ANALYSIS OF TRITERPENOIDS IN ETHANOL EXTRACT OF GOTULA LEAVES (*Centella asiatica* (L) Urban) WITH N-HEXANE ELUENT AND ETHYL ACETATE BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Background: Centella Asiatica (L.) Urban, commonly known as gotu kola, contains various bioactive compounds, one of which is triterpenoid. This compound plays an important role in pharmacological activities such as wound healing and anti-inflammatory effects. Identifying these compounds is essential as a basis for developing herbal medicines. Objective: To identify the presence of triterpenoid compounds in 70% ethanol extract of gotu kola leaves from Tawangmangu using Thin Layer Chromatography (TLC) method. Method: The 70% ethanol extract was analyzed using TLC with two eluent systems: n-hexane:ethyl acetate (4:1) and ethyl acetate:n-hexane (4:1). Detection was based on spot color observation and Rf values. Results: The eluent n-hexane:ethyl acetate produced purple-red spots with an Rf value of 0.75, while the eluent ethyl acetate:n-hexane produced purple spots with an Rf value of 0.77. The appearance of these colors indicates a positive result for triterpenoids. Conclusion: The 70% ethanol extract of Centella Asiatica leaves tested positive for the presence of triterpenoid compounds. Implication: These findings support the potential of gotu kola leaves as a basic material for phytopharmaceutical formulations containing triterpenoids and encourage further research on the isolation and biological activity of these compounds.

Keywords: *Triterpenoids, Centella asiatica, Thin Layer Chromatography*

INTRODUCTION

Indonesia has Lots source riches beneficial nature for life human. Source riches natural is plants that can utilized For health and beauty. In ancient times, herbal concoctions were used For maintenance face, hair, and body. When That Not yet There is technology sophisticated, so that Good man and also Woman only depends on the source Power natural from environment around For maintenance beauty (Dewayanti, 2014). One of the plant the is Centella *asiatica* (L.) Urban).

Centella *asiatica* (L.) Urban) is one of the abundant wild plants grow on plantations and have diverse benefit For treat various problem health. Properties and benefits Centella *asiatica* (L.) Urban) among others because Centella *asiatica* (L.) Urban) contains a number of

nutrients and components substance chemistry that has effect therapeutic. Herbs Centella *asiatica* (L.) Urban) is selected as material main Because including one of plant featured (Swintari, 2017). In addition that, has Lots products processed drugs become cream, ointment, medicine pimple as well as skincare that contains material from Centella *asiatica* (L.) Urban) in Indonesia. Benefits in a way scientific from Centella *asiatica* (L.) Urban) has Lots studied in animals try and conclude that Centella *asiatica* (L.) Urban) can used as antioxidant, antigastritis, antitumor, healing wounds, immunomodulators, antiproliferation, antibacterial and so on (Belwal, 2019). Content bioactive in herbs Centella *asiatica* (L.) Urban) which has activity antibacterial reason pimple namely flavonoids, tannins, saponins and others (Sutardi, 2016).

Solvent is one of factor chemicals that can influence quality extract. Selection solvent based on different polarity aiming For to obtain the best solvent. Good solvent capable extract in amount big and extract compound chemistry with good (Chotimah, 2019), as well can give influence to content the chemicals produced. The influence solvent to content chemistry show difference method drying affect the yield extract in n- hexane solvent, but No affect the solvent ethanol 96% and ethyl acetate, while difference solvent extraction on each -mas. Solvents can also be give influence to yield the resulting compound (Azzahra et al., 2022). Research Kemit, (2016) shows that interaction between type solvent and time maceration influential very real against total flavonoids ing method drying influential to yield extract.

In order to use it optimally, it is necessary known adequate information about group compound chemicals contained in plant drug said. Part from plant this is what often happens used as drug that is the leaves. However, for know content compound chemistry plant (*Centella asiatica* (L.) Urban), and considering condition geographical each area different possibility There is difference in content compound chemicals found in plants gotu kola.

METHOD

1. Determination

   Determination leaf *Centella asiatica* (L. Urban) in research This conducted at the Hall Big Research and development Plant Drugs and Medicines Traditional (B2P2TOOT), Jl. Raya Lawu No.11 Kalisoro, District Tawangmangu, Regency Karanganyar, Province Central Java is done with objective For prove truth materials used in the research. Determination done with match characteristic features morphology plant with reference in literature.
2. Harvesting and Drying Material

Samples used that is leaf *Centella asiatica* (L.) Urban) which still fresh and colorful green, with age 3-4 months, free from insects, fragments animal or dirt animals, no contain mushrooms, or signs impurity other, no contain other toxic and hazardous materials, places grows at an altitude of 1,200-1,800 meters above sea level surface sea, with intensity light 30-40%, then sample separated from stem Then washed with running water use remove soil and dirt others attached to pegagan (*Centella asiatica* (L.) Urban) after That dried drain. Drying leaf *Centella asiatica* (L.) Urban) which has cleaned up done with method oven at a temperature of 40 º C-50 º C. The sample that has been dry refined with using a blender until become powder. Sieving powder use sieve size 60 mesh. Powder simple Then saved in receptacle clean and closed meeting.

