

## Analysis of Chemical Content, Cytotoxic and Anti-Bacterial Activity Essential Oil of *Lantana Camara* Linn Leaves from Various Regions

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**ABSTRACT.** *Lantana camara* Linn is a family of Verbenaceae that grows in tropical and subtropical areas and spread in about 50 countries in the world. This plant contains essential oils with different contents based on the difference in the place of growth. The purpose of this study was to isolate and determine the chemical content of the essential oil of *L. camara* Linn leaves obtained from Agam, Tanah Datar, Kampar Regency, Padang Panjang City and to determine its cytotoxic and antibacterial activity. Isolation was carried out by hydro distillation method and chemical content analysis using Gas Chromatography-Mass Spectrometry (GC-MS). The high yield from the isolation of essential oil from leaves of *L. camara* Linn obtained from Tanah Datar Regency was 0.095% (w/w). The results of the analysis of the essential oil content with the main compounds for all Regency and City being caryophyllene with the high area percent was Tanah Datar Regency (19.74%). Cytotoxic activity test using the Brine Shrimp Lethality Test (BSLT) method showed that *L. camara* Linn essential oil was highly toxic with an LC<sub>50</sub> value of 28.34 µg/mL from Padang Panjang City. Antibacterial activity test by disc diffusion method showed that *L. camara* Linn essential oil had moderate antibacterial activity againsts *Staphylococcus aureus* and *Escherichia coli* bacteria, with high clear zone from Agam Regency was 12.9 mm and Tanah Datar Regency was 7.8 mm, respectively, at concentration of 100%.

**Keywords:** Antibacterial, cytotoxic, essential oil, *Lantana camara* Linn

### INTRODUCTION

*Lantana camara* Linn is a family of Verbenaceae with about 650 species spread in various countries worldwide, both tropical and subtropical at an altitudes up to 2000 meters (Ved et al., 2018). Traditionally this plant can treat of variety rheumatisms, ulcers, tetanus, malaria, tumor, cancer, chicken pox, asthma, fevers, cough and high blood pressure (Omoriegie et al., 2015). This plant has bioactivity such as antibacterial (Seth et al., 2012), anticancer (Kalita et al., 2012), cytotoxic (Ediruslan et al., 2015), anti-inflammatory, and antioxidant (Suryati et al., 2016). Leaves of this plant are rich essential oil. The essential oils are composed of hydrocarbons terpenes and their oxygenated derivatives (Shriniwas & Subhash, 2017). Essential oil content of a plant will be different at different topography. The composition, quality and quantity of essential oils can be influenced by several factors such as differences in climate, topography and plant age (Mann & Kaufman, 2012).

Yuliani (2013) reported the main chemical constituents of *L. camara* Linn leaf essential oil from Deli Serdang Regency, North Sumatra, namely 3-cyclohexen-1-ol,  $\alpha$ -terpinol, benzenethanol, phenol,

and hexadecanoic acid compounds (Yuliani, 2013). Suryati et al. (2021) have also reported the chemical content of *L. camara* Linn essential oil from Padang City, West Sumatra, namely isocaryophyllene,  $p$ -cimene,  $\beta$ -cubebene,  $\alpha$ -pinene, dan  $\beta$ -elemene (Suryati et al., 2021). Currently, there is not much information about the chemical content and cytotoxic and antibacterial activity of *L. camara* Linn leaves essential oil obtained from regions with different topography. Therefore, in this study, an analysis of the essential oil content of *L. camara* Linn leaves was carried out which was obtained from four different regions, namely Agam Regency, Kampar Regency, Padang Panjang city, and Tanah Datar Regency and examined the differences in the cytotoxic and antibacterial activities of isolated essential oils.

### EXPERIMENTAL SECTION

#### Material

The materials used in this research are dry powder from the leaves of the *L. camara* Linn plant from each location, Agam Regency (000°01'34" - 000°28'43" South Latitude - 990°46'39" - 1000°32'50" East Longitude), Tanah Datar Regency (00°17"-00°39"

South Latitude and 100°19"-100°51" East Longitude), Padang Panjang City (100°20"-100°30" East Longitude and 0°27"- 0°32" South Latitude), and Kampar Regency (00°27'00" South Latitude and 100°28'30" – 101°14'30" East Longitude), aquadest, anhydrous copper sulphate (Sigma-Aldrich), methanol (Merck), Paper disk, Nutrient Agar (NA) (Merck), Mueller-Hinton Agar (MHA) (Merck), tween 80, chloramphenicol, seawater, dimethyl sulfoxide (DMSO) were preserved in Cell Culture Laboratory, Faculty of Pharmacy, Andalas University. *Artemia salina* Leach shrimp larvae were purchased from biota laboratory, Sumatra. *Escherichia coli* bacteria and *Staphylococcus aureus* bacteria were purchased from biotechnology laboratory, Faculty of Agricultural Technology, Andalas University.

