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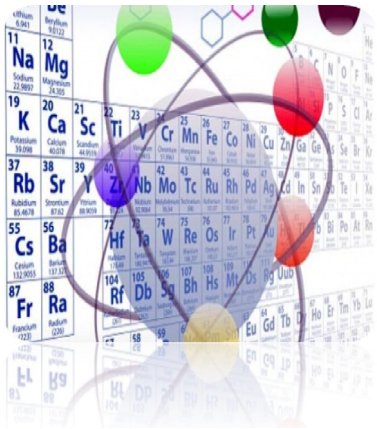

Identification of Active Compounds on *Muntingia calabura L.*Leaves using Different Polarity Solvents

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ABSTRACT

Plant of Muntingia calabura L are often known as "kersen", "seri or "cherry". Leaves of Muntingia calabura L. contains many benefits but its properties are still little known to the public. It contains secondary metabolites which have many uses. This study was aimed to determine the content of secondary metabolites in this leaf. Leaves extracts were obtained by maceration extraction for 3 times 24 hours using polar, semi-polar and non-polar solvents to determine the solubility of secondary metabolite compounds in each solvent. The solvents used were ethanol, ethyl acetate and n-hexane. The leaveswere dissolved a lot in polar solvents, marked by the formation of a dark green color in ethanol-series extracts, the color fades more in semi-polar and non-polar solvents. The three leaves extracts were tested for secondary metabolite contents by phytochemical screening tests. Phytochemical screening was an initial selection stage to detect classes of chemical compounds contained in plant. Phytochemical screening were included alkaloid, terpenoid, steroid, tannin, flavonoidand saponin tests. Based on the results of phytochemical screening tests, the leaf was contained several secondary metabolite compounds, namely flavonoids, saponins, steroids, terpenoids, alkaloids, phenols and tannins.

Keywords: leaves of Muntingia calabura L, secondary metabolite compounds, and phytochemical screening

ABSTRAK

Tumbuhan Muntingia calabura L sering dikenal dengan "kersen", "seri, ataucherry". Daun Muntingia calabura L. mengandung banyak manfaat namun khasiatnya masih sedikit diketahui masyarakat. Daun ini mengandung senyawa metabolit sekunder yang memiliki banyak kegunaan. Penelitian ini bertujuan untuk mengetahui kandungan senyawa metabolit sekunder yang terdapat dalam daun tersebut. Ekstrak daun diperoleh dengan ekstraksi secara maserasi selama 3 kali 24 jam menggunakan pelarut polar, semi polar dan non polar untuk mengetahui kelarutan kandungan senyawa metabolit sekunder dalam masing-masing pelarut. Pelarut yang digunakan yaitu etanol, etil asetat dan n-heksana. Daun ini banyak melarut dalam pelarut polar, ditandai dengan terbentuknya warna hijau tua pada ekstrak seri-etanol, warna semakin memudar pada pelarut semi polar dan non polar. Ketiga ekstrak daun tersebut diuji kandungan metabolit sekunder dengan uji skrining

fitokimia. Skrining fitokimia merupakan suatu tahap seleksi awal untuk mendeteksi golongan senyawa kimia yang terdapat dalam tumbuhan. Skrining fitokimia meliputi uji alkaloid, terpenoid, steroid, tannin, flavonoid dan uji saponin. Berdasarkan hasil uji skrining fitokimia, daun seri mengandung beberapa senyawa metabolit sekunder, yaitu flavonoid, saponin, steroid, terpenoid, alkaloid, fenol dan tannin.

***Kata kunci**:daun Muntingia calabura L, senyawa metabolit sekunder, dan skrinig fitokimia*

I. Introduction

One of the plants that contains many properties and useful as a medicine is the Muntingia calabura L. plant. The plant is widely available in the community, but its use is still very limited. Series plant taxonomies[1]are:Kingdom: Plantae, Division: Spermatophyta, Class: Dicotyledoneae, Order: Malvales, Family: Elaeocarpaceae, Genus: Muntingia, and Species: Muntingia calabura L.

The names of this plant in some countries such as Jamaican cherry, Panama berry, Singapore cherry in English and in Dutch are called Japanse kers, so that they are taken as kersen in Indonesian. But in Aceh, cherry plant is better known as "seri" plant, so in this article uses the term series as Muntingia calabura L.

