ORIGINAL ARTICLE

Phenolics compounds, flavonoids, and antioxidant activity methanol extract of arum manis leaves (*Mangifera indica* L. Var. Arumanis)

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Abstract

Aim: Arumanis is one of Indonesian mango varieties that have traditionally been used to prevent various non-infectious diseases such as diabetes, cancer, and other degenerative diseases. The active ingredients contained in Arumanis leaves (*Mangifera indica* L. Var. Arumanis) have the ability to stabilize free radicals which is one of the factors causing these diseases. **Materials and Methods:** In this study, the extract was used to validate the potential of this plant as a source of natural antioxidants. The antioxidant activity of the extract was expressed as inhibitory concentration (IC_{50}), whereas total phenolic and flavonoid contents were each determined using the Folin–Ciocalteu method and Chang method. **Results and Discussion:** The results showed that Arumanis leaves (*M. indica L*. Var. Arumanis) had an IC_{50} value of 48.458 µg/mL. Total phenolic obtained was 1280 mg of gallic acid/g from the extracted compound, and the total flavonoid contents obtained was 1240.1 mg expressed in terms of quercetin equivalent. **Conclusion:** Research data showed that methanol extract of Arumanis leaves (*M. indica* L. Var. Arumanis) was significantly able to free radicals scavenging which correlated with high phenolic and flavonoid contents.

Key words: Flavonoids, free radicals, *Mangifera indica,* phenols

INTRODUCTION

In Indonesia, natural resources in the form of plants have long been used for various purposes, both for daily needs and for medical treatment and health maintenance.[1,2] Traditional medicine is still widely practiced and maintained by Indonesian people because besides being a cultural heritage it also aims to apply the back to nature principle, so this kind of medicine becomes more popular.[3] The development of traditional medicine is now also increasing as more and more new problems are found due to the use of chemical drugs, especially in terms of their side effects.[4]

Indonesia's natural wealth, with approximately 30,000 types of plants and 940 species of which are medicinal plants, makes Indonesia the largest source of medicinal plants in the world. In addition, Indonesia's tropical climate greatly allows various plants to live in fertility. One of Indonesia's natural wealth that can be used as a treatment is *Arumanis* mango (*Mangifera indica* L. Var. Arumanis).[5,6]

Fresh Arumanis mango leaves contain bioactive compounds such as alkaloids, tannins, flavonoids, and phenolics.[7] Methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) has an effect in reducing pain.[8] In addition, Arumanis leaves (*M. indica* L. Var. Arumanis) are also efficacious as antidiabetic, anti-inflammatory, antitumor, antioxidant, antimicrobial, and as imunostimulan.[9] Other studies also stated that the ethanol extract of Arumanis mango leaves can provide an analgesic effect at a dose of 200 mg/kg BB, the anti-inflammatory and antimicrobial effects.[10] Mango flesh and skin have effects as a very strong antioxidant and an antiproliferative.[11] The chemical compound in arum manis is also very much determined by

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the amount of the active substance itself, the solubility and the type of the solvent used. Several studies have shown that the use of different solvents gives different results, especially for antioxidant effects.[12]

Antioxidants found in plants, especially in Arumanis mango leaves, can neutralize free radicals by donating a number of electrons needed to stabilize free radicals. Once free radicals are stable due to receiving electrons from active substances found in plants, these free radicals will become unreactive to cellular DNA.[13] Based on the foregoing, this study was conducted to characterize and evaluate the total phenolic, flavonoids contents, and antioxidant capacity of methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) *in vitro*.

MATERIALS AND METHODS

Chemicals and Standards

Gallic acid, quercetin, Folin–Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1- picrylhydrazyl (DPPH) from Sigma Chemicals, sodium nitrate, aluminum chloride, sodium hydroxide, methanol, and distilled water were used.

Collection and Preparation of Plant Material

Fresh Arumanis leaves (*M. indica* L. Var. Arumanis) were obtained from Simarasok area of Agam Regency, West Sumatra Province, Indonesia. *M. indica* L. Var. Arumanis of vouchered herbarium specimen was prepared and preserved to the Department of Pharmacognosy, Dwi Farma Academy of Pharmaceutical, Bukittinggi, Indonesia.

