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Insecticidal Activity and Phytochemical Profiles of *Avicennia marina* and *Excoecaria agallocha* Leaves Extracts

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Abstract

Toxic bioactive compounds can be obtained from various mangrove plants that have the potential to be developed in agriculture as bioinsecticides. The coast of South Sumatra has relatively abundant Avicennia marina and Excoecaria agallocha vegetation. This study was conducted to analyze the bioactivity of insecticides and the phytochemical profiles of mangrove leaves A. marina and E. agallocha was extracted with methanol, ethyl acetate, and n-hexane from the most toxic extract fraction. Samples were obtained from the Barong River in Sembilang National Park, Banyuasin Regency. The insecticidal activity of the samples was carried out on Gryllus bimaculatus and Tenebrio molitor. Furthermore, the phytochemical profile was analyzed using GC-MS. Based on the results of insecticidal activity on G. bimaculatus, the respective LC_{50} values of A. marina and E. agallocha extracts for the hexane fraction were 12,562 $mg.L^{-1}$ and 15,464 $mg.L^{-1}$, ethyl acetate 9,986 $mg.L^{-1}$ and 10,292 $mg.L^{-1}$, methanol 6,454 $mg.L^{-1}$ and 6,969 $mg.L^{-1}$. Whereas in T. molitor, the LC_{50} values for the hexane fraction were 10,682 $mg.L^{-1}$ and 11,070 $mg.L^{-1}$, ethyl acetate 9,065 $mg.L^{-1}$ and 9,269 $mg.L^{-1}$, methanol 4,799 mg.L-1, and 5,408 $mg.L^{-1}$. The results of GC-MS analysis on the methanol extract of A. marina leaves which contained phytochemical compounds such as alcohol, lauric acid, myristic, linoleic, elaidate, stearate, endogenous, olead, phthalic ester, and siloxane. Based on the insecticide toxicity category, the insecticidal activity of both A. marina and E. agallocha leaves extracts were low and non-toxic. Further research is needed regarding variations in anti-insecticide of mangrove extract measurements in the future studies.

Keywords: *Insecticidal, Mangrove, Phytochemical, Toxicity*

Introduction

One of the main ways to eliminate food and environmental poverty in the world is to make agriculture more environmentally friendly to create a balance between the production of materials for population growth and food security (Zegeye *et al.*, 2022). Problems are the main obstacle to achieving higher crop production (Souto *et al.*, 2021). So far, the control of agricultural pests is still very dependent on synthetic insecticides for land protection and post-harvest crops (Bertomeu-Sánchez, 2019). The use of synthetic insecticides can cause failure in pest control due to unwanted side effects on plants and the rapid evolution of insecticide resistance in agroecosystems (Alfaro-Tapia *et al.*, 2021). It not only impacts plants and the environment, but also humans who are in direct contact with synthetic insecticides that can cause cancer, hypertension, kidney failure, lung damage, and neurological disorders (Krishnamurthy *et al.*, 2020). Therefore, discoveries related to new natural pesticides are urgently needed to prevent damage to crops and not to the environment (Rahman *et al.*, 2021).

The knowledge of scientists about the actual harmful effects of the massive use of synthetic insecticides has increased and is changing the direction of research to find new solutions. The solution that can be given is to look for alternatives to synthetic insecticides that are more natural, environmentally friendly, effective, high efficiency, and have low levels of environmental degradation (Zhu *et al.*, 2022). Alternatives to synthetic insecticides are vegetable insecticides that can be obtained from natural materials such as plants, microorganisms, animals, and certain minerals as natural substances that can control pests with non-toxic and low toxicity mechanisms (De Bernardi *et al.*, 2022). More than 2000 plant species have potential insecticidal activity (Souza *et al.*, 2017).

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Mangrove is one of the plants that have been widely used in the health sector because it has the potential various biological activity from the bioactive compounds it produces (Ulmursida *et al.,* 2017; Sopalun *et al*., 2021). These perfectly vegetated plants grow in intertidal or brackish water and greatly adapt to environmental changes (Abdul-Azeez *et al*., 2022). These natural processes will encourage plants to produce natural compounds in response to environmental changes. These compounds are called secondary metabolites, which are unique and structurally diverse and attract significant attention in organic chemistry and pharmacology (Chen *et al*., 2022). This bioactive compound in mangrove plants has a toxic effect. Toxicity is a reaction caused by natural components extracts that can cause biological damage, including death in the test organism (Rozirwan *et al*., 2022). Toxicity is most often manifested in certain parts of consumption because the toxic constituents in plants generally come from organic compounds and are rarely found in minerals (Elouardi *et al*., 2022). Several researchers have considered the toxic effects of mangroves as potential future drug development, such as raw materials for anti-inflammatory, anticancer, antioxidant, and antimicrobial (Parthiban *et al*., 2021).

