



Journal of Health and Medical Sciences

Sirat, Ni Made, and Senjaya, Asep Arifin. (2021), Chemical Compound of *Terminalia Catappa L.* as Hemostatic Agents in Post Tooth Extraction. In: *Journal of Health and Medical Sciences*, Vol.4, No.4, 16-26.

ISSN 2622-7258

DOI: 10.31014/aior.1994.04.04.189

The online version of this article can be found at:

<https://www.asianinstituteofresearch.org/>

Published by:
The Asian Institute of Research

The *Journal of Health and Medical Sciences* is an Open Access publication. It may be read, copied, and distributed free of charge according to the conditions of the Creative Commons Attribution 4.0 International license.

The Asian Institute of Research *Journal of Health and Medical Sciences* is a peer-reviewed International Journal. The journal covers scholarly articles in the fields of Medicine and Public Health, including medicine, surgery, ophthalmology, gynecology and obstetrics, psychiatry, anesthesia, pediatrics, orthopedics, microbiology, pathology and laboratory medicine, medical education, research methodology, forensic medicine, medical ethics, community medicine, public health, community health, behavioral health, health policy, health service, health education, health economics, medical ethics, health protection, environmental health, and equity in health. As the journal is Open Access, it ensures high visibility and the increase of citations for all research articles published. The *Journal of Health and Medical Sciences* aims to facilitate scholarly work on recent theoretical and practical aspects of Health and Medical Sciences.



ASIAN INSTITUTE OF RESEARCH
Connecting Scholars Worldwide



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

Ni Made Sirat¹, Asep Arifin Senjaya²

^{1,2}Dental Hygiene Department, Poltekkes Kemenkes Denpasar, Bali

Correspondence: Ni Made Sirat, Dental Hygiene Department, Poltekkes Kemenkes Denpasar, Bali.
E-mail: sirat_made@yahoo.com

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 fractionated with distilled water and the lowest content were fractionated with hexane. Meanwhile distilled water is also used to fractionated the highest content of tannins and phenol. While ethyl acetate was used to fractionated the lowest content of tannins and the highest content of flavonoid. The lowest flavonoid in distilled water extract was fractionated using hexane. For the lowest content of phenol was fractionated 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/EA/KEPK/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.* *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 \pm 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 Dragendrof 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 Dragendrof 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 FeCl_3 1% and HCl 2 M. With a solution of FeCl_3 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–Ciocalteu 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 Folin–Ciocalteu was added and incubated for 6 minutes. After the incubation process, 4.2 ml of 5% Na_2CO_3 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).

$$\text{Total phenol} \left(\frac{\text{mg GAE}}{\text{g extract}} \right) = \frac{C \times V \times \text{FP}}{W} \quad (1)$$

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 AlCl_3 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 NaNO_2 solution (10%). After that, it was incubated for 5 minutes and added 0.3 ml of AlCl_3 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 $C \times V \times \text{FP} \times 37$ 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).

$$\text{Total phenol} \left(\frac{\text{mg QE}}{\text{g extract}} \right) = \frac{C \times V \times \text{FP}}{W} \quad (2)$$

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).

$$\text{Total phenol} \left(\frac{\text{mg TAE}}{\text{g extract}} \right) = \frac{C \times V \times FP}{W} \quad (3)$$

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).

$$\text{Antioxidant activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100 \quad (4)$$

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 1: Results of Qualitative Analysis

Code Sample	Saponins	Alkaloid	Tannins	Flavonoid	Triterpenoid
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	Positive (+)	Positive (+)	Positive (+)	Positive (+)	Positive (+)

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 2: Quantitative Analysis of Saponins (mg/100g)

Code Sample	Examination			Average
	1	2	3	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	4.411.76	4.112.35	4.261.76	4.261.96
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	2.980.77	2,892.36	2,936.26	2,936.46
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	190.48	143.06	166.47	166.67
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	4,117.65	3,458.14	3.787.60	3,787.80
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	1,137,44	2,105,46	1,621,15	1,621,35
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	406,50	388,55	397,23	397,43

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)

Code Sample	Examination			Average
	1	2	3	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	1,346.15	1,481.98	1,413.32	1,413.82
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	1,284.40	1,311.98	1,297.44	1,297.94
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	636.36	517.74	576.30	576.80
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	1,636.36	1,961.28	1,798.07	1,798.57
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	1,203.70	1,346.65	1,274.43	1,274.93
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	614.04	708.46	660.50	661.00

