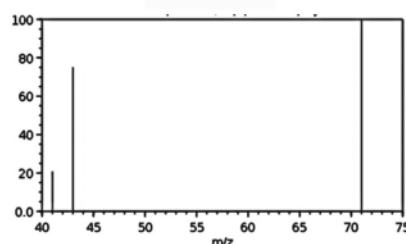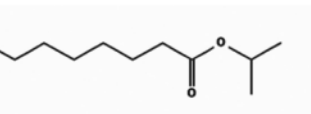
Butan, 2-iodo-3-methyl
Not reported
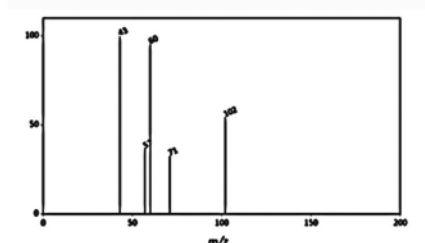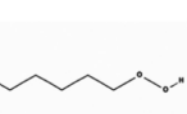
Decanoic acid, 1-methylethyl ester
Antioxidant, Antibacterial, antifungal,
antiviral
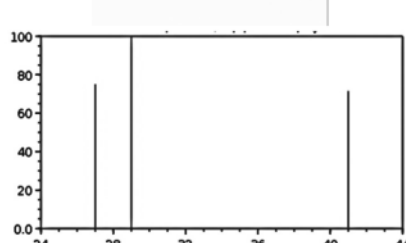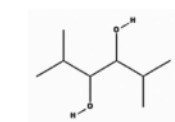
Hydroperoxide, heptyl
Antimicrobial
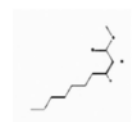
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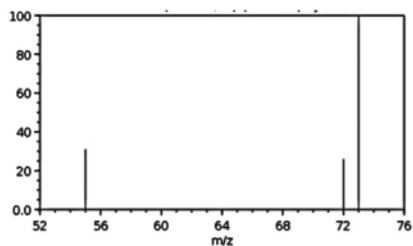
3,4-Hexanediol, 2,5-dimethyl

Not reported
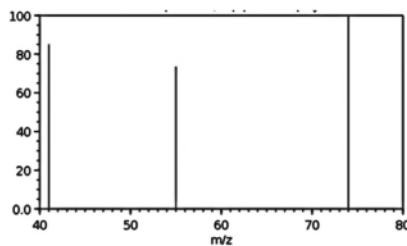
2-Decenoic acid, methyl ester

Flavouring agent
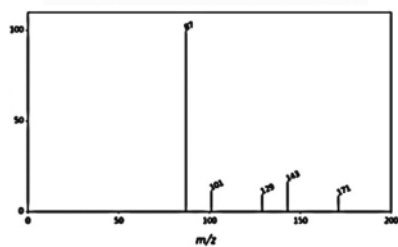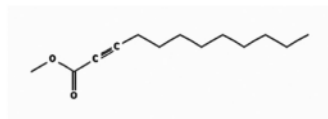
Methyl dodecadienoate

Not reported
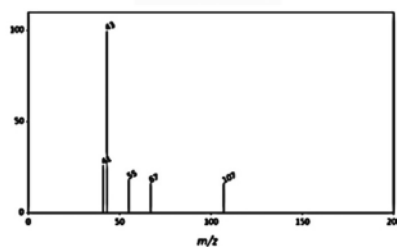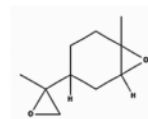
Limonene dioxide 2

Anticancer
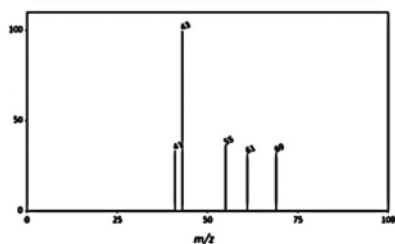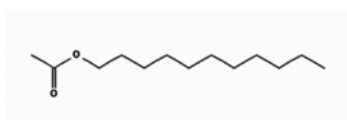
2-Undecenyl acetate