3. Making Extract Leaf *Centella asiatica* (L. Urban)

   Powder simple leaf *Centella asiatica* (L. Urban) extracted with method maceration with use solvent 70% ethanol with 1:10 ratio. Making extract done with do weighing simple as much as 1000 g then soaked with solvent as much as 10 liters (10,000 ml), then keep quiet for 18 hours in the first 6 hours stir once in a while. After that filtered with use paper filter, then repeat Return with give 5000 ml solvent in meserate earlier (FI III ed 2013). Then extract obtained thickened with rotary evaporator with temperature 40 º C (Asiyah, 2019).

$$\%\ rendemen = \frac{Bobot\ ekstrak\ yang\ dihasilkan}{Bobot\ awal\ serbuk\ simplisia} \text{x } 100\ \%$$

   Calculation results yield shown with unit (%), the more tall mark yield so mark the resulting extract the more many. Quality the resulting extract compared to backwards with amount the yield produced. The more tall mark yield so semkin low the quality obtained (Wijaya et al., 2018).

4. Shrinkage Drying

   Shrinkage test drying done with tool *moisture balance.* Turn on *moisture balance* and heat for 10 minutes, after That set tool with press menu, select the method that will be used. Enter sample into the moisture balance then flatten. Close moisture balance then until light dead and note The results. The test was carried out as many as 3x trials, then measure the average. Wait until temperature 30 º C and turn off tools (Yuri, 2016).

$$\text{Susut Pengeringan} = \frac{\text{bobot awal} - \text{bobot akhir}}{bobot\ awal} \text{x } 100\ \%$$

5. Weight Type

   Weight type extract determined to results dilution extract 1% in solvent ethanol in pycnometer. Used pycnometer clean, dry and has calibrated with set weight pycnometer and the weight of the water that has been boiled at 25 º C. Temp set up until extract liquid

more less than 20 ° C, then entered in pycnometer. Set temperature pycnometer that has been filled until temperature 25 ° C. Advantages extract liquid removed and weighed. Subtract weight pycnometer that has been filled. Weight type extract liquid is results obtained with share weight extract and water weight, in pycnometer in temperature 25 ° C (Anam, 2016).

6. Remainder Solvent Organic

   Free test ethanol in extract leaf *Centella asiatica* (L.) Urban) is carried out with How to, Extract ethanol leaf *Centella asiatica* (L.) Urban) plus 2 drops of H_2SO_4 concentrated and CH_3COOH, then heated. No. existence smell typical ester shows results negative (Tenda et al., 2017).

7. Test Tube Compound Triterpenoid

   A number of sample dissolved with chloroform, then added with ±1 ml of acid acetate anhydrous and added 3 drops of H_2SO_4 concentrated. Positive result if formed color red until purple.

8. Test Triterpenoid Compounds with TLC

   Before the separation process with TLC started, the TLC plate is heated in the oven at 110 °C for 30 minutes. Extract leaf star fruit sweet dissolved with methanol Then spotted on the TLC plate that has been marked with line limit elution. Next, to identification use method thin layer chromatography, used mixture eluent ethyl acetate and n- hexane which have been saturated in the chamber with paper filter. Prepare phase n- hexane motion: ethyl acetate (4:1) and ethyl acetate: n- hexane (4:1) with standard comparison of β-sitosterol. The TLC plate that has been spotted with extract leaf gotu kola entered to in the chamber and eluted. The observation process to formation stain done use 254 nm UV light. Positive results for triterpenoids/steroids if arise color purple-red or purple after sprayed reagent anisaldehyde sour sulfate and then Rf value was calculated. (Budi et al., 2020).

RESULTS

1. Determination results according to Hall Big Research and development Plant Drugs and Medicines Traditional (B2P2TOOT) is as following:

   Species: *Centella Asian* (L) Urban.

   Synonyms: *Hydrocotyle asiatica* L.

   Family: Apiaceae

Objective he did determination This is For know that plants used in research This Already Correct so that can avoid from error in collection materials used.

2. Simple wet used 15 kg, obtained simple dry leaf *Centella asiatica* (L.) Urban) as much as 1.5 kg, obtained presentation simple dry to simple wet by 15%.

Figure 1. Powder Gotu kola

3. Shrinkage Yield Drying

Shrinkage drying powder leaf *Centella asiatica* (L.) Urban) obtained an average of 9.60%. The results are fulfil condition that is No more from 10%, so that powder become more durable in storage, no overgrown microbes Good That mold or bacteria. Shrinkage drying also identifies evaporated water content. Shrink drying also provides limitation maximum about the magnitude compounds lost during the drying process.

