

# Antioxidant Activity of Methanolic Extract of *Ficus elastica* L. Leaves

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**Abstract—Objectives:** Natural antioxidants have an important role in the prevention of many diseases and promotion of health. Many natural sources of antioxidants derived from plants that were spread throughout the plant. Phenolic compounds are potent antioxidants. The *Ficus elastica* L. (*F. elastica*) leaf is one of the sources that contain phenolic compounds. This study aims to determine the antioxidant activity of methanol extracts of *F. elastica* leaves which extracted in an acidic environment. Simplisia *F. elastica* leaves macerated in methanol solvent with the addition of HCl 2 N to pH 2. The antioxidant activity test was carried out by the DPPH method. Phytochemical screening results showed flavonoids, tannins, polyphenols, saponins, quinones, monoterpenoids, and sesquiterpenoids. The qualitative tested of antioxidant activity by TLC method showed the presence of antioxidant activity which was marked by the formation of faded yellow spots against the background of the purple TLC plate after being sprayed with a DPPH of 0.2%. These results strengthened by the results of quantitative antioxidant activity testing which shows that the methanol extract of *Ficus elastica* L. leaves has a very high antioxidant potential with an  $IC_{50}$  value of  $7,6906 \pm 0,3 \mu\text{g/ml}$  with an  $IC_{50}$  value of standard compound (ascorbic acid) of  $5,6109 \pm 0,5 \mu\text{g/ml}$ .

**Keywords:** *Ficus elastica* L. leaf, antioxidant, DPPH,  $IC_{50}$

## I. INTRODUCTION

*Ficus* is a huge tropical, deciduous, evergreen tree with more than 800 species. Bark, root, leaves, fruit, and latex of this plant frequently used for the treatment of various illnesses.<sup>11</sup> Different parts of these species have been used in folk medicine for a variety of purposes, due to their antimicrobial, antidiabetic, anticarcinogenic, anti-inflammatory, anthelmintic, mild laxative, hypotensive, antirheumatic, digestive and anti-dysentery drugs and antioxidant activities. [8,12]

*Ficus* species are the rich source of polyphenolic compounds, flavonoids which are responsible for strong antioxidant properties that help in the prevention and therapy of various oxidative stress-related diseases such as neurodegenerative and hepatic diseases. The present review correlates the antioxidant activity of *Ficus* species with its pharmacological activities.<sup>11</sup> Among natural antioxidants, polyphenolic compounds are one of the most abundant and are extensively distributed in the plants, acting as free radical scavengers and antioxidants. An antioxidant is a molecule that

slows or prevents the oxidation of the molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants considered as reducing agents such as thiols, ascorbic acid.[6]

A few studies have reported the antioxidant activity and other pharmacological activities on the common *Ficus* sp., which include *Ficus auriculata* Lour, [4,19] *Ficus benghalensis* L,[10,13,21] *Ficus religiosa* L [14], *Ficus microcarpa* L.[3] and *Ficus. racemose* L,[9,13] *Ficus carica* L[17,5,2,9,6]. However, the antioxidant activity and profiling of phenolic compounds of most of the *Ficus* species have remained unexamined and lack extensive documentation. Most of the researchers investigated the antioxidant activity, phenolic content, and others bioactivities on the leaves of *Ficus* species.[9,15,1] On the contrary, few studies have focused on fruits,[18,10,17] barks[13,19] and others parts[7,21] of the *Ficus* sp., investigating a low number of species or from concrete regions. However, some studies suggest that leaves exhibited higher phenolic content and antioxidant capacity in comparison to other parts of the *Ficus* species, due to the presence of phenolic compounds.[5,2] So, this study aims to determine the antioxidant activity of methanol extracts of *Ficus elastica* L. leaves which have been extracted in an acidic environment.

## II. MATERIAL AND METHOD

### A. Procedure

#### Sample preparations

*F. elastica* plants obtained from Pamarican, Ciamis district, then the determination made at the Herbarium of the School of Biological Science and Technology, Bandung Institute of Technology. Plant parts used are leaves. The leaves are washed thoroughly and then dried by wind. Dried leaves are blended to get Simplicia powder.

#### Characterization of Simplisia *F. elastica*

Raw extraction material characterization is based on examination procedures listed on the Indonesian Herbal Pharmacopoeia including macroscopic examination organoleptically (smell, taste, color and shape), microscopic examination with using a microscope to see the typical fragments on the leaves of *F. elastica*, water-soluble extracts,

ethanol-soluble extracts, total ash content and acid insoluble ash content, water content, and drying shrinkage.

#### Extraction procedures of Antioxidants

Two hundred fifty grams of *Simplicia* were soaked using n-hexane solvent first to remove the waxy coating on *F. elastica* and other fats. The pulp is dried and macerated using methanol in an acidic atmosphere by adding 2N HCl to pH two at room temperature for 3x24 hours.

