Antioxidant of Total Phenolic from Saputangan Leaves (*Maniltoa grandiflora* (A. Gray) *Scheff*)

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Keywords: Antioxidant, Total Phenolic, Thin Layer Chromatography and Spectrophotometer of UV-Visible

Abstract: Phytochemical screening test used 5% $FeCl_3$ reagent showed extract become to black extract that saputangan leaves contained phenolic compound. Saputangan leaves powder was macerated with methanol and got macerate as 100.84 g. Macerate was dissolved with aquadest to remove lipid in saputangan leaves. First partition was used ethyl acetate to get solid extract as 37.03 g. Second Partition was used n-hexane to get solid extract as 18.25 g. Total phenolic was analysed on plate of thin layer chromatography with used chloroform and methanol with 70:30 comparison. Total phenolic has 3 spots that have an Rf of 0.44 ; 0.29 and 0.22. Total phenolic absorbance measured at 516 nm wavelength using a UV-Visible spectrophotometer. Total phenolic solutions were soluted in concentrations of 10, 25 and 50 ppm using methanol p.a as solvent. The absorbance obtained at all three concentrations was 0.202; 0.195 and 0.175. Based on the linear regression equation Y = 1,191 X + 31.87, the IC_{50} value for the total phenolic was 15.22 ppm. IC_{50} value of 15.22 ppm can be categorized as a total phenolic compound as a strong antioxidant.

1 INTRODUCTION

The development of plant production is increasing by the community who more understanding about the benefits of medicinal plants. In developing countries, 65%–80% of population depends upon herbal medicines for primary health care (Oladele and Ayoola., 2015). Different categories of bioactive compounds are being isolated and characterized since the middle of 19th century. Most of these compounds are used as raw material for new medicines or as an active ingredient of existing medicines. Herbal medicines provide rich amount of tannins, alkaloids, flavonoids, phenolic compounds, and so forth, so these can be used in the treatment of several degenerative disorders (Ali *et al,* 2015). Plants are rich and valuable resources of bioactive phenolic. They can be utilized in various fields such as antioxidant, antimicrobial, anti-inflammatory, antitumor, antiviral, analgesic and antipyretic (Salinas *et al* 2017).

Saputangan Plants (*Maniltoa grandiflora* (A. Gray) *Scheff*) is a type of plant that belongs to the genus Maniltoa and Fabaceae family. Saputangan is usually made as ornamental plants that can reduce pollution by absorbing pollutants such as carbon monoxide (Hidayati et al., 2016). In English the handkerchief plant is named as Dove Tree, Ghost Tree, Handkerchief Tree. Historically, the origin of this Saputangan plants came from Fiji. The distribution of saputangan is from the areas of Fiji, Indonesia, Papua New Guinea, Solomon Islands, Tonga and the United States. This plant is a tree with high 5 to 15 m. Stems upright, round, simpoldial branching and brown. Leaves in the form of complex leaves, pinnate leaf reinforcement, oval flat edges, pointed edges and base of leaves. Leaf length of 7 to 14 cm and width of 3-8 cm with stem length between 1 - 1.5 cm and green. It has malae-shaped compound flowers and is located under the leaves and ends of tree trunks. The shape of the flower stalk is round with length as 1-2 cm and green. Cup shaped petals, oval sheath, loose flower crowns and yellow. The fruits of this plant are pods. Kidney shaped seeds, black and small in size. The types of roots include taproots and brownish white (Health Department, 2015).

Phytochemical screening method is done by checking at the color testing reaction using a color reagent. The important thing that plays an important

