PHYTOCHEMICAL SCREENING, CHARACTERIZATION TESTS AND COMPONENT ANALYSIS OF FATTY ACID COMPOUNDS OF LERAK SEEDS (*SAPINDUS RARAK DC*)

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Abstract

Background: The lerak, also known as *Sapindus rarak DC*, is an industrial plant belonging to the Sapindaceae family, native to Southeast Asia and South Asia. Lerak is a type of plant in Indonesia that grows in lowland areas. Lerak plants are considered to be a highly suitable industrial plant for development. Based on the numerous benefits of lerak plants, it is believed that they contain secondary metabolites that are valuable for the advancement of research and the healthcare industry.
Objective: To determine phytochemical screening, characterization and fatty acid compounds of lerak seeds.
Methods: The method used in this study was experimental in the laboratory. Phytochemical screening tests alkaloids, glycosides, flavonoids, tannins, saponins, steroids, and triterpenoids. Analysis of fatty acid compounds using gas chromatography.
Results: The ethanol extract of lerak seeds was found to contain flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. The result of determining the water-soluble essence content of the lerak seed (*S. rarak*) simplicia was 5.03%, while the ethanol-soluble extract content was 12.19%. The lerak seeds used in this study contained the highest percentage of oleic acid, which was 70.5%, and eicosenoic acid, which was 27.4%.
Conclusion: Phytochemical screening lerak seed extract contains secondary metabolites of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. The highest fat content contained in lerak seed samples is oleic acid.

Keywords: *sapindus rarak DC*, secondary metabolites, characterization of simplicia, fat content, lerak seeds.

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1. Introduction

Medicinal plants are botanical species employed in the treatment of diverse human ailments due to their pharmacological effects. These plants contain bioactive compounds that exhibit significant therapeutic properties, offering potential remedies for a multitude of human diseases. According to reports, more than 50% of contemporary pharmaceuticals originate from natural sources.[1]

The lerak, also known as *Sapindus rarak DC*, is an industrial plant belonging to the Sapindaceae family, native to Southeast Asia and South Asia. This plant has a variety of different names in certain cities such as Werak/Lerak (Java), Rerek (Sunda), Kalikea (Jambi), soap fruit (Tapanuli) and lamuran (South Sumatra).[2] There are more than 40 wild species in the genus *Sapindus* (family Sapindaceae) such as *Sapindus mukorossi* or Chinese Soapberry (India, Southern China), *Sapindus saponaria* or Wingleaf Soapberry (Caribbean, Central America), *Sapindus marginatus* or Florida Soapberry (Florida) and *Sapindus drummondii* or Western Soapberry (Southern United States, Mexico).[3] Lerak is a forest plant with a trunk diameter of 1 m and an average height of 10 meters. However, it is known to grow as high as 42 meters. Between 450 and 1500 meters above sea level, this plant grows wild in Java; the stems are white, dirty, and rooted. This plant has unusual pinnate compound leaves with lance-shaped leaflets. The lerak flower has four petals, whitish yellow in color, and appears in clusters (racemes) which are linked at the base. The lerak fruit is brownish yellow, round in shape, and sturdy with a diameter of about 2 cm. Fruit seeds are round, hard, and black in color, and the surface of the fruit is smooth or shiny. The pulp of small apples is sticky and has a pleasant aroma. Lerak fruit has 27% seeds and 73% flesh.[2,4]

Figures 1. Tree of sapindus rarak DC

Saponins, which account for 28% of the chemical composition of the fruit, are composed of alkaloids, polyphenols, antioxidant chemicals, flavonoids, and tannins.[5] The Lerak plant contains saponins, alkaloids, steroids, antiquinones, flavonoids, polyphenols, and tannins in its fruit, bark, seeds, and leaves.[6,7] The high content of saponins in lerak plant is widely used by the community as a preparation for cleaning, such as additives used in hand washing soap, dish washing soap, and laundry soap.[8]

Lipids, often known as fats, are organic molecules that cannot be removed from cells and tissues by polar solvents such as ethanol and chloroform. Essentially, the building blocks of all lipids included fatty acids.[9] Fatty acids are long-chain organic acids, with carbon atoms ranging from 4 to 24. A single carboxyl group, a long nonpolar hydrocarbon tail, and other groups are all present in fatty acids. The

