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ANTIBACTERIAL ACTIVITY AND POLYPHENOLS CONTENT OF METHANOLIC EXTRACT OF *SAPINDUS RARAK*

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ABSTRACT

The study focused on the methanolic extract of lerak fruit (Sapindus rarak DC.). The effect of varying extraction time and temperature on the polyphenols content of lerak extract was evaluated, that is by determining the total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC). The increasing extraction temperature from 30°C to 70°C was found to give increase in TPC from 172 mg GAE/100 mg to 246 mg GAE/100 mg, further increase of extraction temperature to 90°C results in the decrease of TPC. Similar effect was also observed in TFC, where TFC was found to increase as the extraction temperature was increased to 70°C, with TFC of 79 mg QE/100 mg. TSC of lerak fruit extract at 70°C was found to be 17.6 mg/100 g. Antibacterial activity assay on the lerak fruit extract shows the potential inhibitory activity of the extract on the growth of Escherichia coli and Staphylococcus aureus, wherein 95% inhibition efficiency can be achieved after the incubation of the bacteria in media containing 15 wt.% of the extract. The more prominent inhibitory effect of the extract was shown against Escherichia coli than Staphylococcus aureus. Collectively, the results of this study has demonstrated the potential of lerak fruit extract as natural antibacterial agent with foaming ability, which can be used as detergent additive.

ABSTRAK

Penelitian ini berfokus pada evaluasi terhadap ekstrak metanol buah lerak (Sapindus rarak DC.). Pengaruh variasi waktu dan suhu ekstraksi terhadap kandungan polifenol ekstrak lerak ditinjau, yaitu dengan menentukan kandungan fenolik total (TPC), kandungan flavonoid total (TFC), dan kandungan total saponin (TSC). Peningkatan suhu ekstraksi dari 30°C ke 70°C didapatkan memberikan peningkatan TPC dari 172 mg GAE/100 mg menjadi 246 mg GAE/100 mg, peningkatan lebih lanjut suhu ekstraksi hingga 90°C mengakibatkan penurunan TPC. Efek serupa juga diamati pada TFC, di mana TFC ditemukan meningkat seiring dengan peningkatan suhu ekstraksi hingga 70°C, dengan TFC sebesar 79 mg QE/100 mg. TSC ekstrak buah lerak pada suhu 70°C ditemukan 17,6 mg/100 g. Uji aktivitas antibakteri pada ekstrak buah lerak menunjukkan potensi aktivitas penghambatan dari ekstrak terhadap pertumbuhan Escherichia coli dan Staphylococcus aureus, dimana efisiensi penghambatan sebesar 95% dapat dicapai setelah inkubasi bakteri pada media yang mengandung 15% berat ekstrak. Efek penghambatan yang lebih menonjol dari ekstrak ditunjukkan terhadap Escherichia coli dibandingkan Staphylococcus aureus. Secara kolektif, hasil penelitian ini menunjukkan potensi ekstrak buah lerak sebagai zat antibakteri alami dengan kemampuan berbusa, yang dapat digunakan sebagai bahan tambahan deterjen.

Keywords: *Lerak; Sapindus; Saponin; Rarasaponin.*

I. Introduction

Lerak, *Sapindus rarak de candole* (*Sapindus rarak* DC) is a tall tree that can be easily found to grow in tropical countries, such as Indonesia and other Asian countries. lerak is a species of soapberry, it has oval-shaped fruit and the skin of the fruit is yellow-green when it is still unripe, and the color becomes brown to dark brown when the fruit is ripe (Figure 1). The fruits of lerak has hard texture and bitter taste, which make them inconsumable. The fruit pericarps of lerak exhibits foaming ability in water, owing to its high saponin content [1, 2] —And due to this foaming ability, the water extract of lerak fruit has been widely utilized to produce natural-based surfactant. Aglycone and triterpene type sapogenin has been identified to compose the saponins moiety in lerak fruit [2, 3].

