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**Original Article**

# Effect of Ethanol Solvent Concentration on the Characteristics and Antioxidant Activity of *Salak Madu* (*Salacca edulis* Reinw.) Peel Extract

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## Abstract

Excessive free radical exposure can cause cellular damage and trigger diseases such as cancer. Antioxidants are needed to neutralize these effects, with natural sources being a safer alternative to synthetic ones. The peel of salak madu (*Salacca edulis* Reinw.) contains secondary metabolites like flavonoids, saponins, alkaloids, and tannins. This study aimed to evaluate the effect of different ethanol solvent concentrations on the extract characteristics and antioxidant activity of *S. edulis* peel using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A laboratory-based experimental design was employed, combining qualitative and quantitative approaches. The extract obtained with 70% ethanol showed the highest total phenolic content (1.941%) and flavonoid content (0.314%). It also demonstrated the strongest antioxidant activity with an $IC_{50}$ value of 182.070 ppm (moderate category), compared to 220.921 ppm (96% ethanol) and 284.304 ppm (50% ethanol), both in the very weak category. Statistical analysis confirmed significant differences in total phenolic content, total flavonoid content, and antioxidant activity across solvent variations. In conclusion, ethanol concentration significantly influences the bioactive compound content and antioxidant activity of *S. edulis* peel extract.

## Keywords

Antioxidant, DPPH, *Salacca edulis* Reinw., Solvent concentration.

## 1. Introduction

Free radicals are unstable molecules that have one or more unpaired electrons. The presence of excessive free radicals can cause oxidative stress, which triggers cell damage and contributes to various degenerative diseases such as cancer, heart disease, and diabetes (Pratiwi et al., 2023). The body does produce natural antioxidants, but the amount is limited, so it is necessary to obtain antioxidants from external sources (Santi et al., 2021).

Antioxidants stabilize free radicals by donating electrons to them, thereby stopping the oxidative chain reaction that destroys body cells (Pratiwi et al., 2023). Natural antioxidants are preferred because they are safer than synthetic antioxidants, which have the potential to cause toxic effects (Chopipah et al., 2021). Plants are a rich source of natural antioxidants, one of which is *salak madu (Salacca edulis Reinw.),* a local Indonesian variety that contains flavonoids, tannins, and other phenolic compounds, especially in the skin of the fruit (Ginting et al., 2019) (Saputri et al., 2024).

The effectiveness of antioxidant compound extraction

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is influenced by the type and concentration of the solvent. Ethanol is commonly used because it can dissolve various polar and semi-polar compounds (Fatah et al., 2024). Based on literature search, this is the first study to explore the antioxidant capacity of *S. edulis* fruit peel. This study aims to determine the effect of varying concentrations of ethanol solvent (96%, 70%, and 50%) on the characteristics of *S. edulis* fruit peel extract and its antioxidant activity using the DPPH method.

## 2. Methods

### 2.1. Materials

The materials required for this study are *Salacca edulis* Reinw fruit peel (*Salacca edulis* Reinw), ethanol, DPPH (2,2-diphenyl-1-picrylhydrazil), distilled water, chloroform, dilute hydrochloric acid, $CHCl_3$, Dragendorff reagent, magnesium (Mg), concentrated HCl, anhydrous $CH_3COOH$, concentrated $H_2SO_4$, Bouchardat reagent, HCl, NaCl, $FeCl_3$, methanol, ethanol, Folin-Ciocalteu, NaOH, ascorbic acid, aluminum chloride, and sodium acetate.

### 2.2. Procedures

The fruit peel of *Salacca edulis* Reinw was obtained in Saribuana Village and identified at the Department of Pharmaceutical Biology, Gadjah Mada University. The peels were extracted using the maceration method with 50%, 70%, and 96% ethanol solvents. After obtaining the extract, the yield was calculated and organoleptic tests were conducted. Phytochemical screening included alkaloid testing with Dragendrof, flavonoid testing with concentrated HCl and Mg, steroid testing with anhydrous acetic acid and concentrated sulfuric acid, terpenoid testing with Bouchardat reagent, saponin testing with a vigorously shaken solution followed by addition of hydrochloric acid, and tannin testing with $FeCl_3$. The testing of total phenol and total flavonoid content refers to the Indonesian Herbal Pharmacopoeia, 2nd Edition, 2017. A 0.4 mM DPPH solution was prepared using 15.8 mg of DPPH powder in 100 ml of methanol (Eko Murwanto & Santosa, 2015). Antioxidant activity was measured at concentrations of 100, 150, 200, 250, and 300 ppm.