Sinurat, J., Br Karo, R., Siregar, S., Marbun, R. and Shufyani, F.
Antioxidant of Total Phenolic from Saputangan Leaves (Maniltoa grandiflora (A. Gray) Scheff).
DOI: 10.5220/0009974405360542
In *Proceedings of the International Conference on Health Informatics and Medical Application Technology (ICHIMAT 2019)*, pages 536-542
ISBN: 978-989-758-460-2

role in phytochemical screening is the selection of solvents and extraction methods. Phytochemical screening of simplicia powder and samples in wet form includes checking the content of alkaloids, flavonoids, terpenoids, tannins and saponins according to procedures that have been carried out by Harbone (Subramanian *et al*., 2016). The effective parameters in these extraction methods are the type and polarity of solvents and their ratio, time and temperature of extraction and moreover chemical composition and physical characteristics of the samples (Garcia *et al*, 2015). Maceration is a stepwise solid-liquid extraction method which is carried out by leaving the solids submerged in a solvent. The process of immersion in an effort to extract a substance from this natural material can be done without heating (at room temperature), by heating or even at boiling temperatures. After filtering, the residue can be extracted again using a new solvent. A new solvent in this case does not necessarily mean different substances from the previous solvent but can be a solvent of the same substance. This process can be repeated several times as needed. If maceration is done with a water solvent, a further extraction process is needed, it is the water phase extraction obtained with organic solvents. If maceration is directly carried out with organic solvents, the extracted filtrate is collected into one, then evaporated or distilled (Kristanti *et al*., 2018). Partition is the process of separation to obtain components of solutes from their mixtures in solids using an appropriate solvent. It can also be defined as the dispersion of the chemical component of the extract which has been dried in an appropriate solvent based on the solubility of the chemical component and undesirable substances such as insoluble salts. This extraction operation can be carried out by stirring the solid suspension in a container with or without heating (Jiao *et al*, 2015).

Thin Layer Chromatography on a larger layered plate, usually 5 x 20 cm, 10 x 20 cm, or 20 x 20 cm. Usually it takes 30 minutes to an hour of development. In essence, TLC involves two phases, namely the stationary phase or layer properties, and the mobile phase or mixture of developer solvents. The stationary phase can be a fine powder that functions as an absorbent or buffer surface for a liquid layer. The mobile phase can be almost any kind of solvent or a mixture of solvents. The selection of the right mobile phase is a very important step for the success of the analysis with TLC (Sri Atun, 2016). Phenol compounds are the main class of antioxidants in plants. The content of phenolic compounds is widely known as a free radical terminator and in general the content of phenolic compounds is positively correlated to antiradical activity. Phenolic compounds are easily found in plant parts such as stems, leaves, flowers, and fruit. The large variety of groups which may be substituted in the main framework of phenol causes a wide structural variation in phenolic compounds. There are more than 8000 types of compounds included in the group of phenolic compounds and whose structures are known include flavonoids, simple monocyclic phenols, phenyl propanoids, polyphenols (lignin, melanin, tannin) and phenolic quinones (Marinova *et al*., 2015).

Phenolics are one of the major and diverse group of active compounds in the plants which have at least one aromatic ring and one or more hydroxyl groups in their structures (Gharaati *et al*, 2017). In terms of biogenetics, phenol compounds are basically divided into two main types. The first is the phenol compound derived from the shikimat pathway and the second is the phenol compound derived from the acetate-malonate pathway. Another class of phenol compounds derived from a combination of these two biosynthetic pathways is the flavonoid compound (Kristanti *et al*., 2018). Antioxidants are inhibitors of oxidation reactions due to free radicals that can cause damage to unsaturated fatty acids, cell wall membranes, blood vessels, DNA bases, and lipid tissue, causing disease. A plant has antioxidant activity if it contains compounds that are able to ward off free radicals such as phenols and flavonoids. Free radicals occur due to complex chemical processes in the body that can damage the body's immune system. If there are excess free radicals in the body will be able to attack anything that can have implications for the emergence of various degenerative diseases, therefore the formation of free radicals must be prevented or served with antioxidants (Widyastuti, 2015).

Recent research conducted by Sinurat *et al.* (2018) showed that the methyl gallate compound isolated from saputangan leaves had a very strong antioxidant ability with IC_{50} value of 16,136 mg/ml. Lubis *et al.* (2018) who isolated phenolic compounds in the form of methyl gallate from jengkol skin (Archidendron jiringa) which is a Fabaceae family which is proven to have very strong antioxidant power. Previous research was also conducted by Dzoyem *et al.* (2017) regarding the antioxidant, antimicrobial and cytotoxic activity of 8 compounds isolated from Entada abyssinica (Fabaceae) where there are 4 types of phenolic compounds that can act as antibacterial. Based on

this description, the researcher was interested in testing the antioxidant activity of the total phenolic compounds of the saputangan leaves using the DPPH method (2,2-diphenyl-1- picrilhidrazil).

