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Chemical Compound of *Terminalia Catappa L.* as Hemostatic Agents in Post Tooth Extraction

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Abstract

*Terminalia catappa L.* is one of the herbal plants that contain flavonoids that play a role in wound healing including to bleeding after tooth extraction. The aim of our study was to identify the chemical compounds contained in *Terminalia catappa L.* leaves. This type of research was experimental. The sample in this study was the leaves of *Terminalia catappa* number 3-6 from the base, at a tree height of 6 meters picked as much as 6 kilograms. Data analysis qualitatively and quantitatively. The highest content in ethanol extract of *Terminalia catappa L.* of saponins and alkaloids were fractioned with distilled water and the lowest content were fractioned with hexane. Meanwhile distilled water is also used to fractioned the highest content of tannins and phenol. While ethyl acetate was used to fractioned the lowest content of tannins and the highest content of flavonoids. The lowest flavonoid in distilled water extract was fractioned using hexane. For the lowest content of phenol was fractioned using hexane. The results show the highest content of saponins is 3,787.80 mg/100g, the lowest is 166.67 mg/100g. The highest content of Alkaloids is 1,798.57 mg/100g, and the lowest is 576.80 mg/100g. The highest content of tannins is 53,140.72 mg/100g, the lowest is 8,391,803 mg/100g. The highest content of flavonoids is 2,5964.14 mg/100g, the lowest flavonoid is 462.84 mg/100g. The highest content of phenol 29,968.05 mg/100g, the lowest is 225.46 mg/100g. The highest antioxidant activity with AAI (Antioxidant Activity Index) obtains value of 0.36. This shows the moderate antioxidant ability. *Terminalia catappa L.* contain saponins, alkaloids, tannins, flavonoids, and triterpenoids. The active compound of *Terminalia catappa L.* will generally be produced optimally if a polar solvent is used.

Keywords: *Terminalia Catappa L.*, Hemostatic, Tooth Extraction

1. Introduction

Tooth extraction is a common practice in dentistry. The act of tooth extraction can interfere with various complications, such as bleeding or also recognized as post-extraction bleeding (PEB). Post-extraction bleeding happens beyond 8 – 12 hours after dental extraction(Nagraj et al., 2018). Bleeding is indicated by broken blood vessels (arteries, veins or capillaries) due to trauma, which can occur in external or internal blood vessels. Complications that may occur during tooth extraction are bleeding, fracture (crown, root, mandible), dry socket,
swelling, mandibular dislocation, shock, and several other complications. Wound healing is a complex process because there are many different cell interactions with cytokine mediators and the extracellular matrix. The wound healing process is divided into 4 continuous and overlapping phases, namely hemostasis, inflammation, proliferation, and remodeling or maturation phases (Gonzalez et al., 2016).

Traditional medicine is one of the alternative treatments that people choose to treat wounds. Herbal medicine has attracted interest from 80% people worldwide by using it as part of primary healthcare (Ekor, 2014). The reasons for safety and originated from nature become the consideration of people using herbal medicine. In line with the global use of herbal medicine, the concerns of its safety have come into account. The safety of the medicine includes the content inside the herbal medicine itself. However, herbal medicine has become common in its lacking quality control and insufficient knowledge of the herbal medicine towards people. So it is essential to study the information of the herbal medicine for a better understanding of the risks that may associate with the herbal medicine used (Raynor et al., 2011).

One natural ingredient that is often used for healing wounds is the extracts from *Terminalia catappa L.* as they contain flavonoid compounds that can accelerate wound healing. *Terminalia catappa L.* or Indian almonds has become the alternative for the healing of a wound. *Terminalia catappa L.* is proven 97% succeed in reducing the wound area of a mice compared to betadine which is only 81% (Khan et al., 2014) and confirmed by (Nugroho et al., 2019) that reveals *Terminalia catappa L.* is working better than Povidone-iodine in wound healing. Our aim of research was to identify the chemical compounds in *Terminalia catappa L.* with quantitative and qualitative analysis based on the experimental work.

2. Method

The type of research is an experiment in a laboratory with qualitative and quantitative measurement designs. This research has obtained ethical clearance from the Health Research Ethics Commission, Poltekkes Kemenkes Denpasar, Bali, number LB.02.03/EKPK/0598/2021. The experimental steps in this research are as follows:

1. Making Simplisia

*Terminalia catappa L.* taken were mature leaves because the older the leaves affect the content of secondary metabolites. The leaves are dark green, then the leaves were washed under running water. Next, *Terminalia catappa L.* was chopped into smaller pieces. In this study, drying was carried out using an oven at 50°C for 24 hours. After the leaves are dry, the *Terminalia catappa L.* simplisia is made by blending the dried *Terminalia catappa L.* that have been blended were sieved through a 60 mesh sieve.

