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Research Article

TOTAL FLAVONOIDS CONTENT IN ACIDIFIED EXTRACT OF FLOWERS AND LEAVES OF GARDENIA (*GARDENIA JASMINOIDES* ELLIS)

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ABSTRACT

Background: Gardenia (*Gardenia jasminoides* Ellis) contains secondary metabolites which responsible for pharmacological activities such as flavonoids.

Objective: The aims of this study were to determine the total flavonoids content (TFC) in the flowers and leaves of gardenia (*G. jasminoides* Ellis) which macerated with acidified ethanol.

Method: The method was the colorimetric of a colored complex between flavonoid with aluminum chloride.

Results: The results showed that the highest TFC in the flowers (0.078% w/w) was obtained in a mixture solvent of ethanol and acetic acid, whereas in the leaves (0.090% w/w) was obtained in ethanol.

Conclusion: There is no correlation between acidified ethanol and TFC in gardenia flowers and leaves.

Keywords: Maceration, Acidified ethanol, Colorimetric, Aluminum chloride.

INTRODUCTION

Gardenia (*Gardenia jasminoides* Ellis, family Rubiaceae) has shiny green leaves and heavily fragrant white summer flowers. It was used as analgesic, diuretic, larvicide, antihypertensive, antibacterial, anxiolytic, antiplasmodial, antipyretic, treatment of headaches, anti-inflammatory, treatment of hepatic disorders, conjunctivitis, jaundice, epistaxis, hematemesis, pyogenic infections, and skin ulcers [1-3]. Chemical constituents of gardenia are flavonoids, saponins, tannins, steroids, and terpenoids [4].

There is increasing interest to explore the therapeutic potential of phenolic compounds, especially flavonoids. Biological activities of flavonoids in human are antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral activity [5]. The general technique for flavonoids quantification is based on the spectrophotometric determination of complex flavonoid and alumunum chloride, which provides a bathochromic displacement and the hyperchromic effect [6]. This technique was developed for analysis of O-glycoside flavonoids in herbal materials, which need acid hydrolysis and an organic solvent for extraction, then quantified as a complex between flavonoid aglycones and alumunum chloride [7]. The aims of this study were to determine the total flavonoids content (TFC) in the flowers and leaves of gardenia which macerated with acidified ethanol.

METHODS

Materials

The flowers and leaves of gardenia (*G. jasminoides* Ellis) were obtained from Research and Experimental Gardens, West Java, Indonesia, and identified by the Laboratory of Plant Taxonomy, Padjadjaran University, Indonesia. All chemicals were analytical grade (Merck, German).

Methods

Loss on drying

A weighed sample (5 g) was dried on 105°C at atmospheric pressure for 5 hrs, then weighed. Drying and weighing continued with a distance of 1 hr, until a constant weight [8].

$$\text{Loss on drying}(\%)=\frac{\text{Initial weight}-\text{final weight}}{\text{Initial weight}}\times 100$$

Flavonoids identification

A weighed sample (1 g) was extracted with 10 ml of 70% ethanol, then filtered [9].

a. Shinoda test: A few fragments of magnesium ribbon and concentrated hydrochloric acid were added to the ethanolic extract. The appearance of red to pink color after few minutes indicates the presence of flavonoids.
b. Ferric chloride test: A few drops of neutral ferric chloride solution were added to the ethanolic extract. Formation of blackish green color indicates the presence of phenolic compounds.
c. NaOH test: A few drops of sodium hydroxide solution were added to the ethanolic extract. An intense yellow color which disappeared after adding dilute HCl indicates the presence of flavonoids.

Extraction

Five weighed sample (500 mg), each sample was added to 5 ml of 96% ethanol and 96% acidified ethanol (pH 1.0), i.e. hydrochloric acid, sulfuric acid, nitric acid, and acetic acid. The sample was extracted by reflux for 5 minutes, then filtered. The residue is re-extracted with 5 ml of the same solvent. Extracts were poured in a 10 ml volumetric flask and rounded up to the mark with the same solvent.

Determination of maximum wavelength

The extract (1 ml) was added 2 ml of 5% $AlCl_3$ in 10 ml volumetric flask, and rounded up to the mark with distilled water. The mixture was incubated for 15 minutes. The absorbance was measured at 300-500 nm. The same sample without $AlCl_3$ was used as a blank solution [10].

Quantification of TFC

The extract (1 ml) was added 2 ml of 5% $AlCl_3$ in 10 ml volumetric flask, and rounded up to the mark with distilled water. The mixture

was incubated for 15 minutes. The absorbance was measured at the maximum wavelength. The same sample without $AlCl_3$ was used as a blank solution. TFC was calculated as rutin with a modified formula [10].

$$TFC(\%w/w) = \frac{A \times DF}{A_{1\,cm}^{1\%} \times (w \times (1 - ld))}$$

Where, A = absorbance, DF = dilution factor, $A_{1\,cm}^{1\%}$ = specific absorption for rutin-$AlCl_3$ complex (259.4), w = mass of s ample (g), ld = loss on drying.

Statistical analysis

Data analysis was conducted with R software. TFC in flowers and leaves were analyzed using Pearson's correlation.

RESULTS

The samples were yellowish-white flowers and dark green leaves. Loss on drying was 92.28% for flowers, and 91.76% for the leaves.