"Seri" plant has many benefits, it can be processed as syrups, jams and sweets that contains many properties and economic value. Its stem can be used as the basis for furniture manufacture while series leaves are antioxidants and widely used as a remedy for several diseases including anti-tumor, anti-septic, anti-inflammatory and so forth. Antioxidants contained in leaf series are thought to be able to protect the body from diseases caused by free radicals. Its leaf contains several secondary metabolites, one of which is a flavonoid compound. Flavonoids have antioxidant properties, these compounds act as free radical scavengers because they contain hydroxyl groups. Due to it is a reducing agent, flavonoids can act as hydrogen donors to free radicals.[2]

Based on previous studies, it was found that the leaf contained flavonoid compounds, saponins, tannins, triterpenes and steroids, while alkaloids were not detected. Study using methanol as a solvent obtained positive results of the presence of flavonoids, saponins and tannins in the leaf series.[3] Furthermore, the results of phytochemical analysis of leaf extract series showed the presence of flavonoids, steroids / triterpenoids from glycoside compounds. The presence of flavonoids causes the Muntingia calabura L leaves to have anti-diabetic activity.[4]The four solvents used, namely ether, chloroform, ethanol and distilled water. It was found that the content of secondary metabolites was identified in ethanol, including alkaloids, steroids, flavonoids, phenols, quinones, saponins and terpenoids.[5]

Due to the use of series leaves is very much needed precise information about the content of secondary metabolites which can then be used in subsequent research. This study was aimed to determine the content of secondary metabolites contained in technical ethanol, ethyl acetate and n-hexane solvents. Alkahol, however, is a versatile solvent that is good for preliminary extraction, then the material can be macerated in a doze, then filtered.[6]Ethyl acetate is a semi-polar solvent that is volatile, non-toxic, and not hygroscopic. Ethyl acetate is a weak recipient of hydrogen bonds and has a density of 0.897g/cm^3. While n-hexane is an organic solvent which has a density of 0.6548g/mL.

Previous study has not been widely conducted yet to detect the nature of the polarity of the series leaves, so in this study three types of solvents were used to find out what solvents were suitable for obtaining this leaf extracts and examine the content of secondary metabolites of the three extracts. Based on this background, therefore this study was interested in examining the content of any secondary metabolite compounds contained in "seri" leaves in ethanol, ethyl acetate, and n-hexane solvents.

II. Research Methodology

2.1. Tools and Materials

Some tools used in this experiment were glassware, filters, analytical balance and arm balance. The main material used in this study was the Muntingia calabura Lleaves originating from the campus environment of Syiah Kuala University.The leaves used as samples were not too old and not too young, as shown in Fig. 1. Other materials used were technical ethanol, ethyl acetate and n-hexane

as solvents in the maceration extraction process, concentrated sulfuric acid, concentrated hydrochloric acid, 10% ammonia, chloroform, magnesium powder, 10% iron (III) chloride solution.

Figure 1. Sampel of Muntingia calabura L. leaves

2.2. Procedures

Sample Preparation

The Muntingia calabura L leaves were cut into small pieces and then aerated to dry at room temperature, weighed as much as 150gr and divided into three. Macerated was used by three types of solvents namely technical ethanol, ethyl acetate and n-hexane for 3x24 hours. The extraction results were separated using a filter and tested for secondary metabolite content by phytochemical screening tests.

Phytochemical Screening

Alkaloid Test (Mayer and Dragendorff Tests)

In the mayer test, the 3ml of mayer reagent was added to 3 ml of the extract. White deposits indicated the presence of alkaloids.[7] The Dragendorff test was carried out by adding 2mL of concentrated hydrochloric acid to 3mL of extract then 5 drops of Dragendorff reagent were added. Positive results were marked by the formation of brownish orange or orange.[8]

Flavonoid Test

Method 1, the 3mL of extract was added with 1mL of 10% ammonia and 1mL of concentrated sulfuric acid. The absence of yellow indicated the presence of flavonoids.Shinoda test, 1mL of extract was added 5mL of 95% ethanol then added 2mL of concentrated hydrochloric acid through the test tube wall. Furthermore, 0.5gr of magnesium powder was added. The formation of pink deposits indicated the presence of flavonoids.[8]

