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Effect of Extraction Solvent on Total Phenol, Total Flavonoid Content and Antioxidant Activities of Extract Plants *Punica granatum*, *Vitis vinifera* L, *Ficus carica* L. and *Olea europea*

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Abstract. Several plants have high antioxidant compounds. Antioxidant compounds in plants include phenol compound and flavonoids. Plants are extracted with solvents, and different types of ingredients will show different active compounds. The combination of several plant extracts shows a higher compound content compared to a single compound. This study aims to examine the total phenol content, total flavonoids, and antioxidant activity in the combination of *P. granantum*, V. *vitifera*, *F. carica* and *O. europea* extract. The maceration method used is maceration of dry simplicia with methanol 95% solvent, fresh maceration with 95% methanol and dry simplicia with 95% ethanol solvent. Each extract measure of total phenol content and total flavonoid content and antioxidant activity using the DPPH method. The result of the antioxidant test showed the fresh maceration 95% methanol showed highest results with IC50 25.22 with a potent antioxidant activity category, total phenol content 68.43 mg/g, total flavonoid content 295.95 mg/g.

INTRODUCTION

The plants that are efficacious as drugs have essential substances that are very important in determining the work activities of these medicinal plants. One of the active ingredients is flavonoids, which commonly found in plants as glycosides. Flavonoids are natural phenolic compounds that have the potential as antioxidants. Flavonoids contained in all parts of the plant, including the fruit, seeds, leaves, stems, and roots. Antioxidants derived from plants that contain flavonoids are very good for preventing cancer, protecting cell structures, increasing the effectiveness of vitamin C, preventing bone loss, anti-inflammatory and as antibiotics [1].

The active compounds contained in *V. vinifera* are phenolics that have pharmacological effects as anticancer, antifungal, antibacterial and antioxidant [2] Fruit and *P. granatum* peel contain compounds of anthocyanins and flavonoids that have the potential as antioxidants [3]. *F. carica* are fruits that contain abundant polyphenolic compounds and flavonoids. This compound has the potential as an antioxidant and can prevent various oxidative stresses and diseases [4]. *O. europea* contains phenolic compounds and has antioxidant activity potential that is beneficial to health [5].

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One source of natural antioxidants that are commonly found in plant parts, especially in flowers and fruit, one of which is anthocyanin [6]. Reddish purple fruit is thought to contain anthocyanin. The anthocyanin extraction method uses polar solvents because of the polar anthocyanin properties.

In general, in measuring antioxidants, ethanol solvents are most often used. Ethanol is commonly used in anthocyanin extraction because its polarity is almost the same as the anthocyanin polarity so that it is easy to dissolve anthocyanin [7]. The use of solvents to a material must base on the solubility material of the solution and the material of the components to dissolve. Phenolic components can be extracted from plant material using solvents such as water, methanol, ethanol, acetone, ethyl acetate.

The extracting solvent also affects the number of active compounds contained in the extract, according to the concept of like dissolve like, where polar compounds will dissolve in polar solvents, and non-polar compounds will dissolve in non-polar solvents. A solvent such as methanol and ethanol are very widely used solvents and are sufficient for the extraction of phenolic components from natural materials [8].

The chemical components that act as antioxidants are phenolic and polyphenolic compounds. These group compounds are widely available in nature, especially in plants, and can capture free radicals [9]. Research on each antioxidant activity in *P. granantum* extract fruit extracts, *V. vinifera, F. carica* and *O. europea* has widely reported, but studies on antioxidant activity, total phenol and total flavonoid combinations of fruit extracts not yet to research. This study aims to examine the total phenol content, total flavonoids and antioxidant activity in the combination of *P. granantum extract, V. vitifera, F. carica and O. europea*.

EXPERIMENTAL DETAILS

Sample Preparation

Pomegranate ripe fruit, figs, grapes, and olives obtained on the market. After collection of fruit samples, the sample was extracted by drying using 40 ºC cabinet drying for 48 h, then mashing it to powder which then stores at cold temperatures 4 ºC until used

Sample Extraction

Maceration dried simplicia with methanol solvent. Each fruit simplicia with a ratio of 1:1:1 then extracted using methanol at room temperature and shaken with a shaker with a speed of 150 rpm for 2 × 24 h. The extract evaporates at the rotary evaporator at 50 ºC.

