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THE INFLUENCE OF MACERATION TIME AND ETHANOL VOLUME ON TOTAL FLAVONOID CONTENT OF *Luffa cylindrica* FRUIT EXTRACT

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ABSTRACT

Luffa cylindrica is a potential source of bioactive compounds, particularly flavonoids, which are known for their antioxidant and health-promoting properties. However, information regarding optimal extraction conditions for maximizing flavonoid yield from *L. cylindrica* fruit remains limited. Therefore, this study aimed to evaluate the effect of solvent volume and maceration time on the total flavonoid content of L. cylindrica fruit extract. Extraction was performed using the maceration method with 70% ethanol at solvent volumes of 750, 1000, and 1250 mL, and maceration times of 3, 5, and 7 days. Total flavonoid content was determined quantitatively using the aluminum chloride colorimetric method and measured by UV–Visible spectrophotometry, with quercetin used as the standard. Flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g). The results showed that both solvent volume and maceration time significantly affected total flavonoid content ($p < 0.05$). Flavonoid levels ranged from 1.12 to 3.15 mg QE/g, with a clear increasing pattern observed as solvent volume and maceration time increased. The highest flavonoid content (3.15 mg QE/g) was obtained using a solvent volume of 1250 mL and a maceration time of 7 days. In conclusion, increasing solvent volume and extending maceration time effectively enhanced flavonoid extraction from *L. cylindrica* fruit. The maceration method using 70% ethanol is a simple, effective, and economical approach for flavonoid extraction from this plant.

Keywords: *Luffa cylindrical*, Flavonoids, Maceration, Ethanol, Spectrophotometry UV- Vis

ABSTRAK

Luffa cylindrica merupakan sumber potensial senyawa bioaktif, khususnya flavonoid, yang dikenal memiliki aktivitas antioksidan dan manfaat bagi kesehatan. Namun, informasi mengenai kondisi ekstraksi yang optimal untuk memaksimalkan rendemen flavonoid dari buah *L. cylindrica* masih terbatas. Oleh karena itu, penelitian ini bertujuan untuk mengevaluasi pengaruh volume pelarut dan waktu maserasi terhadap kadar flavonoid total ekstrak buah *L. cylindrica*. Ekstraksi dilakukan menggunakan metode maserasi dengan etanol 70% pada variasi volume pelarut 750, 1000, dan 1250 mL serta waktu maserasi 3, 5, dan 7 hari. Kadar flavonoid total ditentukan secara kuantitatif menggunakan metode kolorimetri aluminium klorida dan diukur dengan spektrofotometri UV–Visibel, dengan kuersetin sebagai standar. Kadar flavonoid dinyatakan sebagai miligram ekuivalen kuersetin per gram ekstrak (mg QE/g). Hasil penelitian menunjukkan bahwa volume pelarut dan waktu maserasi berpengaruh signifikan terhadap kadar flavonoid total ($p <$

0,05). Kadar flavonoid berkisar antara 1,12 hingga 3,15 mg QE/g, dengan pola peningkatan yang jelas seiring bertambahnya volume pelarut dan lamanya waktu maserasi. Kadar flavonoid tertinggi (3,15 mg QE/g) diperoleh pada volume pelarut 1250 mL dan waktu maserasi 7 hari. Dengan demikian, peningkatan volume pelarut dan perpanjangan waktu maserasi terbukti efektif dalam meningkatkan ekstraksi flavonoid dari buah *L. cylindrica*. Metode maserasi menggunakan etanol 70% merupakan metode yang sederhana, efektif, dan ekonomis untuk ekstraksi flavonoid dari tanaman ini.