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)

Code Sample	Examination			Average
	1	2	3	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	12,524.59	12,524.69	12,524.49	12,524.59
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	8,481.97	8,301.74	8,391,703	8,391,803
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	10,711.48	10,154.20	10,432.69	10,432.79
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	53,303.04	52,978.51	53,140.62	53,140.72
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	17,795.81	17,682.29	17,738.90	17,739.0
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	3,237.31	3,507.98	3,372.493	3,372.593

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)

Code Sample	Examination			Average
	1	2	3	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	789.17	826.77	820.31	812.08
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	1122.45	1,413,27	1,357,14	1,297,62
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	417,38	440,00	531,14	462,84
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	13,390,11	12,651.18	12,764.82	1,2935,37
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	24,751.55	26,614.91	26,525.97	2,5964.14

Hexane fraction of <i>Terminalia catappa</i> 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* L₂ 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)

Code Sample	Examination			Average
	1	2	3	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	895.84	850.96	961.97	902.92
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	351, 32	272.81	326.23	316.79
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	197.42	209.16	269.8	225.46
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	31.706.51	28.232.18	29.965.46	29.968.05
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	7,422.73	6,401.26	8,499.45	7,441.15
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	1,291.32	1,345.38	1,122.80	1,253.17

Table 6 shows the highest content of total phenol is in the ethanol extract of *Terminalia catappa* L₂ leaves which was fractionated with distilled water. , which is 29,968.05 mg/100g. Meanwhile, the lowest content of total phenol is found in distilled water extract of *Terminalia catappa* L₂ leaves which was fractionated with hexane, in as much as 225.46 mg/100g.

Table 7: Antioxidant Activity

Code Sample	Activity (%) ppm				IC50		Information
	1	2	3	Average	ppm	AAI	
Aquadest fraction of <i>Terminalia catappa</i> L. aquadest	14.47	14.44	14.51	14.47	1.987.01	0.02	Very weak
Ethyl acetate fraction of <i>Terminalia catappa</i> L. aquadest	10.59	10.44	10.57	10.53	2.605.49	0.02	Very weak
Hexane fraction of <i>Terminalia catappa</i> L. aquadest	11.76	11.86	11.86	11.81	2,531.11	0, 02	Very weak
Aquadest fraction of <i>Terminalia catappa</i> L. ethanol	95.74	95.71	95.77	95.74	110.37	0.36	Moderate
Ethyl acetate fraction of <i>Terminalia catappa</i> L. ethanol	25.18	25.99	25.64	25.60	2.115.16	0.02	Very weak
Hexane fraction of <i>Terminalia catappa</i> L. ethanol	25.18	25.99	25.64	25.60	2.115.16	0.02	Very weak

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* L₂ 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 & Neelananarayanan, 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