Fresh maceration with methanol solvent, done by blending the mixture of fresh fruit which then macerated with methanol solvent. Each fruit simplicia with a ratio of 1:1:1 then extracted using methanol at room temperature and shaken with a shaker with a speed of 150 rpm for 2 × 24 h. The extract evaporated at the rotary evaporator at 50 ºC.

Maceration of dried simplicia with 96% ethanol solvent. Each fruit simplicia with a ratio of 1:1:1 then extracted using ethanol at room temperature and shaken with a shaker with a speed of 150 rpm for 2 × 24 h. The extra evaporated at the rotary evaporator at 50 ºC.

Concentrated of Macerate

Concentrate of macerate is carried out using a rotary evaporator equipped with a vacuum pump. Solvent evaporation can be carried out below the boiling point of the solvent, and the evaporation process can take place faster. Evaporation of methanol solvents can be carried out below the boiling point at 55 °C. This process is carried out at this temperature to keep the active compounds not damaged due to heating.

Total Phenol Content

Total Phenolic Content (TPC) of the extract was determined using the Folin-Ciocalteu method [10]. The prepared extract (200 µl) was mixed with 1.5 ml of the Folin-Ciocalteu reagent that previously diluted to ten-fold with distilled water, and allowed to stand at 200 °C for 5 min. Sodium bicarbonate solution 1.5 ml (60 g/L) were added to the mixture. After 90 min at 22 °C, absorbance was measured using a UV spectrophotometer at wavelength 725 nm. Total phenolics content were quantified by a calibration curve from measuring the absorbance of a known concentration of

gallic acid (GA) standard (20-150 mg/L). The concentrations expressed as milligrams of gallic acid equivalents (GA) per 100 g dry plant.

Total Flavonoid Content

The total flavonoid content (TFC) of each extract was investigated using the aluminium chloride colorimetry method [11]. In brief, the extracted sample diluted with methanol until 100 mg/ mL. The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The calibration curve prepared by diluting quercetin in methanol (0-100 mg/mL). The mixture keeps at room temperature for 30 minutes. Then the absorbance of the mixture was measured at 415 nm using a UV-VIS spectrophotometer. TFC expressed as milligram quercetin equivalent per gram.

In Vitro Test Antioxidant Activity

The antioxidant activity of extracts based on the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical determined according to the method described by Singh *et al.* [12] with modifications. Briefly, the dilution series (three different concentrations) formulated extracts prepare on a 96-well plate. The reaction mixture consisted of 0.1 mL extract with a solution of 0.2 mL DPPH (0.15 mM in 80% methanol solution). The mixture is shaken hard and left for 30 minutes at room temperature in the dark. Ascorbic-acid as a positive control. The absorption of the resulting solution was measured spectrophotometrically at 517 nm, and percent inhibition activity was calculated using equation 1:

$$Scavenging\ activity\ (\%) = \frac{A\ control - A\ sample}{A\ control} \times 100\ \% \quad (1)$$

Where A is the control is the absorbance reaction and, sample A is the absorbance of the extract

Data Analysis

The data obtained were analyzed using descriptive qualitative and quantitative descriptive methods.

RESULT AND DISCUSSION

In principle, the free radical deterrent method is a measure of the deterrence of synthetic-free radicals in polar organic solvents such as methanol at room temperature by a compound that has antioxidant activity. The process of scavenging free radicals is through the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals so that free radicals capture one el-electron from antioxidants. The synthetic free radicals used are DPPH, this compound reacts with antioxidant compounds by taking hydrogen atoms from anti-oxidant compounds and getting electron pairs.

The existence of an antioxidant which can donate electrons to DPPH produces yellow, which is a specific feature of the DPPH radical reaction [13]. Compounds that can counteract radicals generally are donors of hydrogen atoms (H), so that the H atom can be captured by DPPH radicals to change into its neutral form.

Based on the results of the study showed that the highest antioxidant activity in fresh methanol extract. This is consistent with research on mangosteen peel extract has a high total phenolic content, and antioxidant activity, with the highest content of total phenolic and antioxidant activity in dry sample methanol extract, followed by wet sample methanol extract, dry sample water extract, and sample water extract wet [14].

The difference in the number of antioxidants is closely related to the difference in flavonoid content. The more flavonoids contained, the higher the total antioxidant. The result was in line with the research of Harizu and Hazrin where anti-oxidant activity from dried samples *P. niruri* was lower than fresh samples [15].