The application of secondary metabolites in mangroves to overcome problems in agriculture is an exciting study. One of the existing problems is fighting agricultural pests. Plants with potential for bioactivity usually contain bioactive compounds such as essential oils, fatty acids, alkaloids, glycosides, flavonoids, and esters (Sakul, 2017). These compounds are known to have chemical properties that can affect insects through antifeedant mechanisms, contact toxicity, and reduction of fecundity and fertility (Tak *et al*., 2017). Mangroves of *A. marina* and *E. agallocha* are known to have toxic activity and potential as insecticides. Several previous studies revealed that testing of *A. marina* leaf extract against mosquito larvae revealed a *Aedes aegypti* of 0.164 mg.L^{-1} and *Culex quinquefasciatus* LC_{50} of 0.197 mg.L^{-1} (Karthi *et al*., 2020). Furthermore, tests of *E. agallocha* Pradeepa *et al.* (2015) stating that the toxic methanol and *E. agallocha* leaf extract were tested on *A. aegypti* and *C. quinquefasciatus* larvae, which resulted in 100% mortality. A methanol extract of *E. agallocha* leaves with a concentration of 0.05% was tested against the larval pest *Crocidolomia pavonana*, causing a mortality of 86.25%. Based on these studies, the leaf extract of *A. marina* and *E. agallocha* strongly affect *mosquito* larvae, and *caterpillar* larvae (Melo *et al*., 2015). The toxicity of mangrove leaves of *A. marina* and *E. agallocha* leaves, as evidenced against these test organisms can be predicted to be that the extract is also toxic to test insects, which is greater than larvae. So far, there have been no studies using insects as test animals of Crickets (*Gryllus bimaculatus*) and caterpillars (*Tenebrio molitor*) (Irwan *et al*., 2021). Sembilang National Park which is located on the coast of the Banyuasin regency South Sumatera, is an area that has various types of mangroves (Rozirwan *et al*., 2022a; Rozirwan *et al*., 2022c), including the two most dominating *A. marina* and *E. agallocha*. Research on the insecticide toxicity of mangrove leaves extracts of *A. marina* and *E. agallocha* has never been carried out. This research is an interesting topic to be studied to prove its activity as an insecticide. In the future, its potential can be developed as an alternative source of insecticides from natural materials to replace synthetic insecticides.

Materials and Methods

Samples of *A. marina* and *E. agallocha* leaves were taken in September 2021 at the Barong River, Sembilang National Park, Banyuasin Regency, South Sumatra (Figure 1.). This sampling location had coordinated on 2.160000 S° and 104.896389 E°. the location had also much biodiversity, such as mangrove vegetation, macrozoobenthos community, and migratory birds (Rozirwan *et al*., 2022a; Fitria *et al*., 2023). Mangrove guidebooks referred to (Giesen *et al*., 2007). *E. agallocha* and *A. marina* samples were plucked from the fresh leaves using the purposive sampling method (Rahman *et al*., 2020).

Environmental quality measurement

Environmental parameters were measured at the sampling location. These parameters include temperature, salinity, dissolved oxygen, and acidity (Ramses *et al*., 2020; Almaniar *et al*., 2021). All environmental parameters were measured using portable tools.

Plant maceration and extraction

Stratified maceration had been used in this study with solvents polarity: methanol (polar), ethyl acetate (semi-polar), and hexane (non-polar) (Pontes *et al*., 2020). Mangrove leaves were placed in a glass jar and macerated with the three solvents in a ratio of 1:4 (w/v) the first maceration with hexane for 2 x 24 h and the next were ethyl acetate and methanol. The filtrate was filtered and the solvent was replaced using a new hexane solvent every 24 h (Rozirwan *et al*., 2022b). The final filtrate of each solvent obtained was evaporated using a rotary evaporator at a temperature of 40°C (Rozirwan *et al*., 2023).

