

COLORIMETRIC METHOD FOR TOTAL PHENOLIC AND FLAVONOID CONTENT DETERMINATION OF FIG (*Ficus carica* L.) LEAVES EXTRACT FROM WEST JAVA, INDONESIA

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ABSTRACT

Fig (*Ficus carica* L., Moraceae) contains phenolics, flavonoids, tannins, sesquiterpenes, and organic acids. These secondary metabolites content creates an opportunity to utilize fig as a functional food or standardized herbal preparations. This study aimed to determine the total phenolic and flavonoid content of fig leaves extract by the colorimetric method. In this study, fig leaves from Ciwidey District, West Java Province, Indonesia were used. Fig leaves were extracted with 70% ethanol by the maceration method. Folin-Ciocalteu method was used to determine total phenolic content and AlCl_3 method was used to determine total flavonoid content. Fig leaves extract had total phenolic content of 2.52 ± 0.243 mg GAE/g simplicia and total flavonoid content of 2.028 ± 0.007 mg RE/g simplicia. Fig leaves from Ciwidey District, West Java, contain good total phenolic and flavonoid content, so they have the potential to be developed into standardized herbal preparations.

Keywords: Ciwidey District, Maceration, Folin-Ciocalteu Method, AlCl_3 Method

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INTRODUCTION

Fig (*Ficus carica* L.), belonging to the Moraceae family, is the earliest cultivated fruit tree and an important crop worldwide.¹ The fruit, leaves, and root fig are used in traditional medicine to treat the disorder of cardiovascular, digestive (loss of appetite, colic, and diarrhea), respiratory (cough, bronchial problems, and sore throat), and as an antispasmodic and anti-inflammatory remedy.² Various parts of fig contain phenolics, flavonoids, tannins, sesquiterpenes, and organic acids.³

Phenolic compounds are important components of the color, flavor, and aroma of fresh fruits, vegetables, and their products.^{4,5} Flavonoids are phenolic compounds with a three-ring system, composed of 15 carbon atoms in the form of C6-C3-C6. Phenolic compounds in the plants are presented in free and bound forms through ether, ester, or acetal bonds.⁶ Phenolic compounds have antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antimicrobial activities.^{4,5} These compounds act as an antioxidant by scavenging free radicals, donating a hydrogen atom or an electron to other compounds, and quenching singlet oxygen.⁷ The stability, nature, and antioxidant activity of extractable phenolic compounds depend on the matrix, temperature, pH, presence of enzymes, presence of inhibitors or enhancers of absorption, and other related factors.⁸ This study was conducted to determine the total phenolic and flavonoid content of fig leaves extract collected from Ciwidey District, West Java Province, Indonesia. This was because the fig phenolic content is influenced by the cultivar, varies significantly from one fruit part to another.⁹ This study was conducted to determine the total phenolic and flavonoid content and compared to fig leaves extract from other places.

EXPERIMENTAL

Fig leaves were collected from Ciwidey District, West Bandung Regency, West Java Province, Indonesia. Plants were identified in the Bandungense Herbarium, School of Life Sciences and Technology, Bandung

Institute of Technology with No. 883/I1.CO2.2/PL/2021. All chemicals were analytical grade and purchased from Merck (Germany), i.e. AlCl_3 , gallic acid, rutin, and Folin-Ciocalteu reagent.

Sample Preparation

The fresh fig leaves were washed, then dried, and weighed. A total of 5 g of simplicia was dried in an oven at 105 °C for 5 h. The simplicia was re-weighed to a constant weight.¹⁰ Water content of simplicia was determined using toluene distillation.¹⁰

Extraction

A total of 750 g of simplicia was macerated with 70% ethanol for 24 h, stirred occasionally. The solvent was changed twice, every 24 h. All extract was collected and concentrated using a rotary evaporator, then calculated the yield.¹¹

Phytochemical Screening

Simplicia and extract were screened with the Farnsworth method. Phytochemical screening was conducted to detect phenolics, flavonoids, tannins, alkaloids, triterpenoids, sesquiterpenes, steroids, and saponins groups.¹²

Determination of Total Phenolic Content

Gallic acid (1 mg/mL) was pipetted 0.2, 0.4, 0.6, 0.8, and 1.0 mL into a 1.0 mL volumetric flask, added 1 mL of Folin-Ciocalteu reagent, then incubated for 5 min. Then 4 mL of 7.5% Na_2CO_3 solution was added, and filled with distilled water. The solution was incubated for 40 min at room temperature, then the absorbance was measured at 650 nm. A total of 1 mL of 1 mg/mL extract was reacted with the same procedure for gallic acid.¹³⁻¹⁵