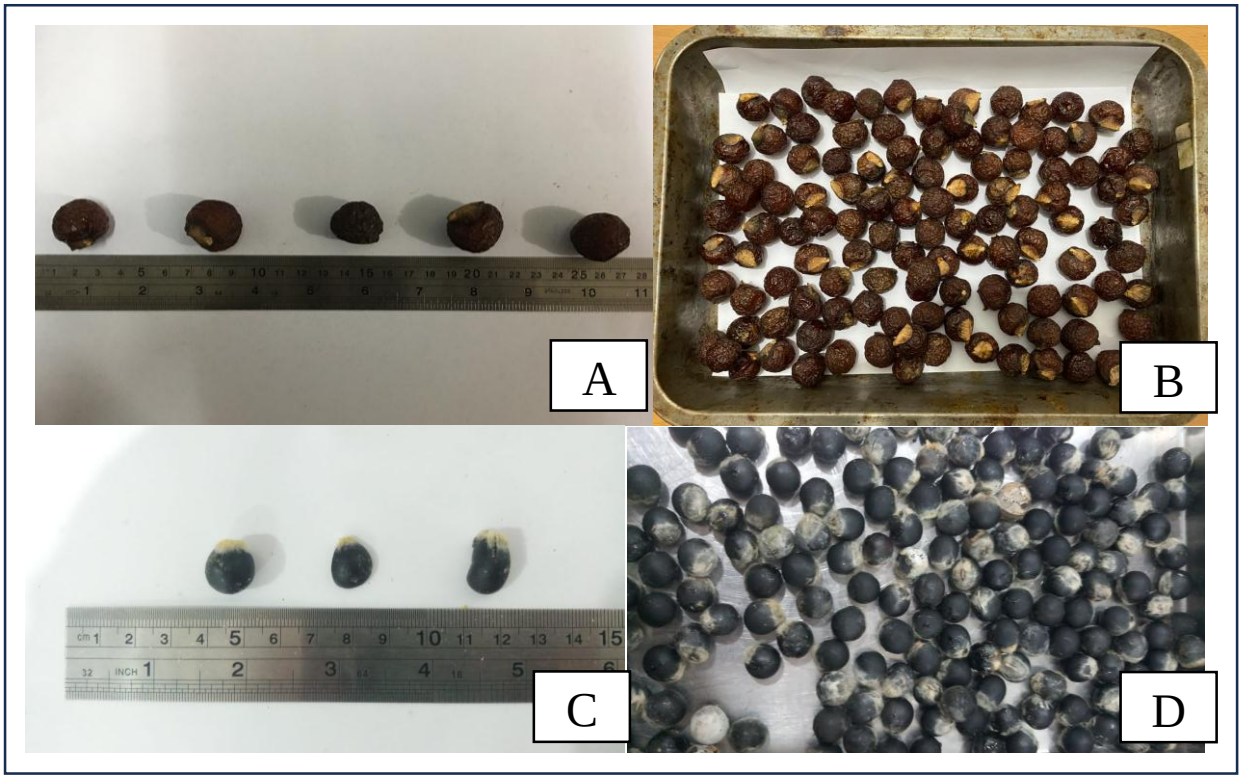
most sense is that lipids have an oily or fatty appearance yet are insoluble in water.[10]
Glycerol and three acids make up the fat in lerak seeds. Triglycerides are a type of fat, with roughly 70% of glycerides having no constituents. Specifically, oleodipalmitin (POP), oleodistearin (SOS), and oleopalmistearin (POS) are monounsaturated. In extremely small proportions, lerak fat also contains di-unsaturated triglycerides. Triglycerides are long-chain fatty acid esters of glycerols. It is tasteless, odorless, and colorless. Part a mixture of two or three distinct fatty acids makes up triglycerides. Triglycerides are hydrolyzed to produce three fatty acid molecules and one glycerol molecule.[11,12]

In this study, phytochemical screening tests and characterization tests of lerak seed simplicia were carried out to determine the quality of simplicia. Phytochemical identification tests carried out included testing for alkaloids, tannins, saponins, flavonoids, glycosides, steroids, and triterpenoids. The simplicia characterization tests carried out were the determination of water content, ethanol-soluble extrat, content acid-insoluble ash content, total ash content and water-soluble extract content, and the last test was the determination of the fat content contained in lerak seeds.

2. Methods

Materials

Lerak seeds were collected from lerak cultivation in Sriwedari village, Laweyan sub-district, Surakarta city, Central Java province, Indonesia. Taxonomic examination or plant identification of the lerak fruit was conducted at the Plant Systematics Laboratory, Herbarium Medanense (MEDA), Universitas Sumatera Utara, Medan, Indonesia. Screening and simplicia characterization tests were conducted at the Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. All other solvents and chemicals used were analytical grade, and they were procured from commercial sources and used as such without further treatment.



