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## PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT OF TREMBESI FRUIT SEEDS (*Samanea saman*)

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**Abstract:** Trembesi (*Samanea saman*) is a fast-growing plant from Central America and Northern South America. the Trembesi Tree is an easily recognize tree because it has an umbrella-shaped canopy with a canopy diameter greater than its height, the trembesi fruit is blackish-brown when ripe, with seeds embedded in the flesh of the fruit. This study aims to determine the class of compounds contained in the ethanol extract of trembesi fruit seeds extraction by maceration method using 70% ethanol solvent. The analysis uses univariate that present data with calculation results. The extract results were obtained by weight of 91% with an amendment value of 18.20%. The phytochemical screening were carried out with color tests and emphasized by the TLC test. The trembesi fruit seed ethanol extract containing alkaloid, flavonoids, saponins, and tannins compounds.

**Keywords:** Trembesi seed (*Samanea saman*), Phytochemical screening

### INTRODUCTION

Trembesi (*Samanea saman*) is a fast-growing plant from Central America and Northern South America. The Trembesi Tree is an easily recognize tree because it has an umbrella-shaped canopy with a canopy diameter larger than its height (Nuroniah and Kosasih 2010). The flowers of this plant are white with a pink spot on the top, length of the flower reaches 10 cm from the base of the flower to the tip of the flower. Trembesi flowers produce nectar that can attract insects that are useful in the process of pollination. Trembesi fruits are blackish-brown when ripe, with seeds embedded in the flesh of the fruit (Setiawan *et al.*, 2019).

The trembesi plant can be used as an ingredient in traditional medicine. Based on the research of Nafi'ah *et al.* (2015) mentioned that trembesi leaf extract is used as an alternative to diarrhea, fever, abdominal pain, and headaches. The compounds contained based on the research of Setiawan *et al.*, (2019) stated that trembesi leaf extract contains active compounds, namely alkaloids, flavonoids, tannins, saponins, and steroids. Based on the description above, researchers are interested in scientifically proving the content contained in the seeds of trembesi fruit (*Samanea saman*). It is not yet known about the content of compounds from the extract of the trembesi plant.

Based on the description above, researchers are interested in scientifically proving the content contained in trembesi fruit seeds with color tests and thin layer chromatography (TLC).



## 1 METHODS

### Extraction Process

Trembesi fruit seeds were taken from Malang, East Java. Seeds of trembesi fruit are taken when they are fully ripe (physiologically ripe). The viscous ethanol extract of trembesi fruit seeds (*Samanea saman*) is carried out by the maceration method. The fine *Simplicia* is weighed as much as 500 grams and put into a vessel, and then add 70% ethanol solvent until the sample is submerged (1:10), closed, and left for 3-5 days in a place protected from light. Stirred over and over again, sprinkled, and squeezed. then separate the deposits obtained and be concentrated (Marjoni, 2016).

### Phytochemical screening

#### Alkaloids

A sample of 100 mg, supplemented with 5 ml of HCl 2M and heated in a water bath while stirring, then cooled. NaCl 0.5 grams of powder is added, stirred, and filtered, then add HCl 2M to a certain volume then add Wagner reagent if a precipitate of alkaloid-containing material is formed (Mustarichie *et.al.*, 2011).

#### Flavonoids

A sample of 100 mg was dissolved in absolute ethanol ad with 2 drops of concentrated HCl observed color warm on a water bath for 15 minutes, then observed changes that occurred. The appearance of a red or violet color indicates the presence of flavonoid compounds (Mustarichie *et.al.*, 2011).

#### Saponins

The saponin test 100 mg sample is put in a test tube. Add 1 ml of aquadest, then shake and settled, if foam is formed that does not disappear for 30 minutes, then the material contains saponins (Mustarichie *et.al.*, 2011).