Insecticide activity test

The test insects used to refer to (Irwan *et al*., 2021), using crickets (*G. bimaculatus*) and caterpillars (*T. molitor*); each test treatment was ten tails. Furthermore, the concentration of the insecticide test solution modified the research (Khan *et al.*, 2017). Insecticide activity test used stock solution 10,000 mg.L^{-1}. This solution was prepared from 1 g of each fraction and dissolves it in 1 mL of dimethyl sulfoxide, then adding aquades up to 99 mL. The test concentration was 10,000, 8,000, 6,000, 4,000, 2,000 and 1,000 mg.L^{-1}. The jar containing insects was sprayed with 5 mL extract solution (Table 1.).

Gas chromatography-mass spectroscopy analysis

The components of the phytochemical profile contained in the leaf extract of *A. marina* could be analyzed using GC-MS. The data bank in the Wiley 7 library had been used as a comparison data for the results of the analysis graph spectrum (Rozirwan *et al.*, 2022b)

Percentage of yield value and extract weight

The percentage of yield value and extract samples using the formula (1), and formula (2) used for the calculation of the percentage of weight.

$$\%\,\text{Yield value} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (1)$$

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

Percentage of mortality

The mortality categories according to Rozirwan *et al.* (2022b) were non-toxic (50%), moderately toxic (50%-75%), and high toxic (75%-100%). The percentage of deaths was analyzed based on the number of test insects that died after 24 h using the formula (3).

$$\%\,\text{Mortality} = \frac{\text{Number of dead individuals}}{\text{number of test individuals}} \times 10 \quad (3)$$

Percentage of LC_{50}

LC_{50} values were obtained from data analysis of test concentrations, total individual trials, and mortality. There was to know before determining of LC_{50} value was the log 10 concentration test and mortality probit value. The standard linear regression equation was used to analyze LC_{50} values with Microsoft Excel 2019 (4). Based on Rozirwan *et al.* (2022b), LC_{50} value categories included non-toxic (> 1000), weak toxic (500–1000), moderate toxic (100–500), and very toxic (< 100).