Tannin and Phenol (Iron chloride Test)

A total of 5 drops of 10% iron (III) chloride solution was added to 3mL of the extract. If blue or green precipitate was formed, it indicated the presence of phenol and tannin.[7]

Steroids (Libermann Burchard Reaction)

As much as 3mL of extract was added into 1mL of chloroform and a few drops of concentrated sulfuric acid slowly through the cold tube, a reddish-brown precipitate found on the bottom of the reaction tube indicated the presence of steroids.[7]

Saponins (H_2O)

As much as 3mL of extract was put into a test tube then added 2mL of distilled water. The formation of froth indicated the presence of saponins.[7]

Terpenoids (Salkowski)

As much as 3mL of extract was added 1mL of chloroform and a few drops of concentrated sulfuric acid carefully through the walls of the test tube. The formation of reddish brown-brown sediment indicated terpenoids.[7]

III. Results and Discussion

Sample Preparation

The results of maceration extraction using three solvents produced a series of leaf extracts in a thick green ethanol solvent.The green color faded on ethyl acetate and n-hexane solvents. This showed that the leaf samples were more bound to polar solvents.

Phytochemical Screening

Phytochemical screening in Muntingia calabura L leaves were done with one repetition for each test of the three solvents. The content of secondary metabolite compounds in the leaves through phytochemical screening for the test were of alkaloids, flavonoids, tannins, phenols, saponins, steroids and terpenoids showed the following results as in Table 1.This table shows that the content of secondary metabolites contained in series leaf extracts including alkaloids, flavonoids, tannins, phenols, steroids, saponins and terpenoids.

Alkaloids

Based on phytochemical screening results, the three series leaf extracts tested showed the presence of alkaloid compounds, this was seen from the formation of dark green solution and white sediment at the bottom of the test tube from the series leaf extract in technical ethanol and ethyl acetate.

Table 1. Phytochemical Screening Test Results of series leaf extracts in three solvents

Test	Testing Method	Solvent					
		Technical Ethanol		Ethyl Acetate		n-hexane	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Alkaloids	Mayer Test	+++	+++	++	++	+	+
	Dragendorff	-	-	-	-	-	-
Flavonoids	Test Method I	+++	+++	+++	+++	-	-
	Shinoda Test	-	-	-	-	-	-
Tannin	Iron Chloride Test	++	++	++	++	+	+
Phenol	Iron Chloride Test	++	++	++	++	+	+
Steroids	Libermann-Burchard	+++	+++	+++	+++	+++	+++
Saponin	H_2O	++	++	+++	+++	+++	+++
Terpenoids	Salkowski	+++	+++	++	++	++	++

Information:
(-) = Not detected, (+) = weak intensity, (++) = strong intensity, (+++) = Very strong intensity

However, in n-hexane extract only two layers were formed, the upper layer was light green and the lower layer was white, as can be seen in Fig. 2.

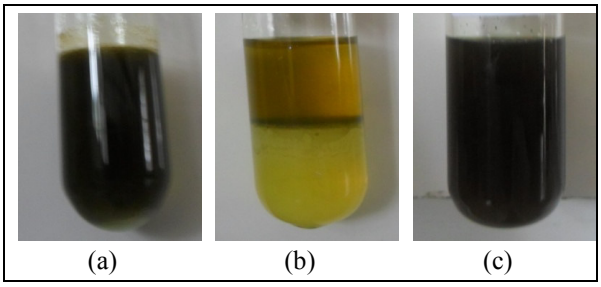


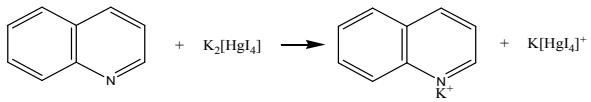
Figure 2. Phytochemical screening results of alkaloid compounds with Mayer's reagents: (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

The formation of white deposits was thought to be due to the nitrogen in the alkaloids reacting with K^+ metal ions from potassium tetraiodomercurat (II). The reaction can be seen in Fig. 3.

$$HgCl_{2\,(s)} + 2KI_{(aq)} \rightarrow HgCl_{2\,(aq)} + 2KCl_{(aq)}$$

$$HgI_{2\,(aq)} + 2KI_{(aq)} \rightarrow K_2[HgI_2]_{(aq)}$$

Potassium tetraiodomercurate (II)



Potassium-alkaloid deposits

Figure 3. Estimated reaction of the Mayer test with an alkaloid compound

An alkaloid compound test using Dragendorrf reagents showed negative results on all three extracts. This was characterized by the absence of orange deposits in the three extracts but brownish and brick red deposits formed on the bottom of the tube. Alkaloid compound test resulted using Dragendorff reagents can be seen in Fig. 4.