The analysis included determining the total phenol content, total flavonoids, and $IC_{50}$ value through linear regression. The data were then analyzed descriptively and tested for normality (Shapiro-Wilk), homogeneity (Levene's test), and comparability using One Way ANOVA and Least Significant Different (LSD) follow-up test with a significance level of $p<0.05$.

## 3. Results and Discussion

The results of the determination showed that the fruit peel used was of the species *Salacca edulis* Reinw which has the synonyms *Salacca zalacca* (Gaertn.) Voss and *Salacca rumphii* Wall, belonging to the Arecaceae family. The analysis of the extract characteristics was carried out through yield measurements and organoleptic observations, such as color, odor, taste, and texture, to determine the effect of solvent concentration variations on the quality of the *S. edulis* fruit peel extract produced. Table 1 shows the characteristics of ethanol extract *S. edulis* fruit peel. From Table 1, all three concentrations of *S. edulis* fruit peel extract showed similar aromas, namely the distinctive aroma of *S. edulis* peel with a thick texture and a bitter taste. Extracts obtained using 70% and 50% ethanol have the same physical characteristics, namely a dark brown color. Meanwhile, extracts produced using 96% ethanol show a difference, namely a yellowish brown colour.

**Table 1.** Characteristics of ethanol extract *S. edulis* fruit peel

| Solvent | Organoleptic | | | | Yield (%) |
|---|---|---|---|---|---|
| Concentration (%) | Colour | Aroma | Taste | Texture | |
| 50 | Dark chocolate | Distinctive smell | Bitter | Thick | 12.57 |
| 70 | Dark chocolate | Distinctive smell | Bitter | Thick | 11.14 |
| 96 | Yellowish brown | Distinctive smell | Bitter | Thick | 2.98 |

The variation in color may be influenced by the different polarity levels of the solvent concentrations used (Kristiani & Susanti, 2023). This condition allows for the extraction of polar compounds, such as tannins, in greater quantities, resulting in a darker-colored filtrate (Fauziyah et al., 2022). The percentage yield obtained shows that the extract with 50% ethanol solvent produced the highest yield of 12.57%, followed by 70% ethanol at 11.14%, and 96% ethanol with the lowest yield of 2.98%. This difference may be attributed to the use of varying concentrations of ethanol in each solution. The solubility of a compound is fundamentally influenced by the polarity of the solvent it is placed in. Generally, compounds tend to dissolve most readily in solvents that possess a similar polarity to their own. Therefore, as the ethanol concentration changes, the solvent's overall polarity shifts, affecting how well the compound can dissolve (Fatah et al., 2024). The higher yield observed in the 50% ethanol extract can be attributed to the principle of "like dissolves like" in chemistry. With a polarity level higher than that of pure ethanol or pure water alone, this specific solvent mixture is exceptionally effective at extracting a broad range of compounds (Susiloningrum & Mugita Sari, 2021). It readily dissolves polar substances such as proteins, carbohydrates, and various polysaccharides that are abundant in the plant material.

Consequently, this enhanced solubility allows for the extraction of a greater total mass of soluble components, leading to a significantly higher overall yield (Padmawati et al., 2020).

**Table 2.** Phytochemical screening of ethanol extracts from *S. edulis* fruit peels

| Solvent Concentration (%) | Alkaloids | Flavonoids | Steroids | Terpenoids | Saponins | Tannins |
|---|---|---|---|---|---|---|
| 50 | + | + | - | - | + | + |
| 70 | + | + | - | - | + | + |
| 96 | + | + | - | - | + | + |

Preliminary phytochemical screening of the ethanol extract obtained from S. edulis fruit peel revealed the presence of several bioactive secondary metabolites, including alkaloids, flavonoids, saponins, and tannins. The presence of alkaloids was specifically confirmed by a positive reaction with Dragendorff's reagent, which resulted in the immediate formation of a characteristic brick-red precipitate (Fajrin & Susila, 2019). Flavonoid compounds are detected through a color change to reddish brown after the addition of concentrated HCl and magnesium powder, which hydrolyzes flavonoids into aglycones (Fadhila & Etika, 2023). Saponins are identified by the formation of stable foam when the solution is shaken, due to the interaction of hydrophilic and hydrophobic groups in the saponin structure (Elfira et al., 2024). The tannin test yielded a positive result, which was clearly indicated by the appearance of a distinctive blackish-green coloration immediately following the addition of $FeCl_3$, a reaction that occurs due to the formation of a stable coordination complex between the tannin compounds and the $Fe^{3+}$ ions (Harahap et al., 2024).

