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Original Research Article

A Comparative Study of Phytochemical Screening and DPPH Radical Scavenging Activity of *Ficus carica* Linn. Leaves Extracts

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ABSTRACT

This study focused on two extraction solvents and the examination of antioxidant with the most reliable antioxidant activity test. Furthermore, this study aimed to compare the phytochemical components and antioxidant activity of two *Ficus carica* Linn. extracts using the local (Indonesian) *Ficus carica* Linn. plant as the sample. The extraction was conducted by maceration technique using two organic solvents, namely methanol, and ethanol. The phytochemical screening of these extracts was conducted on several metabolite classes, namely alkaloids, flavonoids, steroids, tannins, glycosides, and saponins. In addition, the total phenolic and flavonoid compounds were determined with gallic acid and quercetin, respectively for standards. The antioxidant activity assay was performed using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method with ascorbic acid as the comparative standard. The phytochemical screening of the extracts showed positive results for all tested metabolite classes, however, the methanol extract showed a negative result to alkaloids compounds. The total phenolic and flavonoid compounds were 33.93 ± 0.31, 40.76 ± 0.23 mg GAE/g and 26.28 ± 0.20, 24.35 ± 0.31 mg QE/g for both ethanol and methanol extracts, respectively. Furthermore, the assay showed that both extracts had strong antioxidant activity, however, the methanol extract is slightly higher. The result also showed that methanol extract of *F. carica* Linn. leaves has fewer phytochemical compounds but demonstrates higher antioxidant activity compared to the ethanolic extract.

Keywords: Fig, *Ficus carica*, antioxidant, phytochemical screening, DPPH.

Introduction

The utilization of plants as health therapy and for promoting beauty has been explored for a century. Plants used as the main therapy in ancient times have evolved into an alternative or complementary product in the last decades. However, the harmful side effects of synthetic drugs have forced researchers to consider plants as safe therapies for treating illness as well as promoting health and beauty. The phenolic compound is mostly explored among the three major classes of phytochemical components, which include terpenoids, alkaloids, and phenolic compounds.[1] This group of phytochemicals is known to have many biological activities, including antioxidants.[2,3] The yield extraction of this phenolic compound is influenced by the polarity of the solvent due to the solubility of this compound.[4] Furthermore, the amount of phenolic compound extracted will determine the biological activity.[3] Therefore, it is necessary to know which solvent gives the best yield value of the phenolic compound.

Most phenolic compounds are soluble in a polar solvent, such as methanol and ethanol, however, their usage is different. Ethanol is known to have less toxicity compared to methanol, [5] thus the utilization of methanol extract is limited. Methanol extract is mostly formulated into a topical dosage form that appears to be safer than oral dosage. In this study, *Ficus carica* Linn (*F. carica* Linn), also known as fig was chosen as the plant sample.

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Empirically, it is used as a remedy for certain diseases such as anti-inflammatory, cardiovascular, respiratory, and antispasmodic.[6] Several studies have been conducted to examine the biological activities of fig leaves, such as antioxidant, antimicrobial, anticancer, hepatoprotective, antipyretic, hypoglycemic, anti-hyperlipidemic, and antimutagenic.[7-13] Some reports also showed that the antioxidant activity of this plant dissimilar with different solvent extraction.[14,15,16] This study was developed to compare the phytochemical compounds of *F. carica* Linn leaves that was originated from Binjai city, Indonesia with two different extraction solvents which have almost similar polarity. Then, the antioxidant activity of the two extracts was tested using the most reliable assay which is the DPPH method.

Materials and Methods

The plant sample used in this study was *Ficus carica* Linn leaves were collected in July 2020 from Binjai City, North Sumatera Province, Indonesia. The plant sample was identified by Eka Karya Botanical Garden Characterization Laboratories, National Research and Innovation Agency (Bali-Indonesia) with voucher number 1617-77020-1. The extraction solvents used were 96% ethanol and absolute methanol (Smart Lab, Tangerang, Indonesia). 2,2-diphenyl-1-picryl-hydrazyl-hydrate, and Folin-Ciocalteu, which are products from Sigma-Aldrich, Switzerland. Furthermore, gallic acid and quercetin were obtained from E. Merck, Germany and the other chemical reagents were purchased from Bratachem company, Indonesia. All chemical reagents used were of analytical grade with no further purification.

Preparation of plant sample

The leaves of *F. carica* Linn. were washed to remove dust and unwanted materials and dried at room temperature to reduce water content for microbial growth prevention. Afterward, the dried leaves were ground into fine powder.

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Characterization of dried powder
F. carica Linn. leaves dried powder (FLDP) was characterized for contents of water, total ash, total acid insoluble ash, water-soluble substances, and ethanol soluble substances. All the characterization parameters were conducted based on the standard procedure described in Indonesian Herbs Pharmacopoea.[17]

Preparation of extracts
FLDP was macerated with ethanol and methanol solvent with a 1 to 10 (FLDP:solvent) ratio. Approximately 500 grams FLDP and 5 liters of solvent were used to complete of each maceration process. The maceration process was conducted for 24 hours and then the solvent was added to the residue until a clear solvent was obtained. The extracts were then evaporated using a rotary evaporator (Heidolph, Germany) until thick extracts were obtained, which then were weighed to calculate the extraction yield.[17] This procedure was carried out in triplicate and then combined. Afterward, the extracts were referred to as MEFC (methanol extract of *F. carica* Linn.) and EEFC (ethanol extract of *F. carica* Linn.).

