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ENVIRONMENTAL HEALTH RISK ANALYSIS OF LEGUNDI LEAF ESSENTIAL OIL TOXICITY (*VITEX TRIFOLIA L.*)

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Abstract

Introduction: *Caring for the environment means participating in preserving the environment as well as possible, one concern for the environment is regarding the development of the use of natural materials. This is done to ensure the safety of consumers' use of herbal medicinal products or food products. One of the plants that are often used for traditional medicine is the legundi plant (Vitex trifolia L.). Legundi leaf essential oil is used for humans for environmental health and food products.* ***Methods:*** *Samples of legundi leaves were collected in stages from the Sukawati Gianyar area. Furthermore, essential oil isolation was carried out using the maceration method, then a shrimp larvae toxicity test was carried out, then analysis was carried out by Gas Chromatography-Mass Spectroscopy (GC-MS).* ***Results and Discussion:*** *Toxicity test analysis show that the essential oil of legundi leaves has an LC_{50} value of 51,541 ppm, so it can be said that legundi leaf essential oil is toxic. GC results obtained eight chromatogram peaks. Of the eight peaks, there was one peak of the compound with 41.77% of the high area, namely the compound furan-2-carboxaldehyde.* ***Conclusion:*** *Legundi leaf essential oil is toxic, so this finding is important for developing science in environmental health and food product development. Environmental Health is used to increase public knowledge in the field of the utilization of food products.*

INTRODUCTION

Caring for the environment means participating in preserving the environment as well as possible, by maintaining, managing, restoring, and protecting the environment. Caring for the environment is an attitude and action that is always attempted to prevent damage to the surrounding environment and foster efforts to repair the natural damage that has occurred (1). One concern for the environment is the development of the use of natural materials.

The development of research using the use of natural ingredients as medicine and as ingredients for food products has long been carried out with various samples of natural ingredients and tested to produce information on the compounds contained in these natural ingredients and produce innovative new products. Many studies have been carried out to develop or utilize Indonesia's natural resources as raw materials in developing herbal medicines. However, until now only a few studies of herbal medicines and food products can be used in health facilities because they must meet safety requirements, and benefits and be standardized.

The important thing that must be done by using natural ingredients as raw materials for drugs and food products is to know the toxic effects of the compounds contained. Herbal medicines that have been studied as immunomodulators or immune enhancers include bitter leaf, temulawak rhizome, legundi leaf, ginger, orange, and echinacea rhizome (2). Herbal medicine is widely used by the community for the treatment and prevention of disease. Many studies have been conducted on standardization, pre-clinical trials, and clinical trials of

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herbal medicines (3). Therefore, it is necessary to do a toxicity test to there is a level of damage caused by a compound to biological materials.

Toxicity testing is usually carried out on a product candidate to meet a region or country's distribution and licensing requirements. This is done to ensure the safety of using herbal medicinal products or food products for consumers (4). One of the plants often used for traditional medicine is the legundi plant (*Vitex trifolia L.*) presented in Figure 1.

(Source: Private Collection)

Figure 1. Legundi Plants

Vitex trifolia L. is an example of an efficacious plant in traditional medicine known as Legundi. The classification of legundi plants is the Spermatophyta division, the Angiospermae sub-division, the Dicotyledoneae class, the Lamiales tribe, the Verbenaceae family, the Vitex genus, and the *Vitex Trifolia type L.* (5).

Legundi plants contain compounds such as flavonoids, alkaloids, terpenoids, tannins, saponins, sterols, carbohydrates, proteins, amino acids, and essential oils (6). In legundi, the content that produces a lot of essential oil compounds is in the leaves. The essential oil content in the leaves ranges from 80.83%, namely the citronella compounds found in citrus leaves (7). Legundi plants have a distinctive aroma like spices. Legundi leaves contain essential oils, namely alkaloids, flavonoids, sesquiterpenes, terpenoids, glycosides, and other compounds (8). The chemical constituents of legundi leaves include essential oils (*l-pinen, kamfen, terphenyl acetate*), diterpene alcohol, *aukubin*, agnostic, viteksikarpine (*casticin*), orientin, *isoorientin, and luteolin* 7-glucoside which have analgesic, diuretic, diaphoretic, antipyretic, carminative properties, insecticides, and anthelmintics. In addition, legundi leaves also contain phytochemicals such as flavonoids, saponins, and alkaloids (9).

