

Phytochemistry Screening and Antioxidant Activities of Extract Pomegranate, Grape, Fig, and Olive in the Various Solvent

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Abstract. *The active compounds of grape, pomegranate, olive, and fig have anthocyanins that potential as antioxidant are flavonoids. Flavonoids have potential as antioxidant to prevent and therapy various oxidative stress and related diseases. This study aimed to examine the antioxidant activity of a combination of pomegranate, grape, fig, and olive extracts using the DPPH (diphenyl-2-picrylhydrazyl) method. The maceration method used was maceration of dry *Simplicia* with methanol 95% solvent, fresh maceration with 95% methanol and dry *Simplicia* with 95% ethanol solvent. The results of the phytochemistry test showed several compounds found in the extract combination pomegranate, grapes, fig, and olives such as polyphenol, flavonoid, tannin, steroid/triterpenoid. The result of the antioxidant test showed the fresh maceration 95% methanol showed higher results with the IC50 of 25.22 with a potent antioxidant activity category.*

Keywords: *antioxidant activity, DPPH, pomegranate, grapes, fig, olive*

Citation

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INTRODUCTION

Antioxidants are compounds binding free radicals and molecules that can inhibit oxidation reactions. ROS are oxygen-containing, a highly reactive species. ROS is very important for biological functions and is produced continuously during normal cell metabolism. Moderate ROS ratios such as hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) can act as cellular messengers. However, the production of excessive ROS can cause oxidative stress and harm to biological cells and molecules (Held, 2012 ; Fu et al., 2014 ; Patel et al., 2018).

ROS usually removed from the body undergo an antioxidant mechanism. However, antioxidant mechanism dysfunction can cause

an imbalance between the formation of ROS and its deterrence. Previous research has shown that continuous ROS exposure can induce skin aging to undergo the destruction of the antioxidant system, formation of wrinkles, and melanogenesis. (Boligon, 2014; Shahidi & Zhong, 2015).

The active compounds of grape are believed to have pharmacological effects such as anti-inflammatory, anticancer, antifungal, anti-bacteria, and antioxidant. Grape phenolics are usually high in the grape peel. The phenolic is classified into two groups: flavonoids and nonflavonoids. The flavonoids include flavan-3-ols (catechin), flavonols (quercetin), and anthocyanins. They have a high concentration of flavonoids, linoleic acid, and polyphenols due to their high antioxidant capacity (Kata-

linic et al., 2013 ; Rathi & Rajput, 2014 ; Burin et al., 2014)

Pomegranate has anthocyanins that are potential as antioxidant flavonoids. Flavonoids and tannins are abundant in the peels of wild species compared to cultivated fruits. Fig/*Ficus* species are a rich source of polyphenolic compounds such as flavonoids. Flavonoids are potential for antioxidant activities to prevent and therapy various oxidative stress and related diseases (Misbah et al., 2013). The olive fruit is rich in phenolic compounds that have antioxidant activity and health benefits. The classes of phenolic compounds in olive fruits include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (Zaouay et al., 2012 ; Hmid et al., 2017 ; Zhang et al., 2019).

The antioxidant activity of the compound combined with several extracts is thought to have higher activity compared with a single extract (Ghimeray et al., 2015). The findings in this study were that the combination of pomegranate extract: grape: fig: and olive in fresh maceration methanol solvent showed the highest antioxidant activity. Several plants in the study are thought to have active compounds like antioxidants and some supporting research for antiaging compounds. This study aimed to study the bioactive compounds and the antioxidant activity of a combination of pomegranate extract, grapes, tin, and olives.

MATERIALS AND METHODS

This research was conducted in July-September 2020. This research is a qualitative and quantitative descriptive study with sample testing to obtain qualitative data, phytochemical content, and quantitative data on antioxidant activity.

Sample Preparation

Pomegranate ripe fruit, figs, grapes, and olives were obtained from the market. After the collection of fruit samples, the sample was extracted by drying using 40°C cabinet drying for 48 hours, then mashing it to powder which was then stored at cold temperatures 4°C.

