



Preliminary Studies on the Content of Phytochemical Compounds On Skin of Salak Fruit (*Salaccazalatca*)

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ABSTRACT

Salak (*Salaccazalatca*) is a native plant of Indonesia. Some Indonesians use zalacca as a traditional medicine. This plant belongs to the Palmae family. The purpose of this study is to reveal the structure of the active components that are efficacious and increase the economic value and efficacy that exist in the skin of zalacca (*Salaccazalatca*). The extraction method chosen was the maceration method using 96% ethanol solvent. A total of 1 kg of fresh zalacca skin (*Salaccazalatca*) is dried using the sun-heating method and produces 625 g of dried zalacca skin. The extraction-maceration process uses 100 grams of Implicial. Simplisia is soaked and stirred occasionally in 96% ethanol for 3 days and filtered until a thick extract is obtained. Thick extract obtained was 10.69 g. The percentage of yield obtained was 10.69%. Phytochemical screening carried out is alkaloids, steroids, triterpenoids, saponins, tannins, and flavonoids by using chemical reactions and using thin layer chromatography. The mobile phase used in thin layer chromatography is toluene: ethyl acetate (9.3: 0.7). Determination of the mobile phase is done based on a trial and error approach to obtain a clear line of movement. The stationary phase uses silica gel GF 254. Based on phytochemical analysis, the skin of salak fruit (*Salaccazalatca*) contains alkaloids, triterpenoids, saponins, tannins, and flavonoids. In addition, based on analysis using thin layer chromatography it produces Rf 0.1875 and is thought to contain terpene alcohol.

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1. Introduction

Salak plants (*Salaccazalacca*) are native plant of Indonesia. Some Indonesian people use Salak as traditional medicine. Particularly in Indonesia, Salak has been widely used as a product that is useful as traditional medicine such as cholesterol lowering. Some parts used as treatment in Indonesia are flesh of fruit, Salak seeds, and Salakskin.⁽¹⁾

According to Dhyana Putri et al⁽²⁾, the results of phytochemical tests showed skin of Salak contains flavonoids and tannins and a little alkaloid. Flavonoids can reduce glucose levels in the blood. Ethanolic extract of zalacca contains secondary metabolites of alkaloids, polyphenolates, flavonoids, tannins, quinones, monoterpenes and sesquiterpenes. The extract of zalacca skin has antioxidant activity with IC50 value of 229.27 ± 6.35 (µg/mL)⁽²⁾.

Based on Nuranti's research⁽³⁾, Salak skin extract can be beneficial for reducing cholesterol levels. This research was conducted by in vitro testing of male Swiss Webster mice induced by a high-fat diet. The results of phytochemical screening on zalacca skin extracts contain alkaloids, polyphenols, flavonoids, quinones, tannins, triterpenoids and steroids⁽³⁾. Based on these descriptions, the authors are interested in researching the phytochemical of Salak skin fruits.

2. Method

Materials

Vessel for maceration complete with cover and stirrer, analytical scales, ovens, glassware, aluminum foil, mori cloth, waterbath, porcelain cups, test tubes, tube racks, dropper pipettes, filter paper, chamber, silica gel GF 254 nm, UV light 254 nm and 366 nm.

The materials used in this study were the skin of Salak fruits, 96% ethanol, dilute ammonia (10%), Chloroform, HCl 2 N, Ether, Sulfuric Acid, aqueous Chloride Acid, 10% Iron (III) chloride solution, Mg Powder, Amyl Alcohol, Ethyl Acetate, Vanilin 10%, Lieberman-Burchard reagents, Mayer reagents and Dragendorff reagents.

Methods

Determination

Determination of the *Salaccazalacca* plant aims to ensure and ensure that the ingredients used are really *Salaccazalacca*. Determination of plants is carried out at the Plant Taxonomy Laboratory, Faculty of Biology, University of Jenderal Soedirman, Purwokerto.

Sampling

Salak fruit skin that is used for research comes from old Salak fruits. The weight of fresh skin of Salak fruits are 1

kg.

Drying method

Salak fruit skin is washed and dried in direct sunlight with a black cloth covered so that the active substances contained are not damaged by direct sunlight. Furthermore, dried Salak fruit skin is blended to form a coarse powder.