Polyphenols are naturally occurring compounds in fruits, vegetables, and most of plant materials. Polyphenols are the umbrella term of a set of bioactive content in plants, that is including phenolics, flavonoids, and other

type of polyphenols. The polyphenols content in plants is widely studied owing to their positive health benefits, such as antioxidant activity [4, 5]. The pericarps of lerak fruits also containing some polyphenols, however, not much of the study evaluating the polyphenols of lerak. Most of the study is performed to assessing the saponin content of lerak. In this work, the polyphenols content of lerak fruit extract was evaluated. Furthermore, the effect of the polyphenols content of lerak fruit extract on its antibacterial activity was also studied.

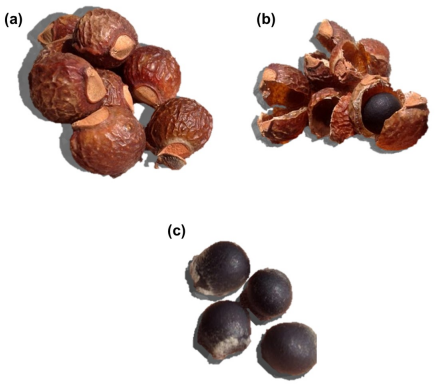

Figure 1. (a) Ripe lerak fruit. (b) Cut section of lerak fruit. (c) Seed of lerak fruit.

Methanol has been widely employed as the solvent for extracting saponin from lerak fruit. Methanol has been widely utilized as solvent for the extraction of bioactive compounds from many natural products [6]. Furthermore, the use of methanol has been reported to yielded highest saponin content [7]. Furthermore, methanol also is the most effective solvent to extract other secondary metabolites from plants, such as phenolic and flavonoid [5, 8]. Herein, methanol is chosen as the solvent for the extraction of lerak fruit. Then polyphenol content of the lerak fruit extract is evaluated by evaluating the total phenolic and total flavonoid content.

II. Research methodology

II.1. Chemicals

Methanol (99.5% purity, technical grade) was purchased from a local chemical distributor Nusakimia Surabaya (Surabaya, East Java, Indonesia). Mueller-Hinton Agar, gallic acid (≥98% purity), quercetin (≥ 95% purity), Folin-Ciocalteu's phenol reagent, $AlCl_3$ (98%, anhydrous) were purchased from Sigma-Aldrich, Singapore. All chemicals were directly used without further purification.

II.2. Plant material and extract preparation

Lerak fruits were obtained from a local strore in Surabaya, East Java, Indonesia. The fruits were oven-dried at 70°C until the moisture content is less than 10%. The dried fruits were crushed into small pieces and then extracted 1:10 (w/v) with technical grade methanol by means of maceration. To minimize solvent evaporation, Teflon-capped bottles were used as extraction vessels and the caps were tightly closed to avoid pressure buildup. Fresh solvent is added intermittently to make up evaporated solvent especially for extraction at high temperatures. The extraction was performed at varied temperature of 30, 50, 70, and 90°C and time. The obtained extract was collected and the solvent was evaporated by using rotary evaporator at 70°C until almost all solvent is being evaporated. The concentrated extract was then further dried by employing freeze-drying procedure using a FreeZone 2.5L Benchtop Freeze Dryer (Labconco, Kansas, MO), and the obtained powder was stored in -20°C refrigerator.

II.3. Polyphenols content evaluation

The phytochemical content of the extract was evaluated by determining the total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC). The TPC was determined by using Folin-Ciocalteu reagent and the result is expressed as the gallic acid equivalent (GAE) [9]. TFC was determined by using $AlCl_3$ reagent, and the result is expressed as quercetin equivalent (QE) [10]. TSC was determined by means of spectrophotometric procedure using a Genesys 150 UV-Vis spectrophotometer (Thermo Fisher Inc. Indonesia), the saponin groups were detected by UV-light at a wavelength 277 nm, and pure saponin was used to prepare the calibration curve [1].

II.4. Antibacterial activity assays

II.4.1. Determination of growth inhibition efficiency

Escherichia coli and *Staphylococcus aureus* microorganism were used to evaluate the antibacterial activity of the extract. To evaluate the effect of extract concentration on the growth inhibition of the bacteria, extract powder was dissolved in sterile nutrient broth at various concentration. Subsequently, 100 µL of bacteria suspension was added into 3 mL broth containing extract at various concentration of 1, 5, 10, and 15 %wt., and then the mixture was incubated at 37°C incubator for 24 h. The concentration of the bacteria was calculated by determining the optical density measured using spectrophotometer at a wavelength of 600 nm. The inhibition efficiency was then calculated according to equation (1).

$$Inhibition\ eff.\ (\%) = [(A_0 - A_N)/A_0] \times 100 \quad (1)$$

where, A_0 is the absorbance at 600 nm for bacteria suspension without extract addition; and, A_N is the absorbance at 600 nm for bacteria

suspension with addition of extract at certain concentration.

