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Phytochemical Profile and Antioxidant Activity of Ficus carica L. Leaf Extracts Prepared Using Different Solvents

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Abstract

This research investigated two extraction solvents and evaluated antioxidant potential using the most dependable assay for antioxidant activity. Additionally, the study sought to assess the phytochemical constituents and antioxidant properties of extracts from locally sourced (Indonesian) Ficus carica Linn. leaves. Maceration was employed for extraction with two organic solvents: methanol and ethanol. Phytochemical screening of the extracts targeted several secondary metabolite groups, including alkaloids, flavonoids, steroids, tannins, glycosides, and saponins. Total phenolic and flavonoid contents were quantified using gallic acid and quercetin as reference standards, respectively. Antioxidant activity was measured via the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay, with ascorbic acid serving as the reference compound. Screening results indicated the presence of all examined metabolite classes in the extracts, except for alkaloids, which were absent in the methanol extract. Total phenolic and flavonoid levels were 33.93 ± 0.31 and 40.76 ± 0.23 mg GAE/g, and 26.28 ± 0.20 and 24.35 ± 0.31 mg QE/g for the ethanol and methanol extracts, respectively. Both extracts exhibited potent antioxidant effects, with the methanol extract displaying marginally superior activity. Findings further revealed that the methanol extract of F. carica Linn. leaves contained fewer phytochemicals yet showed greater antioxidant capacity than the ethanol extract.

Keywords: Fig, Ficus carica, Antioxidant, Phytochemical screening, DPPH

Introduction

Plants have been employed in therapeutic and cosmetic applications for centuries. Traditionally central to healing practices, they have in recent decades become popular as alternative or adjunctive options. Concerns over the adverse effects of synthetic medications have prompted increased interest in plant-based remedies as safer alternatives for disease treatment, health maintenance, and aesthetic enhancement. Among the primary phytochemical categories—terpenoids, alkaloids, and phenolics—the phenolic group has received the most attention [1]. These compounds are recognized for diverse biological effects, particularly antioxidative properties [2, 3]. Extraction efficiency of phenolics depends on solvent polarity, as it affects compound solubility [4]. The quantity of extracted phenolics directly influences the resulting biological activity [3]. Consequently, identifying the optimal solvent for maximum phenolic yield is essential.

Most phenolics dissolve readily in polar solvents like methanol and ethanol, though their applications differ. Ethanol is considered less toxic than methanol [5], restricting methanol extracts primarily to topical formulations, which are deemed safer than oral administration. In the present work, Ficus carica Linn. (commonly called fig) was selected as the study material. Traditionally, it has been applied for conditions requiring anti-inflammatory, cardiovascular, respiratory, and antispasmodic effects [6]. Numerous investigations have explored the biological properties of fig leaves, including

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antioxidant, antimicrobial, anticancer, hepatoprotective, antipyretic, hypoglycemic, anti-hyperlipidemic, and antimutagenic activities [7-13]. Certain studies have reported variations in antioxidant performance depending on the extraction solvent used [14–16]. This investigation was designed to evaluate phytochemical profiles of F. carica Linn. leaves sourced from Binjai city, Indonesia, using two solvents of comparable polarity, and to determine antioxidant activity of the resulting extracts with the widely accepted DPPH assay.

Materials and Methods

The study utilized Ficus carica Linn. leaves harvested in July 2020 from Binjai City, North Sumatra Province, Indonesia. Botanical identification was performed by the Characterization Laboratories of Eka Karya Botanical Garden, National Research and Innovation Agency (Bali, Indonesia), under voucher number 1617-77020-1. Extraction solvents comprised 96% ethanol and absolute methanol (Smart Lab, Tangerang, Indonesia). 2,2-Diphenyl-1-picryl-hydrazyl-hydrate and Folin-Ciocalteu reagent were sourced from Sigma-Aldrich, Switzerland. Gallic acid and quercetin standards were supplied by E. Merck, Germany, while other reagents were acquired from Bratachem, Indonesia. All reagents were analytical grade and used without additional purification.

Preparation of plant sample

F. carica Linn. leaves were cleaned to eliminate dirt and debris, then air-dried at ambient temperature to minimize moisture and inhibit microbial contamination. The dried leaves were subsequently pulverized into a fine powder.

Characterization of dried powder

The dried leaf powder of F. carica Linn. (FLDP) was analyzed for moisture content, total ash, acid-insoluble ash, water-soluble extractives, and ethanol-soluble extractives. All tests followed protocols outlined in the Indonesian Herbal Pharmacopoeia [17].