Essential oils or also called essential oils are extra natural commodities from plant species, which are volatile at room temperature and have an odor like the original plant. Essential oils are usually colorless, especially when freshly obtained from isolation, but will darken over time due to the oxidation process (10). Essential oils are one of natural ingredients that are easily obtained because they are found in various types of plants and are also easily biodegradable (11).

Essential oils are hydrophobic, volatile, and aromatic compounds that give plants a distinctive aroma or odor. It is usually considered a by-product of secondary metabolites and is a by-product of plant metabolism. Essential oils are extracted from various botanical sources, many of which are members of the family *Lamiaceae* (12). The components of essential oils are divided into two groups, namely volatile and nonvolatile residues. Volatile (90–95%) consists of monoterpenes and sesquiterpenes, aliphatic aldehydes, alcohols, and esters. The residue is not easy to evaporate (1–10%) and consists of flavonoids, fatty acids, hydrocarbons, sterols, carotenoids, and waxes (13).

The use of essential oils for humans, especially for health and food products. Essential oils have a very important role in various industries. The activity of essential oils as a repellent against insects is because insects can smell the essential oil. The volatile nature of essential oils. The essential oil is pressed at a high temperature, the insects will smell the odor which will affect the Odor Receptor Protein in insects. This Odor Receptor Protein will detect the smell of essential oils (14).

The essential oil extraction process can be used by steam distillation or maceration methods. Hexane is a non-polar solvent. Hexane is a non-polar solvent that can dissolve non-polar compounds (15). Before becoming a phytopharmaceutical preparation, every natural ingredient must pass several stages, including experimental pharmacology tests, toxicity tests, clinical trials, quality tests, and other tests according to requirements for user safety (16).

The study of the toxicity of legundi leaves on shrimp larvae test used the Brine Shrimp Lethality Test (BSLT) method. Brine Shrimp Lethality Test was chosen because it is often used for pre-screening active compounds contained in plant extracts. After all, it is simple, fast, cheap, easy, and reliable, and the results are representative. Toxicity test using BSLT can be determined from the number of deaths on shrimp larvae test because there is the effect of the extract

or compounds of natural ingredients. The test result is expressed as LC_{50} (17).

The toxicity test aims to detect toxicity in a material or substance and obtain toxic data after being given a compound acutely so that it can determine the target organ and its sensitivity (18). Legundi leaves, apart from being insecticidal, are also antifeedant and prolong the larval stage. The best concentration (0.50%) of ethyl acetate extract of legundi leaves can kill larvae by 85.33% and causes a decrease in larval feeding activity by 83.21%.

So far, no reports have been found on toxicity tests with shrimp larvae on *Vitex trifolia L.* species. Based on the data above, it is necessary to research to test its toxicity to Artemia salina Leach shrimp larvae to determine which compounds are the most toxic.

METHODS

Material

The research material was Legundi leaves (*Vitex trifolia L.*) taken in the br. Lantangidung, Batuan, Sukawati, Gianyar areas of Bali. Determination was carried out at the National Innovation Research Agency, Bedugul-Bali.

Material Preparation

Legundi leaf samples (*Vitex trifolia L.*) were collected gradually from the Sukawati Gianyar region. Furthermore, the collected leaves were thoroughly washed using water. The leaves of such legundi are cut into small pieces before distillation.

