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Identification of chemical compounds of 70% ethanol extract of chinese betel leaves (Peperomia pellucida) from blora regency

Gigih Kenanga Sari[1*]
[1]Department of Pharmacy, An Nuur University, Indonesia

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ABSTRACT

Indonesia is rich in various plants, one of which is the Chinese Betel (Peperomia pellucida). This study aims to identify the chemical compounds of 70% ethanol extract from Chinese betel leaves in Blora Regency. The identification method is by laboratory observation with Thin Layer Chromatography (TLC). The results of the TLC test with the highest or best values are saponins, tannins, triterpenoids which can be seen by looking at the difference between the high points of the stains on the sample and the comparator. The active content in saponins, tannins and triterpenoids functions as a source of antibacterials, increasing the immune system. Flavonoids also have anti-inflammatory content (anti-inflammatory on the skin), as antioxidants and as histamine. Saponins as antibacterials with a mechanism that reacts with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that damage the porins and can inhibit the growth of antibacterials.

1. Introduction

Indonesia has long known and used medicinal plants as an effort to overcome health problems. Knowledge about medicinal plants is based on experience and skills that have been passed down from one generation to the next. One of the medicinal plants in Indonesia is Chinese betel (Peperomia pellucida).

The Chinese betel plant (Peperomia pellucida) is a wild plant belonging to the Piperaceae family. This plant has fibrous roots that are embedded in the soil surface (shallow) and are

[1]* ***Corresponding Author:***
Gigih Kenanga Sari,
Department of Pharmacy,
An Nuur University,
Indonesia.
Email: gigihkenangasariapt@gmail.com
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white. The stem of the Chinese betel plant (Peperomia pellucida) has a stem height of 20 to 40 cm, erect, branched, round, about 5 mm thick, juicy, and soft, pale green or light green in color. Branches with nodes similar to betel plants. Chinese betel (Peperomia pellucida) has a single leaf shape, sitting spirally, oval, 1-4 cm long. The width of the Chinese betel leaf (Peperomia pellucida) is about 0.5-2 cm, heart-shaped and about 4 cm long, pointed tip, incised base, flat edge, curved bones, smooth surface, soft and green in color. Chinese betel flowers (Peperomia pellucida) are arranged in a series of spikelets 1-6 cm long, green in color, located at the end of the stalk and the fruit is round, with a pointed tip, very small with a diameter of less than 1 mm arranged like a pepper, oval in shape and green when young and brown when ripe [1].

The Chinese betel plant (Peperomia pellucida) contains chemical compounds of alkaloids, tannins, saponins, flavonoids, calcium oxalate, fats and essential oils of polyphenyls, cardenolides, steroids, triterpenoids and carbohydrates. Various studies have been conducted and show that the Chinese betel plant (Peperomia pellucida) has analgesic, antipyretic, anti-inflammatory, hypoglycemic, antifungal, antimicrobial, anticancer, antioxidant, antidiabetic and antibacterial activities [2].

The Chinese betel plant (Peperomia pellucida) has traditionally been used to treat several diseases, such as abscesses, boils, acne, skin inflammation, kidney disease and stomach ache. Apart from that, Chinese betel (Peperomia pellucida) is also used to treat colic, fatigue, gout, headaches, rheumatism and joint pain.

2. Method

2.1 Chinese Betel (Peperomia pellucida)

Samples of Chinese betel plants (Peperomia pellucida) were obtained from Dukuh Bulumanis, Dalagan Village, Todanan District, Blora Regency. Plants are selected that are homogeneous so that the phytochemical levels are the same. To select samples, plants that are still fresh green are taken, and plants are taken in the morning, when the plants are undergoing photosynthesis. The plants are randomly removed one by one, amounting to 10 kg, then the wet sorting stage is carried out, the aim is to separate the simplicia from impurities/contaminants (soil, grass, gravel and insects) from the samples to be used. After the wet sorting stage, the simplisia is washed using running water, then the simplisia that has been washed clean is drained and aired indoors for approximately 3 days so that when chopping there are not many substances that come into direct contact with the cutting tool and the resulting simplisia is good, after it is half dry, then chop it (reduce the size of the simplicia) to make the drying process easier.

The drying process is carried out with the help of sunlight that does not come into direct contact with the plants (covered with a black cloth for shade) which lasts for 7 days. With such drying conditions, it is hoped that the content of secondary metabolite compounds in the sample will not be damaged [3]. Drying simplicia aims to obtain simplicia that is not easily damaged during storage by reducing the water content and preventing rotting of simplicia caused by bacteria [4].

2.2 Powder

Making powder by collecting samples, wet sorting, washing, chopping, drying, dry sorting.