2 METHODS

This research was conducted in the natural product of organic chemistry laboratory, pharmacy faculty of Institut Kesehatan Medistra, Lubuk Pakam. The study was conducted in the period April-August 2018. The Saputangan leaves obtained from the environment around the Universitas Sumatera Utara. Materials: Saputangan leaves powder, Methanol p.a., Ethyl acetate, n-Hexane, Chloroform p.a., Aquadest, reagent of 5% $FeCl_3$ and DPPH (2,2-diphenyl-1-picrilhidrazil). Equipment: Macerator, Separate Funnel (Schoot Duran), Rotary evaporator (Heidolph), Steaming waterbath (Memmert), TLC plate, Chamber, Incubator (Memmert) and UV-Vis Spectrophotometer (Shimadzu). Saputangan leaves that have dried and blend become powder.

This research was carried out sequentially in laboratory with the research scheme. Process started from maceration and screening test, then continued to evaporate solvent. Solid extract is soluted by water to remove the lipid. Then filtrated the fraction that soluted in water. Filtrate is partitied with ethyl acetate conducted with n-hexane by separate funnel. TLC is done to analysis of total phenolic compound and measure of antioxidant activity. The scheme of research is showed in Figure 1.

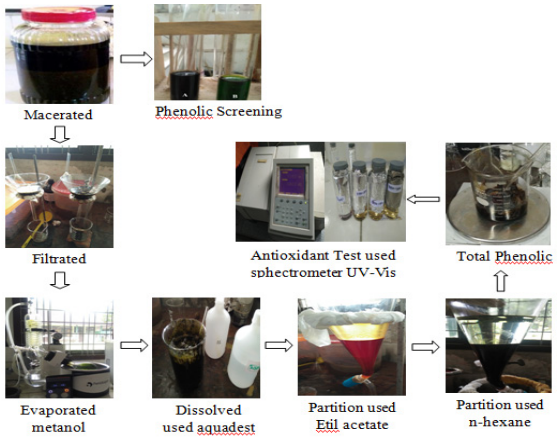
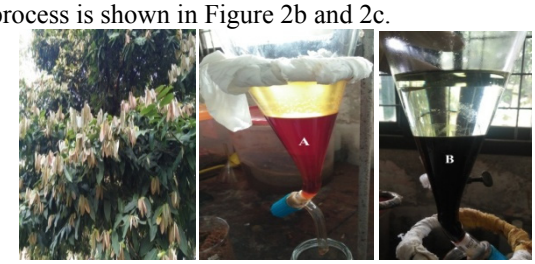


Figure 1: Scheme of Research.

Phytochemical Screening: This research was conducted in the laboratory of organic chemistry of natural materials, Department of Chemistry, Faculty of Mathematics and Natural Sciences to determine the presence of phenolic compounds in the leaves of the Saputangan plant. A preliminary test was carried out, phytochemical screening where 10 g fresh leaves of saputangan plant that had been blended with a blender macerated with methanol and then filtered. The filtrate was tested by adding 3 drops of 5% $FeCl_3$ reagent solution, forming a black precipitate if saputangan extract is positive contained phenolic compound (Eko, 2015).

Maceration of Saputangan Leaves: Sample as 1000 g of Saputangan leaves powder which had been dried and finely macerated for ± 24 hours with methanol as much as 5 liters at room temperature. Macerate was filtered and a extract of saputangan leaves was obtained. Maceration was repeated using methanol as a solvent until the methanol extract obtained gave a negative test result with 5% $FeCl_3$ reagent. The methanol extract obtained was concentrated by rotary evaporator at a temperature of 60°C with a rotation of 80 rpm. In Figure 2a is shown the saputangan leaves. Saputangan is a tree with high 5 to 15 m. Stems upright, round, simpoldial branching and brown. Leaf length of 7 to 14 cm and width of 3-8 cm with stem length between 1 - 1.5 cm and green.

