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Toxicity Test of Moringa (Moringa Oleifera Lam) Essential Oil with the Brine Shrimp Lethality Test (BSLT) Method

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A B S T R A C T

Moringa oleifera is an herbal plant that is found in the tropics and Indonesia. Bioactive compounds in clover are found in many parts of the leaves. The study aims to test the toxicity of the essential oils of moringa leaves against the larvae of Artemia salina Leach and to find out the composition of the essential compounds of the constituents of the oil of moringa leaves using the gas-mass spectrometer chromatography method. (GS-MS). The results of the study of the essential oil on the leaves of the Moringa Oleifera Lum were toxic to the larvae of the moringa leaves, Artemia salina Leach, with an LC_{50} value of 178.58 ppm. GC results on moringa leaves obtained fifteen (15) peak compounds. The potential of the leaves of moringa as an herbal plant that can be a pesticide, antibacterial, and anticancer

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INTRODUCTION

Plants generally contain many kinds of chemical compounds. Chemical compounds in plants are formed and broken down through two metabolic systems, namely primary metabolism and secondary metabolism. Primary metabolic processes involve primary metabolites such as carbohydrates, proteins, lipids, and nucleic acids. While secondary metabolism produces products in the form of secondary metabolites, such as alkaloids, flavonoids, tannins, terpenoids, steroids, and others, Several studies have shown that the compounds contained in Moringa oleifera L have potential as drugs and have bioactivity, including anti-inflammatory (Mulyani et al., 2023), antifungal (Munira et al., 2021), antibiotics (Hutomo et al., 2023), and anticancer (Zahrah Qanitah et al., 2023), as well as antioxidants (Puspitasari et al., 2023).

The results of the study (Jusnita & Syurya, 2019) show that parts of Moringa contain compounds that function as antitumor (Nurpati Panaungi & Sakka, 2022), antipyretic (Wijaya & Lina, 2023), antiepileptic (Wahyudi Satriyo Wicaksono, 2023), antifertility (Munawir et al., 2023), diuretic (P. F. Isna Primadana, Laila tul Mapuh, 2023), antihypertensive (Styowati et al., 2023), and antidiabetic (Allisandra, 2023).

Moringa leaves (Moringa oleifera) are herbal plants that are often found among people, especially in tropical areas, and are widely spread in Indonesia (Muhammad Ayyub Amin, Anwar, 2018). Moringa is a traditional medicinal plant that is proven to be efficacious for the community. This plant has high antioxidant activity with active substances in it that are beneficial to health. Most of the active compounds in Moringa are found in the leaves (Salim & Eliyarti, 2019). Moringa plants contain very high nutrition (Putri Safrida Rahmawati, 2021).

Moringa plants (Moringa oleifera) in particular have a high content of protein, fat, minerals, and vitamins (Samodra et al., 2023). Moringa leaves contain a high amount of protein (around 27%), vitamin content including vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B6, vitamin C, and vitamin E, minerals such as calcium, iron, magnesium, phosphorus, and potassium, antioxidants such as flavonoids, polyphenols, and ascorbic acid, which help protect the body from damage caused by free radicals, nine types of essential amino acids that the body needs, as well as oleic and linoleic and linoleic and fatty acids, which are omega 9 and 6 and are useful for lowering cholesterol levels, anti-inflammatory, preventing heart disease, and cancer (Paramita, 2023).

Previous research on the extraction of Moringa leaves (Moringa oleifera Lamk.) showed high antioxidant activity in vivo and in vitro processes. In addition, Moringa leaves (Moringa oleifera Lamk.) are rich in phytochemicals, carotenes, vitamins, minerals, amino acids, flavonoids, and phenolic compounds (Jusnita & Syurya, 2019). Moringa leaves contain flavonoids, tannins, steroids, saponins, and alkaloids, which shows that they have antioxidant power (Tutik et al., 2018). Moringa leaf extract has an IC50 value of 50.59 ppm (Riskianto et al., 2021), both of which are stated to have strong antioxidant abilities. Due to its synergistic nature, combining two or more types of plants that contain antioxidants will produce greater potential (Septiawan et al., 2021).

Taking into account the aforementioned description, the aim of this study was to evaluate the toxicity of Moringa leaf essential oil (Moringa Oleifera Lum) on the larvae of the shrimp Artemia salina Leach and to identify the essential component composition of the essential oil of Moringa leaves (Moringa Oleifera Lum) using the gas chromatography-mass spectrometer (GS-MS) method.