Isolation of Essential Oils by the Maceration Method

Legundi leaves were separated from the stalk by cutting with a knife. The leaves of the legundi were cut into small pieces. Furthermore, legundi leaves were weighed and then dried for approximately 3 days. After drying, legundi leaves were blended into a fine powder. Then the legundi leaves were extracted with hexane in a ratio of 1:5 of the weight of the leaves. Then in maceration for 24 hours. The next step was to filter with a sieve cloth and vacuum filter. Afterward, a rotary evaporator with a temperature of 45°C functions for evaporation was used. Oil was put in a bottle. Furthermore, it was tested using the Gas Chromatography Method-Mass Spectrometer (GS-MS) to determine the compounds' content in legundi leaves. Following that, a toxicity test on shrimp larvae was performed, and a value (LC_{50}) was calculated to determine whether the legundi leaves were toxic or not (19).

Toxicity Test of Shrimp Larvae Against Artemia Salina Leach

The essential oil of legundi leaves obtained was tested for toxicity using the shrimp larvae. In the toxicity test, a shrimp larval growth medium was first made by filtering seawater to taste. The seawater was put into an aquarium that had a hollow bulkhead with one part of the aquarium made light while the other part was made dark. Eggs of Artemia salina Leach were inserted in the dark part, next the aquarium was kept in a place that had lighting and was oxygenated.

A toxicity test was carried out by preparing 10 test tubes, for each sample of young and old leaves needed 9 test tubes and 1 test tube as a control. Weigh 10 mg of legundi leaf essential oil in each test tube, then dissolve it in 2 mL of ethanol. Then the solution obtained was pipetted as much as 5 L, 50 L, and 500 L respectively into a test tube and the solvent was evaporated for 24 hours. After that, put 1 mL of seawater into each test tube, 50 L of Dimethylsulfoxide (DMSO), 10 shrimp larvae, and a yeast drop. Seawater was added to a volume of 5 mL to obtain solutions with concentrations of 1 ppm, 10 ppm, 100 ppm, and 1000 ppm respectively. As a comparison or control solution, pipette 1 mL of seawater, 50 L of Dimethylsulfoxide (DMSO), and a total of 10 shrimp larvae were then added with seawater to a volume of 5 mL. Each of the above test tubes was covered with aluminum foil with a small hole and repeated 3 times. The number of dead larvae was recorded after 24 hours. Then, the dead shrimp larvae were counted and the value (LC_{50}) was calculated by graphing the % probit with the concentration log (20).

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

Analysis was performed using GC-MS obtained from legundi leaf essential oil to determine the chemical components of legundi leaf essential oil (*Vitex trifolia L.*). Identification was carried out by comparing the mass spectrum obtained from essential oils with the mass spectrum of standard compounds with known mass spectrum in a database programmed on the GC–MS tool (21).

Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The GC-MS tool was used to identify several bioactive compounds that can be seen from the peak of the chromatogram as the identification of data from chromatography and Mass Spectrometry (MS) seen from the mass spectrum with each molecular weight of the bioactive compound (22).

RESULTS

Isolation of Essential Oils by the Maserary Method

The results of the isolation of essential oil of legundi leaves are presented in Table 1. The result of the legundi leaf maceration extract was 6.8 grams with a powder weight of 100 grams which had physical properties, namely light green, sour-smelling, and an amendment value of 6.8%.

Table 1. Observation of Legundi Leaf Thick Extract

Sample	Extract Color	Simple Weight (g)	Extract Weight (g)	Yield (%)
Legundi Leaves	Light green	100 grams	6.80 grams	6.8 %

The components of the constituent compounds of the essential oil of legundi leaves were identified using the GC-MS tool after the results of the essential oil of legundi leaves were obtained. The shrimp larvae were then tested for toxicity.

Toxicity Test of Essential Oil against Artemia Salina Leach Shrimp Larvae

The results of the toxicity test of legundi leaf essential oil on shrimp larvae are presented in Table 2. Toxicity test results showed an LC_{50} value of 51,541 ppm in legundi leaf essential oil. It can be inferred that legundi leaf essential oil is toxic. The toxicity category of materials based on the value of LC_{50} is divided into three categories: very toxic has an LC_{50} value <30 ppm, toxic has an LC_{50} value of 30-1,000 ppm, and non-toxic has an LC_{50} value > 1,000 ppm.