Determination of Total Flavonoid Content

Rutin (1 mg/mL) was pipetted 0.2, 0.4, 0.6, 0.8, and 1.0 mL into a 10 mL volumetric flask, added 0.3 mL of 5% NaNO_2 and 0.3 mL of 10% AlCl_3 solution, then incubated for 6 min. Then 2 mL of 1 N NaOH solution was added, and filled with distilled water. The solution was incubated for 15 min at room temperature, then the absorbance was measured at 510 nm. A total of 1 mL of 1 mg/mL extract was reacted with the same procedure for rutin.^{15,16}

RESULTS AND DISCUSSION

Sample Preparation

Fig leaves were taken from a 3-month-old fig plant and collected from the Ciwidey District, West Java Province, which has 1148 m in altitude. The lowest temperature and relative humidity are 10 °C and 20%, while the highest is 30 °C and 70%, respectively.¹⁷ The condition of the Ciwidey district, which meets the needs of fig cultivation, causes fig to grow well. Fig cultivated in most Mediterranean-type climates,¹⁸ in marginal soils under rain-fed conditions with improved cultural practices. Now, its cultivation is enhanced by irrigation,¹⁹ along with agro-techniques to increase fruit quality and yield.²⁰ Fig leaves were dried to obtain 1 kg of dried simplicia, then powdered to facilitate the extraction of secondary metabolites from fig leaves. Loss on drying must be determined because it is needed in the calculation of total flavonoids total. The result of loss on drying fig leaves simplicia was $9.64 \pm 0.16\%$. This value met the requirements of the Indonesian Herbal Pharmacopoeia, which is not more than 10% for leaves.²¹

The water content of fig leaves was $6.02 \pm 0.24\%$. This value met the water content requirements for leaves, which is not more than 8%.²⁰ High water content causes the simplicia to be easily overgrown by microorganisms and the occurrence of enzymatic reactions that can decompose the secondary metabolites in the simplicia.²²

Extraction

A total of 750.05 g of fig leaves produced 77.93 g of concentrated extract, so the yield was 10.39%. The fig leaves extract was dark green with a slight characteristic odor. This value met the general extract yield requirements based on the Indonesian Herbal Pharmacopoeia, which is not less than 10%,²¹ and indicated that the secondary metabolites can be extracted properly using maceration.

Phytochemical Screening

The secondary metabolites in the simplicia and extract were analyzed for their compounds by color test using specific reagents.^{12,23,24} The extracted phytochemicals compounds depend on nature, the origin, the processing degree, moisture content, and particle size of the plant material.²⁵ Alkaloids contain nitrogen in a cyclic system and various substituents, such as amine, amide, phenol, and methoxy groups, so tend to be semipolar. Triterpenoids have a cyclic structure in the alcohol form, so tend to be semipolar. Saponins are triterpene glycosides that have a polar tendency to glycosidic bonds.¹² Phytochemical screening on simplicia and fig leaves extract showed positive results for flavonoids, tannins, phenolic compounds, saponins, alkaloids, steroids, and terpenoids. The results were the same as Sharma *et al.*²⁶

Alkaloid identification using a color test was aimed at the nitrogen atom which is covalently bonded with potassium metal ion to form a precipitated potassium-alkaloid complex with a different color. Identification with Mayer's reagent produces a yellow precipitate, while Dragendorff's and Wagner's reagents produces a precipitated brownish to yellowish.¹²

Flavonoid identification using the Shinoda test containing magnesium metal and concentrated hydrochloric acid will reduce the benzopyrone core to form flavylum salts. This causes the hydrolysis of flavonoid glycosides into flavonoid aglycones which are observed from a color change to red or orange. This result predicted the flavonoid was flavan-3,4-diol, flavanone, or isoflavone groups.¹²

Phenol group identification in flavonoids was conducted using NaOH solution which was observed from a color change to yellow. The reaction between flavonoids and NaOH solution forms a salt and quinoid structure in ring B which causes a longer conjugated double bond thus shifted to a longer wavelength.²⁷

Saponin identification was observed from foam formation. The foam proves the presence of glycosides which are capable to produce foam in water due to hydrolysis into glucose and aglycones. Saponins contain glycosyl as polar groups, while steroids and triterpenoids as nonpolar groups. Compounds containing polar and nonpolar groups are surface-active compounds. When shaken vigorously with water, saponins form micelles.¹²

Tannin identification using FeCl₃ reagent will form green, red, purple, or black colored complexes.¹² Simplicia and fig leaves extract formed a green solution.