Figures 2. The *S. rarak* fruit has a diameter of approximately 3 cm (A,B). The seeds of *S.rara*k have a diameter of around 1.5 - 2 cm (C,D).

Preparation of Lerak Seed Ethanol Extract (*S. rarak*)

Lerak seed (*S. rarak*) simplicia weighing up to 1000 grams was soaked in 96% ethanol for five days using a ratio of 1 part simplicia to 10 parts solvent. It was then concentrated using a rotary evaporator at 40°C, filtered, and remacerated for two days using 25 parts of a 96% ethanol solvent.[13]

Phytochemical Screening

Phytochemical screening was carried out on lerak seed (*S. rarak*) simplicia including alkaloids examination, tannin examination, saponin examination, glycoside examination, flavonoida examination, steroid/triterpenoid examination.[14]

Identification of Alkaloids

Lerak seed (*S. rarak*) simplicia powder, 2N HCl solution and distilled water were combined, and the mixture was heated in a water bath before filtering and collecting the filtrate three times into a test tube. Mayer, Dragendroff, and Bouchardat were added to test tube one for each reactant. When a precipitate is observed in two out of the three reagents, it indicates a positive result for the presence of alkali.[15]

Identification of Saponnins

Lerak seed (*S. rarak*) simplicia powder was added to a test tube along with hot water, agitated rapidly for 10 seconds, and then allowed to froth for at least 10 minutes up to a height of 1 to 10 cm without losing any of its quality when 2N HCl solution was added.[16]

Identification of Tannins

The lerak seed (*S. rarak*) simplicia powder was macerated with distilled water for a duration of 15 minutes, followed by filtration. Subsequently, the filtrate was diluted until it became colorless, and two drops of 10% FeCl3 solution were added. The presence of blue and green hues in the filtrate indicated a positive result for the presence of tannins..[17]

Identification of Flavonoids

The lerak seed (*S. rarak*) simplicia powder was dissolved using hot water and subsequently cooked and immediately filtered. The filtrate was then mixed with magnesium powder, concentrated HCl solution, and millimeter alcohol. If the resulting amyl alcohol layer exhibited a red or orange-yellow coloration, it indicated the presence of flavonoids.[18]

Identification of Steroids/Triterpenoids

The simplicia powder of lerak seeds (*S. rarak*) was subjected to maceration using an N-hexane solution and subsequently filtered. The resulting filtrate was evaporated, and the remaining residue was treated with Liebermann-Burchard reagent by adding it through the cup wall. A color change from red to blue-green in the solution indicated a positive presence of triterpenoids or steroids.[19]

Identification of Glycoside

A small amount of concentrated H2SO4 was then gradually introduced via the test tube wall after the lerak seed (*S. rarak*) simplicia powder had been dissolved in ethanol solvent and subsequently subjected to evaporation in anhydrous acetic acid. The appearance of a blue or green tint in the filtrate indicates a positive presence of glycosides..[20]

Simplicia characterization test

Examination of lerak seed (*S. rarak*) simplicia characteristics involves conducting organoleptic, macroscopic, and microscopic examinations, as well as determining the water content, water-soluble extract, ethanol-soluble extract, total ash content, and acid-insoluble ash content.[21]

Determination of Total Ash Content

The silicate crucible was ignited and leveled before placing the sample in it. The charcoal was gently heated until it burned completely, then cooled and weighed. The phytate was introduced into the crucible, steamed, heated until a constant weight was achieved, and reweighed. The ash content of the air-dried material was determined by performing these steps and calculating the result.[22]

Determination of Acid Insoluble Ash Content

The ash collected for the determination of ash content was boiled in 25 ml of dilute hydrochloric acid for 5 minutes to dissolve soluble components. The insoluble portion was then carefully collected. The mixture was

subsequently filtered using either a slate glass crucible or ash-free filter paper. Afterward, it was washed with hot water, heated until a constant weight was achieved, and then weighed. The acid-insoluble ash content of the air-dried material was calculated based on these procedures.[22,23]