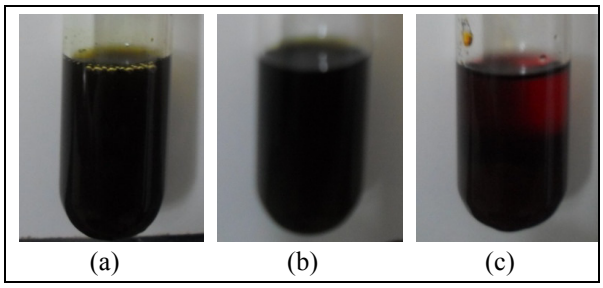


Figure 4. Phytochemical screening results of alkaloid compounds with Dragendorff reagents (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

Flavonoids

Flavonoids are secondary metabolites that have many benefits. Flavonoids are often good reducing compounds, they inhibit many oxidation reactions, both enzymes and nanoenzymes. Flavonoids act as good reservoirs of hydroxide and superoxide radicals and thus protect membrane lipids against damaging reactions.[9]

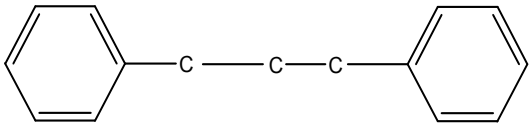


Figure 5. Flavonoid structure

Based on the results of phytochemical screening tests, each extract was reacted with 10% ammonia and concentrated sulfuric acid. The positive results were found in ethanol and ethyl acetate extracts, characterized by the formation of a brownish-green solution. Whereas in the n-hexane solvent the

negative results were obtained because of the formation of orange or orange deposits. The results of the flavonoid compound test using method 1 can be seen in Figure 6.

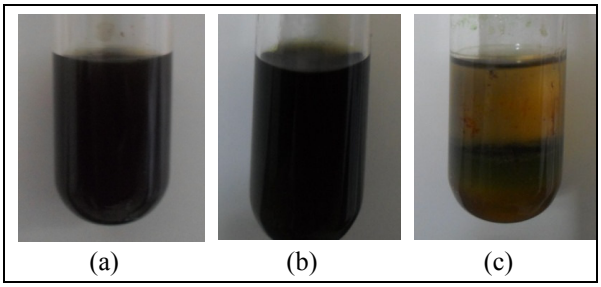


Figure 6. Phytochemical screening results of flavonoid compounds by method 1: (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

The Shinoda test to determine the content of flavonoid compounds in the leaves showed negative results on all three extracts. The results showed a brick red solution was formed after the addition of magnesium powder, while positive results were obtained if a pink solution was formed. The results of the study can be seen in Fig. 7.

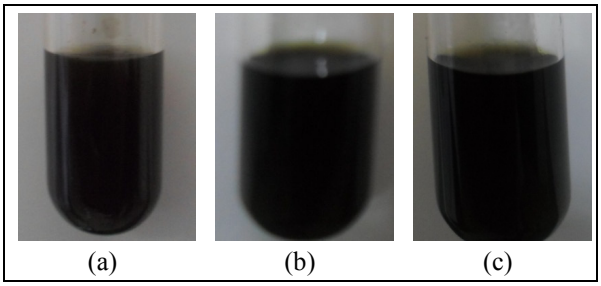


Figure 7. Phytochemical screening results of flavonoid compounds in the Shinoda test : (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

The pink precipitate that was supposed to be formed results from the coordination covalent bond between the magnesium ion and the OH phenolic group of flavonoid compounds. The equation of reaction is stated as follows:

$$Mg_{(s)} + 2HCl_{(aq)} \rightarrow MgCl_{2\,(aq)} + H_{2(g)}$$

$$MgCl_{2\,(aq)} + 6ArOH_{\,(aq)} \rightarrow [Mg(Oar)_6]^{4-}{}_{(s)} + 6H^{+}{}_{(aq)} + 2Cl^{-}{}_{(aq)}$$

Pink

Tannin and Fenol

Based on the results of phytochemical screening tests on tannin compounds, all three extracts showed positive results. This was indicated by the formation of a black-green solution after the addition of $FeCl_3$ to the three macerates. Once the addition of 1% $FeCl_3$ solution, it was estimated that this solution reacted with one of the hydroxyl groups present in tannin compounds.