Terpenoid and steroid identification with Lieberman-Burchard reagents formed reddish-orange color for terpenoids and blue for steroids. The color change occurs due to the terpenoids or steroids oxidation through the formation of conjugated double bonds.¹² Simplicia and fig leaves extract gave positive results on steroids and negative results on terpenoids. This was observed from the absence of a brown ring at the solution boundary when concentrated sulfuric acid was added to the terpenoid test and a bluish-green color was observed in the steroid test.

Determination of Total Phenolics Content

Phenolic compounds react with the Folin-Ciocalteu reagent to form a blue complex which the absorbance was measured at visible wavelengths. The phenolic-hydroxy group reduces the heteropoly-(phosphomolybdate-phosphotungstic acid) in the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex. This reaction occurs in an alkaline environment so that proton dissociation occurs in phenolic compounds into phenolic ions.²⁸

Gallic acid was used as a standard, due to gallic acid is the best standard among the phenolic standards in the Folin-Ciocalteu method.²⁹ Gallic acid and the Folin-Ciocalteu reagent produced a blue molybdenum-tungsten complex with the maximum absorbance at 639.8 nm (Fig.-1). This result was the same as Singleton *et al.*²⁸ and Kaur and Kapoor.¹³

Five concentrations of standard gallic acid were measured for absorbance and a calibration curve was made with a correlation coefficient of 0.9989 (Fig.-2). This value met the ICH criteria³⁰ and indicate that the instrument response is proportional to the concentration. A total of 50 mg of extract was dissolved in 50 mL of distilled water to make 1 mg/mL. Then 1 mL of the extract solution was reacted with 1 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% Na₂CO₃, diluted with distilled water to the mark in a 10 mL volumetric flask to make 100 µg/mL. The absorbance of 100 µg/mL extract was of 0.369 ± 0.001, so total phenolic content was 2.427 ± 0.006 µg GAE/mL. Total phenolic content in 1 mg extract was 24.27 ± 0.006 µg GAE/mg extract or 24.27 ± 0.07 mg GAE/g extract which calculated as gallic acid. In this study, the yield

was 10.39%, so 10.39 g extract was produced from 100 g simplicia, so total phenolic content in simplicia was 252.165 ± 24.34 mg GAE/100 g simplicia. These results are similar to those of Ivanov *et al.*³¹ i.e. 2.5 mg GEA/g simplicia.