50 g of fresh Arumanis mango leaves (*M. indica* L. Var. Arumanis) were extracted using maceration method with methanol as the solvent for 72 h a day at room temperature. The residues were extracted twice with the same fresh solvent. The extracts were separated from the residues by filtering first through several layers of muslin cloth for coarse filtration and then through Whatman No. 1 filter paper. The filtered extracts were concentrated and solvents were evaporated under reduced pressure at 40°C, using a rotary evaporator. The dried crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator (−8°C), until it was used for analyses.[14]

Scavenging Activity (DPPH) Assay

The free radical scavenging activities of the extracts were determined using DPPH free radical scavenging method according to Molyneux, 2004.[15] DPPH in oxidized form gives a deep violet color in methanol. An antioxidant compound donates the electron to DPPH, thus causing its reduction and in reduced from its color changes from deep violet to yellow. Diluted sample (0.2 mL) and DPPH working solution (50 µM) were added to a microcentrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature (23°C). The absorbance was then measured against methanol at 515 nm in 3 mL cuvettes using a spectrophotometer. The decrease in absorbance of a sample was calculated in comparison to a blank sample (0.2 mL methanol and 3.8 mL DPPH). The relative decrease in absorbance was then calculated as follows:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control})] \times 100$$

Determination of Total Phenolic Contents (TPCs)

The TPCs of the extracts were determined by the Folin–Ciocalteu colorimetric method, described by Singleton *et al.*, with modifications.[16] The extract samples, appropriately diluted, were mixed with the Folin–Ciocalteu reagent. After 8 min, the solution of 10% sodium carbonate (Na_2CO_3) was added and the tubes were kept in the dark at room temperature (approximately 23°C) for 2 h. After this time, the absorbance was determined at 765 nm in a spectrophotometer and compared with a calibration curve of gallic acid $\hat{Y} = 0.6225 + 0.004975x$, $R2 = 0.971$. The results were expressed as gram of gallic acid equivalent (GAE) per kilogram of dry sample (mg/g GAE of extracted compound).

Determination of Total Flavonoid Contents (TFCs)

The TFCs were determined using the colorimetric method described by Zhishen *et al*. with modifications.[17] Aluminum chloride complex forming assay was used to determine the TFCs of the extracts quercetin was used as standard and flavonoid contents were determined as quercetin equivalent (QE). A calibration curve for quercetin was drawn for this purpose. From the standard two quercetin solution, the dilutions of (100, 150, 200, 250, and 300 mg/mL) concentrations were prepared in methanol. 0.8 mL of each of the quercetin dilution was mixed with 5 mL of distilled water and then with 0.3 mL of 5% sodium nitrate and allowed to stand for 6 min. Then, 0.3 mL of 10% aluminum chloride solution was added and allowed to stand for 5 min after which 4 mL solution of 4% sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on UV spectrophotometer. TFC was calculated as QE (mg QE/g).

RESULTS AND DISCUSSION

Determination of Antioxidant Activity

The extraction of Arumanis mango leaves (*M. indica* L. Var. Arumanis) was performed by maceration method using methanol solvent. The maceration method is chosen because it can extract compounds well and can prevent

the decomposition of unstable compounds against heating. The principle of extraction by maceration is the diffusion of solvent into plant cells that contain active compounds which cause osmotic pressure in cells to be different from the outside conditions. The active compounds are then pushed out due to osmotic pressure inside and outside the cells.[18]

Phytochemical screening of extracts was performed using a test tube, i.e., reacting the sample with a specific reagent solution to determine the secondary metabolite contents.[19] In Table 1, the results of the phytochemical screening of methanol extract of *Arumanis* mango leaves (*M. indica* L. Var. Arumanis) are shown in Table 1.

Based on the results of phytochemical screening examination, it was found that methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) contained secondary metabolites, namely alkaloids, flavonoids, phenolic, and tannin.

Antioxidant activity testing on methanol extract of Arumanis leaves (*M. indica* L. Var. Arumanis) was performed using DPPH free radical scavenging method. The principle of this free radical scavenging method is the measurement of synthetic free radicals capturing in polar organic solvents such as methanol at room temperature by a compound that has antioxidant activity.