Partition of Saputangan Leaves: Patition of the saputangan was carried out on the distilled water filtrate in a 500 ml separating funnel using ethyl acetate solvent so that the bottom layer was obtained in the form of distilled water and the top layer was in the form of ethyl acetate. Then the ethyl acetate layer is taken and continued with repeated partitions of the aquades filtrate. The ethyl acetate extract obtained was concentrated by a rotary evaporator at 60°C with a rotation of 40 rpm and evaporated until the solvent evaporated. Then partitioned repeatedly with n-hexane and evaporated by rotary evaporator at 60°C with a rotation of 30 rpm. The partition process is shown in Figure 2b and 2c.



(a) (b) (c)

Figure 2: (a) Saputangan Leaves, (b) Partition in Ethyl Acetate, (c) Partition in n-hexane.

Thin Layer Chromatography: Total phenolic obtained from the partition extraction process were

analyzed using the Thin Layer Chromatography (TLC) method using the Merck 60F254 silica gel stationary phase and the Chloroform:Methanol at a ratio of 90:10, 80:20, 70:30 and 60:40 v/v. To see the change in elucidation, thin layer chromatograms were marked with upper and lower limits using a pencil. The total phenolic compound is dropped at the lower limit of the thin layer plate, then put into a chamber that containing the eluent and allowed to move to the upper limit. Thin layer chromatograms were irradiated with ultraviolet light to see the spots of the compound, then marked with a pencil and calculated the Rf value. Furthermore, the thin layer was fixed with a 5% $FeCl_3$ solution producing a black spot on the thin layer chromatogram showing positive containing phenolic compounds. Observed the color of the stain that arises and calculate the price of Rf obtained. Rf range from 0.00 to 1.00. Separate components are good if the value of Rf is different from at least 0.1. The same thing is done in each eluent comparison used to determine the results of the separation of the thin layer chromatogram. Stains that arise are calculated using the factor retention formula as follows: Rf formula is *stain distance to the lower limit per eluent distance to the lower limit*.

Antioxidant Test: 0.3 mM DPPH solution was prepared by dissolving 2.957 mg of DPPH powder in methanol p.a in a 25 mL measuring flask, then homogenized so that the solution to be formed was violet. Total phenolic prepared in 100 ppm as main solution, by dissolving 1 mg of total phenolic with methanol p.a solvent in a 10 ml measuring flask. Then the 100 ppm main solution soluted in variation of solution with concentrations of 10, 25 and 50 ppm. 1 ml of DPPH 0.3 mM was added with 2.5 ml of methanol p.a as a blank solution. 1 ml of 0.3 mM DPPH solution was added with 2.5 ml of total phenolic with a concentration of 10 ppm, homogenized in a test tube and left for 30 minutes in a dark room. After that measured absorbance with a maximum wavelength of 516 nm. The same work procedure was carried out to test the antioxidant total phenolic compounds by concentrations of 25 ppm and 50 ppm.

3 RESULTS

Phytochemical screening is using 5% $FeCl_3$ reagent where previously the sample was dissolved with methanol solvent in repeatedly. In this case, the extract was became black precipitate after dropped 5% $FeCl_3$ whereas the extract was previously green. The screening result is tested in test tube. The black precipitate is meant saputangan leaves contained phenolic. The following will be displayed screening result in Figure 3a. Maceration process is treated to the powder sample of saputangan leaves in macerator. Maceration is treated repeatly to maximize the extract that resulted. Sample as solid extract was macerated in methanol solvent was obtained at 100.84 g. This method was carried out by inserting suitable plant powders and solvents into a tightly closed inert container at room temperature. The principle of maceration method is based that samples soaked using organic solvents will break down the walls and cell membranes due to pressure differences found outside and inside the cell so that secondary metabolites contained in the cytoplasm will dissolve into organic solvents. The extraction process is stopped when an equilibrium is reached between the concentration of the compound in the solvent and the concentration in the plant cell (Yeon-Ju et al., 2015).