Kata kunci: *Luffa cylindrica*, Flavonoid, Maserasi, Etanol, Spektrofotometri UV–Vis

INTRODUCTION

Luffa is a climbing plant belonging to the Cucurbitaceae family and is widely recognized as a potential source of herbal medicinal ingredients. This plant has a broad geographical distribution and is cultivated in various regions of the world due to its adaptability to diverse environmental conditions. Although it is generally grown in tropical and subtropical climates, Luffa species have been reported in countries such as India, Southeast Asia, China, Japan, and several regions in Africa (PV & Shenoy, 2024; Swamy, 2023; Shendge & Belemkar, 2018).

One of the most widely cultivated species is Luffa cylindrica, which has long been grown as a garden plant and occurs naturally in many tropical regions. This species is known to possess diverse nutritional properties that are beneficial for human health. Proximate analysis has shown that *L. cylindrica* seeds contain high levels of protein (33.55–35.83%) and carbohydrates (13.67–29.51%), which play an important role as sources of energy. In addition, young fruits of *L. cylindrica* are reported to be rich in vitamins A and C, which function as natural antioxidants and support immune system health. Essential minerals such as calcium, magnesium, and iron have also been identified, contributing to various physiological functions of the human body (Ogunyemi et al., 2020; Nagar et al., 2024; Azeez et al., 2013).

Beyond its nutritional value, *L. cylindrica* contains various bioactive compounds that contribute to its functional properties. The fruit has been reported to contain carotenoids and flavonoids, which are widely associated with antioxidant activity and cellular protection (Folake Lucy & Babajide Abidemi, 2012). From an amino acid profile perspective, *L. cylindrica* seeds are rich in essential amino acids such as arginine, as well as non-essential amino acids including alanine, glycine, cystine, glutamic acid, hydroxyproline, leucine, and serine (Onigemo et al., 2020; Folake Lucy & Babajide Abidemi, 2012). Furthermore, oil extracted from the seeds contains saturated fatty acids such as palmitic, stearic, and myristic acids, along with a high proportion of polyunsaturated linoleic acid, which dominates the lipid profile (Shadrach et al., 2023). These characteristics highlight the potential of *L. cylindrica* as a raw material for functional food and nutraceutical development.

Flavonoids are a major class of bioactive compounds that play an important role in protecting the body from oxidative damage caused by free radicals. Their antioxidant activity contributes to the prevention of blood cell aggregation, stimulation of nitric oxide production for vasodilation, and inhibition of cancer cell proliferation. The primary mechanism of flavonoids involves scavenging free radicals that can damage cellular components (Xi et al., 2022). However, flavonoids are known to be thermolabile compounds that are susceptible to degradation when exposed to high temperatures, making extraction conditions a critical factor in preserving their bioactivity.

Previous studies have emphasized the importance of optimizing extraction parameters such as solvent concentration, extraction time, temperature, and solid-to-solvent ratio to obtain maximum flavonoid yield. The

use of 70% ethanol has been widely recommended due to its effectiveness in extracting polar and semi-polar compounds, while mild extraction conditions help prevent flavonoid degradation (Amini et al., 2024). Cold extraction techniques such as maceration are therefore commonly applied, as they are simple, cost-effective, and suitable for thermosensitive compounds (Azwanida, 2015; Chen et al., 2024; Adeyeni & State, 2023; Reninta et al., 2022).

Although several studies have reported the presence of flavonoids in *L. cylindrica*, most of the available literature focuses on qualitative phytochemical screening or general nutritional profiling. Quantitative determination of total flavonoid content, particularly in relation to extraction optimization, has received limited attention. In addition, studies that specifically examine the combined effect of maceration time and solvent volume on flavonoid yield in *L. cylindrica* fruit are still scarce. Most extraction optimization studies have been conducted on other plant species, resulting in a lack of specific and consistent data for *L. cylindrica*. Consequently, the optimal extraction conditions required to maximize flavonoid content in *L. cylindrica* fruit remain unclear.

Therefore, this study aims to quantitatively evaluate the effect of maceration time and ethanol solvent volume on total flavonoid content in Luffa cylindrica fruit extract using the maceration method. Flavonoid levels were determined using UV–Vis spectrophotometry to identify the optimal extraction conditions that can improve extraction efficiency and support the utilization of L. cylindrica as a source of natural bioactive compounds.