The results of the reaction between DPPH free radical scavenging by antioxidant compounds can be known through DPPH color changes from dark purple to yellow. DPPH color changes are caused by the resonance structure of DPPH free radical scavenging structures. The decrease in color intensity that occurs is related to the electron numbers of DPPH free radical scavenging that captures hydrogen atoms from antioxidant compounds. This color change from DPPH is also one of the bases for the measurement on UV-visible spectrophotometers.

DPPH radical scavenging activity assay aims to assess the ability of DPPH as a stable free radical and when it reacts with its stability antioxidant compounds it will decrease and change to diphenylpicryhidrazine compounds. Free radical, initially, is in purple color, in the presence of antioxidants will turn into light yellow.[20]

Free radical scavenger provides strong absorption at wavelength of 515 nm. Methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) at the small concentrations tested, i.e., 2.5 µg/mL, 5 µg/mL, 7.5 µg/mL, 10 µg/mL, and 12.5 µg/mL, experienced color changes from purple to yellow after incubation for 30 min at room temperature. After incubation, absorbance measurements were then performed using a spectrophotometer at a wavelength of 515 nm. The absorbance value obtained was used to calculate the value of antioxidant activity of the sample against DPPH (IC_{50}). The antioxidant activity data of methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) can be shown in Table 2.

Table 2 shows that there is a decrease in absorbance values ranging from 0.761 to 0.730 for each increase in the extract concentration. Scavenging activity of Arumanis mango leaves (*M. indica* L. Var. Arumanis) occurs due to the presence of polyphenols and flavonoids. The antioxidant activity of polyphenols and flavonoids can be seen and is known from the decrease in DPPH radical absorbance values at various concentrations and the increase in inhibition percentage values produced. Visually, antioxidant activity can also be observed with the occurrence of DPPH purple color changes to yellow after 30 min of incubation.[21] The value of the activity of free radical reduction is expressed as IC_{50} which is the amount of concentration of the test compound which can reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging activity.[22]

The IC_{50} values of methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) were obtained based on the calculation results of the linear regression equation. In Table 2, the linear regression equation obtained is $\hat{y}$=2.259+0.9852x and r=0.981. The coefficient of y in

Table 1: Results of phytochemical screening

Secondary metabolites	Reagent	Information
Alkaloids	Mayer and Dragendorff	+
Flavonoids	HCl and Mg	+
Tannin	$FeCl_3$	+
Phenolic	$FeCl_3$	+
Saponin	Aquades	-

Table 2: Antioxidant activity of methanol extract of Arumanis leaves

Concentration (µg/mL)	Absorbance	% inhibition	Equation ($\hat{y}$ = a+bx)	IC_{50} (µg/mL)
Blanko	0.785	0	$\hat{y}$=2.259+0.9852x r=0.981	48.458
2.5	0.761	3.015		
5	0.751	4.331		
7.5	0.742	5.478		
10	0.736	6.285		
12.5	0.730	6.964		

the equation is calculated as the value of IC_{50}, while the coefficient x is the concentration of the extract that the values will be determined. The value of x obtained is the amount of concentration needed to reduce 50% of DPPH free radicals. The value of r=0.981 which is approximately +1 (positive) illustrates the existence of a linear relationship between extract concentration and antioxidant activity. Increased extract concentration will be followed by the increase in antioxidant activity. This can be seen from the relationship curve of methanolic extract concentration of Arumanis mango leaves (*M. indica* L. Var. Arumanis) against percentage of inhibition as shown in Figure 1.

Increases in the absorbance values in the extract indicate that the extract concentration added affects the ability of the extract to absorb free radicals. The percentage of inhibition of free radical activity will increase along with the increasing concentration. The extract shows high phenol levels so that it has strong antioxidant activity. Phenol compounds extracted from methanol are thought to be phenol compounds that have function as antioxidant. The number of phenolic compounds and molecular structures plays an important role in antioxidant activity. IC_{50} value of methanol extract of Arumanis mango leaves (*M. indica* L. Var. Arumanis) based on the results of calculation was 48.458 µg/mL. A substance with IC_{50} values between <50 µg/mL is categorized as very strong antioxidant. Antioxidant activity will increase with the increasing hydroxyl groups and will decrease in the presence of glycoside groups.[23]

The hydroxyl (-OH) groups in phenol compounds bind directly to aromatic hydrocarbon groups.[24] Antioxidant activity of phenol compounds is formed because of the ability of phenol compounds to form phenoxide ions which give one electron to free radicals as shown in Figure 2.