2.3 Making Extracts

Chinese betel powder (Peperomia pellucida) was extracted using the maceration method using 70% ethanol solvent with a ratio of 1:10. Making the extract is done by weighing 1 kg of simplicia then soaking it in 10 liters (10,000 ml) of solvent, then leaving it for 18 hours, stirring occasionally for the first 6 hours. After that, filter it using filter paper, then repeat again by adding 5000 ml of solvent to the macerate [5]. Then the extract obtained was thickened using a rotary evaporator at a temperature of 40℃ [6].

$$\% \text{ Yield} = \frac{(\text{Weight of extract produced})}{(\text{Initial weight of simplicia powder})} \times 100\% \quad (1)$$

The yield calculation results are shown in units (%), the higher the yield value, the greater the extract value produced. The quality of the extract produced is inversely proportional to the amount of yield produced. The higher the yield value, the lower the quality obtained [7].

2.4 Extract Testing

2.4.1 Ethanol Free Test

The thick extract is put into a test tube, add acetic acid and sulfuric acid then heated. An extract is said to be ethanol free if it does not have the characteristic ester odor of ethanol [8], [9].

2.4.2 Drying Shrinkage

The porcelain exchange tower is heated at 105℃ for 30 minutes. Weigh out 2 grams of extract, then spread the extract evenly in a porcelain saucer by shaking the curve until it forms a layer 5-10 mm thick. Put it in the oven, open the lid and dry at 105℃ until the weight remains [8].

$$\text{Drying Loss} = \frac{(\text{initial weight} - \text{final weight})}{(\text{Initial weight})} \times 100\% \quad (2)$$

2.4.3 Specific Gravity

The specific gravity of the extract was determined from the results of 1% extract dilution in ethanol solvent in a pycnometer. A clean, dry and calibrated pycnometer is used by determining the weight of the pycnometer and the weight of water that has been boiled at a temperature of 25℃, the temperature is adjusted until the liquid extract is approximately 20℃, then placed in the pycnometer.

Set the temperature of the filled pycnometer to 25℃. The excess liquid extract is discarded and weighed. Subtract the weight of the empty pycnometer from the weight of the filled pycnometer. The specific gravity of the liquid extract is the result obtained by dividing the weight of the extract and the weight of water, in a pycnometer at a temperature of 25℃ [10].

2.4.4 Water content

Weigh 3 grams of extract and put it in a container that has been tarnished, then dry at a temperature of 105℃ for 5 hours and weigh carefully. Drying is continued, then weighed again at an interval of 1 hour until the difference between the 2 weighers is no more than 0.25% .

$$\text{Water Content} = \frac{(W2-W1)}{W} \times 100\% \quad (3)$$

W: weight (g) of the sample

W1: weight (g) of the porcelain cup of the sample before heating.

W2: weight (g) of the porcelain cup and sample after heating.

2.5 Thin Layer Chromatography Chemical Compound Test

Prepare a silica gel stationary phase GF_{254}/TLC plate with a length of 6 cm and a width of 3 cm. Wash with methanol and activate in the oven for 10 minutes at 105℃. Dissolve 10 ml of extract with 1 ml of ethanol, then spot on the stationary phase.

2.5.1 Identification of Flavonoid Compounds

is glacial acetic acid : butanol: water in the ratio (1:4:5). The steam stain visible agent used was ammonia with a comparison standard of quercetin and then observed with UV light. A positive reaction shows the formation of a yellow-brown stain, after spraying ammonia in visible light, UV 254 nm and 366 nm [11].

2.5.2 Identification of Saponin Compounds

The mobile phase used is chloroform: methanol: water (13:7:2). The stain detector used is Lieberman-Buchard. The comparison standard used is sapogenin. If a green color occurs after spraying Lieberman-Buchard then the results are declared positive for saponin [12].

2.5.3 Identification of Tannin Compounds

Prepare the mobile phase n- butanol: stearic acid: water in the ratio (4:1:5) with 5% F_eCl_3 stain reagent. The comparison standard used is catechin. If a blue-black stain forms after spraying 5% F_eCl_3, it is declared positive for tannin [13].

2.5.4 Identification of Triterpenoid Compounds

Prepare the mobile phase n- hexane: ethylacetate (4:1) with the reference standard β-sitosterol. Sulfuric acid anisaldehyde stain appearance. A positive result for triterpenoids/steroids is if a purple-red or violet color appears after being sprayed with sulfuric acid anisaldehyde reagent [14].

2.5.5 Identification of Alkoloid Compounds

Prepare the mobile phase ethyl acetate: methanol: water (6:4:2) with the reference standard piperine. The appearance of the dagrendroff reagent stain will appear brown or orange after spraying the dagrendroff reagent, indicating the presence of alkaloids in the extract.