#### Apparatus

The tools used are a set of clevenger apparatus, GC-MS-QP-2010 (Agilent Technology 7890A/5975C), aerator, incandescent lamp, petri dish, autoclave, watch glass, magnetic bar, hot plate, incubator, analytical balance, caliper, spirit lamps, ose needles, and some glass utensils commonly used in research.

#### Essential Oil Isolation

The dry leaf mass from each region (1000 g) was isolated by the hydro distillation method using a Clevenger apparatus (Seth et al., 2012). The dried leaves are put into a distillation flask and then added aquadest until the volume is 2/3 parts of the flask. Distillation was carried out for 7 hours at 100 °C. The water in essential oil was removed by adding anhydrous copper sulphate. The isolated essential oil was weighed and its specific gravity determined.

#### Analysis of Isolated Essential Oils Using GC-MS

The isolated essential oil was analyzed using GC-MS-QP-2010 equipped with a triple-axis as a detector. The sample was injected as much as 0.1 µL with a split ratio of 200:1 without using a solvent with the injector and detector temperatures of 250 °C – 280 °C. The column used is a capillary column HP-5MS 5% phenyl methyl silox (30 m x 250 µm x 0.25 µm) with a flow rate of 1.2 mL/min with a pressure of 0.8 atm. The carrier gas used is Ultra High Purity (UHP) helium gas, which flows at a speed of 240 mL/minute.

The column temperature was set at 80 °C for 1 minute, then increased to 100 °C at a speed of 2 °C/min, then raised again to 140°C at a rate of 3 °C/min and finally grew to 170 °C at a pace of 4 °C/min. The readable m/z value of MS is 45-500 AMU with ionization energy of 70 eV and a scanning time of 3 seconds with a source temperature of 230 °C. Parameter settings for GC-MS, recording and data processing were carried out using the GC-MS Agilent Mass Hunter software. The analyzed compounds were identified using comparisons with data from National Institute of Standards and Technologies (NIST) 14.

#### Cytotoxic Activity Test

Cytotoxic activity test was carried out using the Brine Shrimp Lethality Test (BSLT) method on *Artemia salina* Leach shrimp larvae. The isolated essential oil was made into a solution with various concentrations (100; 50; 25; 12.5 and 6.25 µg/mL). Each solution with various concentrations was left for 24 hours to evaporate the solvent, then 50 µL of DMSO and 2 mL of seawater were added to each solution and homogenized using a vortex. A total of 10 larvae of *Artemia salina* Leach shrimp were put into each solution and added with seawater to a volume of 5 mL. After 24 hours, the dead shrimp larvae were counted. The number of dead shrimp larvae in each solution was used to calculate the LC<sub>50</sub> value through probit analysis and regression equations (Santoni et al., 2016; Meyer et al., 1982).

$$\% \text{ mortality} = \frac{\text{total dead shrimp larvae}}{\text{total shrimp larvae}} \times 100\%$$

#### Antibacterial Activity Test

The antibacterial activity test was carried out using the disc diffusion method using *E. coli* and *S. aureus* bacteria. Nutrient Agar (NA) and Mueller-Hinton Agar (MHA) medium were dissolved in distilled water and heated at 120 °C while stirring and then sterilized by autoclave at 121 °C for 15 minutes. Then the medium is poured into test tubes and petri dishes. The test solutions concentrations were 100%, 75% and 25% using dimethyl sulfoxide (DMSO), aquadest and tween 80. Chloramphenicol (0.2%) was used as a positive control and a mixture of DMSO and tween 80 as a negative control. The test solutions with concentrations of 100%, 75% and 25% were pipetted 10 µL and dripped onto disc paper diameter 6 mm, then placed on the surface of MHA medium and incubated for 24 hours at 37 °C.

## RESULTS AND DISCUSSION

#### Analysis of Essential Oil Isolated from *Lantana camara* Linn

The results of the isolation of essential oils from dry leaves of *L. camara* Linn from each region showed different amounts of mass, volume, density, and yield (Table 1). This difference can be caused by different topography, altitude, climate and rainfall.