THEORETICAL REVIEW

The Moringa plant (Moringa oliefera) is an herbaceous plant that has phenolic compounds in the form of flavonoids, tannins, terpenoids, alkaloids, and saponins (Rivai, 2020). Flavonoids are natural components and belong to a group of phenolics found in plant chloroplasts (Ademiluyi et al., 2018). The flavonoid group is known to have high antioxidant activity, is very beneficial for health, and is often used in the drug, cosmetic, pharmaceutical, and nutraceutical industries (Sadiah & Indiarto, 2022). Flavonoids are antioxidative, anticarcinogenic, antimutagenic, anti-inflammatory, and able to modulate the function of major cellular enzymes. Several studies report that moringa (Moringa oleifera) has a good antioxidant effect on the body to overcome and prevent oxidative stress in various degenerative diseases (Padayachee & Baijnath, 2020).

In the process of separating bioactive compounds, the choice of method for separating compounds is important to note because, in this separation process, it will be determined how much yield is produced. The extraction or separation of chemical compounds from plant sources is the beginning of the process of isolating bioactive compounds present in plants, whether in leaves, seeds, roots, or stems. Extraction is used to produce chemical compounds that are soluble in solvents. Several types of extraction can be used in the process of separating bioactive compounds from plants to find out the yield that will be produced, namely cold extraction methods consisting of maceration, percolation, and soxhletation and hot extraction methods, namely reflux methods (Ellyn Dasrinal, 2022).

Essential oils are oils that come from plants. Essential oils are also called essential oils or essential oils, which are usually produced by plants (Pratiwi & Utami, 2018). The need for essential oils grows yearly as modern sectors like perfume, cosmetics, food, medicine, aromatherapy, and medical emerge. The substances that make up vital oils are generally classified into two groups, namely: Hydrocarbons, namely compounds belonging to the hydrocarbon group formed from the elements hydrogen (H) and carbon (C) (Ni Luh Putu Putri Setianingsih, I Wayan Suirta, 2013). The types of hydrocarbons contained in essential oils mainly consist of terpene compounds, paraffins, olefins, aromatic hydrocarbons, and oxygenated hydrocarbons, namely compounds belonging to the oxygenated hydrocarbon group formed from the elements carbon (C), hydrogen (H), and oxygen (O), compounds of alcohols, aldehydes, ketones, oxides, esters, and ethers (Sabrina et al., 2023).

In a study conducted by Rajebi (2023), gas chromatography-mass spectroscopy was used to identify moringa seed oil. The results obtained from the examination using GC-MS from Moringa seed oil consisted of lauric acid, palmitoleic acid, palmitic acid, stearic acid, arachidic acid, and oleic acid (Rajebi et al., 2023).

The initial testing method uses brine shrimp of the Artemia salina Leach type and will later obtain an LC50 (Lethal Concentration 50) value, which is the substance's concentration at which 50% of test animals will perish.. If the LC50 value is <1000 ppm, the substance has biological activity; if the LC50 value is <30 ppm, the substance shows bioactivity as a compound that inhibits and stops the growth of cancer cells (Ni Luh Putu Putri Setianingsih et al., 2023).

METHODOLOGY

Material

The research material was moringa leaves (Moringa Oleifera Lum), which were collected in Br. Lantangidung, Batuan, Sukawati, Gianyar Bali.

Material Preparation

Moringa leaf samples (Moringa Oleifera Lum) were collected in stages from the Sukawati area of Gianyar. Furthermore, the leaves that have been collected are washed thoroughly with water. Every time you do the distillation, the moringa leaves are cut into small pieces.

Isolation of Essential Oils by the Ethanol Maceration Method

Moringa leaves are separated from the stem by cutting with a knife. Moringa leaves are then cut into small pieces. Then the moringa leaves were weighed and dried for approximately 3 days. After drying, the moringa leaves are blended to creating a fine powder. Then, ethanol was used to extract the Moringa leaves at a ratio of 1:5 by weight of the leaves. then 24 hours of maceration. After that, it was vacuum- and cloth-filtered. A 45°C rotary evaporator was then used to perform the evaporation process. After measuring it, the oil was poured into a bottle. The composition of the chemicals in Moringa leaves was then examined using the gas chromatography-mass spectrometer (GS-MS) method. The value (LC50) was then obtained following a toxicity test on Artemia salina Leach shrimp larvae to ascertain whether Moringa leaves were harmful were toxic or not to shrimp larvae (Artemia salina Leach), then analyzed by GC-MS.