Figure 2. Shrinkage Results Drying

Table 1. Shrinkage Results Drying

Replicat ion	Weighing (g)	Water content (% w/v)
1	3.004	9.60
2	3.004	9.62
3	3.004	9.63
	Average	9.60

4. Yield Results Extract Gotu kola

Extract thick produced in the study This as much as 277.42 g. So that obtained yield extract by 27.74%. Characteristics physique colored green yellowish, thick and smelly typical *Centella asiatica* (L.) Urban). Yield value related with the amount content compound chemicals contained in the simple drug.

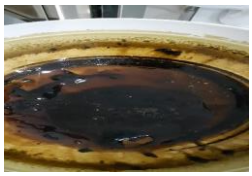

Figure 3. Extract Thick Gotu kola

Table 2. Yield Results Extract Gotu kola

Powder pegagan (g)	Extract Thick (g)	Yield (%)
1000	277.42	27.74

5. Determination Results Weight Type

Result of testing weight the average type is 0.975. Weight type that is weight from a substance air with a temperature of 25 ° C is divided with weight of the volume of water at the same temperature. The weight value type influenced by content compound chemistry on the test material.

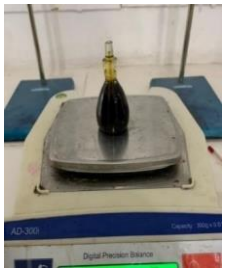

Figure 4. Weight Test Type

Table 3. Weight Test Type

Replication	Weight pycnometer empty (g)	Weight pycnometer + water 25 ° C	Weight pycnometer + extract 25 ° C	Weight type
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

6. Free Test Results Ethanol

Free test ethanol aiming For know extract No contain ethanol with characteristics No smell the characteristic ester odor of ethanol., so that can concluded that obtained extract that can used For study stage next. Free test ethanol done For to free extract from ethanol so that

obtained pure extract without contamination, besides That ethanol nature antibacterial and antifungal so that No will cause positive fake on sample

Free Test Results Ethanol

Table 4. Results of the Independent Test Ethanol

Sample	Treatment	Results	Library
Gotu kola *(Centella asiatica (L.) Urban)*	Extract gotu kola + H_2SO_4 + CH_3COOH is heated	No There is characteristic ester odor in the extract	No There is distinctive ester odor from ethanol (Tenda et al., 2017).

7. Test Tube Compound Triterpenoid

Triterpenoids are important compounds present in *Centella asiatica* (L.) Urban). One of the part of triterpenoids is asiaticoside which has function For strengthen cells skin and improve repair skin, stimulating cell blood and immune system and can functioning as antibiotics experience.

Figure 6. Triterpenoid Tube Test

Table 5. Content Test Chemical Compounds of Gotu Kola

Chemical Compounds	Information	Extract Results
Triterpenoid	Produce color red	+

Information:

(+): contains compound

(-): No contain compound

Part other of triterpenoids is brahmoside that works For to smoothen flow blood and is an important protein For cell brain. Triterpenoids can functioning For stimulate formation of important fats and proteins For health skin. Besides That can change analyte and proline become collagen that works For nurse skin. Function other that is speed up healing wound post-surgery, acne, and blemishes black on the skin (Sutardi, 2016).

8. Test Triterpenoid Compounds with TLC

Research results on TLC extract test *Centella asiatica* (L.) Urban) shows that gotu kola contain triterpenoid compounds.

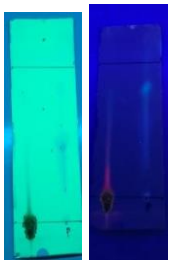

Figure 7. Test Triterpenoid Compounds with TLC

Table 6 Results of Thin Layer Chromatography Test of Triterpenoid Compounds

Eluent	Positive Results	Research result
n- hexane: ethyl acetate (4:1)	β-sitosterol comparison standard. Appearance stain anisaldehyde sour sulfate. Positive results for triterpenoids/steroids if arise color purple-red or purple after sprayed reagent anisaldehyde sour sulfate	Rf value of sample = 0.75
Ethylacetate: n-hexane (4:1)	β-sitosterol comparison standard. Appearance stain anisaldehyde sour sulfate. Positive results for triterpenoids/steroids if arise color purple-red or purple after sprayed reagent anisaldehyde sour sulfate	Rf value of sample = 0.77

CONCLUSION

Extract 70% leaf ethanol *Centella asiatica* (L.) Urban) positive contain triterpenoid compounds. TLC results with eluent N- hexane and ethyl acetate the ratio (4:1) shows color purple-red and Rf value of 0.75. And the TLC results with eluent ethyl acetate and N- hexane the ratio (4:1) shows color purple with Rf value 0.77.

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