3. Results and Discussion

The results of the wet weight of 10 kg of Chinese betel were obtained by a dry weight of Chinese betel of 1300 gr. The percentage of dry weight to wet weight was 13%. The results of this dry weight are not much different from the results of previous research where the drying results from wet Chinese betel plants were 11%, which shows that the extract produced meets the requirements.

1 kg of Chinese betel powder was used in the extraction process with 10 liters of 70% ethanol filter fluid.

Table 1. Rendement Results

Chinese Betel Powder (gr)	Condensed Extract (gr)	Yield (%)
1000	194.83	19.48

The thick extract produces 194.83 gr. So the extract yield was 19.48%. The higher the percentage of simplicia yield, the better it is because more compounds are isolated [7]. The results of calculating the yield of Chinese betel extract were found to be greater than the previous extract yield which was 17.92%. The characteristics of Chinese betel extract are blackish brown in color, distinctive aroma, distinctive aroma and thick consistency.

3.1. Ethanol Free Test Results

Chinese betel extract was tested for ethanol free. The ethanol-free test aims to ensure that the extract does not contain ethanol which has antibacterial activity from Chinese betel extract and the results of Chinese betel extract are free from the solvent, namely ethanol, which is indicated by the absence of the typical ester smell of ethanol.

Table 2. Ethanol Free Test Results

Procedure	Results	References
Extract + H_2SO_4 + CH_3COOH heated	There is no ester smell typical of ethanol	There is no ester smell typical of ethanol (Yuri, 2016).

3.2. Drying Shrinkage

Determination of drying shrinkage aims to ensure that the powder is truly dry. From the research data above, the average drying loss of Chinese betel powder is 9.74%, indicating that Chinese betel powder meets the requirements, because the drying loss level of the

powder should not be more than 10%, because levels less than 10% cause the cells to die, the enzymes will not active, and bacteria and fungi do not grow so the powder lasts longer. Drying shrinkage that is too high in powder will facilitate the growth of fungi and bacteria as well as chemical growth that can damage the powder [8].

Table 3. Drying Shrinkage Test Results

No	Weighing (g)	Water content (%w/v)
1	3,000	9.70
2	3,000	9.74
3	3,000	9.78
	Average	9.74

According to the existing literature, the results of determining drying losses were carried out using the oven method at a temperature of 105℃, producing powder of 8.98% of the fixed weight.

3.3. Results of Determining Specific Gravity

The result of the specific gravity of Chinese betel is 0.976. Measurement of the specific gravity of Chinese betel extract was determined using a pycnometer. Specific gravity is the weight of an air substance at a temperature of 25℃ divided by the weight of the volume of water at the same temperature. The specific gravity value is influenced by the chemical compound content in the test material.

Table 4. Results of Determining Specific Gravity

No	Empty pycnometer weight (g)	Pycnometer weight + 25℃ water	Pycnometer weight + extract 25℃	Specific gravity
1	16.67	36.62	36.15	0.976
2	16.67	36.65	36.17	0.975
3	16.67	36.63	36.20	0.978
	Average			0.976

3.4. Water Content Results

The results of the water content of Chinese betel simplicia are 8.6%, meaning that Chinese betel meets the requirements for good water content, namely not exceeding 10%. This result was obtained after 5 hours of drying and waiting for it to cool so that the weight became permanent. The purpose of determining the water content in a simplicia is to ensure that there is no residual water left after the drying process [10]. The water content results obtained were higher than the resulting water content results, namely 2.4%.

Table 5. Water content results

Initial Weight (gr)	Final Weight (gr)	(%) End
3	2.76	8.6 %
3	2.76	8.6 %
3	2.76	8.6 %
Average		8.6 %

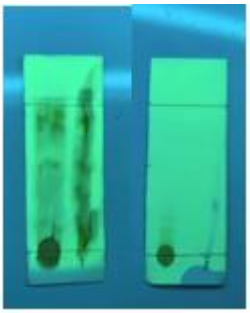
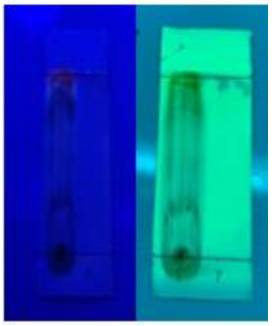
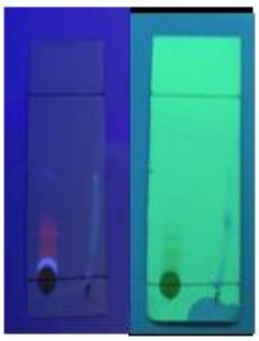
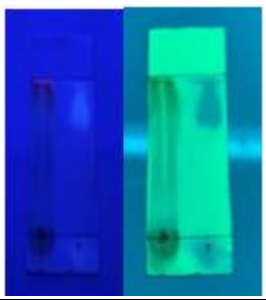
3.5. Thin Layer Chromatography Test Results

The results of research on the TLC test of Chinese betel extract show that Chinese betel extract contains triterpenoid compounds, saponins, flavonoids, alkaloids and tannins. Several factors influence the thin layer chromatography (TLC) profile, namely the chromatography system in the mobile phase and stationary phase, the suitability of the solvent for the target compound in the extract, the weighing quantity of the extract, and the selection of the appropriate visualization method.