Toxicity test on Artemia Salina Leach Shrimp Larvae

Artemia salina Leach shrimp larvae were used to test the essential oil from Moringa leaves for toxicity. In the toxicity test, sufficient saltwater was filtered before being used as the growth medium for shrimp larvae. In an aquarium with a perforated partition, sea water is added, and one side of the aquarium is rendered bright while the other side is made dark. The aquarium is kept in a location with a light and is darkened before the Artemia salina Leach eggs are placed there. given oxygen.

The toxicity test was carried out by preparing 5 test tubes; for Moringa leaves, 4 test tubes and 1 test tube were needed as controls. Weighed essential oil from 10 mg of Moringa leaf was then dissolved in 2 mL of ethanol. Additionally, the resulting solution was pipetted into test tubes with volumes of 50 liters, 250 liters, 500 liters, and 2500 liters, respectively, and the solvent was allowed to evaporate for 24 hours. After that, add 10 shrimp larvae of Artemia salina Leach, 50 L of dimethylsulfoxide (DMSO), 1 mL of saltwater, and a drop of yeast to each test tube. Following that, 5 mL of saltwater was added to create solutions with concentrations of 100 ppm, 500 ppm, 1000 ppm, and 5000 ppm, respectively. Pipette 1 mL of seawater, 50 L of dimethylsulfoxide (DMSO), and 10 Artemia salina Leach shrimp larvae to create a comparative or control solution 5 mL of seawater should be added. The aluminum foil used to cover the test tubes above was punched with a few holes and used three times. Artemia salina Leach shrimp larvae were monitored for mortality after 24 hours, and the number of dead larvae was noted. Then the calculation of the dead Artemia salina Leach shrimp larvae was carried out, and the value (LC50) was calculated by taking a % probit graph.

Analysis by Gas Chromatography-Mass Spectroscopy (GC-MS)

The Moringa leaf essential oil obtained was then analyzed by GC-MS to determine the chemical components of Moringa leaf essential oil (Moringa Oleifera Lum). Identification was carried out by comparing the mass spectrum obtained from the essential oil with the mass spectrum of a standard compound that has a known mass spectrum in a database that has been programmed on the GC-MS tool.

RESULTS

Results of Essential Oil Isolation by Maceration Method

Table 5.1 displays the outcomes of the extraction of the essential oil from Moringa leaves. Moringa leaves were 6.27 grams with a powder weight of 100 grams, which had physical properties, namely being light green in color, smelling sour, and having a yield value of 6.27% (w/w). The process of macerating Moringa leaves with an evaporator can be seen in table 1.

Table 1. Observation of Moringa Leaves Condensed Extract

Sample	Extract Color	Simplicia Weight (g)	Extract Weight (g)	Yield (%)
Moringa Leaves	Light green	100 grams	6.27 grams	6,27%

The Moringa The components of the essential chemicals that make up Moringa leaf essential oil are then identified using GC-MS after the leaf essential oil has been collected. Additionally, studies for toxicity on Artemia salina cockle shrimp larvae.

Essential Oil Toxicity Test on Artemia Salina Leach Shrimp Larvae

The results of the toxicity test of Moringa leaf essential oil on Artemia salina Leach shrimp larvae are presented in Table 5.2.

Table 2. The Results of the Toxicity Test of Moringa Leaf Essential Oil on Artemia Salina Leach Shrimp Larvae

Concentration (ppm)	**x**	**Number of test larvae (tail)**	**Number of Dead Larvae (tail)**				**Percent death (%)**	**Probit Value**
	Logs (ppm)		**I**	**II**	**III**	**Average**		
5.000	3.70	10.00	7	9	9	8.333	83	5.95
1.000	3.00	10.00	10	9	9	9.333	93	6.48
500	2.70	10.00	7	6	7	6.667	67	5.44
100	2.00	10.00	4	3	4	3.667	37	4.67
LC_{50} = 178.58 ppm (Toksic)								

Note: LC50 = Concentration that causes 50% mortality of shrimp Artemia salina Leach larvae.