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

Concentration (ppm)	x	Number of Test Larvae (tails)	Number of Dead Larvae (tails)				Percentage of Deaths	Probit Value
	Log (ppm)		I	II	III	Average		
1,000	3.00	10.00	9	10	9	9.33	93.33	6.48
100	2.00	10.00	4	5	5	4.67	46.67	4.90
10	1.00	10.00	2	1	2	1.67	16.67	4.01
1	0.00	10.00	1	0	3	1.33	13.33	3.87
***LC 50 = 51,541 ppm (Toxic)**								

**LC_{50} = Concentration that causes 50% mortality of Artemia salina Leach shrimp larvae.*

The data analysis on the toxicity test of legundi leaf essential oil against Shrimp larvae was using the Least Square Regression Method (Probit Method) Calculation LC_{50} method with the Least Square Regression Method method (Probit Method). A graph of the value of probit against the LC_{50} is presented in Figure 2.

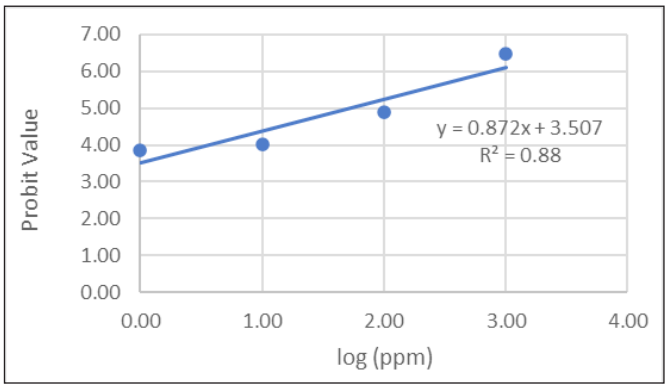

Figure 2. Graph of Probit Value against LC_{50}

Analysis of Legundi Leaf Essential Oil with Gas Chromatography-Mass Spectroscopy (GC-MS)

Obtained essential oil of legundi leaves from the steam distillation process was then analyzed for the components of the compounds contained in it using a GC-MS tool. Gas Chromatography results obtained eight chromatogram peaks. Of the eight peaks, there was one peak of the compound with 41.77 % of the high area, namely the compound furan-2-carboxaldehyde, and seven peaks of other essential compounds with a fairly small % area as shown in Figure 3.

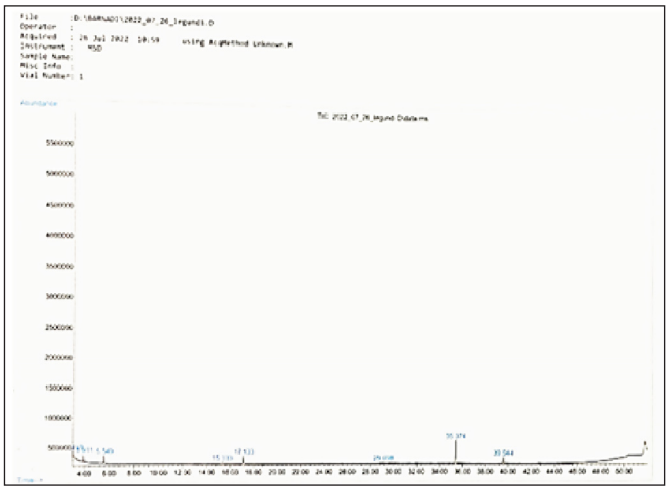

Figure 3. Chromatogram of Legundi Leaf Essential Oil

In the chromatogram, the peaks of compounds belonging to the essential oils class were further identified with a mass spectrometer, where each compound has a specific mass fragmentation pattern. Identification was done by comparing the mass spectrum of each peak with compounds that were already known and programmed in the database so that it can be suspected that the essential compounds constitute the essential oil of legundi leaves. Estimates of essential compounds based on the GC-MS database (WILEY229. LIB) can be seen in Table 3.