$$y = a + bx \quad (4)$$

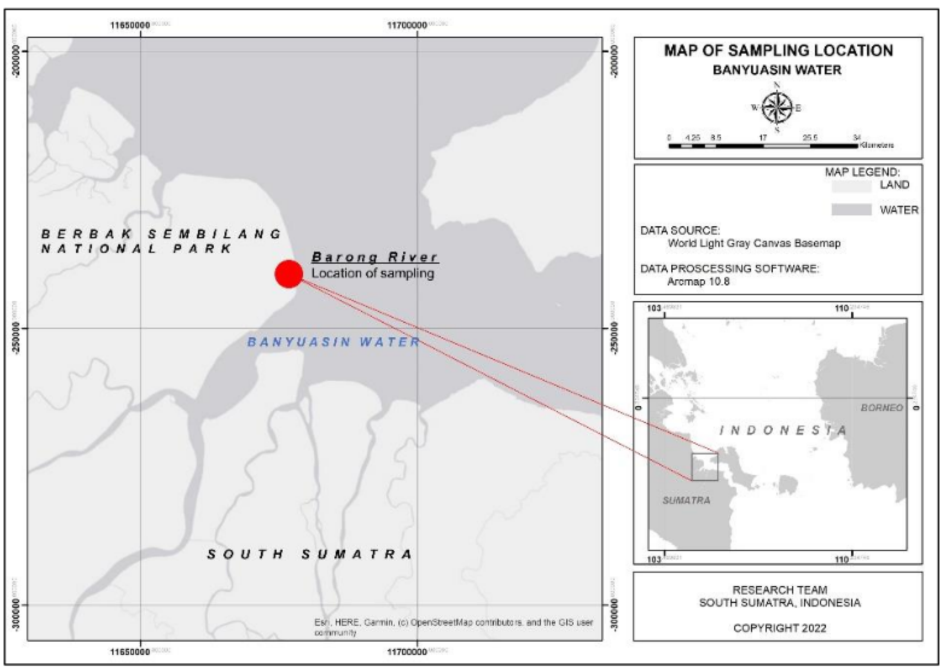


Figure 1. Map of sampling locations for *A. marina* and *E. agallocha*

Results and Discussion

Description of mangrove A. marina and E. agallocha leaves

Leaves morphological observations were done to determine the characteristics between *A. marina* and *E. agallocha* leaves (Figure 2.). Barong Kecil River is one of the areas included in the Sembilang National Park and is located in wetlands, and is dominated by mangrove forests (Ratmoko *et al.*, 2021). In this region, mangrove plants of various genera, such as *E. agallocha* and *A. marina*. Mangrove habitat is very decisive for growth and development such a muddy substrate conditions (Saputra *et al*., 2021) and it was also reported that sedimentary mangroves also has antibacterial potential (Lalitha *et al.*, 2021). A selection of old leaves is more appropriate to be sampled than young leaves. According to Sumartini *et al.* (2022), the type and level of leaf maturity had an influence on phytochemical compounds, such as the old leaves of *Rhizophora* sp. contain more phenolic, and flavonoid compounds that function as antioxidants.

Environmental characteristics

The mangroves *A. marina* and *E. agallocha*, discovered at the sampling site, coexist. Both were associated with other mangrove species and grow on muddy substrates. Ecologically, mangrove growth was influenced by several parameters of the aquatic environment, such as temperature, salinity, pH, and DO water (Table 2.).

Mangrove growth in an area is strongly influenced by environmental conditions. Based on (Table 2.) the parameters of the water quality of the sampling location of this study were still within normal limits, good mangrove growth is in the water salinity does not exceed 34 psu, DO >5 mg.L^{-1}, temperature range of 28-32°C, and pH 7-8.5. The temperature obtained is optimal for mangrove growth because mangroves were intolerant of temperatures below 0 °C and high temperatures, which could cause delays in the tree deposition process (Noor *et al.*, 2015). Salinity is also an important component in the mangrove growth and development system because if there is excessive salt content, it would certainly inhibit the growth and development of mangroves and could even cause death of mangroves (Sudhir *et al.*, 2022). In addition, the pH value obtained was classified as neutral and suitable for the sustainability of mangrove life (Wahyuni *et al.*, 2018). The dissolved oxygen parameter indicates the value that low at low tide and rising on the high tide. Almaniar *et al.* (2021) the quality of environmental parameters in mangrove forests was influenced by nutrient and anthropogenic sources.

Table 1. Preparation of test solution

Concentration (mg.L^{-1})	Extract solution (mL)	Saline water (mL)	Final volume (mL)
10,000	100	0	100
8,000	80	20	100
6,000	75	25	100
4,000	67	33	100
2,000	50	50	100
1,000	50	50	100
0 as control (-)	-	50	100

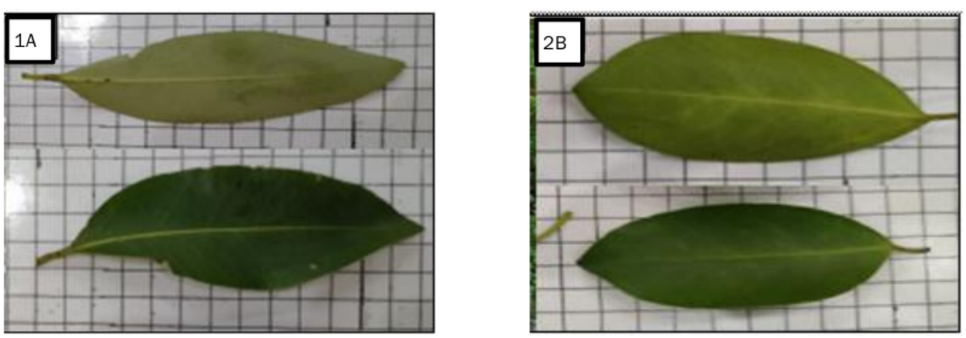


Figure 2. Morphology of mangrove leaves, (1A) *A. marina*, (2B) *E. agallocha*.

Extract characteristics of A. marina and E. agallocha mangrove leaves

The weight loss percentage of the wet and dry sample leaves was 67.9% for the *A. marina* sample and 72.4% for the *E. agallocha* sample (Table 3.). These results indicated that the moisture content of the leaves of *A. marina* was lower than *E. agallocha.*

The removal of the water content in the sample was carried out by drying until the water content was completely lost because the range of compounds contained in the sample would be better if the sample is dry. Meanwhile, the extraction process of *A. marina* and *E. agallocha* leaf samples was carried out using different solvents (Table 4.). The selection of this stratified maceration aims to obtain the best extract results with high toxicity. Maceration with different samples and solvents would certainly affect the value of the weight percentage of the extract obtained. According to Gori *et al*. (2021), graded maceration is very effective and accurate for used in exploration activities for bioactivity compounds.