#### Tannins

The sample is added hot aquadest, then stirred and cooled. Add 5 drops of 10% NaCl then filtered. The Filtrate is divided into 3 parts, A, B, C. Filtrate A blank, Filtrate B added 3 drops of reagent FeCl<sub>3</sub>, and Filtrate C added gelatin solution. If a precipitate forms on filtrate C, there are tannins. If a green color is formed in filtrate B indicates the presence of hydrolyzed tannins, if brownish-green is formed in B indicates the presence of hydrolyzed tannins (Mustarichie *et.al.*, 2011).

#### Terpenoids and steroids

The sample is added 3 drops of C<sub>4</sub>H<sub>6</sub>O<sub>3</sub> to dry, then add 1 drop of concentrated H<sub>2</sub>SO<sub>4</sub> and observe the discoloration. Terpenoids are positive if there is a change in red or purple color But when green color is formed it means positive steroids (Siadi, 2012).

### Thin Layer Chromatography (KLT) Test

#### Alkaloids

Identification of alkaloid compounds of butanol motion phase: acetic acid: water (4:1:5) with



Dragendorff reagent. A positive reaction is shown in the formation of red, the brown spots indicating the presence of alkaloid group compounds (Widyarningsih *et.al.*, 2016).

#### Flavonoids

Identification of flavonoid compounds of the mobile phase of glacial acetic acid: butanol: water (1:4:5), with the appearance of ammonia vapor stains. The positive reaction was shown to be blue after being vaporized by ammonia on observations with visible light at UV 366nm confirming the presence of flavonoid content (Putu *et.al.*, 2017).

#### Saponins

The identification of the mobile phase saponin compound used is chloroform, methanol, and water (13:7:2), with the appearance of Liberman-Buchard reagent stains accompanied by heating at 105 °C for 5 minutes. The positive reaction of saponins is shown by the presence of brown or purple stains (Harborne cited Suharto *et.al.*, 2012).

#### Tannins

Identification of tannin compounds of butanol motion phase: acetic acid: water (14:1:5), with stain imaging Reagent FeCl<sub>3</sub> 5%. A positive reaction is shown by the formation of purple stains (Sriwahyuni, 2010).

#### Terpenoids

Identification of n-Hexane phase flavonoid compounds: ethyl acetate (7:3), with Liberman-Buchard reagent. Positive reactions are shown bluish-green in color. on observations with visible light at UV 366nm (Anam, 2015).

#### Steroids

The identification of the mobile phase steroid compound used is chloroform: methanol (9:1), with the appearance of a Liberman-Buchard reagent stain accompanied by heating at 105 °C for 5 minutes. Positive reactions from steroids are indicated by the presence of blue-green stains (Kristiani *et.al.*, 2008)

## RESULT AND DISCUSSION

### Extraction results

The choice of extraction method is an aspect that needs to be considered because the separation process will determine how much amendment will be produced, the higher the yield value, the more extract results you get (Armando, 2009 in Syamsul *et.al.*, 2020). Flavonoids are compounds that are included in thermolabile so the usage of this maceration method so as not to damage the thermolabile flavonoid compounds. The maceration method is considered economical, and easily to do with simple tools. The powder used for the extraction process is 500 g with 70% ethanol solvent as much as 5 L (1: 10), and obtained extract results weighing 91 grams then calculated the yield results. The amendment value obtained in this study was 18.20%. The amendment is a ratio of the weight of the extract produced with the weight of *Simplicia* as a raw material multiplied by 100%. The higher amendment value indicates that the extract produced from the extraction process is greater (Chairunnisa *et.al.*, 2019).

### Phytochemical screening

Phytochemical screening aims to analyze bioactive content useful for treatment and reference for other researchers (Marjoni, 2016).



## Colortest

The color test is also known as a preliminary test that aims to determine the presence of compounds contained in an extract by adding certain reagents according to the tests carried out on the determination of the compound.