Table 3. Peaks of Volatile Compounds on Chromatogram Based on WILEY229.LIB Database on Legundi Leaves

Compound Peak	Retention Time	% Area	Suspected Compound	Compound Group
Peak 1	3.12	-0.35	9-Borabicyclo [3.3.1] nonane $C_{16}H_{26}B_2$	Sesquiterpene
Peak 2	3.91	9.78	Bicyclo [3.1.0] hexane C_7H_{16}	Monoterpene
Peak 3	5.55	13.64	Eucalyptol $C_{10}H_{18}O$	Monoterpene
Peak 4	15.33	5.08	Benzoic Acid $C_7H_6O_2$	Monoterpene
Peak 5	17.13	13.63	Caryophyllene $C_{15}H_{24}$	Sesquiterpene
Peak 6	29.10	4.46	1.5-Bis (5-methylthio-1,3,4-thiadiazole-2-ylthio) $C_3H_4N_2S_3$	Monoterpene
Peak 7	**35.37**	**41.77**	**furan-2-carboxaldehyde $C_5H_4O_2$**	**Monoterpene**
Peak 8	39.54	11.98	2.8-dimethyl-1-napthalenyl $C_{20}H_{30}O_5$	Sesquiterpene

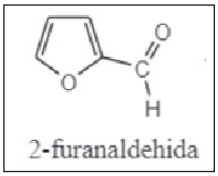

Figure 4. Furan-2-Carboxaldehyde Compound

There were six peak compounds from legundi leaves obtained from the results of Gas Chromatography-Mass Spectroscopy (GC-MS) analysis, where there was one peak of essential compounds with a relatively large abundance, namely furan-2-carboxaldehyde compounds (41.77%) presented in figure 4. Gas Chromatography-Mass Spectroscopy (GC) results on legundi leaves obtained six peaks. The identified results of MS essential compounds are 9-Borabicyclo [3.3.1] nonane; Bicyclo [3.1.0] hexane; Eucalyptol; Benzoic Acid; Caryophyllene; 1,5-Bis (5-methylthio-1,3,4-thiadiazole-2-ylthio); furan-2-carboxaldehyde; 2,8-dimethyl-1-napthalenyl.

DISCUSSION

Isolation of Essential Oils by Maceration Method

The resulting yield was <10%. The more values produced, the higher the yield value produced from a material. Therefore, the yield can be used as a parameter to determine the effectiveness of a process.

There is an interaction between the extraction time of 48 hours with fresh and dry ingredients because the longer the extraction time, the quantity of the extracted components will increase because the chance of contact with the ingredients and the solvent will be greater so that more and more components will be extracted until it reaches the saturation point of the solution. The use of dry samples also affects the value of the extracted yield. This is because drying will damage the cell walls of the material so that the extraction process becomes more effective (23).

The more effective the extraction process, the more weight of the sample is extracted so that the yield of the extract will be higher. The amount of extract yield can be caused by cell wall thickness, cell membrane, and the influence of genetic factors from the extracted sample. The interaction at 36 hours of maceration with fresh and dry ingredients is because the longer the extraction time, the longer the contact of the material with the solvent will last until both will occur by diffusion of mass deposition until there is an equilibrium concentration of the solution inside and outside the extracted material. The amount of yield depends on the solubility properties of the bioactive components contained in the sample (24).

Extraction is a separation process in which the components undergo mass transfer from a solid to a liquid or from a liquid to another liquid which acts as a solvent (25). Soaking the sample in filter liquid is a maceration method. The filtered liquid enters the cell cavity containing the active substance and will penetrate the cell wall. The concentrated solution is pushed out when the active substance dissolves due to the difference in concentration between the solution outside the cell and inside the cell. The difference in concentration between the solution outside the cell and inside the cell occurs because the concentrated solution is pushed out when the active substance dissolves (26).

Dissolving active substances based on their solubility properties Like Dissolved Like, Polar compounds dissolve in polar solvents and non-polar compounds dissolve in non-polar solvents is the principle of maceration. There is a difference in concentration inside the cell and outside the cell at the interface between the simplicia and the solvent. This difference in concentration can result in a diffusion process, where substances move from a higher concentration to a lower concentration. This event occurs repeatedly until an equilibrium concentration exists inside and outside the cell (27).