#### Phytochemical screening of *F. Elastica* leaves

Phytochemical screening was carried out to determine the class of compounds contained in the crude material of *F. elastica* powder and methanol extract of leaves of *F. elastica* L., the contents examined were flavonoid compounds, polyphenols, tannins, saponins, quinones, alkaloids, steroids, triterpenoids, monoterpenoids, and sesquiterpenoids.

#### Total antioxidant capacity

##### DPPH free radical scavenging assay

##### Preparation of DPPH Master Solution

DPPH solution 500 µg/mL made by weighing as much as 50 mg DPPH powder dissolved in methanol p.a to a volume of 100 mL. Pipette as much as 1 mL and add methanol p.a to 5 mL, so that a concentration of 100 µg/mL obtained.

##### Determination of Maximum DPPH Wavelength

Solution DPPH 100 µg/mL input into the cuvette, then the wavelength is determined using UV-Vis Spectrophotometry in the wavelength range of 400-800 nm.

##### Making Vitamin C Master Solution

50 mg of vitamin C dissolved in 100 mL methanol p.a. To obtain a concentration of 500 µg/mL (master solution) and from the master solution, a dilution made into a series of concentrations.

##### Preparation of Samples of the Master Extract

The acidified *F. elastica* leaf methanol extract weighed as much as 50 mg. Dissolved with 100 mL methanol up to the mark so that a concentration of 500 µg/mL (master solution) obtained. From the mother liquor, dilution made into several series of concentrations. *F. elastica* leaf methanol extract made 5, 6, 7, 8, 9 and 10 µg/mL.

##### Determination of Operating Time

Operating time is carried out utilizing 1 mL of the standard solution of vitamin C added 1 mL of DPPH 100 µg/mL solution then shaken, and the absorbance observed at the maximum wavelength obtained from the time interval of 5 minutes for 60 minutes to obtain a stable absorbance. Repeated for the Operating time of the acidified methanol extract of leaves of *F. elastica* L. leaves.

#### Determination of Vitamin C Antioxidant Activity and *F. elastica* methanol extract

1 mL of acidified methanol extract of leaves of *F. elastica* and Vitamin C with various concentrations added 1 mL of 100 µg/mL DPPH solution, then shaken strongly. The mixture of the solution stored in a dark place during operating time. Then the absorbance is measured at the maximum wavelength of DPPH absorption using UV Visible spectrophotometry.

#### B. Data Analysis

##### IC<sub>50</sub> Value Determination of Leaf Methanol Extract *F. elastica*

The antioxidant activity of the sample and comparison is determined by the amount of DPPH radical uptake resistance by calculating the percentage of DPPH absorption inhibition using the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance DPPH} - \text{Absorbance sample}}{\text{Absorbance DPPH}} \times 100$$

Percent value of damping obtained made curve towards the concentration of the test or comparison solution. The calculation results entered into the linear regression equation with the concentration of the sample as the x-axis and the value of antioxidant damping as the y-axis. From the linear regression equation, IC<sub>50</sub> values obtained. The IC<sub>50</sub> value is the extract concentration of *F. elastica* leaves that can reduce or inhibit radicals by 50%. The IC<sub>50</sub> value determined from the relationship curve between the percent attenuation to the sample. The stronger the antioxidant activity of the sample, the smaller the IC<sub>50</sub> value..

### III. RESULTS

The results of the determination show that the sample used *F. elastica* plant with the name of the species *Ficus elastica* L. *F. elastica* leaf extraction in this study was carried out by the maceration method using n-hexane solvent first to remove the waxy coating on *F. elastica* leaves and other fats. The pulp is dried and then maceration using methanol as a solvent in an acidic atmosphere, by adding HCl 2 N to pH 2 (hydrolysis process). Hydrolysis is carried out to break the glycoside bonds between the sugar component (glycone) and secondary metabolites (aglycone). The catalyst used is HCl 2 N to accelerate the hydrolysis reaction. HCl is a strong acid that easily releases H<sup>+</sup> ions completely in water. The more H<sup>+</sup> protons released, the easier it is to release glycoside bonds. The recovery yield of the hydrolysis of the *F. elastica* Leaf extract was 53.56%.

##### The Characterization of *Simplicia F. elastica*

Microscopic examination results of *F. elastica* leaves can be seen in Figure 1.



Figure 1. *F. elastica* Leaf Fragments (a) parasitic type stomata (b) hair covering (c) parenchyma (d) upper epidermis.

*Ficus elastica* Lour leaves have an elongated shape; the tip is taper with a flat edge with a reddish-green top surface, a reddish underneath surface, odorless and tasteless. The results of simplicia quality inspection and methanol extract of *F. elastica* leaves can be seen in table 1.