METHODOLOGY

Tools and Materials

This study employed an experimental research design involving extraction of *Luffa cylindrica* fruit followed by quantitative determination of total flavonoid content.

The instruments used included an analytical balance (Ohaus EP 214), glassware (Iwaki), containers, flannel cloth, filter paper, drying oven (Memmert), blender (National), 40-mesh sieve, rotary evaporator (IKA HB 10 Basic), water bath, micropipette, measuring pipette, chamber, and a UV–Vis–NIR spectrophotometer (Shimadzu UV-3600 Plus), as shown in Figure 1.

Picture 1. Spectrophotometry UV-Vis-3600 plus s-Shimadzu

The materials used in this study consisted of Luffa cylindrica fruit (Figure 2), ethanol 96% (pro-analysis), sulfuric acid (H_2SO_4, pro-analysis), sodium hydroxide (NaOH, pro-analysis), and distilled water.

Picture 2 Fruit *Luffa cylindrical*

Fresh *Luffa cylindrica* fruit was washed with running water, sliced into small pieces, and dried using a hot air oven at 50 ± 2 °C for 48 hours until a constant weight was obtained. The dried material was then ground using a blender and sieved through a 40-mesh sieve to obtain uniform simplicia powder. The final moisture content of the dried simplicia was below 10%, which is considered acceptable for phytochemical extraction to prevent

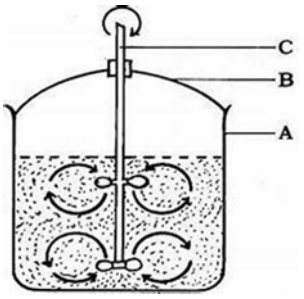
microbial growth and degradation of bioactive compounds. The dried simplicia was stored in an airtight container at room temperature prior to extraction.

Maceration Extraction

The extraction process was carried out using 70% (v/v) ethanol as the solvent, selected due to its effectiveness in dissolving polar and semi-polar compounds, including flavonoids, as well as its relative safety and environmental friendliness compared to other organic solvents (Azwanida, 2015).

Extraction of Luffa cylindrica fruit was performed using the maceration method with variations in solid-to-solvent ratios of 1:7.5, 1:10, and 1:12.5 (w/v). A total of 100 g of dried simplicia powder was macerated separately in 750 mL, 1000 mL, and 1250 mL of 70% ethanol. The maceration process was conducted for 3, 5, and 7 days at room temperature in a closed container, as illustrated in Figure 3.

During maceration, the mixture was stirred periodically to enhance mass transfer and extraction efficiency. After completion of the maceration period, the extract was filtered using filter paper to separate the filtrate from the plant residue.



Picture 3. Reactor Maceration: (a) Maceration vessel, (b) Closed vessel, (c) Mixer

Solvent Evaporation

The filtrate obtained from maceration was transferred into a beaker and concentrated by evaporating the solvent using a water bath at 60 °C for approximately 3 hours until a thick extract was formed and the ethanol odor was no longer detected. This step aimed to remove residual solvent that could interfere with further analysis.

After evaporation, the thick extract was weighed to determine extraction yield. To confirm the absence of residual ethanol, a qualitative test was conducted by adding two drops of the extract into a test tube, followed by the addition of two drops of concentrated sulfuric acid (H_2SO_4) and acetic acid, then heating the mixture. The absence of an alcohol odor indicated that the extract was free from residual solvent (Reninta et al., 2022; Dangi, 2018). If ethanol odor was still detected, evaporation was continued.

Color Identification Test

Qualitative identification of flavonoids was performed using a color reaction test. Two drops of the extract were added into a test tube, followed by 2–4 drops of 10% sodium hydroxide (NaOH) solution. A color change from yellow to brownish-yellow indicated the presence of flavonoid compounds. This color reaction is associated with flavonoids such as quercetin, kaempferol, and rutin commonly found in plant extracts (Bhagat, 2021; Ulfah et al., 2024; Kurnianto et al., 2021).