2. Preparation of Ethanol and Distilled Water/Aquades Extract

The powder of *Terminalia catappa L.* leaves was then macerated with ethanol and aquades (with a ratio of 1:5) for 3 days (72 hours) at room temperature (20–25 °C). Maceration is an extraction step, which a process of soaking the ingredients (sample) is using a solvent that is suitable for the active compound to be taken with low heating or without a heating process. Maceration is a process of extracting or withdrawing active compounds based on differences in the polarity of the active compounds in the extract.

The filtrate was obtained by filtering with Whatman No.1 filter paper (Muhammad & Mudi, 2011; Filho et al., 2017). The pulp obtained was then macerated again with 1000 ml of ethanol 2 times. The obtained filtrate was combined and then evaporated using a vacuum rotary evaporator (Iwaki, Japan) at a temperature of 40°C. The evaporation results obtained crude extract of *Terminalia catappa L.* ethanol and crude extract of distilled water of *Terminalia catappa L.* The crude extract obtained in the form of a paste is assumed to be at a concentration of 100%.

Ethanol is a universal solvent that can attract polar compounds (polar -OH group) and non-polar (CH₂-CH₃) groups so that it can attract some of the active compounds contained in both polar and non-polar plants. The macerate obtained was filtered and the solvent was evaporated using a rotary evaporator (Bahrin et al., 2018). The purpose
of using a rotary evaporator is that to evaporate the solvent at low temperatures with the help of a vacuum so that the extract is not damaged by high temperatures (Santoso et al., 2021).

3. Extract Fractionation

The extraction process may use three types of solvents with different polarity levels. Polarity or polarity is the separation of electric charges leading to molecules or chemical groups having an electric moment. Polar molecules contain polar chemical bonds based on the electronegativity between the bonded atoms. Atoms have the power to attract electrons. Electronegativity is the amount of “pull” an atom exerts on an electron. With regard to the polarity of the solvent, there are three classes of solvents (Kopeliovich, 2017; Abarca-vargas et al., 2016):

a. Polar

Polar solvent has a high degree of polarity, suitable for extracting polar compounds from plants. Polar solvents tend to be universally used because though they are polar, they can still extract compounds with lower polarity levels. Examples of polar solvents are: water, methanol, ethanol, acetic acid.

b. Semi-polar

Semi-polar solvents have a lower level of polarity than polar solvents. This solvent is good for obtaining semi-polar compounds from plants. Some of semi-polar solvents are: acetone, ethyl acetate, chloroform.

c. Non-polar

Non-polar solvent is almost completely non-polar. This solvent is good for extracting compounds that are completely insoluble in polar solvent. This compound is good for extracting various types of oil. Some of the examples are hexane and ether.

Fractionation is the process of separating the components in an extract based on their level of polarity i.e. separating the fractions for polar, semi-polar and nonpolar compounds. The purpose of fractionation is to separate the components of the active compound from the resulting extract (Purwaningsih et al., 2020). In this study, the polar solvent for the fractionation used water, while for semi-polar solvent used ethyl acetate; and a non-polar solvent with hexane.

The thick ethanol extract or distilled water extract (coarse extract) of Terminalia catappa L. was then partitioned using methanol, ethyl acetate, and hexane, with a composition of 4 mg crude extract and 200 ml ethanol and 200 ml hexane. First, the mixture was shaken in a separating funnel so that it was evenly mixed. Then it was allowed to stand for a while until the separation between the ethanol phase and the hexane phase was seen. The two phases were separated and the solvent in each phase was evaporated in a vacuum rotary evaporator to obtain an extract of the ethanol phase and the hexane phase. The ethanol extract, hexane fraction and ethyl acetate fraction of Terminalia catappa L. leaves obtained were then examined. (Herli & Wardaniati, 2019).