Antioxidants of phenol compounds (FI-OH) react with free radicals (FI-OH•) to form ROOH and a phenol radical (FI-OH•) which is relatively unreactive. Furthermore, phenol radical compounds (FI-OH•) can react again with free radicals (ROO•) to form compounds that are not radical. DPPH is a free radical compound capable of reacting with compounds that can donate hydrogen atoms. Polyphenol compounds in methanol extract of Arumanis mango leaves (polyphenols/PhH) donate hydrogen atoms to DPPH, causing DPPH to experience a change in color intensity.[26]

Determination of Total Phenolic Compounds

One of the natural antioxidants is gallic acid (3,4,5-trihydroxybenzoic acid) which is a phenolic compound and has strong antioxidant activity. Determination of TPCs can be done using Folin–Ciocalteu reagent based on the reduction strength of phenolic hydroxy groups. All phenolic compounds including simple phenols can react with Folin–Ciocalteu reagent. The presence of aromatic nuclei found in phenol compounds (phenolic hydroxy groups) can reduce phosphomolybdate-phosphotungstate and change it into blue molybdenum. The TPCs in plants are expressed in GAE, namely the equivalent amount of milligrams of gallic acid in 1 g of sample.[27,28] The results of calibration of gallic acid calibration on Folin–Ciocalteu reagent can be shown in Figure 3.

TPCs were calculated based on of GAE (mg GA/g extract) using the regression equation $\hat{Y}$=0.6225+0.004975x with a value of R^2=0.971 means that 97.1% of absorbance is influenced by concentration, while the rest is influenced

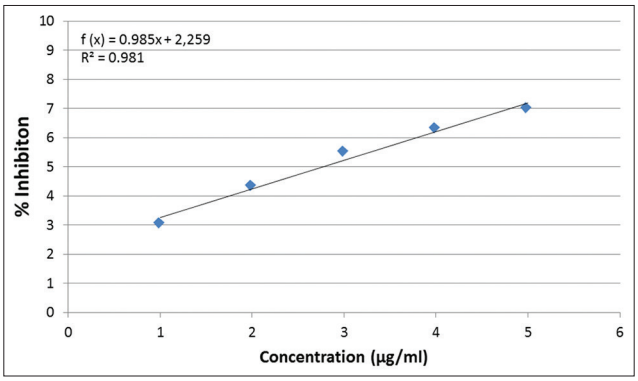

Figure 1: Linear regression curve of methanol extract of Arumanis mango leaves

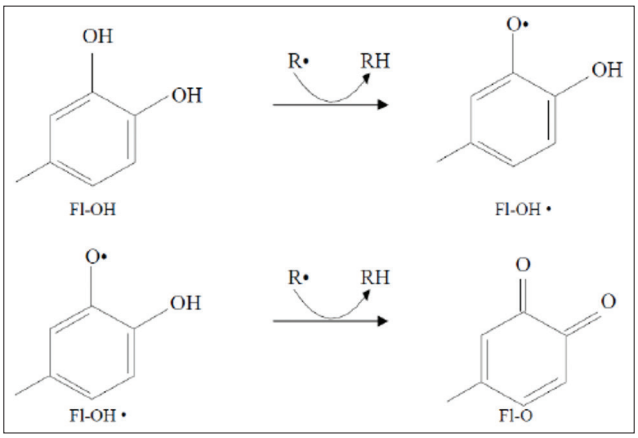

Figure 2: Mechanism of radical absorbance capacity by flavonoids.[25]

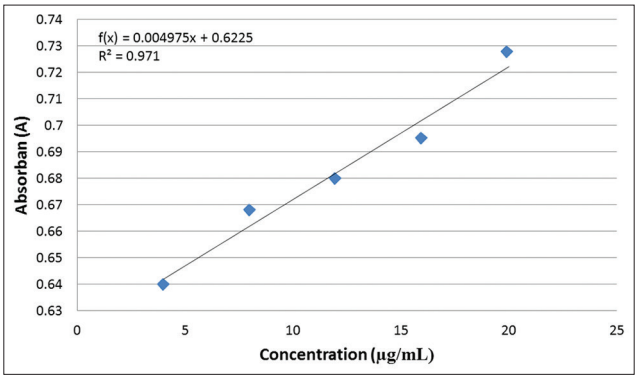

Figure 3: Calibration curves of gallic acid in reagent Folin–Ciocalteu

by other factors such as temperature, light, storage, and chemicals.