The results of the analysis of essential oils by GC-MS on the four isolated essential oils also showed different amounts and main components (Table 2, Table 3 and Table 4). These data were different from previous reported by Yuliani (2013) and Suryati (2021). Yuliani obtained 12 compounds with the main components, namely 3-cyclohexen-1-ol, terpinol, benzeneethanol, phenol and hexadecanoic acid (Yuliani, 2013) and Suryati as many as 38 compounds with main components namely isocaryophyllene, p-cymene, β-cububene, β-element and α-pinene (Suryati et al., 2021).

**Table 1.** Type of experiment of essential oils from different region

| Difference              | Agam<br>Regency<br>(L1) | Kampar<br>Regency (L2) | Padang Panjang<br>City (L3) | Tanah Datar<br>Regency (L4) |
|-------------------------|-------------------------|------------------------|-----------------------------|-----------------------------|
| Oil mass (g)            | 0.93                    | 0.78                   | 0.64                        | 0.95                        |
| Oil volume (mL)         | 1.05                    | 0.88                   | 0.71                        | 1.10                        |
| Specific Gravity (g/mL) | 0.95                    | 0.91                   | 0.91                        | 0.86                        |
| Yield (% w/w)           | 0.09                    | 0.08                   | 0.06                        | 0.10                        |

**Table 2.** Results of chemical content analysis of essential oils

| No | GC-MS<br>Resulting compounds  | L1          |    | L2          |    | L3          |    | L4          |    |
|----|---|-------------|----|-------------|----|-------------|----|-------------|----|
|    |   | Area<br>(%) | SI | Area<br>(%) | SI | Area<br>(%) | SI | Area<br>(%) | SI |
| 1  | (-)-Cis- $\beta$ -elemene   | -           | -  | 3.10        | 91 | -           | -  | 2.42        | 91 |
| 2  | (+)-Eremophilene  | -           | -  | -           | -  | -           | -  | 0.68        | 97 |
| 3  | (+)- $\gamma$ -cadinene   | -           | -  | -           | -  | -           | -  | 0.91        | 95 |
| 4  | (3R,4aS,8aS)-8 $\alpha$ -Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene | -           | -  | -           | -  | 0.40        | 93 | -           | -  |
| 5  | (E)- $\alpha$ -bergamotene  | -           | -  | -           | -  | 0.32        | 99 | -           | -  |
| 6  | (E)- $\beta$ -caryophyllene   | -           | -  | -           | -  | 0.81        | 99 | -           | -  |
| 7  | 1,5,7-trimethyl-1,2,3,4-tetrahydronaphthalene   | -           | -  | 0.43        | 50 | -           | -  | -           | -  |
| 8  | 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-1H-                                   | -           | -  | -           | -  | 3.85        | 93 | -           | -  |
| 9  | cycloprop[a]naphthalene   | -           | -  | 1.31        | 90 | -           | -  | -           | -  |
| 10 | 1H-cycloprop[e]azulene  | -           | -  | 1.19        | 87 | -           | -  | -           | -  |
| 11 | 1S,2S,5R-1,4,4-Trimethyltricyclo[6,3,1,0(2,5)]dodec-8(9)-ene  | 2.45        | 80 | -           | -  | -           | -  | -           | -  |
| 12 | 2,2,3-trimethyl-hexane  | -           | -  | 1.18        | 39 | -           | -  | 1.51        | 98 |
| 13 | 2-carene  | -           | -  | -           | -  | -           | -  | 0.20        | 93 |
| 14 | 2-ethyl-oxetane   | -           | -  | 1.19        | 86 | -           | -  | 1.56        | 95 |
| 15 | 2-methyl-tetrahydro-furan   | -           | -  | -           | -  | -           | -  | 1.42        | 99 |
| 16 | 3,4-dimethyl-3-cyclohexenylmethanal   | -           | -  | -           | -  | -           | -  | 0.73        | 89 |
| 17 | 3-carene  | -           | -  | 0.33        | 95 | 0.22        | 93 | -           | -  |
| 18 | 4-ethyl-o-xylene  | -           | -  | -           | -  | 0.27        | 70 | -           | -  |
| 19 | 4-terpineol   | -           | -  | -           | -  | 0.45        | 76 | 0.28        | 76 |
| 20 | 8-isopropenyl-1,5-dimethyl-1,5-cyclodecadiene   | -           | -  | 2.20        | 95 | 0.63        | 93 | -           | -  |
| 21 | Abulnesene  | -           | -  | 0.48        | 92 | -           | -  | -           | -  |
| 22 | Alloaromadendrene   | 0.78        | 99 | -           | -  | -           | -  | -           | -  |
| 23 | Aristolochene   | 1.40        | 44 | -           | -  | -           | -  | -           | -  |
| 24 | Aromadendrene   | -           | -  | 1.28        | 99 | -           | -  | 0.93        | 99 |