4. Phytochemical Testing

The phytochemical testing was conducted following the testing and color change in (Herli & Wardaniati, 2019).

a. Alkaloid

The sample was added with chloroform and ammonia, left for ± 5 minutes. Subsequently, H₂SO₄ 2M was added. Then, it was shaken until 2 layers of acid are formed. The acid layer was put in 3 test tubes and each of the Mayer, Wagner and Dragendorf reagents was added. Positive results were indicated by the formation of a white precipitate on the Mayer reagent, a brown precipitate on the Wagner reagent and an orange precipitate on the Dragendorf reagent.
b. Flavonoid Test

In flavonoid test, the samples were heated for ± 5 minutes and added concentrated HCl and 2 small pieces of Mg. The reaction is said to be positive if there is a red color change to orange. A few isolates were dissolved in ethanol then added 10% NaOH reagent, the reaction is positive if there is a specific color change.

c. Triterpenoid and steroid testing

The samples were added with chloroform, anhydrous acetic acid and concentrated sulfuric acid. Positive results formed orange/purple color for triterpenoids and green color for steroids.

d. Saponin Test

Samples were heated for ± 5 minutes, cooled and shaken. A positive result for the formation of stable foam/foam that does not disappear for 2-3 minutes indicates the presence of saponins.

e. Tannins Testing

The samples have added a solution of FeCl₃ 1% and HCl 2 M. With a solution of FeCl₃ 1%, there will be a color change to green-black, whereas with HCl 2 M will be indicated by a color change to red.

5. Quantitative Examination

Measurement of chemical substances contained in *Terminalia catappa* L. leaves was carried out in the laboratory of the Faculty of Food Technology, Udayana University and the Laboratory of Agricultural Analysis, Faculty of Agriculture, Warmadewa University. The phytochemical test procedure was as follows:

a. Total phenol

Determination of total phenol was performed using the Folin–Ciocalteau method. A total of 0.01 g of extract was diluted into 5 ml of citrate phosphate buffer according to treatment. A sample of 0.1 ml was pipetted and 0.3 ml of 70% ethanol was added. After that, 0.4 ml of Folinciaocalteau was added and incubated for 6 minutes. After the incubation process, 4.2 ml of 5% Na₂CO₃ was added and then vortexed and incubated for 90 minutes. The absorbance was read at a wavelength of 760 nm. The readings are compared with a standard curve made using gallic acid. Calculation of total phenol is calculated using the following equation (1).

\[
\text{Total phenol} \left( \frac{\text{mg GAE}}{\text{g extract}} \right) = \frac{C \times V \times FP}{W}
\]

Where C is the sample concentration from linear regression (mg/L); FP is Dilution factor; V is sample volume (L) and W is sample weight (g).

b. Total flavonoids

Determination of total flavonoids using a spectrophotometer with the AlCl₃ method refers to (Singh, Verma, & Singh, 2012). A total of 0.01 g of extract was diluted into 5 ml of citrate phosphate buffer according to treatment. A total of 1 ml of sample was mixed with 4 ml of distilled water and added 0.3 ml of NaNO₂ solution (10%). After that, it was incubated for 5 minutes and added 0.3 ml of AlCl₃ solution (10%) and 2 ml of NaOH solution (1%), then immediately tested with a spectrophotometer at a wavelength of 510 nm. The flavonoid concentration in the test sample was calculated as C x V x FP W 37 from the calibration standard prepared using the quercetin standard and expressed as quercetin equivalents in mg QE/g extract. The total flavonoid calculation is calculated using the equation (2).

\[
\text{Total phenol} \left( \frac{\text{mg QE}}{\text{g extract}} \right) = \frac{C \times V \times FP}{W}
\]
c. Total tannin

Determination of total tannin extract was analyzed using the Folin-Denis method. A total of 0.01 g of extract was diluted into 5 ml of citrate phosphate buffer according to treatment. The sample that has been diluted in a pipette is 0.25 ml, then 0.25 ml of Folin-Denis reagent is added, then vortexed and 2 ml of 5% Na2CO3 is added. The solution was vortexed and then incubated for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 725 nm. The readings were compared with a standard curve using tannic acid. The total tannin in the sample was expressed as the equivalent of tannic acid in mg TAE/g extract. Total tannins were calculated using equation (3).

\[
\text{Total phenol (mg TAE/g extract)} = \frac{C \times V \times FP}{W}
\]

(3)

d. Test for saponin content

The test for saponin content was weighed 100 mg of the sample and added 2 ml of 25% H2SO4. Then it was autoclaved for 120 minutes, at 110°C. Next, it was extracted with ether and then dried the filtrate. Then 1 ml of water was added and vortex extraction for 5 minutes. Add 50 µl of anisaldehyde, shake and let stand for 10 minutes. Add 2 ml of 50% sulfuric acid and heat with a water bath at 60°C for 10 minutes. Then add water to a volume of 10 ml with a measuring flask. It was diluted 10 times and read the absorption at a wavelength of 435 nm.