Phenolics in fruits and vegetables have attracted a lot of attention because of their potentials for antioxidant activity. Phenolic compounds experience a complex redox, a reaction with phosphotungstic acid and phosphomolybdate present in Folin–Ciocalteu reagents. However, some chemical groups of amino acids, proteins, organic acids, sugars, and aromatic amino acids can react with reagents so it affects observations.[20] One method to minimize this effect is using drying process. Drying process aims to remove ascorbic acid, protein, and sugar which can interfere with the withdrawal of active substances.

The results showed that Arumanis mango leaves (*M. indica* L. Var. Arumanis) have high TPCs of 1280 mg/g EAG, using a standard curve of gallic acid (R^2=0.971). This gives an illustration that the polar compounds contained in Arumanis mango leaves (*M. indica* L. Var. Arumanis) can be dissolved well in methanol.[29] The total phenol contents in the sample were determined by the Folin–Ciocalteu method based on the ability of phenolic compounds in the extract to react with the phosphomolybdate-phosphotungstate acid contained in Folin–Ciocalteu reagent. This reaction produces a blue molybdenum-tungstate compound. The higher the intensity of the blue solution, the greater the total phenol contents in the sample.

The reaction between phenolic compounds with Folin–Ciocalteu reagent occurs only in alkaline conditions. To make the alkaline condition, 10% of Na_2CO_3 was used so that the protons contained in phenolic compounds can dissociate into phenolic ions. In alkaline conditions, hydroxyl groups in phenolic compounds react with folin reagents to form a blue complex with structures that are unknown and can be detected by a spectrophotometer. The blue color formed is directly in line with the concentration of phenolic ions formed.

Determination of Total Flavonoid

Determination of total flavonoid levels on Arumanis mango leaves (*M. indica* L. Var. Arumanis) was performed by the modified, Zhishen, 1999, method using routine as standard solution at a wavelength of 415 nm. Routine standard solution was mixed with aluminum trichloride and sodium acetate as special reagents that form yellow complexes. Blank measurements are carried out without the addition of aluminum trichloride [Figure 4].[30]

From the making of the calibration curve, the linear regression equation $\hat{Y}$=0.5954+0.0002x was obtained. From the regression equation, total flavonoid level obtained from *Arumanis* mango leaf extract (*M. indica* L. Var. Arumanis) of 1240.1 mgQE/g was calculated as quercetin. Furthermore, data validation parameters are calculated which are useful to prove that these parameters are eligible in their use.

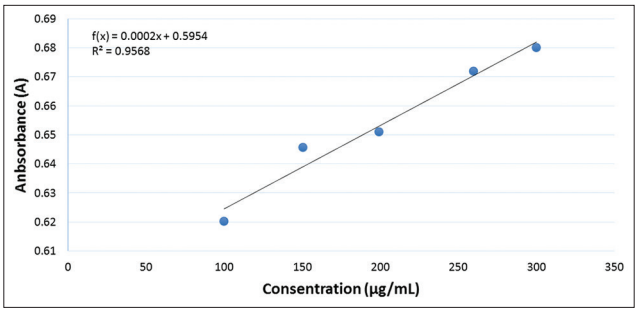

Figure 4: Standard curve of quercetin (total flavonoid contents)

The main purpose of data validation is to ensure that the analytical method used can provide valid and reliable results (high level of trust). The results indicate that there is a positive relationship between the total phenol and flavonoid contents on antioxidant activity so that Arumanis mango leaf (*M. indica* L. Var. Arumanis) has the potential as a source of natural antioxidants that can be developed into pharmaceutical products.

CONCLUSION

Antioxidant was extracted from Arumanis leaves (*M. indica* L. Var. *Arumanis*) and showed that it has high antioxidant activity and its phenolic contents may prove to be a better substitute in place of synthetic antioxidants in extending the shelf life of food product by preventing the peroxide formation in fat- and oil-containing products. The total phenolic and flavonoid contents of Arumanis leaves (*M. indica* L. Var. Arumanis) can be used as a source of natural antioxidant. In addition, natural antioxidant is safe and has health benefits for those who consume it.

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