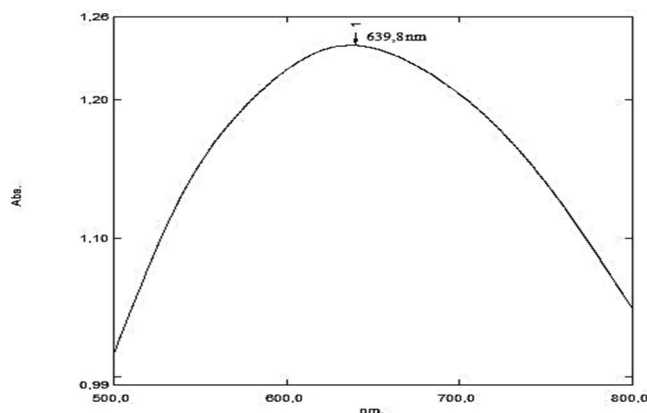


Fig.-1: Spectrum of Gallic Acid and Folin-Ciocalteu Complex

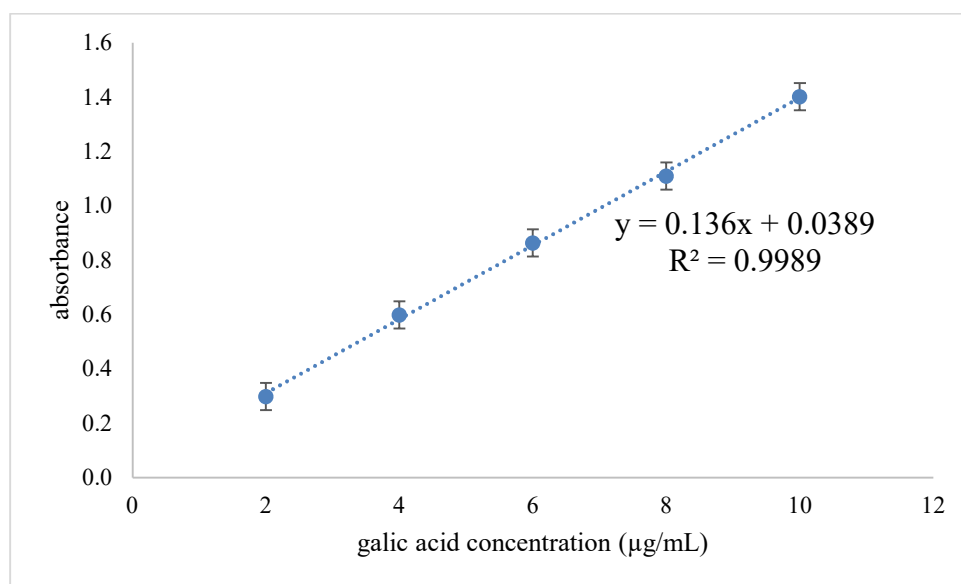


Fig.-2: Standard Gallic Acid Calibration Curve (n = 3)

Determination of Total Flavonoids Content

Total flavonoids content was determined from the absorbance of a brownish-yellow complex of flavonoid aglycone and AlCl_3 with rutin as a standard. Rutin and AlCl_3 reagent produced a brownish yellow complex with the maximum absorbance at 418 nm (Fig.-3). This wavelength was the same as Fernandes *et al.*³² Rutin is the main flavonoid glycoside found in fig.³³ Rutin can be converted into aglycone form, i.e. quercetin, through hydrolysis of one terminal rhamnose.³⁴ This was the reason for rutin selection as the standard in this study.

A total of 50 mg of fig leaves extract was equivalent to 481.2 mg of fig leaves simplicia with a loss on drying was 10.64%. The absorbance of 300 $\mu\text{g/mL}$ extract was 0.593 ± 0.008 with a dilution factor of 33.33.

Total flavonoid content which calculated by $\text{TFC} = \frac{A \times \text{DF}}{A_{1\text{cm}}^{1\%} \times (w - l_d)}$ ^{14,30} was 2.028 ± 0.007 mg RE/g simplicia.

This result was higher than Ivanov *et al.*³¹ i.e. 1.6 mg QE/g simplicia. This was because of the differences in plant origin and the standards. In Ivanov *et al.*³¹ fig leaves were collected from Plovdiv, Bulgaria and the standard was quercetin. The specific absorptivity of rutin and quercetin are different, i.e. 259.4 and 500, respectively.³⁵ Different standards made different calculations because flavonoids were equalized to different compounds. The result of this study was lower than Trifunski *et al.*³⁶ i.e. 2.62 ± 0.003 mg RE/g

simplicia. This was due to differences in growing places. In Trifunski *et al.*,³⁶ fig leaves were collected from Arad, Romania. During growth, the fig will synthesize flavonoids at different levels, depending on morphology and increasing leaves age.³⁷

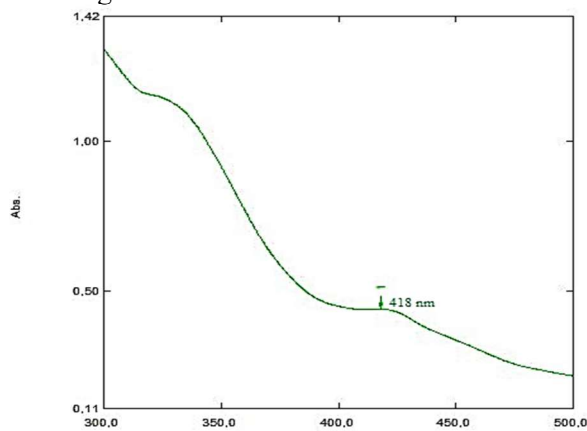


Fig.-3: Spectrum of Flavonoids and AlCl_3 Complex

CONCLUSION

Fig leaves yield 10.39% concentrated extract which is dark green with a slight characteristic odor. Simplicia and fig leaves extract contain flavonoids, tannins, phenolic compounds, saponins, alkaloids, steroids, and terpenoids. Fig leaves extract had total phenolics content of 2.52 ± 0.243 mg GAE/g simplicia and total flavonoids content of 2.028 ± 0.007 mg RE/g simplicia.

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