Table 1. Result of phytochemical screening test

Phytochemical compounds	Reagents	Result of reaction	Result
Alkaloid	Pereaksi Wager	Brown precipitate	Positif(+)
Flavonoid	HCl pekat	Red	Positif(+)
Saponin	Aquadest	Stable foam	Positif(+)
Tanin	Larutan $\text{FeCl}_3$	Brownish green	Positif(+)
Terpenoid	$\text{C}_4\text{H}_6\text{O}_5 + \text{H}_2\text{SO}_4$	Red or purple	Negatif(-)
Steroid	$\text{C}_4\text{H}_6\text{O}_5 + \text{H}_2\text{SO}_4$	Green	Negatif(-)

## Alkaloids

The purpose of adding NaCl before the addition of reagents is to remove proteins (Endarini, 2019). Based on research by Fajrin & Susila, (2019) stated that the sediment is potassium-alkaloid. Wagner's reagent content, iodine reacts with the I ion of potassium iodide which produces I<sub>3</sub><sup>-</sup> (brown) ions. On the



metal ions, K<sup>+</sup> will form a coordinate covalent bond with nitrogen where the alkaloids will form a complex compound of potassium-alkaloids that precipitate.

#### Flavonoids

The purpose of adding concentrated HCl is to hydrolyze and break the glucoside bond by hydrolyzing O-glycosyl which is replaced with H<sup>+</sup> from acid because it has electrophilic properties. The Heating process acceleration the hydrolysis reaction (Susiloningrum, 2020).

#### Saponins

The onset of foam indicates the presence of glycosides that can form foam in water that is hydrolyzed into glucose and its aglicon compounds.

#### Tannins

The purpose of adding NaCl is to increase salting from gelatin tannins (Martiana et al., 2005). The formation of a blackish-green color after adding FeCl<sub>3</sub> 1% because tannins will react with Fe<sup>3+</sup> ions that form complex compounds (Setyowati et al., 2014).

#### Terpenoids and steroids

The purpose of adding C<sub>4</sub>H<sub>6</sub>O<sub>3</sub> is because it has a polarity that corresponds to the class of steroid compounds. The steroids are lipid-derived compounds that are not hydrolyzed (Illing et al., 2017). The addition of H<sub>2</sub>SO<sub>4</sub> aims to hydrolyze water and react with acetyl derivatives and form a color solution. The discoloration is formed due to oxidation in terpenoid compounds and steroids through the formation of conjugated double bonds (Sulistiyarini et al., 2019).

#### Thin Layer Chromatography (TLC) Test

The affirmative test aims to ascertain the compounds contained in the extract carried out by thin-layer chromatography. The data obtained are in the form of R<sub>f</sub> values and chromatogram colors that can be seen visually, and under UV light.





## Alkaloids

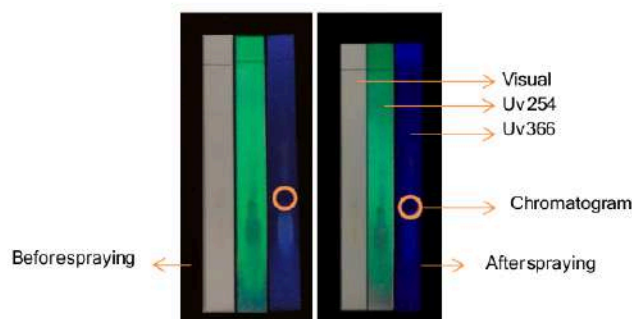


Figure1. Chromatogram of alkaloid on TLC Test

TLC test results of positive alkaloid compounds with brown chromatogram stains after spraying with Dragendorff reagents observed stains on UV 366 rays. The result of the Rf value of 0.36 results meets the requirements of the predetermined alkaloid Rf value of 0.07-0.62 (Harborne, 1996). Alkaloids are generally alkaline and easily soluble in organic solvents, difficult to dissolve in water, but alkaloids in the form of salts can be soluble in water (Sirait, 2007 in Dewi *et al.*, 2021).