Analysis of the Least Squares Regression Method (Probit Method) toxicity test data for Moringa leaf essential oil on Artemia salina leach shrimp larval LC50 calculation. The Probit Method (least squares regression) is presented in Table 3

Table 3. Transformation of Percentage to Probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

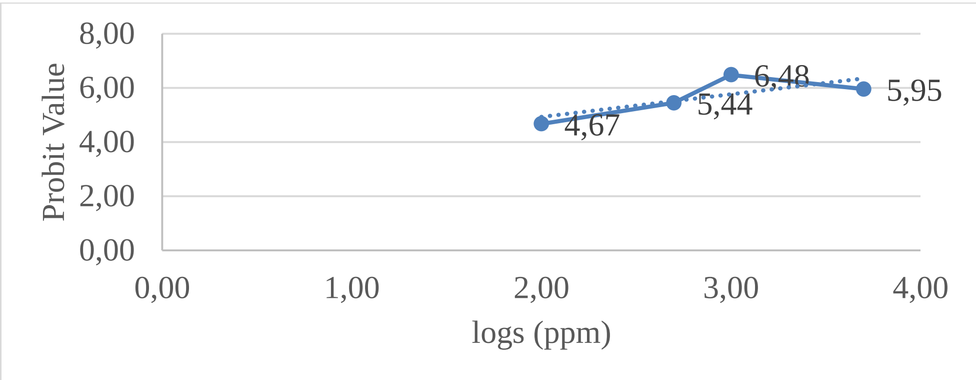
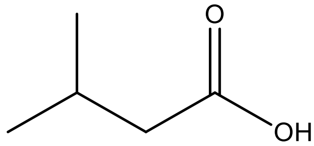
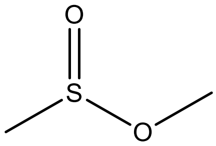
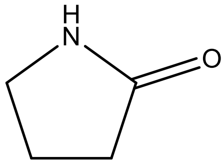
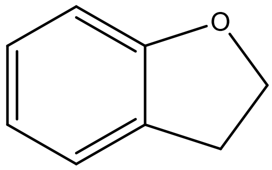
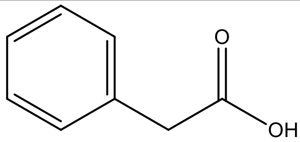
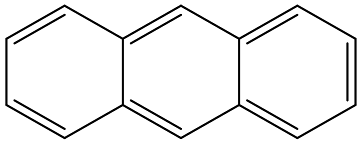
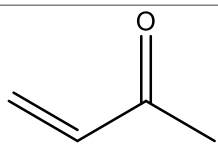
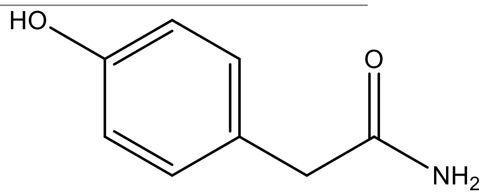


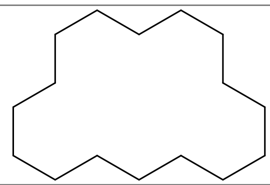
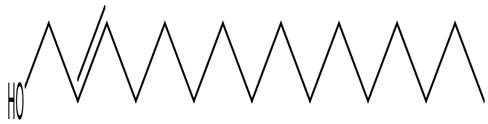
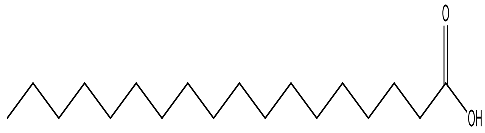
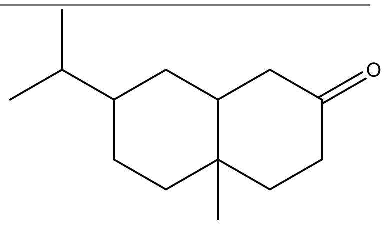
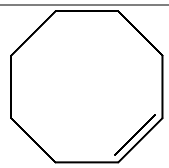
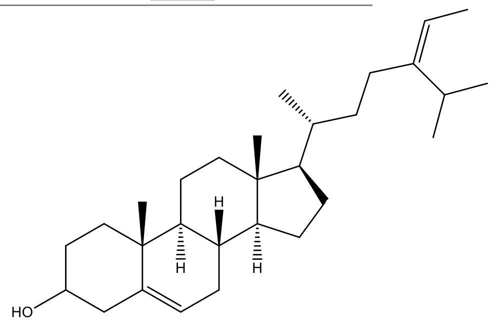
Figure 1 Graph of Probit Value to LC_{50}

Analysis of Moringa Leaf Essential Oil with GC-MS

Moringa leaf essential oil employing a gas chromatography-mass spectrometer (GC-MS) to be examined for the components of the chemicals contained within after the maceration process. The chromatograms produced by the GC revealed fifteen (15) peaks. with compounds, according to Willey.Lib database. Estimates of volatile compounds based on the GC-MS database (WILEY229.LIB) can be seen in Table 4.5.