Maceration Process

In this study, Salak skin was extracted using 96% ethanol solvent. The extract was made using maceration method, which is 100 g of coarse powder of Salak skin put into a glass beaker and then added 96 ml of ethanol as much as 500 ml, covered and allowed to soak for 3 days protected from light (every day stirring for at least 30 minutes). The extract is then filtered using mori cloth so that it is obtained filtrate.

Phytochemical analysis

• Alkaloid Examination

A 2 ml extract solution was evaporated on a porcelain cup until it got a residue. The residue dissolved with 5 ml of diluted ammonia (10%) and added with 2 ml of chloroform and filtered. The filtrate was added with 2N HCl then shaken out to form 2 layers. There were 2 layers, namely 2N HCl layer and Chloroform layer. The layer containing 2 N HCl was taken using a 3 ml pipette and then divided into 3 test tubes. The first tube functions as a blank. The second tube is added Dragendorff reagent as much as 3 drops, if orange, yellow to brown precipitate is formed indicating the presence of alkaloids. The third tube was added by Mayer reagent as much as 3 drop, if a white to yellowish sediment was formed indicating the presence of alkaloids.⁽⁴⁾

• Steroid and Triterpenoid Examination

A sample of 2 ml (w/v) was extracted with ether then evaporated to dryness. Lieberman-Burchard reagent is deposited on the residue. The formation of purple color indicates triterpenoid group content, whereas if green-blue color is formed, it indicates the presence of steroid group compounds.⁽⁵⁾

• Saponin Examination

A sample of 2 ml was dissolved with 10 ml of distilled water in a test tube. After it chilled the filtrate in the test tube is shaken vigorously for about 30 seconds. The reaction will stop or be in a resting phase if the reaction temperature is equal to room temperature. Formation of foam with a height of at least 1 cm and persistent for 10 minutes and not lost on the addition of 1 drop of dilute hydrochloric acid shows saponins content.⁽⁵⁾

• Tanin Examination

The 2 ml extract solution was reacted with 10% iron

(III) chloride solution, if dark blue, black blue or greenish black showed the presence of tannin compounds⁽⁴⁾.

- *Flavonoid Examination*

Sample 2 ml of sample was added 0.1 mg of Mg powder, 1 ml of concentrated HCl and 1 ml of Amyl Alcohol then shaken strongly. The presence of flavonoids is indicated by the presence of red, yellow or orange in the Amyl Alcohol layer.⁽⁵⁾

Thin Layer of Chromatography (TLC) analysis

The extract was bottled 1 time on a 245 nm GF silica gel plate which had been activated at 100 °C for 3 minutes, bottling 1.5 cm from the bottom edge and the eluent distance from the top edge was 0.5 cm. The mobile phase propagation distance is 8 cm and then the silica gel plate is inserted into the chamber containing the mobile phase, namely toluene : ethyl acetate (9.3:0.7). The saturated mobile phase is used as much as 10 ml. Fulfillment of the mobile phase is done by inserting filter paper into the chamber, after the mobile phase propagates to the creeping limit then the mobile phase has been saturated. The plates are removed, dried in the air and then detected by UV light with wavelengths of 254 nm and 366 nm.⁽⁶⁾

3. Result and Discussions

Determination

The results of the determination state that the plant used is *SalaccaZalacca*, with the following details:

Family: Arecaceae.

Genus: *Salacca*

Species: *Salaccasalacca* (Gaertn.) Voss

Yield

Salak fruit peel extract yield is not more than 10.73%⁽⁷⁾. The extract yield obtained in this study was 10.69% or less than 10.73% due to the possibility of taking too long to evaporate. According to Yuliantiet al⁽⁸⁾ the length of the extraction process was very influential on the extracts produced.

Phytochemical analysis

- *Result of alkaloid*

The results of phytochemical analysis of Salak skin are presented in **Table 1**. Phytochemical analysis results showed that the Salak skin contains alkaloid compounds⁽⁷⁾. In this study, Salak skin positive contains alkaloid compounds due to the formation of yellow precipitate in the Dragendorf reagent test and white precipitate in the Mayer reagent test. This is in line with research conducted by Marlinaet al⁽⁹⁾. Marlinaet al⁽⁹⁾ conducted a study on Phytochemical Screening and Analysis of Thin Layer Chromatography Components Chemistry of Siamese Pumpkin (*Sechium edule Jacq. Swartz.*) In Ethanol Extract.