Determination of Water Soluble Extract Content

Lerak seed (*S. rarak*) simplicia weighing up to 4 g was macerated with 100 ml of water-chloroform in a stoppered flask, and the mixture was shaken continuously for the initial six hours. The maceration process lasted for a total of 24 hours, after which the mixture was allowed to stand undisturbed for 18 hours. Subsequently, the mixture was filtered. The filtrate was divided into 20 ml portions and added to a tareed evaporating cup. The filtrate was subjected to evaporation in a water bath until complete dryness was achieved. The residual material was subjected to heating at 105°C, followed by baking for an hour. Afterward, it was transferred to a desiccator for a duration of ten minutes and then weighed. The procedure is repeated until a steady weight is achieved. The initial weight of the extract is used to compute the proportion of chemicals that dissolve in water.[23]

Determination of Ethanol Soluble Content

In order to prevent the ethanol from evaporating, s the samples were macerated in a plugged flask with 100 ml of 95% ethanol for 24 hours. The flask was continuously shaken during the initial 6 hours and then allowed to rest undisturbed for the subsequent 18 hours. The filtrate was transferred in a volume of 20 ml to an evaporating cup that had been tareed and dried by evaporation in a water bath. In the oven, the residue was cooked to a constant weight at 105°C. After being placed in a desiccator for 10 minutes, it was weighed again. Up until a steady weight is achieved, repeat the procedure. In comparison to the initial extract weight, the percentage of chemicals dissolved in ethanol was computed.[7,21]

Determination of Moisture Content

A volume of approximately 200 ml of toluene was combined with 2 ml of distilled water within the flask. Subsequently, the mixture underwent distillation for a duration of two hours until complete evaporation of the water droplets occurred. After cooling toluene for 30 minutes, the water content of the moisture meter was measured to the closest 0.0 ml. The flask was then gently heated for 15 minutes while 5 grams of carefully weighed green round eggplant simplicia was added. As soon as the toluene reaches its boiling point, distillation commences at an initial rate of approximately 2 drops per second. This rate is maintained until the majority of the water content has been eliminated, at which point the distillation rate is increased to 4 drops per second. The interior of the cooler should be cleaned with a tube brush attached to a copper wire that has been dampened with toluene after all water has been distilled. distilling for an additional five minutes. The receiving tube should be allowed to cool to room temperature. If water droplets are stuck to the walls of the receiving tube, the tube is massaged with a rubber band tied to a copper wire and wet with toluene to make the drops fall. Read the water volume when toluene and water are completely separated. Determine the percentage of water content.[24]

Determination of fat content

The sample is put into a 250-mL closed Erlenmeyer flask, then added to 95% ethanol and 0.5% phenolphthalein (PP) indicator, then titrated with a 0.1 N NaOH standard solution until a pink color appears and does not disappear within 15 seconds.[23]

3. Result and Discussion

Phytochemical Screening

Phytochemical screening tests can be seen in table 1 below.

Table 1. Results of secondary metabolite testing of Lerak seeds

No	Secondary Metabolities	Reagent	Result
1.	Alkaloids	Dragendroff	+

		Bouchardat Meyer	+ +
2.	Flavonoids	Powdered Mg+ Amyl Alcohol + HCl	+
3.	Glycosides	Molish+H_2SO_4	+
4.	Saponnis	Hot water	+
5.	Tannis	$FeCl_3$	+
6.	Steroids/Triterpenoids	Lieberman-Bourchat	+

Based on qualitative phytochemical analysis, the ethanol extract of lerak seeds was found to contain flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. The phytochemical constituents identified are consistent with the findings of the study conducted by Artha et al. Additionally, this study discovered other active compounds, namely quinones, steroids, and triterpenoids. Each of these phytochemical compounds exhibits its own mechanism of action as antibacterial agents, as demonstrated in previous research.[25]

The analysis of secondary metabolites revealed the presence of a positive alkaloid group, demonstrated by the precipitation of a white precipitate in the Meyer reagent and a reddish brown precipitate in the Wagner and Dragendorff reagents.[15,25] If two or three reagents produced positive results, the alkaloids were considered positive. Positive results from an investigation utilizing FeCl3 on Lerak seed simplicia included a shift in hue to vivid green, red, purple, and black . The presence of saponin components in Lerak seed simplicia was evidenced by the formation of a persistent foam upon agitation with hot water and 2N HCl.[16]

Both were determined to include flavonoids, since the analysis of the flavonoids also yielded favorable results, with a noticeable color change occurring, especially a reddish black hue.[26] When the Lieberman-Bouchard reagent was used, the reaction resulted in a red-purple hue (indicating the presence of positive triterpenoids), as opposed to a green-blue color (indicating the presence of positive triterpenoids). Both showed successful outcomes with triterpenoids and steroids. The roles and advantages of each secondary metabolite found in plants vary, making them excellent for use as herbal and natural remedies with few adverse effects.[14,19]

Simplicia characterization test

The results of the characterization of the (*S. rarak*) simplicia powder, which included examination of the water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content, fulfilled these requirements, as shown in table 2.