Table 4. Essential Compounds in Chromatograms Based on the WILEY 229.LIB Database on Moringa leaves

No.	Compound Name	Retention Time	% Area	Similarity (Quality) %	Compound Image
1	Iso-Valeric Acid	5.456	0.29	80	
2	Methanesulphinic Acid Methyl Ester	6.673	0.06	83	
3	2-Pyrrolidinone	10.109	0.55	87	
4	2,3-Dihydro-Benzofuran	12.978	0.14	83	
5	Benzeneacetic Acid	13.777	0.80	87	
6	Anthracene	21.390	0.13	84	
7	3-Buten-2-One	21.500	0.25	87	
8	Nonadecane	21.753	0.07	86	
9	4-Hydroxyphenylacetamide	22.655	0.10	81	

10	Cyclohexadecane	23.614	0.18	86	
11	2-Hexadecen-1-Ol	27.584	0.53	87	
12	Octadecanoic Acid	27.662	0.54	89	
13	7-Isopropyl-4a-Methyloctahydro-2(1H)-Naphthalenone	33.543	0.40	80	
14	Cyclooctene	23.965	0.05	89	
15	Stigmasta-5,24(28)-Dien-3-Ol	41.141	0.19	83	

DISCUSSION
Results of Essential Oil Isolation by Maceration Method

Both extracts were prepared using the maceration method, which relies on the process of withdrawing the active compound with the appropriate solvent. With the maceration method, the active compounds are taken without requiring high heating or any heating process at all. Maceration has the advantage of ensuring the integrity of the extracted active substance without damage, and in this soaking process, there is a breakdown of the cell wall along with the cell membrane because there is a pressure ratio between the outside and inside of the cell. This is because the compounds in the cytoplasm break down and then dissolve in the organic solvent used (Chairunnisa et al., 2019). What is taken into consideration is that the time used to extract is quite long; it cannot be used for materials containing solid ingredients, for example, benzoin, wax, and tyrax; more liquid solvents are used (Husni et al., 2019).

Essential Oil Toxicity Test on Artemia Salina Leach Shrimp Larvae

The results of this toxicity test showed that Moringa leaf essential oil had an LC_{50} value of 178.58 ppm, so it can be said that Moringa leaf essential oil is toxic. The toxicity category of the substance based on the LC_{50} value is divided into three categories: very toxic with an LC_{50} value < 30 ppm, toxic with an LC_{50} value of 30-1000 ppm, and non-toxic with an LC50 value > 1000 ppm.

The majority of plants have active components that can be employed to stave off assaults from phytophagous insects. These active substances are broken down into a variety of groups, such as repellents, feeding inhibitors, poisons, and growth regulators. While protecting against phytophagous insects is the main purpose of these active substances, many of them are also efficient against biting mosquitoes and other Diptera, especially when the volatile components are released. Some of these substances deter hematophagous insects from feeding on human blood (Kuswidono, 2011).

From The highest concentration, specifically the concentration of 30%, exhibits the highest protective power, as can be observed from the data. This can be explained by the fact that moringa leaf extract has more volatile components at higher concentrations, and as a result, more volatile components bind to shrimp larvae's odorant receptor proteins. (Palawe et al., 2021).

Aquadest extract (room temperature) and hot distilled water (70^{O}C) of Moringa leaves had a toxic effect on Artemia salina Leach larvae, with LC_{50} values lower than 1.000 ppm, namely 265.977 ppm and 163.979 ppm, respectively. The best extract in this study was shown to be based on a lower LC_{50} value, namely the hot distilled water extract (70^{o}C) of 163.979 ppm. The compounds contained in the hot distilled water extract (70^{o}C) of Moringa leaves include alkaloids, flavonoids, tannins, and triterpenoids. From these results, it can be seen that the potential of Moringa leaves as an herbal plant has antipesticide, antibacterial, and anticancer properties.

Analysis of Moringa Leaf Essential Oil with GC-MS

In the chromatogram, the peaks of the compounds included in the essential oil group were then identified with a mass spectrometer, where each compound has a specific mass fragmentation pattern. Identification was carried out by comparing the mass spectra of each peak with compounds that are already known and programmed in the database so that the essential compounds that make up the essential oil of Moringa leaves can be suspected.