Different solvents were used in the leaves extraction processes of *A. marina* and *E. agallocha*. The results showed that the weight of the extract produced by the samples of *A. marina* and *E. agallocha* leaves was the highest in the methanol extract at 7.08 g and 5.21 g, respectively. Each type of mangrove extracted using graded solvent had a different yield value (%). This yield value indicated the percentage of material remaining from the extraction and determined the effectiveness of the solvent in attracting and separating bioactive compounds. Based on (Table 6.), which showed the increasingly polar properties of solvents, the higher the percentage yield value. This statement was following the results of the extraction where the methanol extract in polar solvents in *A. marina* was 2.83%, and in *E. agallocha* it was 2.08%. Generally, secondary metabolites of *A. marina* were polar, so the methanol extract percentage was quite large. Based on these properties, the active compound is dissolved in a solvent that has the same polarity (Soedirga *et al.*, 2020).

Determination of mortality

The percentage values of mortality testing of *A. marina* and *E. agallocha* leaves extracts against *G. bimaculatus* (Table 5.) for the methanol, ethyl acetate, and n-hexane fractions, respectively, were at a concentration of 10,000 $mg.L^{-1}$, 8,000 $mg.L^{-1}$, 6,000 $mg.L^{-1}$, 4,000 $mg.L^{-1}$, 2,000 $mg.L^{-1}$, 1000 $mg.L^{-1}$, and negatif control (0 $mg.L^{-1}$).

Table 2. Environmental quality parameters

Environmental quality	Score	Measuring Instrument
Salinity (psu)	20.30	Handrefractometer
Dissolved Oxygen ($mg.L^{-1}$)	9.63	DO/Temp Meter Gondo
Temperature (˚C)	33.00	DO/Temp Meter Gondo
Acidity (pH)	7.50	pH Meter HI 83141

Table 3. Depreciation percentage of weight

Sample leaves	Sample weight (g)		Depreciation percentage (%)	Weight percentage (%)
	Wet	Dry		
A. marina	1,000	321	67.9	32.1
E. agallocha	1,000	276	72.4	27.6

Table 4. Percentage of extract

Sample leaves	Solution	Extract weight (g)		Depreciation percentage (%)	Weight percentage (%)
		Dry powder	Crude extract		
A. marina	Methanol	250	7.08	97.2	2.83
	Ethyl acetate	250	5.23	97.9	2.09
	N-hexane	250	2.63	98.9	1.05
E. agallocha	Methanol	250	5.21	97.9	2.08
	Ethyl acetate	250	4.56	98.2	1.82
	N-hexane	250	3.87	98.5	1.55

A. marina and *E. agallocha* leaves were extracted using graded solvents of methanol, ethyl acetate, and hexane, which were then tested on *G. bimaculatus* with 6 test concentrations showing different mortality for each extract fraction. The methanol fraction showed a high mortality value compared to the ethyl acetate and hexane fractions in both A. marina and *E. agallocha.* Subsequent mortality tests were also carried out on *T. molitor* test insects for leaves extracts of *A. marina* and *E. agallocha* (Table 6.).

Mortality in *T. molitor* was carried out as comparison data on whether there was a significant difference or directly proportional to the mortality of *G. bimaculatus*. Based on the results of the *T. molitor* mortality test for 24 hours of treatment with the same extract and test concentration, the percentage results were directly proportional to the mortality of *G. bimaculatus*. The methanol extract fraction showed the highest mortality compared to the fractions of ethyl acetate and n-hexane. The percentage mortality in *G. bimaculatus* methanol extract with a concentration of 10,000 mg.L^{-1} showed the highest results in both *A. marina* and *E. agallocha* (Table .5). Likewise with *T. molitor* which showed the percentage results were directly proportional to the mortality of *G. bimaculatus* (Table 6). According to (Permatasari *et al*., 2021), the greater the concentration of extracts, the higher the mortality rate and this illustrates that each different concentration affects the percentage of deaths produced. Based on the category of percentage mortality, this study was included in the moderate toxic. Then it was clarified by (Permatasari and Asri, 2020), the standard of botanical pesticides for biological control was stated to be effective toxic if the mortality value was 80%, if the mortality value was 50%, the extract was not effectively used as a vegetable insecticide.