Antimicrobial
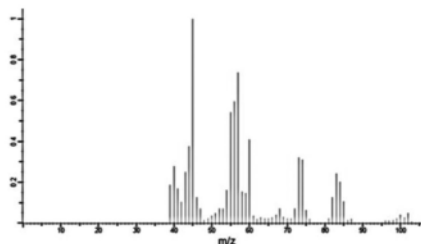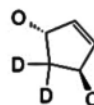
4-Cyclopentene-2,2-D2-1,3-diol, trans
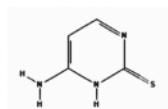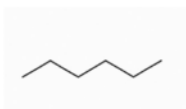
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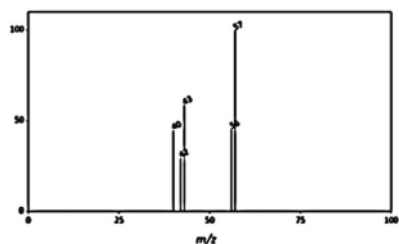
Hexane
Not reported
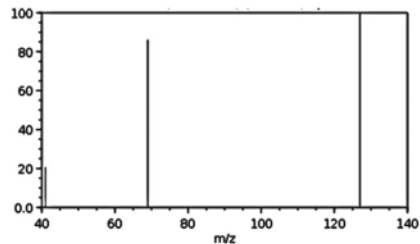
Thiocytosine
Antitumor, Antiviral
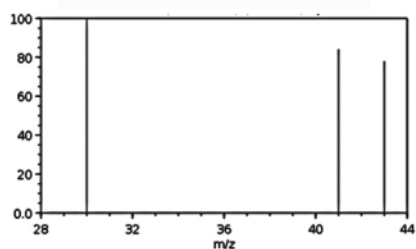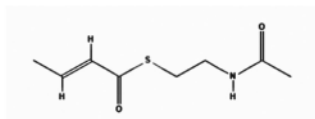
2-Butenethioic acid, s-[2-
(acetylamino)ethyl] ester
Antioxidant
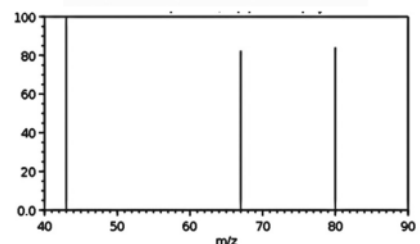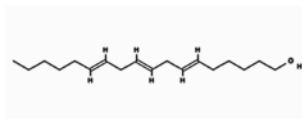
6,9,12-Octadecatrien-1-ol
Anticancer
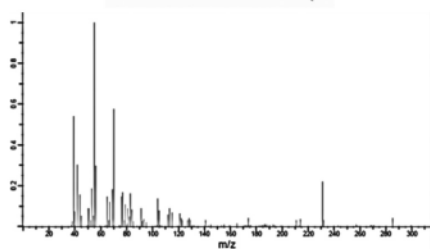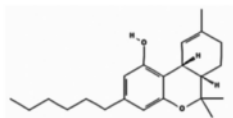
3-n-Hexyl-.delta.9-tetrahydrocannabinol
Anticancer
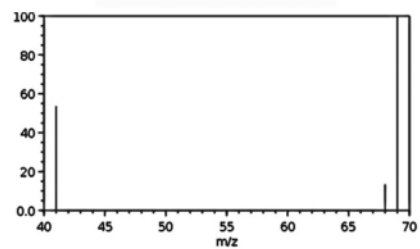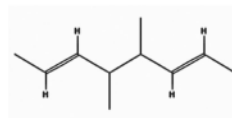
2,6-Octadiene, 4,5-dimethyl
Not reported