After maceration, a partition was carried out using ethyl acetate to obtain a solid extract of 37.03 g. The last partition was carried out using n-hexane to partitied the non polar compound from phenolic compound. Finally, the solid extract after the last partititon is 18.25 g. The extract saputangan leaves from the partition was contained total phenolic because it reacted positively to the $FeCl_3$ reagent when we have screening again. In the liquid-liquid partition process, two phases of solution have differences soluble in solubility. The shaking of the separating funnel during partition aims to expand the contact surface area between the immiscible solvents. The solvent requirement for the partition method has polarity which is suitable for the extracted material and must be separated after shaking. Extract of total phenolic after many process as black extract is shown in Figure 3b.

Thin layer chromatography (TLC) analysis was performed on total phenolic compounds obtained using chloroform : methanol eluent 70:30 v/v. Based on the results of TLC analysis, it can be concluded that the total phenolic compounds contained 3 polar phenolic compounds. The following figure is displayed of the results of TLC analysis of total phenolic compounds. Chromatogram of TLC is shown in Figure 3c.

(a) (b) (c)

Figure 3: (a) Saputangan leaves extract + 5% $FeCl_3$, (b) Extract of Total Phenolic, (c) TLC analysis of total phenolic.

Total phenolic compounds of saputangan leaves were tested for antioxidant activity by the free radical DPPH method to obtain IC_{50} values by a UV-Visible spectrophotometer at a maximum wavelength of 516 nm. The concentration is prepared in many variation, it is 10, 25 and 50 ppm. The absorbance is showed in instrument then converted the absorbance in percentage scale. The measurement is displayed in table 1.

Table 1: Measurement of total phenolic absorbance.

Concentration	Absorbance	% Absorbance
Blank	0.802	-
10 ppm	0.202	74.83 %
25 ppm	0.195	75.72 %
50 ppm	0.175	78.19 %

4 DISCUSSIONS

The green extract became a black extract indicating that extract contained phenolic compounds. It happened because the oxygen group which is bound as a hydroxy releases a pair of free electrons to bind $FeCl_3$ so that the H group that is bound as hydroxy will be released and form an aromatic compound with bound to $FeCl_2$ as a black precipitate and HCl. Figure 4 is regarding the mechanism of reaction.

Thin layer chromatography analysis was performed on total phenolic compounds using chloroform eluent: methanol 70:30 v/v, so that 3 spot spots were obtained. Stain spot is calculated according the Retention Factor formula. Retention factor of Stain spot 1 is 0.44, stain spot 2 is 0.29 and stain spot 3 is 0.20. Total phenolic compounds of saputangan leaves were tested for antioxidant activity by the free radical DPPH method to obtain IC_{50} values by a UV-Visible spectrophotometer at a maximum wavelength of 516 nm. The equation Y = ax + b is used to obtain the value of IC_{50} by entering the value 50 as the Y axis, so value x will be obtained that will represent the value of IC_{50}. Statistical calculation is showed in Table 2 and conducted to complete linear regression equation.

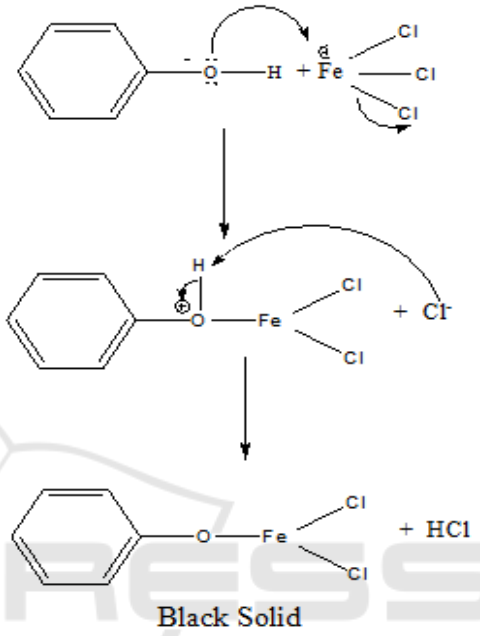


Figure 4: The mechanism of phenolic with $FeCl_3$.