Determination of LC_{50}

The LC_{50} value is basically obtained from linear regression analysis. The LC_{50} value test was carried out to determine the concentration value that was able to cause the death of *G. bimaculatus* insects by 50 % of the total tested insects (Table 7.).

Based on the test results in this study, in addition to testing the *G. bimaculatus*, extracts were also tested on *T. molitor* (Table 8.), it was carried out as comparison data to determine whether there was a difference in the mortality and the LC_{50} obtained.

Table 5. Percentage mortality of *G. bimaculatus*

Test insects	Concentration (mg.L^{-1})	*A. marina* (%)			*E. agallocha* (%)		
		Methanol	Ethyl acetate	N-hexane	Methanol	Ethyl acetate	n-hexane
G. bimaculatus	10,000	53±1.15	30±1.73	17±1.15	47±0.58	27±1.15	10±1.73
	8,000	47±1.53	23±1.53	13±0.58	40±1.00	20±0.00	7±0.58
	6,000	40±1.00	13±1.53	7±0.58	33±0.58	13±1.15	3±0.58
	4,000	23±0.58	7±0.58	3±0.58	20±1.00	7±1.15	3±0.58
	2,000	10±1.00	3±0.58	0±0.00	10±1.00	3±0.58	0±0.00
	1,000	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
	0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Table 6. Mortality percentage of *T. molitor*

Test insects	Concentration (mg.L^{-1})	*A. marina* (%)			*E. agallocha* (%)		
		Methanol	Ethyl acetate	N-hexane	Methanol	Ethyl acetate	n-hexane
T. molitor	10,000	77±1.53	30±1.73	30±1.73	73±1.53	30±1.00	27±1.53
	8,000	70±1.73	23±1.15	20±1.00	67±1.15	23±1.53	17±1.53
	6,000	60±1.73	17±1.15	10±0.00	53±1.53	17±1.53	10±1.00
	4,000	37±0.58	13±0.58	7±0.58	33±0.58	10±1.00	7±1.15
	2,000	27±1.15	7±0.58	3±0.58	23±1.53	3±0.58	3±0.58
	1,000	23±0.58	0±0.00	0±0.00	20±1.73	0±0.00	0±0.00
	0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

Table 7. Classification and determination of LC_{50} (*G. bimaculatus*)

Sample leaves	Solution	Linear regression			LC_{50} (mg.L^{-1})	Category
		a	B	R^2		
A. marina	Methanol	-14,1142	5,0170	0,8238	6,454	Moderate
	Ethyl acetate	-11,9575	4,2400	0,8331	9,986	Moderate
	n-hexane	-15,4042	4,9778	0,8888	12,562	Non-toxic
E. agallocha	Methanol	-13,3975	4,7870	0,8011	6,969	Moderate
	Ethyl acetate	-11,7252	4,1682	0,8231	10,292	Non-toxic
	n-hexane	-13,9887	4,5326	0,8637	15,464	Non-toxic

Table 8. Classification and determination of LC_{50} (*T.molitor*)

Sample leaves	Solution	Linear regression			LC_{50} (mg.L^{-1})	Category
		a	B	R^2		
A. marina	Methanol	0,1611	1,3144	0,8677	4,799	Moderate
	Ethyl acetate	-11,7095	4,2223	0,7606	9,065	Moderate
	n-hexane	-11,4992	4,0954	0,8134	10,682	Non-toxic
E. agallocha	Methanol	-0,1807	1,3878	0,8665	5,408	Moderate
	Ethyl acetate	-11,6561	4,1986	0,7643	9,269	Moderate
	n-hexane	-11,2438	4,0165	0,8016	11,070	Non-toxic

Based on the tests in this study, referring to the LC_{50} toxicity category on (Rozirwan *et al.*, 2022b) the leaves of *A. marina* and *E. agallocha* against *G. bimaculatus*, the smallest value was in the methanol fraction (Table 7). Meanwhile, for the insect *T. molitor* (Table 8) the smallest LC_{50} value was also in the methanol fraction. These results showed that the higher the LC_{50} value indicated the ability of the extract to be non-toxic. Bioactive compounds are toxic if they can cause a deadly effect that can cause the death of more than 50 % in a short time. *G. bimaculatus* and *T. molitor* belong to the major insect groups and are more substantial than test organisms that are often used in toxicity research.