(continued)
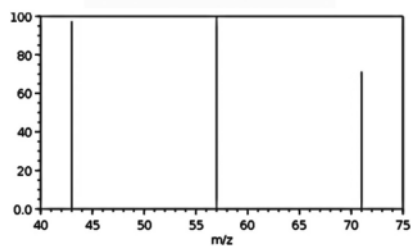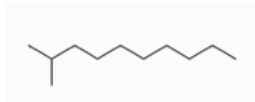
Decane, 2-methyl
Antimicrobial, anti-inflammatory
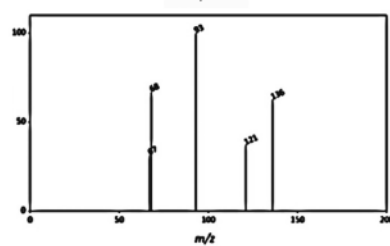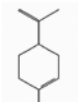
l-Limonene
Anticancer
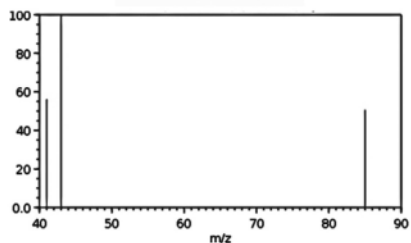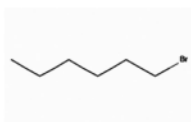
1-Bromohexane
Antimicrobial
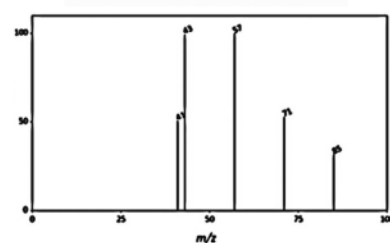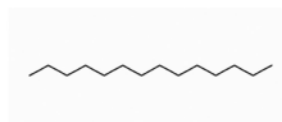
Tetradecane
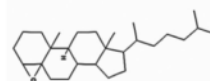Antimicrobial, anticancer
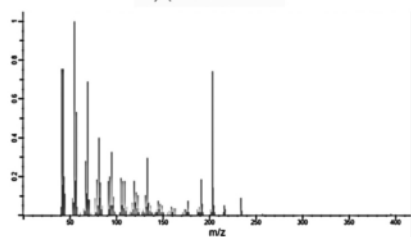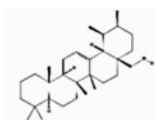
Urs-12-en-28-ol
Anticancer, antitumor
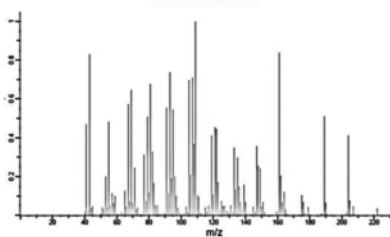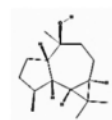
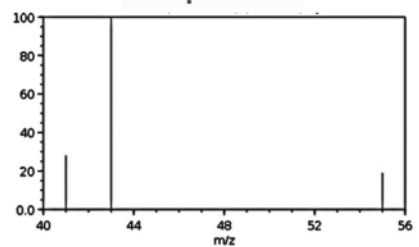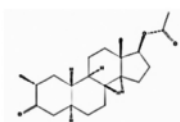Veridiflorol
Antibacterial, antiinflammatory, antioxidant
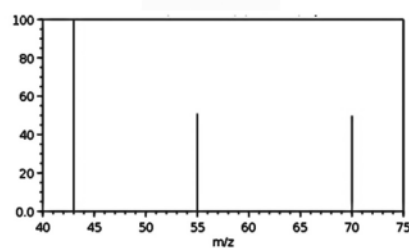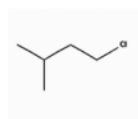
(*continued*)
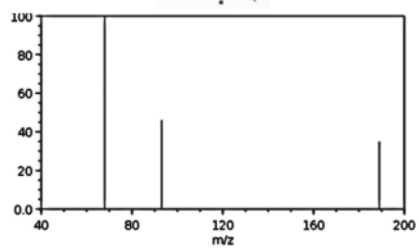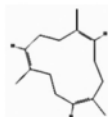
1,5,9-Cyclododecatriene, 1,5,9-trimethyl
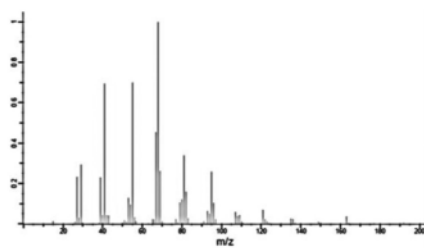
Anticancer
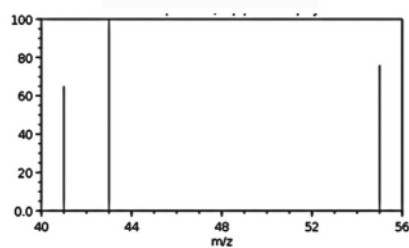
Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.)
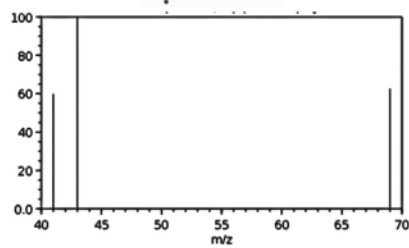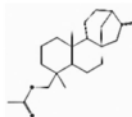
Antiviral

Estran-3-one, 17-(acetyloxy)-2-methyl-,
(2.alpha.,5.alpha.,17.beta.)

Antioxidant

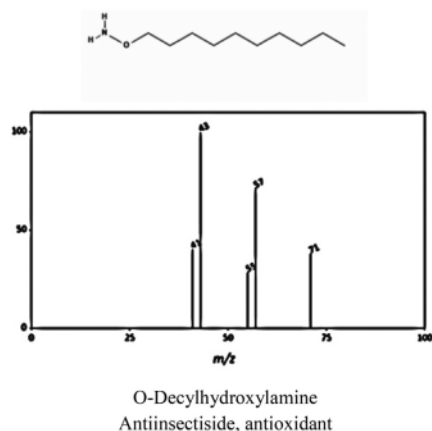1-Chloro-3-methylbutane

Not reported

2,7,12-tetradecatriene

Not reported

Kauren-19-yl-acetate

Antiviral

(*continued*)

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O-Decylhydroxylamine
Antiinsectiside, antioxidant

*Graph and 2D structure image source: (Kim et al., 2021).