Table 2: Statistical Calculation.

X	Y	XY	X^2
0	0	0	0
10	74.83	748.3	100
25	75.72	1893	625
50	78.19	3909.5	2500
ΣX= 85	ΣY= 228.74	ΣXY=6550.8	ΣX^2=3225

Note : X = Concentration (ppm)
Y = Absorbance (%)

The "a value" obtained from the statistical formula is 1.191. This a value will be used as the x-axis in the linear regression equation. The "b value" obtained from the statistical formula is 31.87. This b value is 31.87 will be used as the constant in the linear regression equation. The linear regression equation is Y = ax + b. The "a and b value" are substituted to the linear regression equation as IC_{50} value to be Y = 1.191 X + 31.87. If the concentration is increased, percentage of absorbance

will increase too. Based on the linear regression equation got IC_{50} values as 15.22 ppm. Based on the literature it can be stated that if the IC_{50} value produced is below 50 ppm which indicates that the total phenolic has a very strong antioxidant activity. According to the equation, The R^2 value is 0.461. The correlation between concentration and percentage (%) of absorbance can be seen in Figure 5.

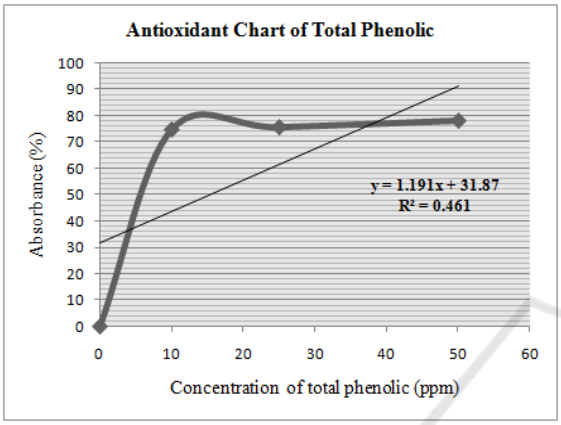


Figure 5: Chart of Antioxidant.

In the antioxidant test, absorbance of DPPH radical is followed by a reversal of absorbance at the maximum wavelength that occurs due to radicals by antioxidants (AH) or reactions with radical species (R.) which are marked by changes the color became pale yellow color, data often given as IC_{50} is an antioxidant needed for 50% of DPPH radical reduction in a certain period of time (15-30 minutes) (Pokorny et al, 2016). The mechanism between DPPH radical and total phenolic will be displayed in Figure 6. The Hydrogen from phenolic compound will stable the DPPH radical, so that phenolic can act as antioxidant.

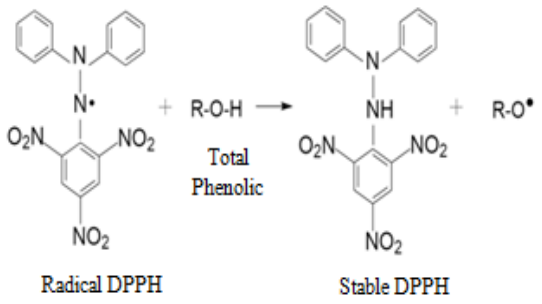


Figure 6: Absorbance mechanism of DPPH radical.

5 CONCLUSION

After maceration and partition, a total phenolic compound is obtained from saputangan leaves as 18.25 g. Results of thin layer chromatography analysis of total phenolics using the chloroform : methanol as eluent showed that total phenolic has 3 spots that have an Rf of 0.44 ; 0.29 and 0.22. The total phenolic compound is able to act as a strong antioxidant by having an IC_{50} value of 15.22 ppm. This antioxidant test was carried out using a DPPH (2,2-diphenyl-1- picrilhidrazil) which was measured using a UV-Visible spectrophotometer at a wavelength of 516 nm.

ACKNOWLEDGEMENTS

Thank you to The Institut Kesehatan Medistra Lubuk Pakam for the laboratory facilities and finance provided for this study and research.

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