**Table 1.** THE CHARACTERIZATION OF SIMPLICIA AND EXTRACT METHANOLIC OF *Ficus elastica* LOUR

Parameter	Characterization	
	Simplicia (%)	Extract (%)
Water Content	2 ± 0	12 ± 0
Drying Shrinkage	6,06 ± 0.0251	34,45 ± 0.1216
Total Ash Content	8,03 ± 0.0321	8,83 ± 0.1258
Acid Soluble Ash Content	1,36 ± 0.0173	1,76 ± 0.1365
Water Soluble Ash Content	3,12 ± 0.1442	4,58 ± 0.0734
Water-soluble extracts	18,58 ± 0.2145	-
Ethanol-soluble extracts	12,77 ± 0.1357	-

Screening of Phytochemical

**TABLE 2.** THE RESULT OF PHYTOCHEMICAL SCREENING OF *F. elastica* LEAVES

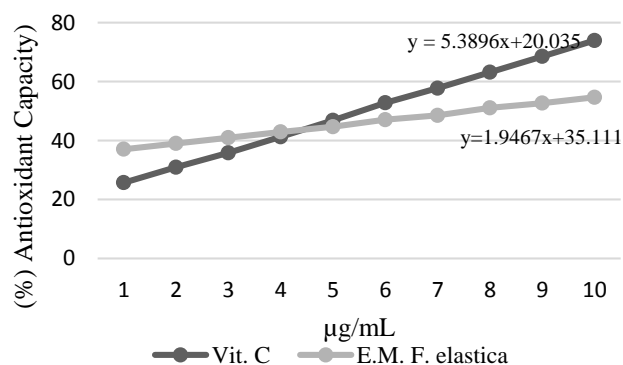
Compound	Simplicia	Extract
Alkaloids	-	-
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+
Monoterpenoids and Sesquiterpenoid	+	+
Steroid Triterpenoid	+	-
Kuinon	+	+
Saponin	+	+

Qualitative Antioxidant Tests Using The Thin Layer Chromatography (TLC) Method

The qualitative test of antioxidant activity on *F. elastica* leaf extract was carried out by the DPPH spray method. The results of thin layer chromatography using silica gel GF254 stationary phase and a mixture of n-hexane and chloroform mobile phases (5: 5) marked by the formation of stains observed n UV light λ 254, λ 366 nm. Thin Layer Chromatography result was then sprayed using a 0.2% DPPH solution in methanol. Chromatogram results after being sprayed with a DPPH of 0.2% will show yellow spots on a purple background. This change indicates that the acidified red rubber leaf extract has a role as an antioxidant that can reduce DPPH free radicals to a reduced form of DPPH-H. Following are the results of the chromatogram pattern of *F. elastica* leaf extract which acidified before and after spraying DPPH 0.2% performance of methanol.

Antioxidant Activity

Based on the measurement results of a 100 ppm DPPH solution obtained maximum absorption is at a wavelength of 516 nm. This wavelength then used in the process of measuring the reduction of extracts and comparing vitamin C to DPPH radicals. Before testing the antioxidant activity of the sample solution, operating time is first performed to determine the stability of the reaction time of the test solution with a radical DPPH solution so that the incubation time can be determined. The incubation time needed for the sample to react completely with different DPPH, for vitamin C requires 15 minutes of incubation time, while for the acidified red rubber leaves methanol extract is 25 minutes. Obtained linear regression equation from leaf methanol extract *F. elastica* is  $y = 1.9467x + 35,111$  with  $r^2$  0,9935. While vitamin C is  $y = 5.3896x + 20.035$  with  $r^2$  0.9989. Test results of antioxidant activity of leaf methanol acidified extract *F. elastica* in Figure 2.



**Figure 2.** Comparison of Antioxidant Capacity

**Table 3.** THE IC50 VALUE OF ACIDIFIED METHANOL EXTRACT OF *Ficus elastic L.* AND VITAMIN C

Sample	IC <sub>50</sub> (µg/mL)	Intensity of IC <sub>50</sub> Value
Acidified methanol Ekstrakt of <i>F. Elastica</i>	7.6906±0.3	Sangat kuat
Vitamin C	5.6109±0.5	Sangat kuat

IV. DISCUSSION

The choice of solvent and extraction method can influence the antioxidant activity. The extraction yield between 100% methanol (C 100) and acidified methanol (CA) also shows a slight difference in yield percentage, where CA produces an extra 3.5% of C 100. Acidified methanol produces extra yield compared to 100% methanol alone due to the combination methanol and acid solvents, where acids can destroy cell membranes and thus dissolve and stabilize more phenolic compounds especially anthocyanins [22]. The polarity of solvents really plays an important role in increasing the solubility of phenols [14]. Methanol was extracted extra phenolic compounds compared to ethanol and water. Methanol and ethanol have similar polarity but give different results, where ethanol is less efficient than methanol. This is because ethanol has a low solvent antioxidant compound caused by the

presence of longer ethyl radicals than methyl radicals in methanol [19].

## V. CONCLUSION

*Ficus elastica* Lour. demonstrated a very good potential property of antioxidants. The extract of acidified methanol has shown highly antioxidant potential.

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