The classic way to detect simple phenol compounds was by adding a solution of 1% iron (III) chloride in water or ethanol to the sample solution, which gave a strong green, red, purple, blue, or black color.[6]Tests for phenol compounds also obtained positive results. The tannin and phenol test results can be seen in Figure 8.

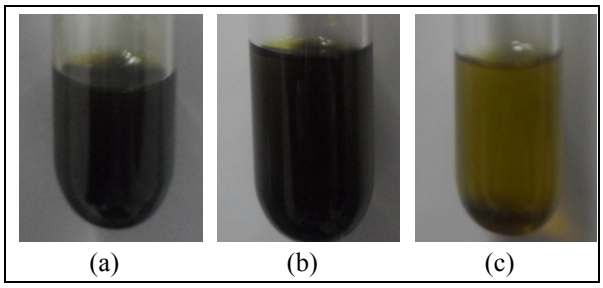


Figure 8. Phytochemical screening results of tannin and phenol compounds with iron (III) chloride solution: (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

The formation of this green color was due to the formation of complex compounds between Fe metals and tannins. Complex compounds were formed because of the coordination covalent bonds between metal and non-metal atoms as can be seen in Figure 9.

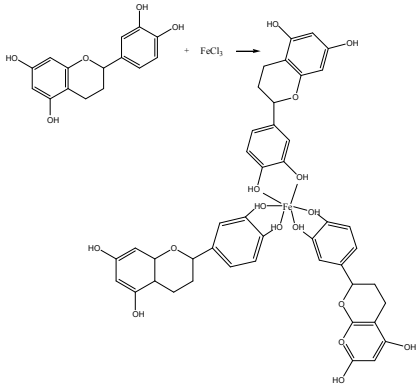


Figure 9. The reaction between tannin and $FeCl_3$

Saponin

Saponins were strong surface-active compounds which gave rise to foaming when boiled in water.[9]The saponins compounds were detected in all three leaf extract series after water was added to produce foam. In the ethanol extract the foam formed was located at the top of the solution while the ethyl acetate and n-hexane extracts were located the foam formed between the water layer and the macerate layer. The addition of water in extracts of ethyl acetate and n-hexane forms two layers, it might be due to water was polar. The saponins test results can be seen in Fig. 10.

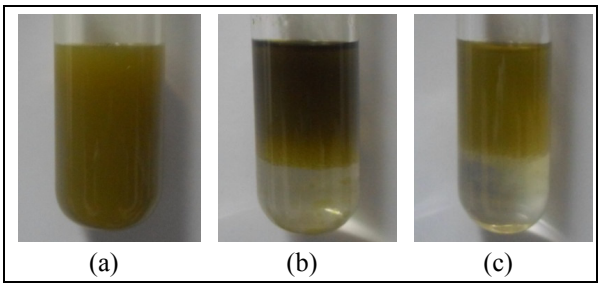


Figure 10. Phytochemical screening results of saponins compounds with water molecules: (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

The emergence of foam in the saponins test showed the presence of glycosides which had the ability to form froth in hydrolyzed water into glucose and other compounds. The reaction can be seen in Fig. 11.

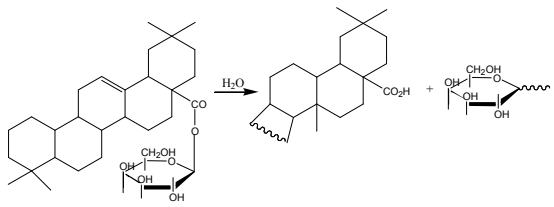


Figure 11. Hydrolysis reaction of saponins in water

Steroids

The structure of steroid core is shown in Fig.12. Based on the results of phytochemical screening tests, positive results were obtained for the presence of steroid compounds in series leaves in all three extracts. It was characterized by the formation of blackish brown sediment in the three extracts used.