Sample Extraction

Maceration of dry fruit simplicia (exocarp, mesocarp, and endocarp) was extracted using 95% methanol using a ratio of 1:1:1 and then shaken at 150 rpm for 2x 24 hours, then the extract was evaporated in a rotary evaporator at 50°C. (Lim et al., 2019)

Maceration of fresh fruit with methanol as solvent was macerated with 95% methanol as solvent. Each fruit with a ratio of 1:1:1 was then extracted using 95% methanol in a shaker at a speed of 150 rpm for 2x 24 hours, the extract was evaporated in a rotary evaporator at a temperature of 50°C.

Maceration of dry fruit Simplicia was extracted with 95% ethanol as solvent. 1:1:1 ratio then shaken at 150 rpm for 2x24 hours, the extract was evaporated in a rotary evaporator at a temperature of 50°C.

Concentrated of Macerate

The concentration of macerate was carried out using a rotary evaporator equipped with a vacuum pump. So solvent evaporation can be carried out below the solvent's boiling point, 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 was carried out at this temperature to keep the active compounds not damaged due to heating.

Phytochemistry Tests

Phytochemistry test (alkaloid, poliphenol, flafonoid, saponin, steroid/ tritenoid) ac-

ording to on Yalavarthi & Thiruvengadarajan (2013).

In vitro test antioxidant activity

Antioxidant activity was tested based on the free radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with modification and positive control using ascorbic acid. 3 types of extracts were diluted to graded concentrations and prepared on 96-well plates. the reaction mixture consisted of 0.1 mL of the extract with 0.2 mL of DPPH solution. The mixture was shaken for 30 minutes at room temperature (Alam et al., 2013).

The absorption of the resulting solution was measured spectrophotometrically at 517 nm, and percent inhibition activity was calculated using the following formula:

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

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

Data Analysis

The obtained data were analyzed using descriptive qualitative based on indicator color changes, and quantitative descriptive methods. The quantitative analysis was statistical methods used relationship curve % of antioxidant activity. Antioxidant activity based on IC50 value based on categories potential activity.

RESULTS AND DISCUSSION

Phytochemistry Test Extract Methanol of Pomegranate, Grape, Fig, and Olive

The results on phytochemical test are presented in Table 1. Qualitative tests of secondary metabolites of extracts included the analysis of flavonoids, alkaloids, tannins, saponins, polyphenols, and terpenoids. The test results showed that there were 5 compounds that showed positive results, namely: flavonoids, tannins, tannins galat, polyphenols, and steroids, while alkaloids, saponins, and triterpene showed negative effects.

Table 1. Phytochemistry test methanol extract of pomegranate, grape, fig, and olive

Test	Result	Standard
Alkaloid test	Green (-)	White sediment (Mayer) Yellow-red sediment (Dragendorff)
Polyphenol test	Blackish blue (+)	Red, green, purple or deep black
Flavonoid test	Orange (++)	Red, yellow or orange
Saponin test	There is no foam (-)	A stable foam form
Tanin test	Blue (+)	Blackish blue
Steroid/	Blue-green (+)	Blue or green
Triterpenoid test	Black- blue (-)	Red or Purple

For relatively dynamic communities, average dominance was due to dominant pollinators in the community. According to Hidayat et al. (2016), high dominance index value also affects the evenness index value.

The higher the dominance index value, the simpler the community structure. Widhiono & Sudiana (2015) reported that chayote plantations in Purbalingga, Central Java had moderate diversity of insects pollinator, rel-

actively dynamic communities, and average dominance. According to Siregar et al. (2014), there are seven interacting influencing factors for insect species diversity in the community. Those factors are community age, environmental and flora community heterogeneity, competition to exploit natural resources, predation, climate stability, flora community productivity, and natural resources.

The chemical compounds in the combination of pomegranate, grape, tin, and olive extract contain compounds such as flavonoids, tannins, tannin galat, polyphenols, and steroids. Pomegranate (*Punica* sp.) has vitamin C which is a well-known potent antioxidant. In general, *Punica* sp. denote to be a good source of natural antioxidants (Zaouay et al., 2012 ; Burin et al., 2014 ; Ghimeray et al., 2015).

Grape extract from whole grapes has a large concentration of flavonoids, linoleic acid, and polyphenols, which are beneficial for health because of their high antioxidant capacity. Grapes contain flavonoid compounds such as quercetin, myristin, and kaempferol which have antioxidant activity.