II.4.2. Disk diffusion assay

Antibacterial activity tests were also carried out using a disc diffusion procedure on Mueller-Hinton Agar (MHA) plates. The bacterial suspension that has been prepared in nutrient broth is spread homogeneously on MHA using sterile cotton. Prior to this test, the extract powder was dissolved in sterile water at various concentrations. Then the sterile disk was dipped into the prepared extract solution and placed on the MHA surface. The zone of inhibition (ZOI) formed after 24 h incubation of MHA plates was evaluated.

III. Results and discussions

III.1. Total phenolic content (TPC) and total flavonoid content (TFC)

Figure 2 shows the effect of extraction time and temperature on the TPC of methanolic extract of Lerak fruit. The increase of temperature from 30°C to 70°C results in the increase of TPC extracted from lerak fruit, that is from 172 mg GAE/100 g to 246 mg GAE/100 g (at 480 min extraction time). However further temperature increase to 90°C lead to the significant decrease of TPC, the TPC content is found to be 130 mg GAE/100 g after 480 min extraction. The increase of extraction temperature from 30°C to 70°C facilitating the breakdown of lignin content in the plant tissue, and therefore, the bound phenolic compounds can be released and the amount of phenolic that can be extracted also increasing. Meanwhile, at 90°C thermal degradation of the phenolic compounds may occur. Furthermore, the decrease of TPC content also can be observed at longest extraction time of 480 min, especially for the extraction performed at high temperature of 70°C and 90°C. The main reason of the TPC decrease is because of the thermal degradation and oxidation process, which are more prominent at higher temperature [4].

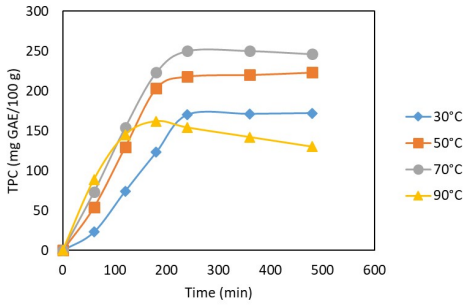

Figure 2. Effect of extraction temperature and time on the total phenolic content (TPC) of lerak fruit extract.

Figure 3 shows the TFC of lerak fruit extracted at different temperature and varied time. Similarly, the TFC of ethanolic extract of lerak fruit was found to increase with increasing temperature from 30°C to 70°C (51 mg QE/100 g to 79 mg QE/100 g, at extraction time of 480 min), and significantly decreased as the extraction temperature increases to 90°C (40 mg QE/100 g). The decrease of TFC is obviously occurred due to the thermal degradation of the compounds, as flavonoids are more sensitive to heat and the degradation of the compounds can be occurred even at low temperature [11].

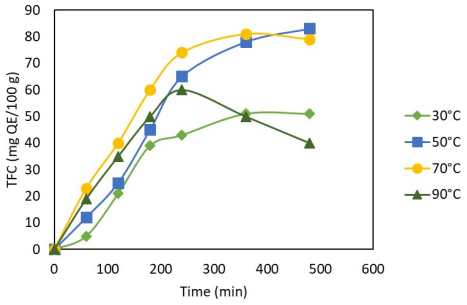

Figure 3. Effect of extraction temperature and time on the total flavonoid content (TFC) of lerak fruit extract.

Based on the results on TPC and TFC content, it can be concluded that extraction time of 240 min is the most suitable condition to obtain extract with optimum TPC and TFC. Prolonged extraction time does not give significant increase in TPC and TFC.