Phytochemical screening
A qualitative phytochemical screening was performed for alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenes compounds to determine the secondary metabolites contained in dried powder and both extracts. The samples were treated with particular detection reagents such as Dragendorf for alkaloids, $AlCl_3$ solution for flavonoids, $FeCl_3$ solution for tannins, Lieberman-Bourchard reagent for steroids/triterpenes. This was followed by a shaken test with the subsequent addition of concentrated acid for saponins.[18,19] All the reagent solutions were prepared based on the procedure stated in Indonesian Herbs Pharmacopoea.[17]

Total Phenolic Content
The total phenolic content was undertaken using the procedure illustrated in Singleton et al with some modifications.[20] Gallic acid was used as the standard compound to determine the maximum wavelength and to generate the calibration curve. A 1.0 ml sample diluted in methanol was taken and added with 7.9 ml of water, then the solution was mixed with Folin-Ciocalteu *reagent (0.5 ml). After 1 minute, 20% Na_2CO_3 (1.5 ml) was added into the mixture, and then incubated for 90 minutes, protected from light. The solution was assessed using spectrophotometer UV-Vis (Shimadzu, Japan) at 744 nm wavelength and the total phenol content was calculated as milligram gallic acid equivalent (mg GAE)/gram MEFC or EEFC.

Total Flavonoid Content
Total flavonoid contents were confirmed by colorimetric method using aluminum chloride as described in Indonesian Herbs Pharmacopoea.[17] Calibration curve and the maximum wavelength was determined using quercetin as standard compound. Furthermore, a 10mg sample was dissolved in a 10 ml methanol solution, and then 2 ml of the standard solution was taken and added with 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1 M CH_3COONa. Subsequently, 2.8 ml of distilled water was added to the solution, after which the mixture was incubated for 30 minutes at room temperature. The solution was then measured for absorbance using a UV-Vis spectrophotometer (Shimadzu, Japan) at a maximum wavelength of 438 nm. The total flavonoid content was stated as milligram quercetin equivalent (mg QE)/gram MEFC or EEFC.

Antioxidant activity assay
The radical scavenging activity of the extracts was developed using the method of Brand-William et al with modification.[21] The standard used was ascorbic acid, which has a very strong radical scavenger activity. Then the diluted extract (in methanol) with different concentrations (50 to 250 ppm) was mixed with a solution of 0.2mM DPPH. After being shaken vigorously, the mixture was placed for 30 minutes in the dark and the absorbance was then measured at 515nm using spectrophotometer UV-Vis (Shimadzu, Japan). Furthermore, the 50% inhibition (IC50) demonstrated was calculated from the graph of extract concentration to the percentage of radical scavenging activity.

Statistical Analysis
Data were shown as means ± S.D. Statistical analysis of the results were carried out using the Microsoft Excel program 2013.

Results and Discussion

Characterization of F. carica Linn. dried leaves powder
The characterization result of FLDP is presented in Table 1. FLDP contained low water content which was below 10%. Based on Indonesia Herbs Pharmacopoea, a plant sample with water content below 10% can decrease the chance of microbial growth.[17] Therefore, it can be stored for a certain of time without fear of contamination by microorganisms. The characterization of dried leaves powder was very important to fulfill a standard sample to be used for extraction. Furthermore, these parametric characterizations are needed to evaluate the *F.carica* dried leaves powder for the next application.

Yield of Extraction
The yield of extraction is affected by a number of factors, such as the characteristic of the chemical constituents, method of extraction, the particle size of the sample, the solvent used, and also the presence of interfering substances.[22] When the sample, extraction method, and particle size are the same, then the solvent used become the main matter that gives the difference. The solvent used in this study was methanol and ethanol which have almost similar polarity. However, slightly different solvent polarity gave significant difference in yield of extraction as shown in Table 2. Furthermore, the sample extracted with methanol demonstrated a higher yield of extraction than ethanol. This indicated that solvent with high polarity gave more yield of extraction. This result is consistent with the reports of Do, *et al* which stated that extraction yield increases with increasing polarity of the solvent used in extraction.[23]

Phytochemical constituents
The results of phytochemical screening showed that the raw material of *F. carica* leaves had alkaloids, flavonoids, glycoside, saponin, tannin, and triterpenes/steroids. The same constituents were detected in the *F. carica* leaves ethanolic extract. However, methanol extract yielded a negative result to the alkaloids group as seen in Table 3. This implies that a slight difference in polarity will influence the component extracted in the solvent. In this study, the methanol extract (MEFC) had fewer components compared to ethanol (EEFC) and the raw material (FLDP).