e. Determination of total alkaloid content

The total crude alkaloids were carried out by liquid-liquid extraction (LLE) method. The filtered extract was concentrated on a hotplate with constant stirring at a temperature of 50°C. The concentrated extract was added with 25 mL of 2% HCl and 25 mL of n-hexane. Then extracted in a 250 mL separating funnel (Pyrex). The hydrochloric acid extract was added with 35% w/w ammonium hydroxide to pH 9, added 25 mL of chloroform and extracted in a 250 mL separating funnel (Pyrex). The chloroform was given twice and evaporated to obtain total crude alkaloid solids.

f. Determination of antioxidant activity

The antioxidant activity was carried out using the DPPH method (1,1-diphenyl-2-picrylhydrazyl) in accordance to research by (Shah & Modi, 2016). A total of 1 ml of 0.1 mM DPPH solution in ethanol was dissolved with 2 ml of extract in a test tube. The solution was vortexed and incubated for 30 minutes in the dark at room temperature. The absorbance was read at a wavelength of 517 nm using a spectrophotometer. The blank used was ethanol. Controls were made according to the treatment given in the sample testing process but without adding a sample. The percentage of free radical scavenging ability (antioxidant activity) is calculated by equation (4).

\[
\text{Antioxidant activity (%) = } \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100
\]

(4)

After testing the antioxidant activity, the IC50 testing was performed. IC50 is the sample concentration required to inhibit 50% of DPPH free radicals. The sample used is extract. The sample concentrations were varied from 0, 100, 200, 300, 400, and 500 mg/L, then the antioxidant activity was measured. IC50 value can be obtained by linear regression equation.

3. Results

1. Qualitative Analysis

The qualitative checks performed on all six chemical compounds of Terminalia catappa L. leaves that has been purified (fractionation). Based on the examination carried out in the laboratory, all Terminalia catappa L. leaves
can be identified as containing: saponins, alkaloids, tannins, flavonoids, and triterpenoids as shown in the Table 1.

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Saponins</th>
<th>Alkaloid</th>
<th>Tannins</th>
<th>Flavonoid</th>
<th>Triterpenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa L.</em> ethanol</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa L.</em> ethanol</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa L.</em> ethanol</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
<td>Positive (+)</td>
</tr>
</tbody>
</table>

2. Quantitative Analysis

Quantitative analysis of the chemical compounds of *Terminalia catappa L.* carried out in the laboratory were saponins, alkaloids, tannins, flavonoids, total phenols, and activity antioxidant. Quantitative examination was repeated three times (Triplo).

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Examination</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>4.112.35</td>
<td>2,936.26</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>1,137.44</td>
<td>1,621.15</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa L.</em> aquadest</td>
<td>190.48</td>
<td>166.67</td>
</tr>
</tbody>
</table>

Table 2 shows the highest content of saponins is found in the ethanol extract of *Terminalia catappa L.* which is fractionated with aquadest/distilled water, i.e. 3,787.80 mg/100g. The lowest saponins were found in aquadest extract of *Terminalia catappa L.* leaves which were fractionated with hexane, which was 166.67 mg/100g.
Table 3: Quantitative Analysis of Alkaloids (mg/100g)

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Examination</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>1,346.15, 1,481.98, 1,413.32</td>
<td>1,413.82</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>1,284.40, 1,311.98, 1,297.44</td>
<td>1,297.94</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>636.36, 517.74, 576.30</td>
<td>576.80</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>1,636.36, 1,961.28, 1,798.07</td>
<td>1,798.57</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>1,203.70, 1,346.65, 1,274.43</td>
<td>1,274.93</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>614.04, 708.46, 660.50</td>
<td>661.00</td>
</tr>
</tbody>
</table>

Table 3 shows the highest content of alkaloids is found in the ethanol extract of *Terminalia catappa* L. leaves which is fractionated with aquadest, i.e. 1,798.57 mg/100g. While the lowest content of alkaloids is found in aquadest extract of *Terminalia catappa* L. leaves which were fractionated with hexane, which was 576.80 mg/100g.