|    |                                   |       |    |           |    |       |    |       |    |
|----|-----------------------------------|-------|----|-----------|----|-------|----|-------|----|
| 25 | Azulene                           | -     | -  | 1.28      | 99 | -     | -  | -     | -  |
| 26 | Bergamotene                       | 0.38  | 99 | -         | -  | -     | -  | -     | -  |
| 27 | Bicyclosesquiphellandrene         | -     | -  | 0.41      | 97 | -     | -  | -     | -  |
| 28 | Biosol                            | -     | -  | -         | -  | 0.17  | 97 | -     | -  |
| 29 | Bicyclosesquipellandrene          | -     | -  | -         | -  | -     | -  | 0.52  | 95 |
| 30 | Borneol                           | 0.19  | 97 | 0.42      | 95 | 0.21  | 95 | -     | -  |
| 31 | Cadina-3,5-diene                  | 1.83  | 95 | 2.61      | 95 | -     | -  | -     | -  |
| 32 | Calamenene                        | 1.62  | 60 | -         | -  | 1.87  | 38 | 0.86  | 60 |
| 33 | Camphene                          | -     | -  | 0.18      | 96 | -     | -  | -     | -  |
| 34 | Caryophyllene                     | 18.38 | 99 | 14.5<br>3 | 99 | 12.23 | 99 | 19.74 | 99 |
| 35 | Cis-3-methyl-2-pentane            | -     | -  | 1.70      | 52 | -     | -  | -     | -  |
| 36 | Cis-3-methyl-pentene              | -     | -  | -         | -  | -     | -  | 2.19  | -  |
| 37 | Cis-muurolo-3,5-diene             | -     | -  | -         | -  | 1.19  | 96 | 2.6   | 95 |
| 38 | Cis- $\beta$ -osimene             | -     | -  | -         | -  | -     | -  | 0.44  | 97 |
| 39 | Cosmene                           | -     | -  | -         | -  | -     | -  | 0.22  | 68 |
| 40 | Cubebanol                         | 1.16  | 96 | -         | -  | -     | -  | -     | -  |
| 41 | Cubenene                          | -     | -  | -         | -  | 0.52  | 97 | -     | -  |
| 42 | Cyclofenchen                      | 0.28  | 93 | -         | -  | -     | -  | -     | -  |
| 43 | Cycloisolongifoline, 8,9-dehydro- | -     | -  | 2.22      | 90 | -     | -  | -     | -  |
| 44 | D-camphore                        | -     | -  | 0.39      | 94 | -     | -  | -     | -  |
| 45 | D-limonene                        | 0.42  | 99 | 1.59      | 99 | 0.90  | 99 | 0.80  | 99 |
| 46 | Element                           | 2.63  | 91 | -         | -  | 2.11  | 91 | -     | -  |
| 47 | Epi-bicyclosesquiphellandrene     | 0.53  | 97 | -         | -  | 0.70  | 96 | -     | -  |
| 48 | Epizonarene                       | -     | -  | -         | -  | 0.14  | 95 | -     | -  |
| 49 | Eremophilene                      | 1.27  | 95 | -         | -  | 1.05  | 97 | -     | -  |
| 50 | Eucalyptol                        | -     | -  | 0.76      | 95 | -     | -  | -     | -  |
| 51 | Germacrene B                      | 7.21  | 99 | 9.54      | 97 | 7.59  | 99 | 10.29 | 99 |
| 52 | Germacrene D                      | 6.86  | 98 | 3.10      | 70 | 9.60  | 98 | -     | -  |
| 53 | Gurjunene                         | -     | -  | 0.19      | 96 | -     | -  | -     | -  |
| 54 | Humulene                          | 3.82  | 97 | 3.71      | 97 | 3.48  | 97 | 3.88  | 97 |
| 55 | Isocadinene                       | 0.32  | 93 | -         | -  | 0.36  | 93 | -     | -  |
| 56 | Isocadinene                       | -     | -  | -         | -  | -     | -  | -     | -  |
| 57 | Isohexane                         | -     | -  | -         | -  | -     | -  | -     | -  |
| 58 | Isospathulenol                    | 4.52  | 86 | -         | -  | -     | -  | -     | -  |
| 59 | Methyleugenol                     | -     | -  | -         | -  | -     | -  | -     | -  |
| 60 | Myrtenyl isobutyrate              | 0.23  | 68 | -         | -  | -     | -  | -     | -  |
| 61 | Neoisolongifoline, 8,9-dehydro-   | 0.38  | 70 | -         | -  | -     | -  | -     | -  |
| 62 | N-hexane                          | -     | -  | -         | -  | 1.69  | 90 | -     | -  |
| 63 | Occidentalol                      | 0.49  | 55 | -         | -  | -     | -  | -     | -  |
| 64 | O-cymene                          | 3.62  | 91 | 3.41      | 91 | 5.66  | 90 | 4.49  | 91 |
| 65 | Sabinene                          | 0.47  | 96 | 1.22      | 96 | -     | -  | -     | -  |
| 66 | Sikloheksana                      | 0.47  | 91 | 0.56      | 91 | 0.78  | 91 | 0.69  | 91 |
| 67 | Spathulenol                       | 4.66  | 96 | -         | -  | -     | -  | -     | -  |
| 68 | Tau-cadinol                       | -     | -  | 1.72      | 96 | -     | -  | -     | -  |
| 69 | Terpineol                         | -     | -  | 0.26      | 86 | -     | -  | -     | -  |