GC-MS analysis of methanol extract A. marina

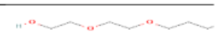
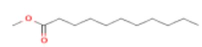
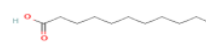
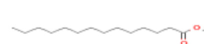
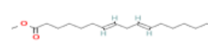
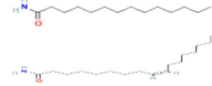
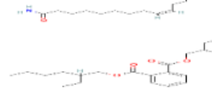
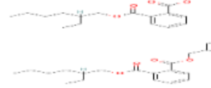
GC-MS analysis was taken based on the higher mortality fraction and LC_{50} values. Based on the test results, the extract with a substantial value is in the methanol fraction of *A. marina* leaves. The graph obtained as many as 14 peak points. The detected compounds were alcohols and organic fatty acids. The secondary metabolites contained in the extract affected the insecticide toxicity in the test (Figure 3.). The compounds identified based on the height of the chromatogram peaks are presented in full in (Table 9). The mass spectrum on the chromatogram graph of the results of the analysis that has been matched is identical to the mass spectrum in the base data library: WILEY.

GC-MS chromatogram analysis of the methanol fraction of *A. marina* leaves clearly showed the presence of 14 secondary metabolites based on the data bank library: WILEY 7. Based on the peaks detected that the main groups of compounds included alcohol, lauric acid, myristic, palmitic, linoleic, elaidate groups, stearate, oleic, phthalate ester, and siloxane. The results of the highest level of toxicity were found in the methanol extract of *A. marina* with the lowest LC_{50} value indicating that there were bioactive compounds that affected the results. Based on the results of GC-MS analysis (Table 9), most of the methanol extracts of *A. marina* leaves contain fatty acid compounds such as Dodecanoic acid, methyl ester, Dodecanoic acid, Tridecanoic acid, 12-methyl-, methyl ester, Hexadecanoic acid, methyl ester, 7,10-Octadecadienoic acid, methyl ester, 13-Octadecenoic acid, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester, 9-Octadecenamide, (Z)-, 1,4 Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, Octasilaxane,1,1,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecame, and Phthalic acid, di(2 propylpentyl) ester.

Fatty acids can be found in green plants and were produced in chloroplasts. In addition to the leaves of *A. marina*, Jaritkhuan and Suanjit (2018) also revealed that the leaves of the mangroves of *Aurantiochytrium mangrovei* and *Aurantiochytrium Limacinum* contains fatty acids. Several studies had revealed that fatty acids reported as antifungal, antibacterial, antioxidant, and anti-inflammatory activities (Sohaib *et al.*, 2022). Futhermore on Hexadecanoic acid, methyl ester (palmitic acid) has antibacterial activity (Karunia *et al.*, 2017). 9-Octadecenamide, (Z)- is oleic acid which is lethal to *A. aegypti* mosquito larvae, these compounds include oleoamides which can cause motor dysfunction and commonly used as sedatives. 1,4 Benzenedicarboxylic acid, bis(2-ethylhexyl) ester is a flatalic acid ester reported as antifungal activity. Phthalic acid, di(2 propylpentyl) ester compound has antimicrobial and anti-inflammatory activity (Chakraborty *et al.*, 2022). Hexadecanamide has anti-inflammatory, antifungal and anti-nociceptive activity (Rahbar *et al.*, 2012). Dodecanoic acid, methyl ester and dodecanoic acid are included in lauric acid. Lauric acid is a saturated fatty acid that can be used as an antimicrobial activity (Li *et al.*, 2022). Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl is a compound that reported as insecticidal activity (Farag *et al.*, 2021). As for the organic acids compounds in Heptadecanoic acid, 16-methyl-, methyl ester of stearic acid, Tridecanoic acid, 12-methyl-, methyl ester of myristic acid, 7,10-Octadecadienoic acid, methyl ester linoleic acid and Trans-13-Octadecenoic acid, methyl ester of elaidic acid reported as antibacterial (Ravichandran *et al.*, 2022). Stearic, oleic, myristic, linoleic and elaidic acids reported as antibacterial (Sani *et al.*, 2022).