Total Flavonoid and Phenolic Contents
The total flavonoid and phenolic contents were conducted to determine the approximate value of flavonoid and phenolic compounds in the extract. The determined flavonoid contents of EEFC and MEFC as shown in Figure 1. are 26.28 ± 0.20 mg QE/g and 24.35 ± 0.31 mg QE/g, respectively. Also, the examined phenolic contents of EEFC and MEFC as shown in Figure 2 are 33.93 ± 0.31 mg GAE/g and 40.76 ± 0.23 mg GAE/g, respectively.

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

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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	IC_{50} (ppm)	Category
Vitamin C	2.6935	Very strong
EEFC	99.1278	Strong
MEFC	92.2137	Strong

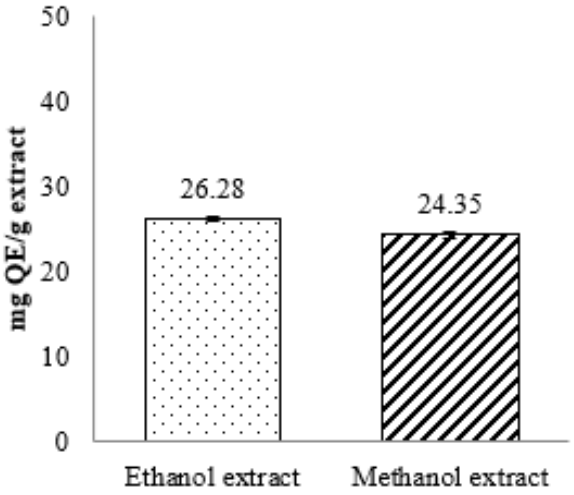

Figure 1: Total flavonoid content in ethanol and methanol extracts of *F. carica* Linn.

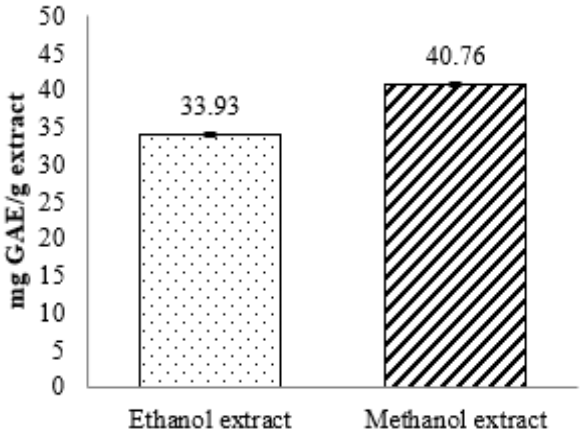

Figure 2: Total phenolic content in ethanol and methanol extracts of *F. carica* Linn.

Flavonoid compound is usually included in the phenolic compound class.[24] Therefore, the value of flavonoid is lower than the total phenolic content and it was shown in both extracts. The EEFC gave a slightly higher flavonoid value than MEFC; however, the result of the phenolic content showed that MEFC had a higher value than EEFC. Based on this difference, it was suggested that MEFC had a lesser amount of flavonoid content, even though it showed higher phenolic content, which is due to the standard compound used. In this study, quercetin and gallic acid were used as the standard compounds to predict the flavonoid and phenolic contents in the extracts, respectively. A phytochemical compound that had a similar structure with quercetin was detected as flavonoid and the one that showed equivalent structure as gallic acid was presented as a phenolic compound. This showed that EEFC possessed a major flavonoid compound as the phenolic class in the extract. However, MEFC has a lower molecular weight of phenolic compounds such as phenolic acids group. In this case, methanol solvent is particularly more effective to extract low molecular weight of the phenolic compound than the ethanol.[25]

Antioxidant activity

DPPH radical scavenging activity had been chosen in many studies to evaluate the antioxidant activity of plant extract.[26,27,28,29,30,31] In this study, the IC_{50} parameter was chosen to determine the DPPH radical scavenging of antioxidant activity. Table 4 shows that MEFC had slightly higher antioxidant activity than EEFC. Also, both extracts had a strong category of antioxidant activity. This shows that the antioxidant activity exhibited was supported by the total phenolic and flavonoid contents of both extracts. In addition, MEFC had a higher total phenolic compound resulting in a slightly lower IC_{50} value, which implies that it shows a little bit stronger antioxidant activity compared to EEFC. However, both IC_{50} of the extracts were lower than the ascorbic acid measurement.

Based on the antioxidant activity result, it is suggested that either methanol or ethanol extract of *F. carica* Linn were suitable as a source of antioxidant. Advance studies are needed to determine the specific compounds responsible for the antioxidant activity and also to formulate the extract into a dosage form.

Conclusion

The phytochemical compounds, total phenolic, and flavonoid contents as well as the antioxidant activity of *F. carica* Linn leaves extract are different with the almost similar polarity of solvent used. Furthermore, the methanol extract of *F. carica* Linn leaves has fewer phytochemical compounds, however, it demonstrates higher antioxidant activity compared to ethanol.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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