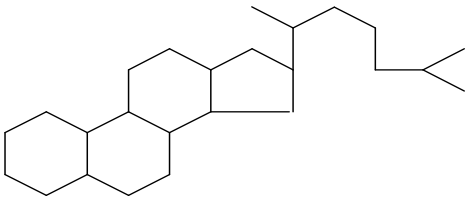


Figure 12. The structure of the steroid core

Terpenoids

The test results of terpenoid compounds in Muntingia calabura L leaves using the salkowski test showed that the leaveswere contained terpenoid compounds in all three solvents. This was marked by the formation of brown-colored deposits. The highest amount of deposition was found in ethanol and n-hexane extracts, while the least amount was in ethyl acetate extract. The results of the terpenoid compound test using salkowski test were obtained as shown in Figure 13.

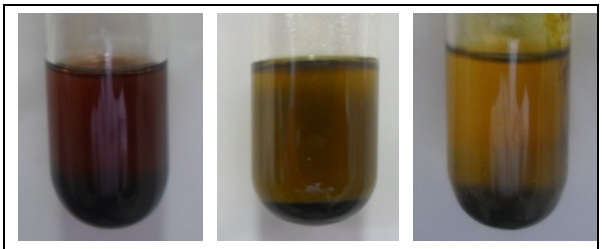


Figure 13. Phytochemical screening results of steroid compounds and terpenoids: (a) ethanol extract, (b) ethyl acetate extract, and (c) n-hexane extract

Based on the research results obtained, the Muntingia calabura L leaves extracts in ethanol solvents were contained alkaloids, flavonoids, steroids, terpenoids, saponins, tannins and phenols. This was in accordance with the results of study[4], in research on secondary metabolite content in Muntingia calabura L leaves. It was found that the leaves extracts in ethanol solvents were contained alkaloids, steroids, flavonoids, phenols, quinones, saponins and terpenoids. The same result was found in ethyl acetate solvents, but the levels were different. Muntingia calabura L leaves extracts in n-hexane solvents showed positive results in the presence of alkaloid, flavonoid, tannin, phenol,

steroid saponins and triterpenoid levels but were lower than ethanol and ethyl acetate extracts.

The results of study[10]also found that three flavones was successfully obtained rather than the raw ethyl acetate extract. Another studywas also conducted to screen the antibacterial activity of methanol extract of Muntingia calabura L leaves.[11]It has been found that thephytochemical tests of extract suggested the presence of flavonoids, tannins, as well as saponins.

Several potential sources of extractsunderutilized fruit were identified. One of which was Muntingia calabura, which was judged to be related to total phenolic and flavonoid content, as well as antioxidant activity in different solventssystems (methanol, ethanol and acetone) and distilled water.[12] The content of phenolics and flavonoids fromM. calabura was found to be 1.356 to 3.872mg equivalent to Tanic acid/g fresh weight (TAE/g fw) and 0.026 to 0.068 routine mg equivalent/g fresh weight (RE/g fw), respectively. This study was also accordance with previous study, whereas from the preliminary phytochemical studies. It was found that the high amount of phenolic, saponins, tannins and flavonoids present in the extract of Muntingia calabura L.[13]

Overall, this study found that Muntingia calabura L leaves extracts were contained the least amount of alkaloids, tannins, phenols, steroids, saponins and terpenoids compared to extracts in ethanol and ethyl acetate solvents. Flavonoids were not detected in n-hexane extract.

III. Conclusion

Based on the results of phytochemical screening tests of Muntingia calabura L leaves samples in positive ethanol solvents, *e.g*., alkaloids, flavonoids, tannins, phenols, saponins, steroids and terpenoids, as well as in ethyl acetate solvents. However, there were more concentrations in ethanol solvents. Whereas, in the n-hexane solvent, the Muntingia calabura L leaveswere contained alkaloids, tannins, phenols, saponins, steroids and terpenoids. However, there were no flavonoid compounds was identified.

Acknowledgement

Acknowledgment was addressed to the FKIP Chemistry Laboratory, Syiah Kuala University, Banda Aceh, which had given permission to conduct this study.

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