The processing of samples has a different effect on the test of antioxidant capacity. Antioxidant compounds are straightforward to change. Various types of the processing result in the loss of antioxidant compounds found in a sample. In the drying process and extraction differences, phenol damage can occur due to heating. Antioxidant activity is shown by Inhibition Concentration (IC), which is the percentage inhibition of antioxidant compounds in scavenging free radicals. If the IC_{50} concentration low, antioxidant activity become strong. IC_{50} on fresh methanol is 25.22 and is different from ascorbic acid control, which is equal to 51.33, indicating that the extract has extreme antioxidant potential.

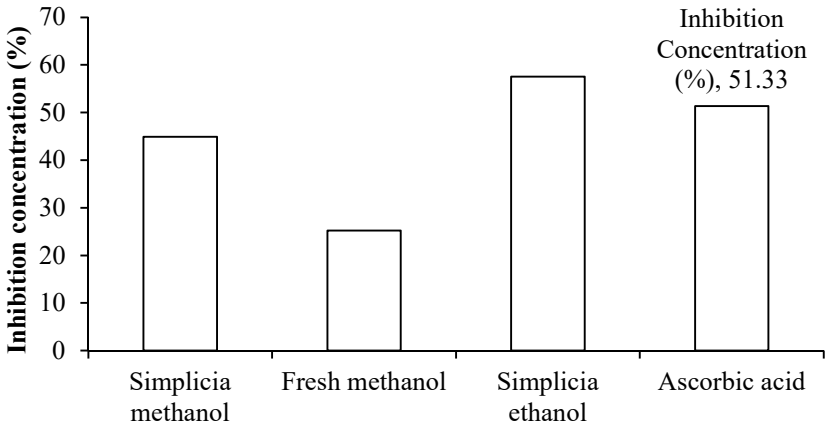

FIGURE 1. Inhibition concentration (%) extract in antioxidant activity test using DPPH methods

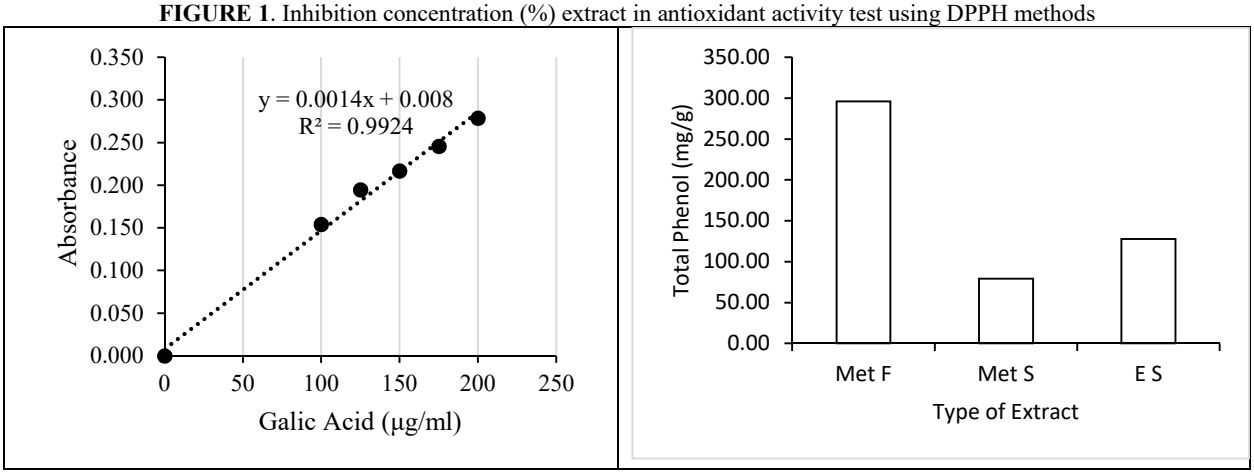

FIGURE 2. Calibration curve obtained from measuring the absorbance of a known concentration of gallic acid (GA) as standard (left side) and Total Phenol Content (mg/g) (right side)

The Folin Ciocalteu method used to test the total phenol content base on the strength of reducing the hydroxyl groups of phenol compounds. All phenolic compounds, including simple phenols, can react with Folin Ciocalteu reagents, although they are not effective radical (antiradical) scavenger [16]. Polyphenols divided into several classes, namely phenolic acid, (hydroxybenzoic acid, hydroxycinnamic acid), flavonoids (flavones, flavonols, flavanones, flavonols, flavanols, anthocyanin), isoflavonoids (isoflavones, kumestan), stilbene, lignans and phenolic polymers (condensed pro-anthocyanidin-tannins and hydrolyzed tannins) [17]. The use of ethanol in liquid-liquid extraction is because ethanol is a polar solvent and not toxic compared to methanol.