Fractionation of viscous extracts was carried out using solvents namely n-hexane, ethyl acetate, and water because they have different polarity levels. Two phases were obtained, namely the non-polar phase and the pattern phase, the n-hexane solvent was non-polar. Therefore, polar phase at the bottom and non-polar phase at the top. Next, the fraction is concentrated. The purpose of fractionation is to separate compounds based

on their polarity (28). Non-polar compounds will enter into non-polar solvents, and vice versa polar compounds will enter polar solvents.

Essential Oil Toxicity Test on Shrimp Larva

Shrimp Mortality Test was using experimental shrimp larvae (Artemia salina nauplii) with a preliminary test/preliminary screening of simple biological activity to determine the acute toxicity of an extract or compound. The number of shrimp larvae deaths due to the influence of the given compound at a predetermined dose is a parameter shown to indicate the presence of biological activity in a compound in Artemia salina Leach. One of the most suitable organisms as test animals to determine the bioactivity of compounds through toxicity tests is the brine shrimp (sea prawns) of the Artemia salina Leach species. This test uses crayfish or nauplii larvae. Some of the advantages of the bioactivity test with the BSLT using shrimp larvae are fast, easy, no special equipment required, simple (no aseptic technique), cheap (no need for animal serum), a large number of organisms, satisfies the need for statistical validation with a small sample, the results are representative and reliable (17).

Table 2 shows that the increase in concentration is directly proportional to the increase in mortality so the killing power is higher if the LC_{50} value is less than 1,000 g/ml (ppm), then a compound is declared to have the potential for acute toxicity. LC_{50} (Lethal Concentration 50) is the concentration of a substance that causes death in 50% of experimental animals, namely Artemia salina Leach larvae. As shown in Table 2, 51,541 g/ml or ppm indicates the LC_{50} value of the hexane extract of Legundi leaves, so it has the potential for acute toxicity according to the BSLT method for hexane extract of Legundi leaves in this experiment, namely in experimental animals with shrimp larvae. The LC_{50} value of the hexane extract which is less than 1,000 ppm indicates that the extract has the potential to be developed.

The mechanism of action of larval death is estimated that the compounds contained in legundi leaf extract by acting as a stomach poison can inhibit the eating power of the larvae (antifeedant). Therefore, the digestive system will be disrupted if these compounds enter the body of the shrimp larvae. Stomach poison attacks the main digestive organs of insects, namely the ventriculus (16).

Alkaloid compounds have the toxin, repellent, and antifeedant characteristics of insects, so they interfere with the growth and development of larvae. In small amounts, alkaloids are only antifeedants and kill larvae slowly due to decreased appetite and will only cause death in some time due to starvation. But in large quantities alkaloids work as contact poisons and digestive poisons that will directly kill larvae and cause death because they attack vital organs such as the nervous system and affect heart activity. Flavonoid compounds have a way of working as respiratory poison and metabolic poison that can directly cause death in a short time. Flavonoid compounds can inhibit the digestive tract of insects and are also toxic. In addition, this compound can inhibit taste receptors in the mouth area of the larvae. This causes the inability to recognize food and the larvae fail to get taste stimulation so the larvae die (29).

The compounds present in the extract can enter the mouth of Artemia salina and are absorbed into the digestive tract through cell membranes, then proceed with distributing toxic compounds into the body of Artemia salina, and the process of damage to metabolic reactions occurs. The anatomical structure of the body of Artemia salina at the nauplii stage is still very simple, consisting of layers of skin, mouth, antennae, digestive tract, and prospective thoracopoda. Toxic compounds can spread quickly into the body of Artemia salina due to changes in the concentration gradient between inside and outside the cells. Within 24 hours it causes 50% of Artemia salina's death, so the metabolic damage effects occur quickly and can be detected. Toxicity test on shrimp larvae or BSLT as a preliminary test is used in a research that leads to a cytotoxic test (17). Based on the results of the study, the potential toxicity level of the ethanol extract of the cat's tail leaf can be seen through a toxicity test of shrimp larvae with the BSLT method so that further bioactivity testing can be carried out.