- Abarca-vargas, R., Malacara, C. F. P., & Petricevich, V. L. (2016). Characterization of Chemical Compounds with Antioxidant and Cytotoxic Activities in Bougainvillea x buttiana Holttum and Standl , (var . Rose) Extracts. *Antioxidants*, 5(45), 1–11. <https://doi.org/10.3390/antiox5040045>
- Babayi, H., Kolo, I., Okogun, J. I., & Ijah, U. J. J. (2004). The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminalia catappa against some pathogenic microorganisms. *Biokemistri*, 16(December), 106–111.
- Bahrin, N., Muhammad, N., Abdullah, N., Talip, B. H. A., Jusoh, S., & Theng, S. W. (2018). Effect of Processing Temperature on Antioxidant Activity of Ficus carica Leaves Extract. *Journal of Science and Technology*, 10(2), 99–103. <https://doi.org/10.30880/jst.2018.10.02.016>
- Ekor, M. (2014). The growing use of herbal medicines : issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4(January), 1–10. <https://doi.org/10.3389/fphar.2013.00177>
- Etienne, D. T., Ah, K. K. C., Yves, N., Constant, K., Adama, C., Daouda, S., ... Marius, B. G. H. (2017). Antioxidants Contents of Terminalia catappa (Combretaceae) Almonds Grown in Côte d ' Ivoire. *Archives of Current Research International*, 10(3), 1–12. <https://doi.org/10.9734/ACRI/2017/37125>
- Filho, A. K. D. B., Fernandes, E. S., & Monteiro, C. D. A. (2017). Phytochemical Characterization of Terminalia catappa Linn . Extracts and Their antifungal Activities against Candida spp . *Frontiers in Microbiology*, 8(April), 1–13. <https://doi.org/10.3389/fmicb.2017.00595>
- Gao, J., Tang, X., Dou, H., Fan, Y., Zhao, X., & Xu, Q. (2004). Hepatoprotective activity of Terminalia catappa L . leaves and its two triterpenoids. *Journal of Pharmacy and Pharmacology*, 56, 1449–1455. <https://doi.org/10.1211/0022357044733>
- Gonzalez, A. C. de O., Andrade, Z. de A., Costa, T. F., & Medrado, A. R. A. P. (2016). Wound healing - A literature review. *An Bras Dermatol*, 91(5), 614–620. <https://doi.org/10.1590/abd1806-4841.20164741>
- Herli, M. A., & Wardaniati, I. (2019). Skrining Fitokimia Ekstrak Etanol dan Fraksi Daun Ketapang yang Tumbuh di Sekitar Univ. Abdurrah, Pekanbaru. *Journal Of Pharmacy & Science*, 2(2), 38–42.
- Irawati, F., & Nita, P. (2012). *Kajian Ekstraksi dari Daun Ketapang*. Universitas Pembangunan Nasional Veteran Jawa Timur.
- Janporn, S., Ho, C., Chavasit, V., Pan, M., Chittrakorn, S., Ruttarattanamongkol, K., & Weerawatanakorn, M. (2014). Physicochemical properties of Terminalia catappa seed oil as a novel dietary lipid source. *Journal of Food and Drug Analysis*, 23(2), 201–209. <https://doi.org/10.1016/j.jfda.2014.06.007>
- Katiki, L. M., Gomes, A. C. P., Barbieri, A. M. E., Pacheco, P. A., Rodrigues, L., Verissimo, C. J., ... Ferreira, J. F. S. (2017). Veterinary Parasitology Terminalia catappa : Chemical composition , in vitro and in vivo effects on Haemonchus contortus. *Veterinary Parasitology*, 246(September), 118–123. <https://doi.org/10.1016/j.vetpar.2017.09.006>
- Khan, A. A., Kumar, V., Singh, B. K., & Singh, R. (2014). Evaluation of Wound Healing Property of Terminalia catappa on Excision Wound Models in Wistar Rats. *Drug Res*, 64(October), 225–228. <https://doi.org/10.1055/s-0033-1357203>
- Kopeliovich, D. (2017). Classification of solvents. Retrieved September 20, 2021, from https://substech.com/dokuwiki/doku.php?id=classification_of_solvents
- Lin, Y.-L., Kuo, Y.-H., Shiao, M.-S., Chen, C.-C., & Ou, J.-C. (2000). Flavonoid Glycosides from Terminalia catappa L. *Journal of the Chinese Chemical Society*, 585(47), 253–256.
- Mininel, F. J., Sérgio, C., Junior, L., Espanha, L. G., Resende, F. A., Varanda, E. A., ... Campaner, L. (2014). Characterization and Quantification of Compounds in the Hydroalcoholic Extract of the Leaves from