|     |                              |      |    |      |    |      |    |      |    |
|-----|------------------------------|------|----|------|----|------|----|------|----|
| 70  | Thymol                       | 0.41 | 95 | 0.93 | 94 | 0.86 | 95 | 0.72 | 95 |
| 71  | Trans- $\alpha$ -bergamotene | -    | -  | -    | -  | -    | -  | 0.42 | 99 |
| 72  | Trans- $\beta$ -ocimene      | -    | -  | 0.48 | 96 | -    | -  | 0.43 | 96 |
| 73  | Valerena-1,10-diene          | -    | -  | -    | -  | 2.56 | 62 | -    | -  |
| 74  | Ylangene                     | -    | -  | -    | -  | 0.15 | 99 | -    | -  |
| 75  | $\alpha$ -amorphene          | -    | -  | 0.24 | 95 | -    | -  | -    | -  |
| 76  | $\alpha$ -calacorene         | -    | -  | -    | -  | 0.27 | 98 | -    | -  |
| 77  | $\alpha$ -copaene            | 0.48 | 99 | 1.88 | 98 | 0.5  | 99 | 0.55 | 99 |
| 78  | $\alpha$ -cubebene           | 0.60 | 97 | 0.46 | 97 | 0.52 | 97 | 0.51 | 98 |
| 79  | $\alpha$ -elemen             | 0.93 | 94 | 4.11 | 95 | -    | -  | -    | -  |
| 80  | $\alpha$ -fernesene          | -    | -  | 0.42 | 91 | -    | -  | -    | -  |
| 81  | $\alpha$ -guaiene            | 0.58 | 99 | 1.28 | 99 | 0.55 | 99 | -    | -  |
| 82  | $\alpha$ -muurolene          | 1.52 | 99 | 1.07 | 99 | 5.18 | 98 | 0.21 | 95 |
| 83  | $\alpha$ -phellandrene       | -    | -  | 0.78 | 87 | -    | -  | 0.29 | 94 |
| 84  | $\alpha$ -pinene             | 1.43 | 97 | 2.82 | 94 | 2.16 | 96 | 2.48 | 95 |
| 85  | $\alpha$ -selinene           | 4.87 | 96 | -    | -  | 1.8  | 99 | 5.12 | 96 |
| 86  | $\alpha$ -terpinene          | -    | -  | 0.45 | 97 | -    | -  | 0.20 | 96 |
| 87  | $\alpha$ -thujene            | 0.19 | 94 | 0.51 | 94 | -    | -  | -    | -  |
| 88  | $\beta$ -bourbonene          | 0.50 | 98 | 0.75 | 98 | 0.54 | 98 | 0.55 | 98 |
| 89  | $\beta$ -copaene             | 2.39 | 98 | 1.71 | 98 | 2.44 | 98 | 2.43 | 99 |
| 90  | $\beta$ -costol              | -    | -  | -    | -  | -    | -  | 6.59 | 70 |
| 91  | $\beta$ -cubebene            | -    | -  | 0.89 | 97 | -    | -  | 0.99 | 97 |
| 92  | $\beta$ -guaiene             | -    | -  | -    | -  | 0.90 | 84 | -    | -  |
| 93  | $\beta$ -helmiscapene        | 2.40 | 99 | -    | -  | -    | -  | -    | -  |
| 94  | $\beta$ -myrcene             | -    | -  | 0.49 | 96 | 0.26 | 96 | 0.30 | 96 |
| 95  | $\beta$ -ocimene             | -    | -  | 0.82 | 97 | 0.24 | 97 | -    | -  |
| 96  | $\beta$ -pinene              | 1.10 | 97 | 2.06 | 94 | 1.68 | 97 | 1.78 | 94 |
| 97  | $\beta$ -selinene            | -    | -  | 1.43 | 99 | 1.91 | 99 | 1.92 | 99 |
| 98  | $\beta$ -thujene             | -    | -  | -    | -  | 0.27 | 93 | 0.86 | 96 |
| 99  | $\beta$ -vatirenene          | 1.20 | 96 | 0.28 | 96 | 1.36 | 97 | 0.47 | 99 |
| 100 | $\gamma$ -bulgarene          | -    | -  | -    | -  | 2,25 | 98 | -    | -  |
| 101 | $\gamma$ -elemene            | 0.53 | 99 | -    | -  | 0.41 | 98 | 0.43 | 98 |
| 102 | $\gamma$ -gurjunene          | 0.70 | 97 | -    | -  | 0.84 | 98 | 0.58 | 97 |
| 103 | $\gamma$ -muurolene          | 2.06 | 97 | 2.11 | 94 | 1.57 | 93 | 1.38 | 94 |
| 104 | $\gamma$ -terpinene          | 0.32 | 83 | 0.42 | 83 | 0.62 | 96 | 1.04 | 97 |
| 105 | $\delta$ -amorphene          | 4.27 | 95 | 4.07 | 95 | 4.08 | 95 | 3.34 | 95 |
| 106 | $\delta$ -elemene            | 0.29 | 99 | 1.09 | 98 | 0.33 | 99 | 0.62 | 98 |