**Table 3.** The TFC, TPC, and $IC_{50}$ values of ethanol extracts from *S. edulis* fruit peels

| Solvent Concentration (%) | TPC (%) | TFC (%) | $IC_{50}$ (ppm) |
|---|---|---|---|
| 50 | 0.849 ± 0.0291 | 0.131 ± 0.008 | 284.304 ± 4.241 |
| 70 | 1.941 ± 0.0554 | 0.314 ± 0.009 | 182.070 ± 10.624 |
| 96 | 1.198 ± 0.035 | 0.230 ± 0.012 | 220.921 ± 3.767 |

TPC = Total Phenolic Content, TPC = Total flavonoid content, IC = *Inhibition Concentration*

Table 3 shows that the total phenol content in the *S. edulis* fruit peel extract varies, namely 0.849% in the 50% ethanol extract, 1.941% in the 70% ethanol extract, and 1.198% in the 96% ethanol extract. This is supported by statistical tests showing significant differences between solvent concentrations and total phenolic content. The results indicate that using 70% ethanol solvent on *S. edulis* fruit peel yields the highest total phenolic content compared to other solvent concentrations. The total phenolic content in an extract is greatly influenced by the polarity of the solvent used during the extraction process (Indra et al., 2019). This finding is in line with research conducted by (Ladeska et al., 2020) which shows that the highest total phenol content in ceguk leaves was obtained using 70% ethanol solvent. This is due to the polarity characteristics of the solvent, whereby the higher the ethanol concentration, the lower the polarity.

The test results showed that 70% ethanol extract had the highest total flavonoid content, which was 0.314%, followed by 96% ethanol at 0.230% and 50% ethanol at 0.131%. This was supported by statistical tests that showed a significant difference between solvent concentrations and total flavonoid content. Research by (Riwanti et al., 2016) shows that *Sargassum polycystum* extract with 70% ethanol solvent has a higher total flavonoid content than 96% or 50% ethanol. This difference is thought to be related to the polarity of the solvent, where the highest flavonoid content is obtained in solvents with moderate polarity (Azizah et al., 2014). 70% ethanol has a higher polarity than 96% ethanol, but lower than 50% ethanol, so polar flavonoid compounds tend to dissolve more easily in 70% ethanol. Variations in ethanol concentration as a solvent affect the polarity of the solvent (Riwanti et al., 2016).

Based on the results of the study, *S. edulis* fruit peel extract with 70% ethanol solvent showed higher antioxidant activity (weak category) compared to 50% and 96% ethanol solvents (very weak category). This difference was supported by statistical tests that showed a significant difference between solvent concentrations and antioxidant activity. These findings are in line with research by (Noviyanti, 2016) who tested the antioxidant activity of Brazilian guava leaves (*Psidium guineense* L.) using 96%, 70%, and 50% ethanol solvents. In that study, the highest antioxidant activity was also obtained in the 70% ethanol extract. This antioxidant activity is related to the secondary metabolites successfully extracted during the extraction process. The differences in the content of secondary metabolites extracted during the extraction process in the three extracts are due to the differences in polarity of each solvent (Moh, 2024). The higher the phenol and flavonoid content, the more influential it is on the $IC_{50}$ value produced (Zuraida et al., 2017). Flavonoids, phenols, and tannins are all part of a larger family known as phenolic compounds, which are defined by a

specific chemical structure where a hydroxyl group (–OH) is attached directly to a carbon atom within an aromatic ring. It is precisely the presence of these hydroxyl groups that enables the compounds to act as antioxidants by readily donating hydrogen atoms to unstable molecules called free radicals. This ability is commonly demonstrated using the DPPH assay, where the deep purple DPPH radical accepts a hydrogen atom from the phenolic compound. This process, known as reduction, neutralizes the unstable DPPH radical and converts it into a more stable, non-radical form, which simultaneously causes the solution to turn pale yellow. (Marbun & Restuati, 2016).

## 4. Conclusions

The highest total phenolic and total flavonoid content were obtained in the 70% ethanol extract. Statistical analysis showed significant differences between the three extract concentrations in terms of total phenol and total flavonoid content. *S. edulis* fruit peel extracts with varying ethanol solvent concentrations of 96%, 70%, and 50% exhibit different antioxidant activities. This is supported by statistical test results showing significant differences between ethanol solvent concentrations and antioxidant activity.

## Supplementary Material

No supplementary materials are associated with this manuscript.

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## Author Contributions

**Ni Kadek Ilda Sugiantari**: conceptualization, data curation resources, and writing – original draft; **I Gusti Ayu Rai Widowati**: methodology, formal analysis, investigation, validation, and supervision; **I Gusti Ngurah Agung Windra Wartana Putra:** software, formal analysis, writing – review & editing, and supervision.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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