Determination of Total Flavonoid Content by UV–Vis Spectrophotometry

Total flavonoid content was determined quantitatively using a UV–Vis spectrophotometer. The extract was diluted appropriately and placed in a cuvette for absorbance measurement at a wavelength of 415 nm, which corresponds to flavonoid–aluminum chloride complex formation.

Measurements were performed in triplicate to ensure accuracy and reproducibility. Flavonoid concentration was calculated using a calibration curve prepared with quercetin as the standard, and results were expressed as mg quercetin

equivalent (QE) per gram of sample or µg/mL of extract.

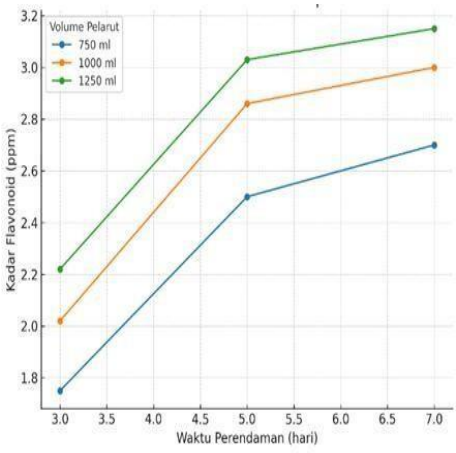
RESULTS AND DISCUSSION

Result

The results of total flavonoid content measurement in Luffa cylindrica fruit extract under different combinations of solvent volume and maceration time are presented in Table 1 and Figure 4. Overall, the flavonoid content ranged from 1.75 ppm to 3.15 ppm, indicating that extraction efficiency was strongly influenced by both solvent volume and maceration duration.

Table 1. Flavonoid levels in Luffa cylindrica extract at varying solvent volumes and maceration times.

Solvent Volume (Ethanol 70%)	Maceration Time (days)	Total Flavonoid Content (mg QE/g DW)
750 mL	3	1.75
750 mL	5	2.50
750 mL	7	2.70
1000 mL	3	2.02
1000 mL	5	2.86
1000 mL	7	3.00
1250 mL	3	2.22
1250 mL	5	3.03
1250 mL	7	3.15



Picture 4. Influence Volume Solvent and Soaking Time on Flavonoid Content of *Luffa cilindrica* Fruit.

At a solvent volume of 750 mL, increasing the maceration time from 3 to 7 days resulted in a gradual increase in flavonoid content from 1.75 ppm to 2.70 ppm. Specifically, flavonoid levels increased from 1.75 ppm (3 days) to 2.50 ppm (5 days) and reached 2.70 ppm (7 days). This trend indicates that longer contact time between solvent and plant matrix enhances flavonoid diffusion.

A similar increasing pattern was observed at a solvent volume of 1000 mL. The flavonoid content increased from 2.02 ppm at 3 days to 2.86 ppm at 5 days, and further increased to 3.00 ppm at 7 days. Compared to the 750 mL solvent volume, the use of 1000 mL ethanol consistently produced higher flavonoid levels at the same maceration times.

The highest flavonoid contents were obtained at a solvent volume of 1250 mL. At this volume, flavonoid levels increased from 2.22 ppm (3 days) to 3.03 ppm (5 days) and reached a maximum of 3.15 ppm at 7 days, which represents the highest value obtained in this study. Conversely, the lowest flavonoid content (1.75 ppm) was observed at the shortest maceration time (3 days) and the lowest solvent volume (750 mL).

Based on these results, a clear extraction pattern can be identified. First, increasing solvent volume from 750 mL to 1250 mL resulted in higher flavonoid content at all maceration times. Second, extending maceration time from 3 to 7 days consistently increased flavonoid levels for each solvent volume. Third, the combination of the largest solvent volume (1250 mL) and the longest maceration time (7 days) produced the most efficient extraction conditions.