Table 4: Quantitative Analysis of Tannins (mg/100g)

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Examination</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>12,524.59, 12,524.69, 12,524.49</td>
<td>12,524.59</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>8,481.97, 8,301.74, 8,391.70</td>
<td>8,391.803</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>10,711.48, 10,154.20, 10,432.69</td>
<td>10,432.79</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>53,303.04, 52,978.51, 53,140.62</td>
<td>53,140.72</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>17,795.81, 17,682.29, 17,738.90</td>
<td>17,739.0</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>3,237.31, 3,507.98, 3,372.493</td>
<td>3,372.593</td>
</tr>
</tbody>
</table>

Table 4 indicates the highest content of tannins is in the ethanol extract of *Terminalia catappa* L. leaves fractionated with aquadest, namely 53,140.72 mg/100g. Meanwhile, the lowest tannin content is found in aquadest extract of *Terminalia catappa* L. leaves which were fractionated with ethyl acetate, which was 8,391.803 mg/100g.

Table 5: Quantitative Analysis of Flavonoids (mg/100g)

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Examination</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>789.17, 826.77, 820.31</td>
<td>812.08</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>1122.45, 1,413.27, 1,357.14</td>
<td>1,297.62</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>417.38, 440.00, 531.14</td>
<td>462.84</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>13,390.11, 12,651.18, 12,764.82</td>
<td>12,935.37</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>24,751.55, 26,614.91, 26,525.97</td>
<td>2,5964.14</td>
</tr>
</tbody>
</table>
Hexane fraction of *Terminalia catappa* L. ethanol | 13,768.32 | 14,363.55 | 13,024.96 | 1,3718.94
---

Table 5 shows the highest content of flavonoids is in the ethanol extract of *Terminalia catappa* L. leaves which was fractionated with ethyl acetate, i.e. 2,5964.14 mg/100g. While the lowest content of flavonoids is aquadest extract of *Terminalia catappa* L leaves which was fractionated with hexane, in amount of 462.84 mg/100g.

Table 6: Quantitative Analysis of Total Phenol (mg/100g)

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Examination</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>895.84</td>
<td>902.92</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>351, 32</td>
<td>367.81</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>197.42</td>
<td>225.46</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>31.706.51</td>
<td>29.968.05</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>7,422.73</td>
<td>7,441.15</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>1,291.32</td>
<td>1,253.17</td>
</tr>
</tbody>
</table>

Table 6 shows the highest content of total phenol is in the ethanol extract of *Terminalia catappa* L. leaves which was fractionated with distilled water, i.e. 29,968.05 mg/100g. Meanwhile, the lowest content of total phenol is found in distilled water extract of *Terminalia catappa* L. leaves which was fractionated with hexane, in as much as 225.46 mg/100g.

Table 7: Antioxidant Activity

<table>
<thead>
<tr>
<th>Code Sample</th>
<th>Activity (%)</th>
<th>IC50 ppm</th>
<th>AAI Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>14.47</td>
<td>1.987.01</td>
<td>Very weak</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>10.59</td>
<td>2.605.49</td>
<td>Very weak</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. aquadest</td>
<td>11.76</td>
<td>2.531.11</td>
<td>Very weak</td>
</tr>
<tr>
<td>Aquadest fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>95.74</td>
<td>110.37</td>
<td>Moderate</td>
</tr>
<tr>
<td>Ethyl acetate fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>25.18</td>
<td>2.115.16</td>
<td>Very weak</td>
</tr>
<tr>
<td>Hexane fraction of <em>Terminalia catappa</em> L. ethanol</td>
<td>25.18</td>
<td>2.115.16</td>
<td>Very weak</td>
</tr>
</tbody>
</table>

Table 7 shows the highest antioxidant activity with AAI (Antioxidant Activity Index) value is 0.36. It is derived from the ethanol extract of *Terminalia catappa* L. leaves which is fractionated with aquadest. The number shows moderate antioxidant ability.
4. Discussion

Our study shows that the whole extract fraction of *Terminalia catappa L.* leaves could be identified as containing: saponins, alkaloids, tannins, flavonoids, triterpenoids, and phenols. The results of this study are in accordance with the opinion of (Muthulakshmi & Neelanarayanan, 2021) that *Terminalia catappa L.* leaves are known to contain chemical compounds such as flavonoids, alkaloids, tannins, triterpenoids, steroids, resins, saponins, quinones, and phenolics(Etienne et al., 2017). *Terminalia catappa L.* is known to contain medicinal compounds such as flavonoids (Lin et al., 2000; Tampemawa et al., 2016), triterpenoids (Gao et al., 2004; Mininel et al., 2014), tannins (Ola et al., 2020), alkaloids (Katiki et al., 2017), steroids (Babayi et al., 2004) and fatty acids (Janporn et al., 2014). The phytochemical test of crude extract of *Terminalia catappa L* leaves in this study was positive for the presence of saponins, tannins, terpenoids, alkaloids and flavonoids.