The correlation between solvent variation and TFC has a p=0.1006 and rho=0.8046 for the flowers, and p=0.5455 and rho=−0.3653 for the leaves.

DISCUSSION

The samples were fresh plants, so the loss of drying was high. Loss of drying should be determined because it affects the sample weight was weighed and the calculation of the TFC.

All flavonoid identification was showed positive results (Table 1). $FeCl_3$ test was the initial test to detect the presence of phenolic compounds, to formed a blackish green complex ions $(Fe(OAr)_6)^{3-}$. Shinoda test was detected the presence of flavan-3,4-diol groups, flavanones, or isoflavones. In the Shinoda test, strong acid was hydrolyzed the glycoside-flavonoid to aglycone-flavonoid, then forma red or orange complex with magnesium. Shinoda's test results for leaves were yellow, which indicates the flavones or isoflavones [11].

Acidified ethanol (pH 1.0) was used for flavonoids extraction optimally [12], which expected can be monitored from extract color (Table 2). Various color extracts were difficult to estimate the TFC, so need quantified by spectrophotometer.

The maximum wavelength differences (Table 3) were caused by heterocyclic substituents containing oxygen and the hydroxyl group distribution. The oxidation differences at the 3-C atom determine the properties and flavonoids types. The differences in the substitution and hydroxylation pattern at the 3-C atom also determine the classification is flavones, flavanones, flavonols, flavonons, isoflavones, aurons, and chalcones [12].

Acidified ethanol (hydrochloric acid, sulfuric acid, nitric acid, and acetic acid) as the solvent was affecting the TFC (Table 4). The highest TFC (0.0785±0.0011% w/w) in flowers was obtained by acidified ethanol with acetic acid. It was suggested because of the flavonoids form was flavonoid O-glycosides, which can be hydrolyzed by acid. The highest TFC (0.0897±0.0001% w/w) in leaves was obtained by ethanol. It was suggested because of the flavonoids form was flavonoid C-glycosides, which cannot be hydrolyzed by acid. The flavonoid-$AlCl_3$ complex formation was influenced by the reaction time, concentration of $AlCl_3$, flavonoids content in the sample, and the chemical structure of polyphenols [7].

The strong acidified ethanol (hydrochloric acid, sulfuric acid, and nitric acid) did not produce high TFC. It was suggested that strong acid hydrolyzed the glycosidic bonds and covalent bonds in flavonoids during the maceration, so the flavonoid degraded into fragments which do not react with $AlCl_3$ [12]. The weak acidified ethanol, i.e. acetic acid, produced the highest TFC in the flowers. It was suggested that weak acid only hydrolyzed the glycosidic bonds in flavonoids during the maceration, so the aglycones flavonoid were reacted with $AlCl_3$ to form the complex flavonoids-$AlCl_3$.

Statistical results showed that there is no correlation between solvent variation and TFC in the flowers (rho=0.8046), and between solvent variation and TFC in the leaves (rho=−0.3653). This is because the flavonoids types in the flowers and leaves are different, suggested the flavonoid O-glycoside in the flowers and flavonoid C-glycosides in the leaves. The strong acid also causes the flavonoids degradation, so the flavonoid fragments cannot react with $AlCl_3$.

Table 1: Flavonoid identification

Test	Color	
	Flowers	Leaves
Shinoda	Red brick	Dark yellow
$FeCl_3$	Blackish green	Blackish green
NaOH	Colorless	Colorless

Table 2: Extract color

Solvent	pH	Extract color	
		Flowers	Leaves
Ethanol	5.0	Light yellow	Light green
Ethanol: HCl	1.0	Dark green	Dark green
Ethanol: H_2SO_4	1.0	Dark green	Dark green
Ethanol: HNO_3	1.0	Dark yellow	Dark yellow
Ethanol: CH_3COOH	1.0	Light yellow	Light yellow

Table 3: Maximum wavelenght (λmax) and absorbance of complex

Solvent	Flowers		Leaves	
	λmax (nm)	Absorbance	λmax (nm)	Absorbance
Ethanol	345.0	0.303±0.001	364.0	0.959±0.002
Ethanol: HCl	406.5	0.383±0.003	400.5	0.312±0.001
Ethanol: H_2SO_4	414.0	0.743±0.013	403.0	0.483±0.007
Ethanol: HNO_3	393.5	0.507±0.001	396.5	0.589±0.012
Ethanol: CH_3COOH	391.0	0.786±0.011	411.0	0.546±0.006

Values are mean±SD (n=3). SD: Standard deviation

Table 4: TFC

Solvent	TFC (percentage w/w)	
	Flowers	Leaves
Ethanol	0.0302±0.0001	0.0897±0.0001
Ethanol: HCl	0.0382±0.0003	0.0292±0.0001
Ethanol: H_2SO_4	0.0742±0.0013	0.0452±0.0006
Ethanol: HNO_3	0.0507±0.0001	0.0551±0.0011
Ethanol: CH_3COOH	0.0785±0.0011	0.0511±0.0006

Values are mean±SD (n=3). SD: Standard deviation,
TFC: Total flavonoids content

CONCLUSION

There is no correlation between acidified ethanol and TFC in gardenia flowers and leaves.

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