Table 1. Result of phytochemical screening analysis

Chemical content	Testing Method	Results	Annotation
Alkaloid	Dragendorff Mayer	Yellow	+
		White	+
Steroid Triterpenoid	Lieberman–Burchard	Purple	- +
Saponin	Aquadest (shaking)	Bubble	+
Tanin	Iron (III) chloride 10% solution	Sedimentation	+
Flavonoid	Mg powder, HCl 2N dan Amyl alcohol	Yellow	+

Positive alkaloid results in the Dragendorff test were characterized by the formation of orange, yellow to brown deposits. The precipitate is potassium alkaloids which are the result of the reaction produced between Dragendorf reagents which bind with alkaloids⁽¹⁰⁾. On the other hand, positive alkaloid results in the Mayer test are characterized by the formation of white to yellowish deposits. It is estimated that these deposits are the result of Mayer reagents that bind with alkaloids through a coordinating bond between N alkaloid atoms and Hg Mayer reagents to produce mercury complex compounds that are non polar precipitating white to yellow⁽¹¹⁾.

The purpose of adding aqueous ammonia (10%) serves to alkalize and precipitate the alkaloids so that the alkaloids can be obtained in the form of salt or free base. Chloroform is used with the aim of being able to attract alkaloid compounds because alkaloids have good solubility in chloroform⁽¹²⁾. The purpose of adding HCl 2 N is to attract alkaloids because alkaloids are alkaline so they are reacted using acidic solvents⁽¹⁰⁾. The results of the alkaloid analysis can be seen in **Fig. 1**.



Fig. 1. Result of alkaloid compound analysis

- *Result of Steroid and Triterpenoid*

The results of phytochemical analysis showed that the Salak skin contained triterpenoid compounds but did not contain steroid compounds. Negative results on steroid compounds can be caused by lack of sensitivity of the tool, the levels of compounds in the skin of the salak fruit is too

little or the skin of the salak fruit does not meet the requirements so that the existing compounds become lost or damaged due to enzymatic reactions or hydrolysis. Positive results on triterpenoid compounds can be caused by tool errors or the influence of compounds that have similar properties and identical atomic structures⁽¹³⁾. This is in line with research conducted by Simaremare⁽¹⁴⁾. Simaremare⁽¹⁴⁾ conducted a study on phytochemical screening of ethanol extract of itchy leaves (*Laportea decumana* (Roxb.) Wedd). The results obtained from this study formed red orange or purple for terpenoids and blue for steroids.

The purpose of the search for using ether is to attract chemical compounds in the sample. This is because ether is an excellent solvent for extraction and for various chemical reactions⁽¹⁵⁾. The Liebermann-Burchard reagent is a mixture of acetic anhydride and concentrated H₂SO₄. When the compound in the extract is dripped by H₂SO₄ through the cup wall, the acetic anhydride will react with the acid so that the C atoms in the anhydride form a carbocation. The carbocation formed reacts with the O atom in the OH group present in steroid compounds or triterpenoids. This reaction is an esterification reaction, namely the formation of ester compounds by steroid compounds or triterpenoids with acetic anhydride. The reaction process that produces color changes in the test using Liebermann-Burchard reagents⁽⁵⁾.

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Fig. 2. Result of steroid and triterpenoid analysis

- **Result of Saponin**

In this study, the salak fruit skin positively contained saponin compounds due to the formation of foam as high as 1.5 cm and persistent for 10 minutes. The foam formation in the saponin compound test is caused by a soapy substance present in the salak skin. Saponin test results are shown by the emergence of foam which is a glycoside that has the ability to form froth in water that is hydrolyzed into glucose and other compounds⁽¹⁶⁾. Test results stating simplicia contain saponins can be seen in **Fig. 3**.



Fig. 3. Result of saponin analysis

This is in line with research conducted by Bintoro et al⁽¹⁷⁾. Bintoro et al⁽¹⁷⁾ conducted research on the analysis and identification of saponin compounds from the leaves of bidara (*Zhizipus mauritania* L.) which produced foam after shaking and adding 2 N hydrochloric acid reagents, the foam formed did not disappear.