Tabel 2. Simplicia characterization

No	**Parameter**	**Result (%)**	**Reference value (%)**
1	Total ash	2,34	≤ 10
2	Acid Insoluble Ash	0,64	$\leq 1,5$
3	Water Content	8,62	≤ 10
4	Water Soluble Content	5,03	≥ 5
5	Ethanol Soluble Content	12,91	$\leq 12,5$

Since the water content of simplicia affects the growth of mushrooms if it has a high moisture content, an examination of the simplicia's water content is performed to determine how much water is contained in the simplicia and to ensure the quality of the simplicia. The water content of lerak seed simplicia was 8.62%, which nevertheless satisfied the guidelines set forth in the 2013 Indonesian herbal pharmacopoeia, which called for a water content of less than 10%.[23] A moisture concentration of more than 10% will encourage microbial growth in simplicia, especially fungal growth, hastening simplicia's demise.[24]

The quantification of extract content in simplicia is carried out utilizing two solvents, specifically water and ethanol. This analytical procedure aims to ascertain the concentrations of polar chemical compounds present in simplicia using the water solvent, while the ethanol solvent is employed to determine the dissolved chemical compounds, encompassing both polar and non-polar compounds.[23] The result of determining the water-soluble essence content of the lerak seed (*S. rarak*) simplicia was 5.03%, while the ethanol-soluble extract content was 12.19%. This shows that lerak seed simplicia contains more non-polar compounds than polar compounds.

The determination of ash content aims to determine the physiological ash content (internal minerals) derived from plant tissues contained in the simplicia. The acid-insoluble ash content shows the amount of silicate, especially the sand present in the simplicia, by dissolving the total ash in hydrochloric acid.[27] Determination of the ash content of the simplicia showed a total ash content of 2.34% and an acid-insoluble ash content of 0.64%.

Fatty Acid Compound Components of Lerak Seeds (*S. rarak*)

The results of laboratory examination of the fatty acid composition of lerak seeds (*S. rarak*) are presented in table 3.

Tabel 3. Results of Lerak Seed Fatty Acid Composition Test (*S. rarak*)

Parameter	Unit	Result	Method
Fatty Acid Composition :			Chromatography Gas
Palmitat acid (C16:0)	%	6.0	
Palmitoleic acid (C16:1)	%	0.6	
Stearic Acid (C18:0)	%	0.4	
Oleic Acid (C18:1)	%	70.5	
Linoleic Acid (C18:2)	%	6.3	
Linolenic Acid (C18:3)	%	2.4	
Asarn Arachidat (C20:0)	%	11.6	
Eicosenoate Acid (C20:1)	%	27.4	

It was found that the lerak seeds used in this study contained the highest percentage of oleic acid, which was 70.5%, and eicosenoic acid, which was 27.4%. The results of this study are consistent with several previous studies. In an in vitro study, it was mentioned that the content of eicosenoic acid, linoleic acid, and oleic acid in soapnut seed extraction can inhibit the process of melanogenesis.[28] A study conducted by Huang et al. found that lerak seeds contained more than 58% oleic acid and more than 25% eicosenoic acid.[29] Another study conducted by Lin et al. found that the fatty acid composition of lerak seeds consisted of monounsaturated fatty acids, namely, oleic acid (>53%) and eicosenoic acid (>20%).[28] Monounsaturated fatty acids are reported to play an important role in inhibiting the transcription of the tyrosinase (TYR) enzyme.[30] A similar study conducted by Qian et al. found that monounsaturated fatty acids such as oleic acid, eicosenoic acid, and linoleic acid can degrade the TYR enzyme at the mRNA enzyme melanogenic level, thereby reducing the formation of the TYR, tyrosinase related protein 1 (TRP-1) , and tyrosinase related protein 2 (TRP-2) enzymes that are involved in the process of melanogenesis.[31]

4. Conclusion

Phytochemical screening lerak seed extract contains secondary metabolites of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. The highest fat content contained in lerak seed samples is oleic acid.

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Author Contribution

All authors have contributed to this research process, including preparation, data gathering, analysis, drafting,and approval to publish this manuscript.

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Ethical Approval
There is no ethical issue.

Conflict of Interest
The authors declare no conflict of interest regarding the publication of this article.

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