The examination of Moringa leaves using gas chromatography-mass spectrometry (GC-MS) revealed that the leaves contain fifteen (15) major components. with relatively large abundances of similar qualities, namely Iso-Valeric Acid, Methanesulphinic acid, methyl ester, 2-Pyrrolidinone, 2,3-Dihydro-Benzofuran, Benzeneacetic Acid, Anthracene, 3-Buten-2-One, Nonadecane, 4-Hydroxyphenylacetamide, Cyclohexadecane, 2-Hexadecen-1-Ol, Octadecanoic Acid, 7-Isopropyl-4a-Methyloctahydro-2(1H) -Naphthalenone, Cyclooctene, Stigmasta-5, 24(28) -Dien-3-Ol.

According to the findings of this study, the essential oil of Moringa leaves has an LC50 value of 178.58 ppm and is harmful to the larvae of the shrimp Artemia salina Leach. According to Syaiful Anwar (2014), if an extract has an LC50 value between 30 and 200 ppm, then the extract has potential as an antimicrobial, while if an extract produces an LC_{50} value of 200–1000 ppm, then the extract has potential as an antipesticide. Based on this statement, the distilled water extract (room temperature) has the potential to be an anti-pesticide, while the hot distilled water extract (70 ^{O}C) has the potential to be an antimicrobial (Anwar et al., 2014).

The mechanism of the reaction of these compounds to the death of Artemia salina Leach shrimp larvae was reported by Putri S. (2023), who found that alkaloid, flavonoid, and triterpenoid compounds at certain levels have the potential for acute toxicity and can cause the death of Artemia salina Leach shrimp larvae. The mechanism of action of these compounds is to act as stomach poisons. Therefore, if these compounds enter the bodies of the larvae, the digestive organs will be disrupted. In addition, these compounds inhibit taste receptors in the mouth area of the larvae. This causes the larvae to fail to get a taste stimulus, so they are unable to recognize their food, and the larvae will starve to death. Artemia salina Leach mortality is thought to be caused by triterpenoid compounds, which are toxic. Triterpenoid compounds can enter the cell membrane of Artemia salina Leach by diffusion. The entry of these compounds can damage membrane permeability and disrupt the biochemical processes of Artemia salina Leach; as a result, Artemia salina will die (Ni Luh Putu Putri Setianingsih et al., 2023).

The results of Moringa leaf extract by maceration method using ethanol solvent contained fifteen (15) compound components with relatively large abundances. Essential compounds generally have several C atoms. The scent and odor of numerous aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones, as well as monoterpenes (C10) and sesquiterpenes (C15), are distinctive. Essential oils, or volatile monoterpene and sesquiterpene chemicals, are present in plants and serve as a key part of the plants' defensive mechanism, especially against pathogenic fungus and herbivorous insect pests. The possibility of Moringa leaves as an herbal plant that can be an anti-pesticide, antibacterial, and anti-cancer

CONCLUSIONS AND RECOMMENDATIONS

The essential oil in Moringa leaves (Moringa Oleifera Lum) is toxic to the larvae of the shrimp Artemia salina Leach with an LC50 value of 178.58 ppm. The GC results on Moringa leaves obtained fifteen (15) compound peaks. The MS results of the volatile compounds identified were Iso-Valeric Acid, Methanesulphinic Acid Methyl Ester, 2-Pyrrolidinone, 2,3-Dihydro-Benzofuran, Benzeneacetic Acid, Anthracene, 3-Buten-2-One, Nonadecane, 4-Hydroxyphenylacetamide, Cyclohexadecane, 2-Hexadecen-1-Ol, Octadecanoic Acid, 7-Isopropyl-4a-Methyloctahydro-2(1H)-Naphthalenone, Cyclooctene, Stigmasta-5,24(28)-Dien-3-Ol. The potential of Moringa leaves as an herbal plant that has the ability as an anti-pesticide, antibacterial and anti-cancer.

The suggestion that needs to be made in this research is to test the antioxidant activity of the essential oil of Moringa leaves (Moringa Oleifera Lum) so that we can get more complete information about the activity and efficacy of the Moringa (Moringa oleifera Lum) plant.

FURTHER STUDY

The results of this study were able to provide answers regarding the toxic compounds contained in Moringa leaves. It is necessary to test the antioxidant, antitumor, and anticancer activities of Moringa leaf essential oil so that we can get more complete information about the activity and efficacy of Moringa (Moringa Oleifera Lum) as a medicinal plant based on its toxicity to Artemia salina Leach shrimp larvae and produce products that can be sold in the market.

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