Information: L1 (Agam Regency); L2 (Kampar Regency); L3 (City of Padang Panjang); L4 (Tanah Datar Regency); SI (Similiarity Index (%))

**Table 3.** Group of essential oil compounds

| Group compounds            | Percentage area (%) |       |       |    |
|----------------------------|---------------------|-------|-------|----|
|                            | L1                  | L2    | L3    | L4 |
| Hydrocarbon monoterpenes   | 18                  | 25.80 | 20.80 | 30 |
| Hydrocarbon sesquiterpenes | 66                  | 54.83 | 67.80 | 60 |
| Oxygenated monoterpenes    | 4                   | 8.06  | 6.70  | 4  |
| Oxygenated sesquiterpenes  | 8                   | 9.67  | -     | 2  |

**Table 4.** The main chemical constituents of essential oils isolated from several regions (L1, L2, L3 and L4)

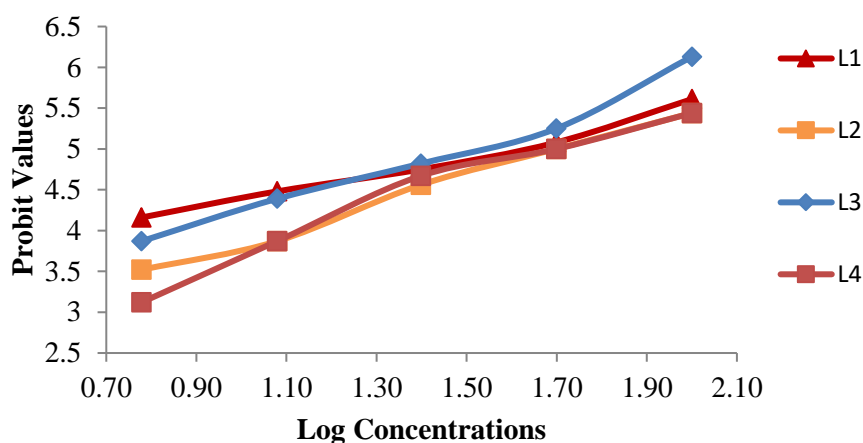
| Area                     | Topography   | Main content of essential oil (percent area)   | The number of compounds obtained |
|--------------------------|--|--|----------------------------------|
| Agam Regency (L1)        | <ul style="list-style-type: none"> <li>Altitude: 0 – 2891 masl</li> <li>Rainfall: 2500-4500 mm/year</li> </ul> | <ul style="list-style-type: none"> <li>Caryophyllene (18.38%)</li> <li>Germacrene B (7.21%)</li> <li>Germacrene D (6.86%)</li> <li><math>\alpha</math>-selinene (4.87%)</li> <li>Spatulenol (4.66%)</li> </ul>               | 50 compounds                     |
| Kampar Regency (L2)      | <ul style="list-style-type: none"> <li>Altitude: 0-1000 masl</li> <li>Rainfall: 283 mm/year</li> </ul>         | <ul style="list-style-type: none"> <li>Caryophyllene (14.53%)</li> <li>Germacrene B (9.54%)</li> <li><math>\alpha</math>-element (4.11%)</li> <li><math>\delta</math>-amorphene (4.07%)</li> <li>Humulena (3.71%)</li> </ul> | 62 compounds                     |
| Padang Panjang City (L3) | <ul style="list-style-type: none"> <li>Altitude: 650- 850 masl</li> <li>Rainfall: 3295 mm/year</li> </ul>      | <ul style="list-style-type: none"> <li>Caryophyllene (12.23%)</li> <li>Germacrene D (9.60%)</li> <li>Germacrene B (7.59%)</li> <li>o-simena (5.66%)</li> <li><math>\alpha</math>-muurolene (5.18%)</li> </ul>                | 59 compounds                     |
| Tanah Datar Regency (L4) | <ul style="list-style-type: none"> <li>Altitude: 200-1000 masl</li> <li>Rainfall: 1750-4000 mm/year</li> </ul> | <ul style="list-style-type: none"> <li>Caryophyllene (19.74%)</li> <li>Germacrene B (10.29%)</li> <li><math>\beta</math>-costol (6.59%)</li> <li><math>\alpha</math>-selinene (5.12%)</li> <li>o-simena (4.49%)</li> </ul>   | 50 compounds                     |