Table 2 shows the highest content is saponin contained in the ethanol extract of leaves of *Terminalia catappa L.* that fractionated with aquadest, in 3787.80 mg/100g (3.78%). While the lowest content of saponins was found in distilled water extract of *Terminalia catappa L.* leaves which was fractionated with hexane, which is 166.67 mg/100g (0.017%). The results of this study indicate that saponins in *Terminalia catappa L.* leaves can be produced optimally when using a polar solvent. Based on their polarity, ethanol and water are polar solvents(Abarca-vargas et al., 2016).

Table 3 shows the highest content of alkaloids found in the ethanol extract of ketapang leaves which were fractionated with aquadest, is 1,798.57 mg/100g (1.79%). While the lowest content of alkaloids is found in aquadest extract of *Terminalia catappa L.* leaves which were fractionated with hexane, in as much as 576.80 mg/100g (0.058%). The results of this study indicate that the alkaloids in the leaves of *Terminalia catappa L.* is able to be created optimally when using a polar solvent.

Table 4 shows the highest content of tannins in the ethanol extract of *Terminalia catappa L.* leaves which were fractionated with aquadest is 53,140.72 mg/100g (53.14%). While the lowest content of tannin was found in aquadest extract of *Terminalia catappa L.* leaves which were fractionated with ethyl acetate, which is 8,391,803 mg/100g (8.39%). The results of this study indicate that the tannins in *Terminalia catappa L.* leaves can be produced optimally when using a polar solvent. Meanwhile, the results of research by (Irawati & Nita, 2012) showed that the best tannin extraction was 12.45% with 85% ethanol as solvent. In this study no fractionation was carried out.

Table 5 shows the highest content of flavonoids is in the ethanol extract of *Terminalia catappa L.* leaves fractionated with ethyl acetate, namely 25,964.14 mg/100g (25.96%). While the lowest content of flavonoids is found in aquadest extract of *Terminalia catappa L.* leaves which was fractionated with hexane, resulting in 462.84 mg/100g (0.046%). The results of this study indicate that the flavonoids in *Terminalia catappa L.* leaves can be produced optimally when using a semi-polar solvent. Based on the polarity, ethyl acetate is a semi-polar solvent (Rahardjo et al., 2019). Tannins and flavonoids are thought to have effects as hemostatic agents/bleeding cessation(Sutopo et al., 2016; Nabavizadeh et al., 2016).

Table 6 shows that the highest content of total phenol is in the ethanol extract of *Terminalia catappa L.* leaves which was fractionated with aquadest, in amount of 29,968.05 mg/100g (29.96%). While the lowest content of total phenol is found in distilled water extract of *Terminalia catappa L.* leaves which was fractionated with hexane, in 225.46 mg/100g (0.022%). The results of this study indicate that the phenol in *Terminalia catappa L.* leaves can be produced optimally when using a polar solvent. Based on their polarity, ethanol and water are polar solvents(Abarca-vargas et al., 2016).

Based on the examination, the highest antioxidant activity with AAI value is 0.36. It is from the ethanol extract of *Terminalia catappa L.* leaves which was fractionated with aquadest. The number indicates a weak antioxidant ability (Punniyakotti et al., 2019). Likewise for the other fractions showed weak antioxidant ability. The antioxidant activity of *Terminalia catappa L.* leaves is greater than vitamin E. The AAI value was obtained from the concentration of DPPH used in the test (ppm) divided by the IC₅₀ obtained (ppm). The value of AAI < 0.5 is...
weak antioxidant, AAI > 0.5-1 is moderate antioxidant, AAI > 1-2 are strong antioxidants and AAI > 2 are very strong antioxidant (Vasi, 2012).

Conclusion

The evidences from this study points towards the idea that the leaves Terminalia catappa L. has proven contain saponins, alkaloids, tannins, flavonoids, and triterpenoids. The active compound of Terminalia catappa L. leaves will generally be produced optimally if a polar solvent is used. Besides, it is noted that Terminalia catappa L. leaves have weak antioxidant abilities.

Examination of chemical compounds in the laboratory requires special reagents. It is recommended for similar research to conduct a wider assessment to various universities and other laboratories.

References


Terminalia catappa Linn. (Combretaceae) and Their Mutagenic Activity. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1–11.