2019). Phenol group is 2,6-Octadiene, 4,5-dimethyl that had not been reported the bioactivity (Souza et al., 2021). Terpenes groups included monoterpenes, sesquiterpenes, diterpenes, triterpenes, and steroids. Monoterpenes include Neomenthyl acetate that reported as antioxidant and antimicrobial (Desam et al., 2019; Shahbazi, 2017), Limonene dioxide 2 as anticancer (Molavi et al., 2020), l-Limonene as anticancer, flavouring additives and aromatherapy (Araújo-Filho et al., 2021; Jongedijk et al., 2016), and Linalyl acetate as anticancer (Gezici, 2018). Sesquiterpenes include 1,5,9-Cyclododecatriene 1,5,9-trimethyl that are reported as anticancer (Jia et al., 2019), Veridiflorol as antibacterial, anti-inflammatory and antioxidant (Trevizan et al., 2016) and Nerol as an anticancer (Wardana et al., 2019). Diterpenes, triterpenes and steroids found only one compound including Kauren-19-yl-acetate that reported as antiviral (Gaspar-Marques et al., 2008), Urs-12-en-28-ol as antitumor and anticancer (Chudzik et al., 2015), and Cholestane 4,5-epoxy-(4.alpha.,5.alpha.) as antiviral (Dembitsky et al., 2018). The alkaloids group include Thiocytosine as antitumor and antioxidant (Sahin et al., 2021), O-Decylhydroxylamine as anti-insecticide and antioxidant (Zaheer et al., 2021), and 2-Benzyloxyethylamine had not been reported the bioactivity. The alcohol groups include 1-(Methylencyclopropyl)-ethanol and 6,9,12-Octadecatrien-1-ol which are reported as anticancer (Higgins et al., 2009; Stading et al., 2021), Triethylene glycol monohexyl ether as a textile industry material (Yu et al., 2020). 4-Cyclopentene-2,2-D2–1,3-diol trans, Cyclonon-4-ynone and 3,4-Hexanediol 2,5-dimethyl had not been reported the bioactivity. Hydrocarbon groups include Tetradecane were reported as antimicrobial and anticancer (Choo et al., 2001), Hexane 1-bromo as an antimicrobial (El Shafay et al., 2016), Decane 2-methyl as antimicrobial and anti-inflammatory (Yakubu et al., 2018), Butane 2-iodo-3-methyl, 2,7,13-Tetradecatriene, Butane 1-chloro-3-methyl, and 5-Methyloctene-1 had not been reported the bioactivities. Minor compounds cannabinoids are 3-n-Hexyl-.delta.9-tetrahydrocannabinol reported as anticancer (Massi et al., 2013), and amines is 2-Butenethioic acid, s-[2-(acetylamino)ethyl] ester reported as antioxidant (Paul and Snyder, 2019).

The phytochemical profile of A. marina leaf extract resulting in this study has been very diverse when viewed from the group of identified compounds. These results may be due to the many environmental influences on the habitat of A. marina. Intrinsic factors in mangrove life found in this study are generally explained by Friess et al. (2012), that ecosystems in the estuary area get environmental influences including changes in temperature, salinity, humidity, water level, nutrient input periodically according to high and low tide conditions. Furthermore, extrinsic factors can include the input of pollution materials in the water and air (Chakraborty et al., 2021). Although individuals get intense levels of stress on an ecological scale, biological processes will produce more and more bioactive compounds. Sepúlveda-Correa et al. (2021) reported that water salinity stress conditions could support the formation of antimicrobial compounds in mangrove forests. In addition, Chan et al. (2021) reported that A. marina species collected from polluted sites have better antimicrobial activity than non-polluted sites. That suggests that more and more environmental influences will trigger compound biosynthesis in reaction to environmental conditions.

5. Conclusions

In conclusion, the toxicity screening results of the brine shrimp lethality assay method of mangrove leaf extract A. marina showed more lethal at semi-polar levels. The identification of bioactive compounds produced good qualitative and quantitative data because it contained groups of potentially bioactive compounds from major groups of fatty acids, phenols, terpenoids, alkaloids, alcohols, hydrocarbons, and minor groups of cannabinoids and amines. Further development is needed to isolate bioactive compounds and in vivo bioassay testing to find potential sources of the latest drugs. As far as we know, this study is the first report of a profile of mangrove A. marina phytochemicals that are biological systems affected by many extrinsic factors. The results of our study are expected to be one of the information for the development of the search for bioactive compounds from natural materials taking into account biological fundamental properties on a more realistic ecological scale.

Declaration of Competing Interest

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

Acknowledgments

References

Acharya, S., Patra, D.K., Pradhan, C., Mohapatra, P.K., 2020. Anti-bacterial, anti-fungal and anti-oxidative properties of different extracts of Bruguiera gymnorrhiza L. (Mangrove). Eur. J. Integr. Med. 36, 101140. https://doi.org/10.1016/j.eujim.2020.101140.

Phytochemical profile

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