Some of the statements above reveal that phytochemical profile of *A. marina* leaves extract has a potential bioactivity. The results of the percentage mortality and LC_{50} test showed that the methanol extract of *A. marina* leaves was a fraction that had a higher mortality and toxicity level even though it was in the very low toxic category compared to the fractions of ethyl acetate and n-hexane. The extract methanol of *A. marina* leaf produces the most dominant bioactive compound is organic fatty acids and shows low and non-toxic activity against test insects.

Table 9. Retention time, peak area, compound name, formula, and structure compound

Ret. time	Peak Area%	Name of Compounds	Formula	Structure Compounds
10.31	3,83	2-(2-Butoxyethoxy)ethanol	$C_8H_{18}O_3$	
13.36	2,43	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	
13.65	2,24	Dodecanoic acid	$C_{12}H_{24}O_2$	
14.66	2,82	Tertbutyloxyformamide, N-methyl- N-[4-(1 pyrrolidinyl)- 2 butynyl]	$C_{14}H_{24}N_2O_2$	
15.10	3,10	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	
17.85	10,16	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	
20.23	2,33	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	
20.31	9,29	Trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	
20.60	7,16	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	
21.25	4,25	Hexadecanamide	$C_{16}H_{33}NO$	
23.30	3,45	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	
24.90	9,06	Phthalic acid, di(2 propylpentyl) ester	$C_{24}H_{38}O_4$	
26.49	15,82	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	
28.28	2,51	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	

*Source 2D structure and Graph image : (Kim *et al.*, 2022).

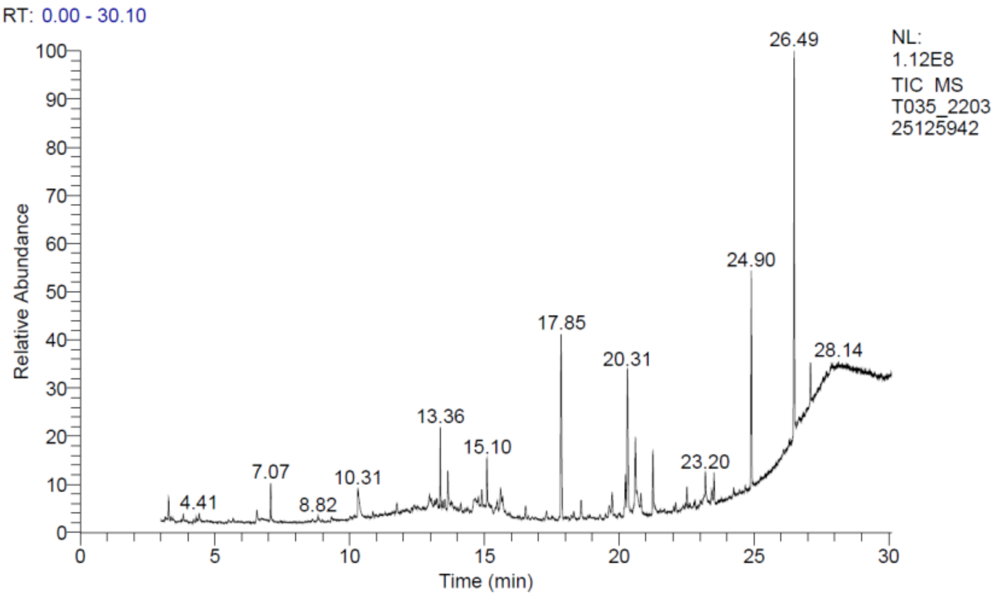


Figure 3. GC-MS chromatogram of methanol extract *A.marina*.

Conclusion

The insecticidal potency of *A. marina* and *E. agallocha* leaves extracts was in the methanol extract fraction in the very low toxic category. This is evidenced by the results of the LC_{50} value of *A. marina* methanol extract testing against *G. bimaculatus* (6.454 mg.L^{-1}) and *T. molitor* (4.799 mg.L^{-1}). Then testing the methanol extract of *E. agallocha* against *G. bimaculatus*. and *T. molitor* of 6.969 mg.L^{-1} and 5.408 mg.L^{-1}. The results of the GC-MS analysis of the most potential extract as an insecticide based on the high toxicity value were in the methanol extract of *A. marina* leaves which were suspected to contain alcohol and organic fatty acids.

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