Based on these data, it is known that the essential oil isolated from *L. camara* Linn leaves have a different percentage of compound area, both qualitatively and quantitatively with differences in the growing regions and topography. Based on the data in **Table 4**, Agam Regency with the highest rainfall and altitude has 50 compounds contained in essential oils and the main compound content of caryophyllene is 18.38%. Tanah Datar Regency with the second highest rainfall and altitude also has 50 compounds in essential oils with the main compound content of caryophyllene of 19.74%. The city of Padang Panjang, which has the third highest rainfall and altitude, has 59 compounds in essential oils with the main compound content of 12.23% caryophyllene. Meanwhile, Kampar Regency with the lowest rainfall and altitude has 62 compounds contained in essential oils with the main compound content of 14.53% caryophyllene. Each area has rainfall and different altitudes produce different amounts of chemical compounds and chemical constituents. So, it can be concluded that topography greatly affects the chemical content in a plant, especially in the essential oil of the plant. Various factors that cause this variation can be distinguished based on intrinsic and extrinsic factors. Intrinsic factors relate to plant interactions with the environment such as soil type and climate, seasonal differences, plant maturity, geographic origin, genetics, and sampling

time. Extrinsic factors are related to the extraction method and sample treatment (Dhifi et al., 2016).

#### Cytotoxic Activity

The cytotoxic activity of isolated essential oils showed highly toxic properties from all regions (**Figure 1**). This data is supported by the presence of monoterpene and sesquiterpene compounds which are dominant in all isolated essential oils such as the presence of  $\alpha$ -pinene and limonene in all isolated essential oils. The cytotoxic potential of monoterpenes and sesquiterpenes has been reported by Dirgantara et. al (2018) acts as stomach poisoning or stomach poison that can inhibit the eating power (antifeedant) of the test animals used (Dirgantara et al., 2018). This is related to the exposure of Raineri (1981) that the toxic compounds present in the sample will enter the digestive tract of the test animals through the mouth. Then the toxic compounds will be absorbed through the cell membrane. A drastic change in concentration inside and outside the cell causes the toxic compounds to be well distributed in the body of the test animal. As a result, there is a metabolic reaction disorder in shrimp larvae (Raineri, 1981). In addition, non-polar and lipophilic essential oils (monoterpenes and sesquiterpenes) can also have an effect on the death of the test animals used by interfering with cell permeability (Bakkali & Idaomar, 2008).