The total phenolic content can produce from several simple molecules, namely phenolic compounds, to complex molecules such as tannins (hydrolyzed tannins and condensed tannins) [18]. Phenolic compounds are reported to react with reactive oxygen compounds, and this is due to one or two hydroxy groups in the aromatic ring, which can act as hydrolytic donors. The study results showed that the most abundant Total Phenol Content in fresh methanol extract was 68.43 mg/g.

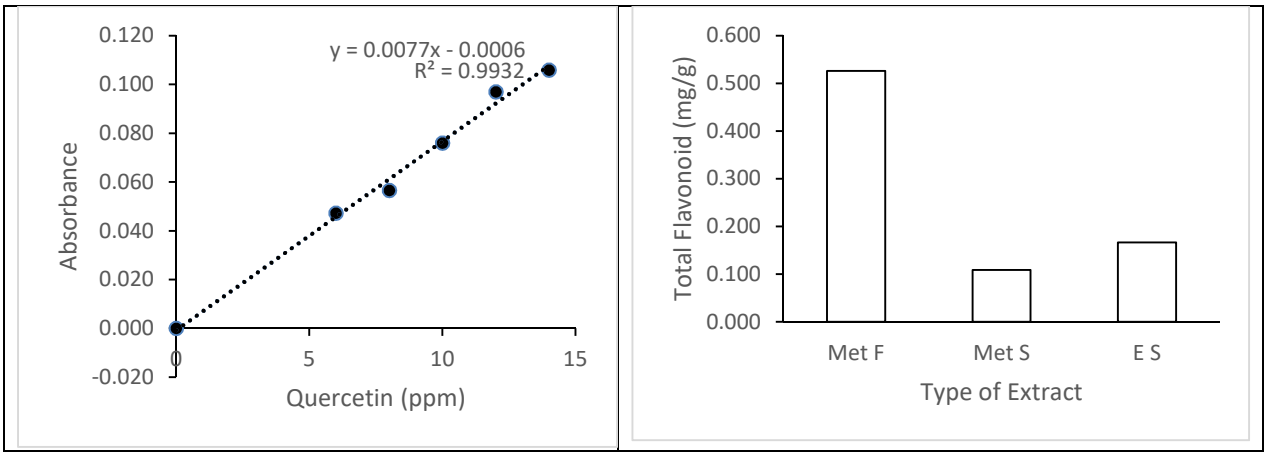

FIGURE 3. The calibration curve obtained from measuring the absorbance of a known concentration of quercetin as standard (left side) and Total Flavonoid Content (mg/g) (right side)

Anthocyanins are natural dyes which are antioxidants found in plants and are derivative compounds of flavylium cations (AH +) (red-forming) [19]. The results of the study showed that the highest Total Flavonoid Content (TFC) found in fresh methanol extract, which was 295.95 mg / g cause the fresh method does not involve heat directly into the fruit material so that the anthocyanin contained is not degraded to colorless, and read with more excellent absorption on the spectrophotometer [20]. The anthocyanin degradation influenced by temperature [21].

The antioxidant activity in the fresh material is higher than the dry method. Hot contact in the drying process affects the low antioxidant activity in a dry way. The low antioxidant activity is comparable to total anthocyanin, the result in line with research [22] where the higher the value of anthocyanin, the greater its antioxidant activity. Heat or light can trigger pre-oxidation. In other words, the sample has donated its H atom to form hydroperoxide, so the reduction of power towards DPPH is getting lower [23]. The results showed that methanol solvents successfully extracted a combination of *P. granantum, V. vitifera, F. carica* and *O. europea* extract and produced higher total phenol, higher flavonoids and antioxidant activity.

SUMMARY

The combination of pomegranate, grape, tin, and olive extract with the extraction of fresh methanol solvent showed the highest total phenol and total flavonoids. The result of the antioxidant test showed the fresh maceration 95% methanol showed highest results with IC50 25.22 with a potent antioxidant activity category, total phenol content 68.43 mg/g, total flavonoid content 295.95 mg/g.

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