The results of the toxicity test for the LC_{50} value of the hexane extract are presented in Table 2. The LC_{50} value was obtained based on calculations using the % probit method by plotting the % mortality with the concentration log. As shown in Table 1, the LC_{50} value of the n-hexane extract was 51,541 ppm. Potential as anticancer and toxic is an extract that has an LC_{50} value of less than 1,000 ppm so both hexane extracts are toxic and have potential as anticancer. Legundi leaf extract has higher toxic properties than the genus Protium serratum Wall with an LC_{50} value of 3.57 ppm, but both genera have the same potential as anticancer agents (30).

Analysis of Legundi Leaf Essential Oil with Gas Chromatography-Mass Spectroscopy (GC-MS)

Based on spectrogram-matched data from each main peak with the research library, the types of chemical compounds that make up the peak can be seen. In addition, the identification of chemical compounds can be done by interpreting the fragments in the spectrogram which are characteristic of a chemical compound (31).

Gas chromatography can read compounds with the lowest concentration so that secondary metabolites in plants can be identified with the results in the form of chromatograms and mass spectra (32). Identification of each peak in the chromatogram was done by matching each peak's MS spectrum with the Wiley database to determine the type of compound (33).

From the laboratory research results, it is known that the LC_{50} value of 51,541 ppm in legundi leaf essential oil is toxic to shrimp larvae. The results of GC on legundi leaves obtained eight peaks. The MS results of the identified volatile compounds were: 9-Borabicyclo [3.3.1] nonane; Bicyclo [3.1.0] hexane; Eucalyptol; Benzoic Acid; Caryophyllene; 1,5-Bis(5-methylthio-1,3,4-thiadiazole-2-ylthio); furan-2-carboxaldehyde; 2,8-dimethyl-1-napthalenyl, where there is one component compound with a relatively large abundance of furan-2-carboxaldehyde (41.77%). The compound 2-furaldehyde, often called furan-2-carboxaldehyde, fural, furfural, and furfuraldehyde, is an aromatic aldehyde compound.

The results of research conducted at the Faculty of Mathematics and Natural Sciences, Tanjungpura University in 2022 showed that there were 41 compounds with 5 main components of legundi leaf essential oil namely 2-ß-Piene, transCaryophyllene, -Ocimene, Cyclohexanol, and Eucalyptol. The results of the repellent activity of Legundi leaf essential oil (*V. trifolia*) against rice lice (*S. oryzae*) showed that the greater the level of rejection, the higher the concentration of essential oil used.

Based on the results of the chemical component analysis of the essential oil of legundi leaves, it appears that the essential oil of legundi leaves has the potential to be developed and studied further in terms of the chemical activity of the chemical compounds that comprise it (31).

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CONCLUSION

Essential oil on legundi leaves is toxic to shrimp larvae with an LC_{50} value of 51,541 ppm. GC results on legundi leaves obtained six peaks. The identified results of MS essential compounds are: 9-Borabicyclo [3.3.1] nonane; Bicyclo [3.1.0] hexane; Eucalyptol; Benzoic Acid; Caryophyllene; 1,5-Bis (5-methylthio-1,3,4-thiadiazole-2-ylthio); furan-2-carboxaldehyde; 2,8-dimethyl-1-napthalenyl.

Based on the results of the analysis of the chemical components of the essential oil of legundi leaves, it shows that the essential oil of legundi leaves has the potential to be developed and studied further regarding the chemical activity of its constituent chemical compounds because legundi leaf essential oil is toxic. These findings are important for the development of science in the field of environmental health and food product development. Environmental Health is used to increase public knowledge in the utilization of food products. It is necessary to do in this study to test the antioxidant, antitumor, and anticancer activity of legundi leaf essential oil (*Vitex trifolia L.)* so that we can get more complete information about the activity and efficacy of the legundi plant (*Vitex trifolia L.*).

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