Preparation of extracts

FLDP was subjected to maceration using ethanol and methanol at a 1:10 ratio (powder:solvent). Each process involved 500 grams of FLDP and 5 liters of solvent. Maceration lasted 24 hours, after which fresh solvent was added to the marc until the liquid appeared clear. Resulting filtrates were concentrated via rotary evaporator (Heidolph, Germany) to yield viscous extracts, which were weighed for yield calculation [17]. The procedure was replicated three times, and extracts were pooled. The products were designated MEFC (methanol extract of F. carica Linn.) and EEFC (ethanol extract of F. carica Linn.).

Phytochemical screening

Qualitative analysis for secondary metabolites was carried out on the dried powder and both extracts, targeting alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenes. Specific reagents were employed for detection: Dragendorff reagent for alkaloids, $AlCl_3$ solution for flavonoids, $FeCl_3$ solution for tannins, and Liebermann-Burchard reagent for steroids/triterpenes. For saponins, a froth test was performed by vigorous shaking followed by the addition of concentrated acid [18, 19]. All detection reagents were prepared according to protocols outlined in the Indonesian Herbal Pharmacopoeia [17].

Total phenolic content

Determination of total phenolic content followed the method described by Singleton *et al*., with minor adjustments [20]. Gallic acid served as the reference standard for establishing the maximum absorbance wavelength and constructing the calibration curve. A 1.0 ml aliquot of the methanol-diluted sample was combined with 7.9 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. After 1 minute, 1.5 ml of 20% Na_2CO_3 was added, and the mixture was incubated in the dark for 90 minutes. Absorbance was measured at 744 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Results were expressed as milligrams of gallic acid equivalent (mg GAE) per gram of MEFC or EEFC.

Total flavonoid content

Total flavonoid content was assessed via colorimetric assay with aluminum chloride, as specified in the Indonesian Herbal Pharmacopoeia [17]. Quercetin was utilized as the standard for calibration curve preparation and wavelength determination. A 10 mg sample was dissolved in 10 ml methanol; 2 ml of this solution was then mixed with 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1 M CH_3COONa, followed by the addition of 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 minutes, and absorbance was recorded at 438 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). Values were reported as milligrams of quercetin equivalent (mg QE) per gram of MEFC or EEFC.

Antioxidant activity assay

Radical scavenging potential of the extracts was evaluated using the DPPH method adapted from Brand-Williams *et al*., with modifications [21]. Ascorbic acid was employed as the positive control due to its potent scavenging ability. Methanol-diluted extracts at varying concentrations (50 to 250 ppm) were added to a 0.2 mM DPPH solution. After vigorous mixing, samples were kept in the dark for 30 minutes, and absorbance was measured at 515 nm on a UV-Vis spectrophotometer (Shimadzu, Japan). The IC_{50} value (concentration required for 50% inhibition) was derived from the plot of concentration versus percentage inhibition.

Statistical analysis

All data were presented as mean ± standard deviation. Statistical processing was performed using Microsoft Excel 2013.

Results and Discussion

Characterization of F. carica Linn. dried leaves powder

Results from the characterization of FLDP are summarized in **Table 1**. Moisture content was below 10%, meeting the criterion in the Indonesian Herbal Pharmacopoeia that limits water content to under 10% to minimize microbial proliferation [17]. This allows safe storage of the powder for extended periods without risk of contamination. Standardization through these parameters is essential to ensure material quality prior to extraction and for potential future applications.

Yield of extraction

Extraction yield is influenced by multiple variables, including the nature of target compounds, extraction technique, sample particle size, solvent choice, and potential interfering substances [22]. When other factors remain constant, solvent selection becomes the primary determinant of differences in yield. Although methanol and ethanol possess comparable polarity, minor variations resulted in notably different yields, as detailed in **Table 2**. The methanol extraction produced a higher yield than ethanol, suggesting that greater solvent polarity enhances overall extract recovery. These findings align with previous work by Do *et al*., which demonstrated that yield increases with higher solvent polarity [23].

Phytochemical constituents

Screening revealed that the unprocessed F. carica leaves (FLDP) contained alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. The ethanolic extract (EEFC) retained all these classes. In contrast, the methanolic extract (MEFC) tested negative for alkaloids, as indicated in **Table 3**. This highlights how even small polarity differences can selectively affect compound extraction. Overall, MEFC exhibited fewer detected phytochemical groups than either EEFC or the original FLDP.

Total flavonoid and phenolic contents

Quantification of flavonoid and phenolic compounds provided estimates of their levels in the extracts. Flavonoid content, illustrated in **Figure 1**, was 26.28 ± 0.20 mg QE/g for EEFC and 24.35 ± 0.31 mg QE/g for MEFC. Phenolic content, shown in **Figure 2**, measured 33.93 ± 0.31 mg GAE/g for EEFC and 40.76 ± 0.23 mg GAE/g for MEFC.