**Figure 1.** Graph of the relationship between probit values and log concentrations of essential oils isolated from L1, L2, L3 and L4

The results  $LC_{50}$  value from regression calculations are 36.04; 51.55; 28.34; and 50.35  $\mu\text{g/mL}$  for Agam Regency (L1), Kampar Regency (L2), Padang Panjang City (L3), and Tanah Datar Regency (L4). The level of toxicity of a compound is said to be non-toxic if the  $LC_{50}$  value is  $>1000 \mu\text{g/mL}$ , low toxic if the  $LC_{50}$  is 500-1000  $\mu\text{g/mL}$ , moderately toxic (medium toxic) if the  $LC_{50}$  is 100-500  $\mu\text{g/mL}$  and high toxic (high toxic)  $LC_{50}$  0-100  $\mu\text{g/mL}$  (Hamidi et al., 2014). Based on these data, it is known that the cytotoxic activity of the essential oil of *L. camara* Linn leaves from each region is highly toxic (Meyer et al., 1982).

**Antibacterial Activity**

The essential oil isolated from *L. camara* Linn leaves from the four regions showed moderate activity in inhibiting the growth of good bacteria *E. coli* or *S. aureus* bacteria. The data on the inhibition zones of essential oils isolated from each region and the controls used are shown in **Table 5**.

Based on the data in **Table 5**, each essential oil of the area has a different inhibition zone. This is caused by differences in the amount and type of chemical content present in the essential oil. According to Davis and Stout in Sudewi & Lolo (2016) the grouping of the inhibitory power of a compound against bacteria

based on its inhibitory diameter was divided into very strong groups with a diameter of  $> 20$  mm, a strong group with a diameter of 10-20 mm and a moderate group with a diameter of 5-10 mm and a weak group with a diameter of 0-5 mm (Sudewi & Lolo, 2016). In the results showed the greatest inhibition zone value at 100% essential oil concentration was from Agam Regency for *S. aureus* bacteria (12.90 mm) and Tanah Datar Regency for *E. coli* bacteria (7.83 mm). For antibacterial activity, essential oils from each region at concentrations of 100%, 75%, and 25% had moderate antibacterial activity against *S. aureus* and *E. coli* bacteria. The different antibacterial activity in this area is due to the difference in the content of the essential oil. Essential oil from Agam and flat soil showed better antibacterial ability due to the presence of  $\alpha$ -selinene and caryophyllene compounds that act as antibacterial agents. Based on the topography of each region, selinene compounds seem to be produced more by plants that grow in areas that have rainfall  $>3295$  mm/year. Selinene compound have been reported has antibacterial activity. Sabinene,  $\gamma$ -terpinene, and limonene compounds has moderate antibacterial activity against *S. aureus* and weak against *E. coli*. (Koroch et al., 2007).

**Table 5.** The results of the antibacterial activity of essential oils isolated from each region

| Area | Inhibition Zone (mm) |      |      |                  |                  |                |      |      |                  |                  |
|------|----------------------|------|------|------------------|------------------|----------------|------|------|------------------|------------------|
|      | <i>S. aureus</i>     |      |      |                  |                  | <i>E. coli</i> |      |      |                  |                  |
|      | 100%                 | 75%  | 25%  | Positive Control | Negative Control | 100%           | 75%  | 25%  | Positive Control | Negative Control |
| L1   | 12.90                | 9.50 | 7.50 | 23.35            | 0                | 7.80           | 7.20 | 6.80 | 21.15            | 0                |
| L2   | 7.95                 | 6.20 | 5.50 | 23.95            | 0                | 6.60           | 5.80 | 5.00 | 21.15            | 0                |
| L3   | 9.45                 | 7.10 | 6.25 | 23.95            | 0                | 6.90           | 5.65 | 5.00 | 21.15            | 0                |
| L4   | 10.43                | 7.76 | 6.03 | 23.35            | 0                | 7.83           | 6.41 | 5.73 | 20.40            | 0                |

## CONCLUSIONS

Analysis of the essential oil content of *L. camara* Linn leaves obtained from four different areas showed that there were differences in both the amount and the main components caused by topographic differences. The test results of cytotoxic activity and antibacterial activity showed the same ability both as cytotoxic and as antibacterial. Essential oils isolated from all tested areas had strong cytotoxic activity against *Artemia salina* Leach shrimp larvae and moderate antibacterial activity against *E. coli* and *S. aureus* bacteria.

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