- **Result of Tannin**

In this research, salak skin contains tannins due to the formation of greenish black. The addition of FeCl₃ was used to determine the presence of phenol groups in the sample. The formation of color changes in the extract after the addition of FeCl₃ is caused by tannins forming complex compounds with Fe³⁺ ions. The presence of tannin will precipitate the protein in gelatin. Tannins react with gelatin to form solid copolymers that are insoluble in water⁽¹⁰⁾. The results of tannin analysis can be seen in **Fig. 4**.

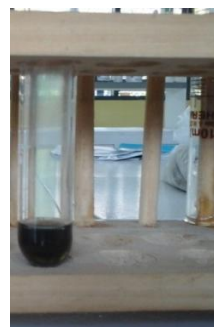


Fig. 4. Result of tannin analysis

This is in line with research conducted by Indarto⁽¹⁸⁾. Indarto⁽¹⁸⁾ conducted a study on qualitative and quantitative tests of groups of organic compounds from the bark and wood of *Artocarpus dadah* Miq plants. Indarto⁽¹⁸⁾ obtained tannin test results from bark and wood sample with FeCl₃

reagent 0.1% shows a positive test that is the color of the solution to yellowish green for bark and greenish brown for woody stems. This happens because of a reduction reaction. Tannins are a class of polyphenol compounds, able to reduce polyphenols iron (III) becomes iron (II). This is also the way classic for detecting phenol compounds, namely by adding a solution of 1% iron (III) chloride in water or ethanol to the sample solution giving rise to green, red, purple, blue or black.

- *Result of Flavonoid*

In this research, salak fruit skin contains flavonoids due to the formation of yellow deposits. The purpose of adding concentrated Mg and HCl powders is to hydrolyze flavonoids to aglycones. Flavonoids will be reduced with Mg and HCl can give red, yellow or orange⁽⁵⁾. The addition of amyl alcohol aims to attract free flavonoids, so that the colorless Amyl Alcohol becomes discolored from the flavonoids. Flavonoid compounds are polar compound, so they dissolve in polar amyl alcohol⁽¹⁹⁾. The result of flavonoid analysis can be seen in **Fig. 5**.

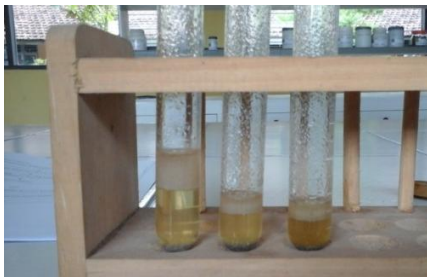


Fig. 5. Result of flavonoid analysis

Thin Layer of Chromatography (TLC) analysis

Thin Layer Chromatography Test (TLC) on extract of Salak skin using UV light 366 nm and 254 nm under the mobile phase, namely toluene: ethyl acetate (9.3 : 0.7) showed a spot stain with Rf 0.1875. In this study stain spots were seen using UV light 366 nm and 254 nm. The spot of staining were suspected terpene alcohol. Thin Layer Chromatography (TLC) test results under 254 nm can be seen in **Fig. 6a**. Thin Layer Chromatography (TLC) test results under 366 nm can be seen in **Fig. 6b**.

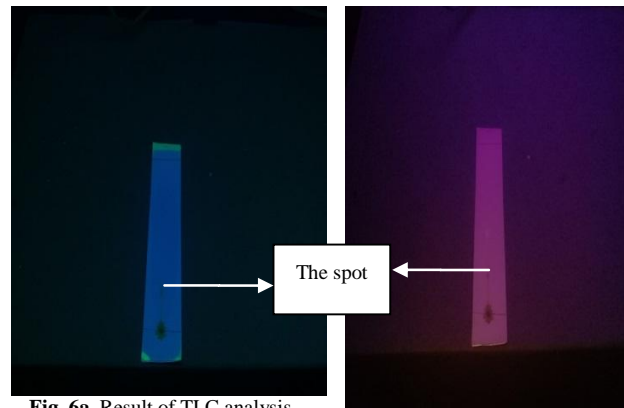


Fig. 6a. Result of TLC analysis under 254 nm

Fig. 6b. Result of TLC analysis under 366 nm

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