III.2. Total saponin content (TSC)

Figure 4a shows the effect of extraction temperature on the TSC of lerak fruit extract. The highest TSC of 22 mg/100 g is obtained at 30°C. At higher temperature, lower TSC was obtained, that is 18 mg/100 g, 17.6 mg/100 g, and 11 mg/100 g, at 50°C, 70°C, and 90°C, respectively. This results suggest that saponin is a heat sensitive compound, and the high temperature can lead to its degradation. As reported in study by Aryanti et al. [1], the bioactive compounds extracted from plants were found to be susceptible to heat degradation.

Figure 4b shows the variation in TSC in lerak extract at constant temperature of 70°C. It can be noted that the highest TSC yield is obtained at the time of 135 min. The prolonged extraction time does not result in the increase of the TSC.

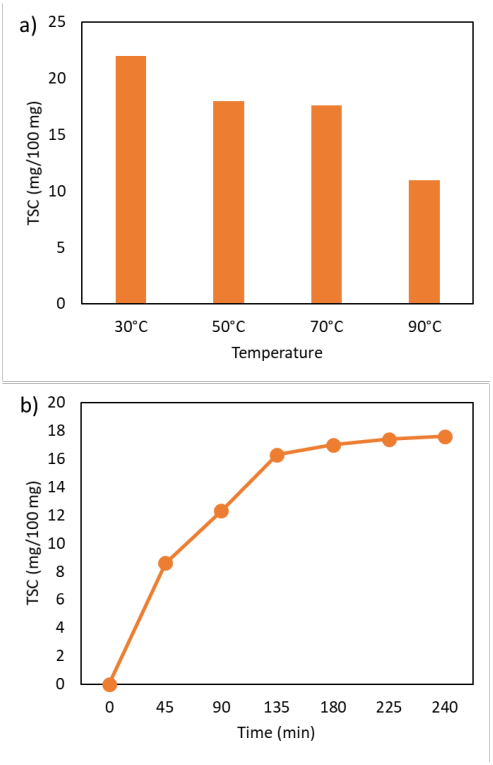

Figure 4. (a) Total saponin content (TSC) of Lerak fruit after 240 min extraction at different temperature. **(b)** TSC of Lerak fruit extracted at different time and constant temperature of 70°C.

III.3. Antibacterial activity assay

The antibacterial activity of the lerak fruit extract was evaluated against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The extract of lerak fruit obtained from extraction at 70°C for 240 min was collected and used for this antibacterial activity assay. As shown in **Table 1**, it can be noted that the extract of lerak fruit show inhibitory effect on the growth of *E. coli* and *S. aureus*. High inhibitory effect of 95% against *E. coli* and *S. aureus* is achieved after incubation of the bacteria with 15 wt.% of extract.

Table 1. Inhibition efficiency of lerak fruit extract on the bacteria growth

Extract conc. (%wt)	Inhibition efficiency (%)	
	E. coli	***S. aureus***
0 (control)	0	0
1	5	7
5	32	31
10	56	51
15	95	95

Table 2 shows the ZOI of lerak fruit against the colony of *E. coli* and *S. aureus*. It can be noted that the lerak extract shows more prominent inhibitory effect on the growth of *E. coli* than *S. aureus*.

Table 2. Zone of inhibition (ZOI) of lerak fruit extract

Extract conc. (%wt)	ZOI (mm)	
	E. coli	***S. aureus***
0 (control)	0	0
1	2	3
5	3	3
10	23	5
15	25	10

This result shows the efficacy of the lerak extract in inhibiting the bacteria growth. As mentioned in a study by Wei et al. [12], saponin content of *Sapindus* is possessing a broad-spectrum antibacterial effect. The antibacterial action of *Sapindus* saponin works by targeting the cell membrane proteins.

V. Conclusions

This study showed the effect of different extraction time and temperature on the total phenolic, total flavonoid, and total saponin content from the methanolic extract of lerak fruit (*Sapindus rarak* DC.). The highest phenolic and flavonoid yield can be obtained by increasing the extraction temperature, however too high extraction temperature may lead to the degradation of the compounds. Prolonged extraction time does not result in significant increase of phenolic and flavonoid content. The total saponin yield is found to be obtained at lower temperature. The antibacterial activity assays show that the lerak fruit extract has inhibitory activity against the investigated bacteria, thus lerak fruit extract can be utilized as natural antibacterial agent.

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