- Terminalia catappa Linn . (Combretaceae) and Their Mutagenic Activity. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1–11.
- Muhammad, A., & Mudi, S. Y. (2011). Phytochemical Screening and Antimicrobial Activities of Terminalia catappa , Leaf Extracts. *Biochemistri*, 23(1), 35–39.
- Muthulakshmi, G., & Neelanarayanan, P. (2021). Evaluation of Antimicrobial Activities of Terminalia catappa Leaves ' Extracts against Bacterial and Fungal Pathogens. *Indian Journal of Natural Sciences*, 11(64), 976–997.
- Nabavizadeh, M. R., Zargarani, A., Moazami, F., Askari, F., Sahebi, S., Farhadpoor, A., & Faridi, P. (2016). Comparison of the Hemostatic Activity of Quercus persica Jaub . & Spach . (Oak) With Ferric Sulfate in Bony Crypts. *Journal of Evidence-Based Complementary & Alternative Medicine*, 21(1), 34–38. <https://doi.org/10.1177/2156587215593378>
- Nagraj, S. K., Prashanti, E., & Aggarwal, H. (2018). Interventions for treating post-extraction bleeding. *Cochrane Database of Systematic Reviews*, (3), 1–22. <https://doi.org/10.1002/14651858.CD011930.pub3>.www.cochranelibrary.com
- Nugroho, R. A., Aryani, R., Manurung, H., Rudianto, & Prameswari, D. (2019). Wound healing potency of Terminalia catappa in mice (Mus musculus). *Eurasia J Biosci*, 13(December), 2337–2342.
- Ola, P. D., Sandri, M. I., Ola, A. R. B., & Kadang, L. (2020). Determination of Total Tanin Contents of Terminalia Catappa L. Leaf Extract and Test of Its Ability as A Complexion Agent of Fe (III). *Chem. Notes*, 1(2), 94–107.
- Punniyakotti, P., Rengarajan, R. L., Velayuthaprabhu, S., Vijayakumar, K., Manikandan, R., Anand, A. V., ... Vijaya, A. (2019). Protective Effect of Terminalia catappa Leaves and Terminalia chebula Fruits on the Enzymatic and Non-enzymatic Anti-oxidant Levels in the Doxorubicin Induced Toxicity Rats. *Pharmacogn J*, 11(2), 346–349. <https://doi.org/10.5530/pj.2019.11.51>
- Purwaningsih, P. P., Darmayasa, I. B. G., & Astiti, N. P. A. (2020). Early Elucidation Of The Resistor Efficacy Of The Ethanol Ekstrakt Of The Leaf Of “Ketapang” (Terminalia Catappa L.) Against The Growth Of Staphylococcus Aureus ATCC25923 Which Is The Cause Of Gingivitis. *Journal of Biological Sciences*, 7(1), 57–64. <https://doi.org/10.24843/metamorfosa.2020.v07.i01.p08>
- Rahardjo, A. P., Fauzantoro, A., & Gozan, M. (2019). Fractionation and Characterization of Semi Polar and Polar Compounds from Leaf Extract Nicotiana tabacum L . Reflux Ethanol Extraction Results. In *AIP Conference Proceedings* (Vol. 030022, pp. 1–9). <https://doi.org/10.1063/1.5023969>
- Raynor, D. K., Dickinson, R., Knapp, P., Long, A. F., & Nicolson, D. J. (2011). Buyer beware ? Does the information provided with herbal products available over the counter enable safe use ? Buyer beware ? Does the information provided with herbal products available over the counter enable safe use ? *BMC Medicine*, 9(4(August)), 1–8.
- Santoso, B., Imaduddin, F., Sukanto, H., Triyono, J., Lambang, R. L., Widodo, P. J., & Siswantoro, D. H. (2021). Mekanika : Majalah Ilmiah Mekanika Procurement and Operation Technical For Meniran (Phyllanthus Niruri) Extraction Equipment. *Mekanika: Majalah Ilmiah Mekanika*, 20(March), 34–43.
- Shah, P., & Modi, H. A. (2016). Comparative Study of DPPH , ABTS and FRAP Assays for Determination of Antioxidant Activity. *International Journal for Research in Applied Science and Engineering Technology (IJRASET)*, 3(6), 636–641.
- Singh, R., Verma, P. K., & Singh, G. (2012). Total phenolic , flavonoids and tannin contents in different extracts of Artemisia absinthium. *Journal of Intercultural Ethnopharmacology*, 1(2), 101–104. <https://doi.org/10.5455/jice.20120525014326>
- Sutopo, T., Bestari, R. S., & Sintowati, R. (2016). The Effect of The 70% Ethanol Extract of Betel Leaf (Piper Bettle L.) on Bleeding Time in Mice Swiss Webster Strain. *Biomedika*, 8(2), 54–61.
- Tampemawa, P. V, Pelealu, J. J., & Kandou, F. E. F. (2016). Uji Efektivitas Ekstrak Daun Ketapang (Terminalia catappa L.) Terhadap Bakteri Bacillus amyloliquefaciens. *Pharmacon*, 5(1), 308–320.
- Vasi, S. M., Stefanovi, O. D., Li, B. Z., & Radojevi, I. D. (2012). Biological Activities of Extracts from Cultivated Granadilla Passiflora Alata. *EXCLI*, 11, 208–218.