## Flavonoid

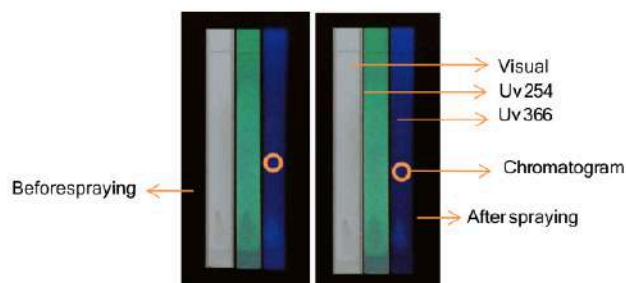


Figure2. Chromatogram of Flavonoid on TLC Test

TLC test results of flavonoid compounds were positive with blue chromatogram stains before and after being steamed with ammonia observed stains on UV 366 rays. The result from the Rf value of 0.43 results meets the requirements of the established flavonoid Rf value of 0.31- 0.98 (Harborne, 1996). Ammonia is a base, flavonoid compounds are acidic compounds, ammonia, and flavonoids occur many reactions that cause a salt formation and form a kinoid structure that makes double bonds longer (Robinson, 1995 in Arnida *et al.*, 2021).



#### Saponin

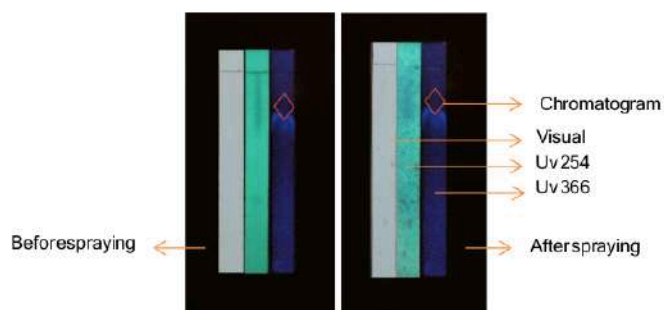


Figure 3. Chromatogram of Saponin on TLC Test

The result of TLC saponin test was positive with brown chromatogram stains after spraying with Liberman-Burchard reagents and then observed stains on UV 366 rays. The result of  $R_f$  value of 0.81 results meets the requirements of the established saponin  $R_f$  value of 0.57-0.92 (Mirza, 2016). Saponins are a form of glycoside from sapogenins so they will be polar. Saponin compounds tend to be attracted by solvents that are semipolar such as methanol (Astarina *et al.*, 2013).

#### Tannins

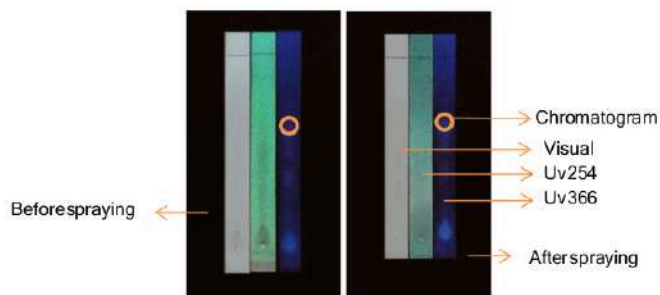


Figure 4. Chromatogram of Tannin on TLC Test

The result of Tannin TLC test was positive with purple chromatogram stains after spraying with a 5%  $\text{FeCl}_3$  reagent, stain spots were observed on UV 366 rays. The result of the  $R_f$  value in Visual, UV 254 UV 366 Chromatogram are 0.59-0.65. The result meets the requirements of the  $R_f$  tannin value that has been set, namely 0.29-0.85 (Mirza, 2016). Discoloration occurs due to the reaction between  $\text{FeCl}_3$  and the phenolic group contained in tannins (Herlianawati, 2007 Dewi *et al.*, 2021).





## CONCLUSION

The results of the phytochemical screening test of compounds contained in the ethanol extract of trembesi fruit seeds are alkaloids, flavonoids, saponins, and tannins. These phytochemical compounds have pharmacological activity, so further research is needed on the pharmacological effects of the ethanol extract of trembesi fruit seeds. It is worth conducting further research on the antidiabetic activity of the ethanol extract of trembesi fruit seeds.

## AUTHOR CONTRIBUTION

O and TS carried out study concept, design and drafting of the manuscript. E and F doing phytochemical screening. F participated in statistical analysis with TS. All authors read and approved the final manuscript

## CONFLICT OF INTEREST

The declare that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest

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