Statistical analysis using two-way ANOVA confirmed that solvent volume had a very significant effect on flavonoid content ($p < 0.01$), while maceration time also showed a significant effect ($p < 0.05$). Furthermore, a significant interaction between solvent volume and

maceration time ($p < 0.05$) indicated that the effectiveness of extraction depended on the combined influence of these two variables. Duncan's Multiple Range Test (DMRT) further demonstrated that the treatment using 1250 mL solvent volume with 7 days maceration resulted in significantly higher flavonoid levels compared to other treatment combinations.

Discussion

The results of this study indicate that both solvent volume and maceration time play an important role in determining the total flavonoid content of *Luffa cylindrica* fruit extract. An increase in solvent volume from 750 to 1250 mL resulted in a consistent increase in flavonoid content at all maceration times. This phenomenon can be explained by the increased solvent capacity, which prevents early saturation and enhances mass transfer and diffusion of flavonoid compounds from the plant matrix into the solvent (Azwanida, 2015; Wang, 2017).

Similarly, extending maceration time from 3 to 7 days significantly increased flavonoid levels, indicating that longer contact between the solvent and plant material allows more effective penetration of ethanol into plant tissues and promotes the dissolution of flavonoid compounds (Dangi et al., 2018). The highest flavonoid content (3.15 mg QE/g DW) obtained at 1250 mL solvent volume and 7 days maceration confirms that sufficient solvent availability combined with prolonged extraction time improves extraction efficiency.

However, excessively long maceration duration may potentially lead to flavonoid degradation due to oxidation or hydrolysis reactions during prolonged solvent exposure, which can reduce compound stability (Kumar & Pandey, 2019). Therefore, optimization of maceration time is essential to achieve a balance between maximizing flavonoid yield and maintaining compound integrity.

The use of 70% ethanol as an extraction solvent proved effective due to its moderate polarity, which enables the extraction of both polar and semi-polar flavonoids. This finding is consistent with previous studies reporting that ethanol–water mixtures are optimal for flavonoid extraction from various plant matrices (Azwanida, 2015; Chen et al., 2024).

These findings are in agreement with previous reports showing that increasing solvent volume and extraction duration significantly enhances flavonoid yield in herbal plant extracts (Patel & Kumar, 2018; Adeosun et al., 2023). Nevertheless, the flavonoid content obtained in this study was lower than that reported for some other plant species, which may be attributed to differences in plant species, plant parts used, extraction methods, and process parameters applied (Kumar & Pandey, 2019).

CONCLUSION

This study clearly demonstrates that both ethanol solvent volume and maceration time significantly influence total flavonoid content in *Luffa cylindrica* fruit extract. The highest flavonoid content (3.15 mg QE/g DW) was achieved using 1250 mL of 70% ethanol with a maceration time of 7 days, representing the optimal extraction condition. Increasing solvent volume and prolonging maceration enhanced solvent–solute interaction and mass transfer efficiency. Therefore, maceration with 70% ethanol is confirmed as an effective, simple, and economical method for extracting flavonoids from *Luffa cylindrica* fruit.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future studies are recommended to further optimize flavonoid extraction from *Luffa cylindrica* by evaluating additional extraction parameters, such as solid-to-solvent ratio, extraction temperature, particle size, and agitation intensity. The application of alternative

extraction techniques, including ultrasound-assisted or microwave-assisted extraction, may also be explored to improve extraction efficiency and reduce processing time. Moreover, qualitative and quantitative identification of individual flavonoid compounds using chromatographic techniques such as HPLC or LC–MS is necessary to better understand the flavonoid profile and bioactive potential of *L. cylindrica* fruit. Further investigation of the antioxidant and biological activities of the extract is also recommended to support its potential application in functional food and pharmaceutical products.

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