Separation of secondary metabolites of flavonoids, saponins, tannins, triterpenoids and alkaloids in samples using a GF_{254} silica gel plate as the stationary phase and irradiated under a UV lamp with a wavelength of 366 nm. The plate was cut to a size of 3 x 6 cm, then activated by oven at 105°C for 15 minutes to remove water trapped on the plate so that the solvent on the plate would come out and form a porous matrix which would attract the developer solution due to the capillary effect. Next, dissolve 2 ml of the sample in 1 ml of 70% ethanol and spot on the plate at the bottom line marked previously. The eluent mixture (mobile phase) must be saturated first for 1 hour to avoid chemical changes so that the atmosphere in the vessel is saturated with steam from the eluent. If it is not saturated, expansion will occur with the solvent surface forming a concave shape and the mobile phase will tend to elute towards the edge of the plate. The resulting spot or stain is sprayed using a spraying reagent as a fluorescent indicator so that the color of the spot can be seen when detected under a UV lamp. The appearance of the spot was observed using a UV lamp with a wavelength of 366 nm. Then measure the distance traveled by the spot using a ruler and calculate the Rf value. The appearance of color at this wavelength is caused by the interaction between UV light and the chromophore group which is bound by ausochrome to the compound that appears to form the spot.

Table 6 . Thin Layer Chromatography Test Results

No	Chemical compounds	Research result
1	Flavonoids	Sample Rf value = 0.6 Comparison standard = 0.62 (+) flavonoids 

2	Saponin	Sample Rf value = 0.68 Comparison standard = 0.91 (+) saponin	
3	Tannin	Sample Rf value = 0.95 Comparison standard = 0.95 (+) tannin	
4	Triterpenoids	Sample Rf value = 0.39 Comparison standard = 0.35 (+) triterpenoids	
5	Alkaloids	Sample Rf value = 0.77 Comparison standard = 0.91 (+) alkaloids	

Separation of flavonoid compounds in Chinese betel extract using the eluent glacial acetic acid: butanol: water in the ratio (1:4:5). The steam stain visible agent used was ammonia with a comparison standard of quercetin and then observed with UV light. A positive reaction shows the formation of a yellow-brown stain, after spraying ammonia in visible light, UV 254 nm and 366 nm [10].
Separation of saponin compounds in Chinese betel extract using chloroform: methanol: water (13:7:2). The stain detector used is Lieberman-Buchard. The comparison standard used is. If a green color occurs after spraying Lieberman-Buchard sapogenin, the results are declared positive for saponin.

Separation of tannin compounds in Chinese betel extract using n- butanol: stearic acid: water in a ratio of (4:1:5) with 5% $FeCl_3$ stain reagent. The comparison standard used is

catechin. If a blue-black stain forms after spraying 5% $FeCl_3$, it is declared positive for tannin.

Separation of triterpenoid compounds in Chinese betel extract using n-hexane: ethyl acetate (4:1) with the reference standard β-sitosterol. Sulfuric acid anisaldehyde stain appearance. A positive result for triterpenoids/steroids is if a purple-red or violet color appears after being sprayed with sulfuric acid anisaldehyde reagent.

Separation of alkoloid compounds in Chinese betel extract using ethyl acetate: methanol : water (6:4:2) with piperine as the standard standard. The appearance of the Dagrendroff reagent stain will appear brown or orange after spraying the Dragendroff reagent, indicating the presence of alkaloids in the extract.

The results of the Rf values of the five extracts according to existing literature show that the Chinese betel extract positively contains flavonoids which are characterized by producing a yellow-brown color. Saponin produces a green color. Tannin produces a blue-black color. triterpenoid compounds that show a red or violet color. And alkaloids that produce brown or orange stains [15].

The TLC test results with the highest or best scores are saponins, tannins, triterpenoids which can be seen by looking at the difference between the height of the stain points on the sample and the comparison. The active ingredients in saponins, tannins and triterpenoids function as a source of antibacterial, improving the immune system. Flavonoids also have anti-inflammatory properties (anti-inflammatory of the skin), as antioxidants and as histamines. Saponin as an antibacterial mechanism reacts with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that cause damage to the porins and can inhibit the growth of antibacterials [16].

4. Conclusion

Based on the results of research using the thin layer chromatography method, it can be concluded that Chinese Betel Leaves (Peperomia pellucida) from Blora Regency contain flavonoids, saponins, tannins, triterpenoids and alkaloids.

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