Table 1. Characterization of dried leaves powder

Parameter	Results (%)
Total ash	10.55 ± 0.72
Total acid-insoluble ash	4.72 ± 0.69
Water content	7.97 ± 1.99
Water-soluble substances	31.54 ± 1.39
Ethanol soluble substances	17.27 ± 1.14

Table 2. Yield value of solvent extraction

Solvent	Yield value (%)
Ethanol	7.80 ± 0.50
Methanol	12.00 ± 0.46

Table 3. Phytochemical screening of dried leaves powder and extracts

Secondary metabolite	FLDP	MEFC	EEFC
Alkaloids	+	-	+
Flavonoids	+	+	+
Glycoside	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Triterpenes/Steroids	+	+	+

+ : presence; - : absence; FLDP: F. carica dried leaves powder; MEFC: methanol extract of F. carica; EEFC: ethanol extract of F. carica

Table 4. Antioxidant activity of F. carica Linn extracts

Sample	IC50 (ppm)	Category
Vitamin C	2.6935	Very strong
EEFC	99.1278	Strong
MEFC	92.2137	Strong

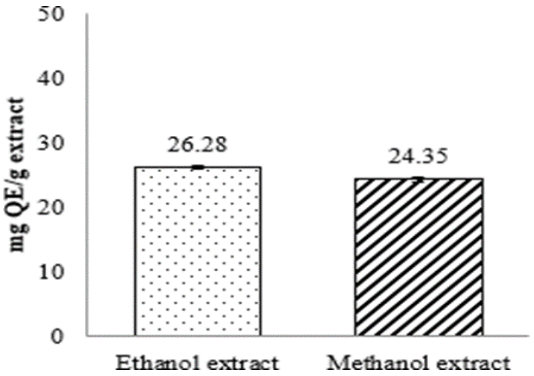

Figure 1. Total flavonoid content in ethanol and methanol extracts of F. carica Linn.

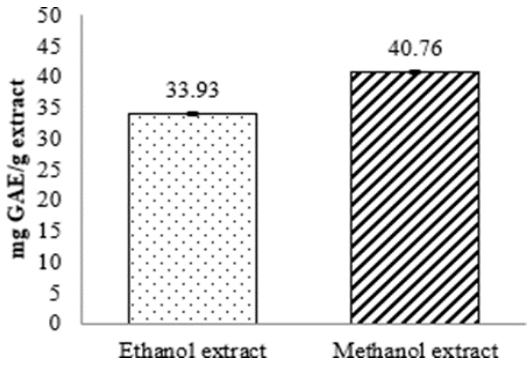

Figure 2. Total phenolic content in ethanol and methanol extracts of F. carica Linn.

Flavonoids are typically classified within the broader phenolic group [24]. Consequently, flavonoid levels were lower than total phenolic contents in both extracts. The ethanolic extract (EEFC) exhibited marginally higher flavonoid concentration than the methanolic extract (MEFC), whereas phenolic quantification revealed higher values in MEFC compared to EEFC. This discrepancy indicates that MEFC contained reduced flavonoid amounts despite elevated overall phenolics, attributable to the reference standards employed. Quercetin and gallic acid served as standards for flavonoid and phenolic estimation, respectively. Compounds structurally akin to quercetin were quantified as flavonoids, while those resembling gallic acid contributed to phenolic totals. Thus, EEFC appeared dominated by flavonoid-type phenolics, whereas MEFC was richer in lower-molecular-weight phenolics, such as phenolic acids. Methanol proved more efficient than ethanol in extracting these low-molecular-weight phenolic compounds [25].

Antioxidant activity

The DPPH radical scavenging assay is widely utilized to assess antioxidant potential in plant extracts [26–31]. Here, IC_{50} values were used to quantify scavenging efficiency. As presented in **Table 4**, MEFC displayed slightly superior antioxidant performance over EEFC, with both extracts falling into the strong antioxidant category. These effects correlated with their phenolic and flavonoid contents. Notably, the higher phenolic level in MEFC corresponded to a lower IC_{50}, indicating modestly enhanced activity relative to EEFC. Nonetheless, both extracts had higher IC_{50} values than the reference ascorbic acid.

Results suggest that extracts from F. carica Linn. Leaves, whether methanolic or ethanolic, represent viable antioxidant sources. Further research is required to isolate specific active compounds and develop suitable pharmaceutical formulations.

Conclusion

Phytochemical profiles, total phenolic and flavonoid levels, and antioxidant properties of F. carica Linn. Leaf extracts varied despite the use of solvents with comparable polarity. Although the methanolic extract contained fewer detectable phytochemicals, it exhibited greater antioxidant activity than the ethanolic extract.

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