The antioxidant activity test of the combined extract of *Aloe barbandesis* (leaf), *Tinospora cordifolia* (stem), *Triticum aestivum* (straw), *Azadirachta indica* (leaf), and *Ocimum sanctum* (leaf) using the DPPH method and ascorbic acid as a positive control. Further analysis to determine the total phenol content using the Folin-Ciocalteu method and the total flavonoid content using the Dowd method. The extract exhibited significant antioxidant activity, total phenolic, and flavonoid content. The extract can be used effectively for medication purposes (Ali & Dixit, 2012). Herbal compounds containing several plant products have a synergistic hepatoprotective effect and can increase their effectiveness. Testing the combination of ethanolic extracts of *Solanum xanthocarpum* (SX) and *Juniperus commu-*

nis (JC) fruit against Paracetamol (PCM) and Azithromycin (AZM) which induces liver toxicity in rats was clearly indicated that SX and JC extract has hepatoprotective potential against AZM and PCM induced liver toxicity due to their synergistic anti-oxidant properties (Singh et al., 2016)

The solvents used in this study were methanol p.a and ethanol p.a. solvents as they are polar and commonly used for extraction. Moreover, both of these solvents were used because the compounds are antioxidants and generally polar. Methanol is a polar solvent that can dissolve polar compounds such as phenol groups. Extraction uses a solution based on component solubility to other components or their polarity in the mixture (Boeing et al., 2014) The 95% ethanol is commonly used in anthocyanin extraction because its polarity is almost the same as the anthocyanin polarity, so it is easy to dissolved (Al-Huqail et al., 2018).

The β -carotene bleaching method showed that the methanol extract has the highest antioxidant activity coefficient (AAC) of 627 ± 40.0 at 200 mg/l by and the longest induction time of 7.0 ± 0.2 h by the Rancimat. The polar solvent of ethanol, methanol, and acetone are commonly used to extract plants. (Yalavarthi & Thiruvengadarajan, 2013).

The type of maceration used in this study was maceration of dry *Simplicia* and fresh extraction, which then tested for antioxidant activity. The extraction used ethanol 70% with the addition of citric acid with a ratio of ingredients and solvents 1: 4 (Rifkowaty & Wardanu, 2016).

Furthermore, antioxidant stability and color tests were carried out from the extract which had the best antioxidant activity and then ANOVA (Variant Analysis) was performed. The best antioxidant activity was obtained from wet method extraction treatment

with yields of 32.6142%, total anthocyanins of 33.3279 mg / 100 gr samples, and antioxidant activity of 95.2434%.

The total phenolic content test in mangosteen peel extract showed the highest results in the dry sample methanol extract (MK), followed by the wet sample methanol extract (MB), dry sample aqueous extract (AK), and wet sample aqueous extract (AB). (Dungir et al., 2012).

Activity test antioxidant used DPPH method

IC50 values of the combination extract pomegranate, grape, fig, and olives presented in Table 2. The IC50 value is the effective concentration of extract needed to reduce 50% of the total DPPH, so the amount of 50 substitutes for the value of Y. After substituting the value 50 for the Y value, X will obtain

the value of IC50.

Table 2 illustrates that IC50 value of all extraction type test samples shows an IC50 value of less than 50. According to the IC50 value parameters in the Table 3, it shows that the extract of pomegranate, grapes, figs, and olives are potent antioxidants (IC50 value <50). The type of fresh maceration extract with methanol solvent showed the highest antioxidant activity of 25.22. Maceration extract *Simplicia* with methanol solvent gave IC50 of 44.94, lower than that with ethanol solvent which give the IC50 of 47.54. Antioxidants expression as compounds that can significantly slow down oxidation, even with lower concentrations than those that can be oxidized (Ghasemi et al., 2019). Those antioxidant compounds such as phenolic acids, flavonoids, polyphenols, carbohydrates, vitamin C, vitamin E, and lycopene.

Table 2. The IC50 extract of pomegranate, grape, fig, and olive in the different solvent

Type of extract	Line Equation	Y value	X value or IC50
Maceration extract <i>simplicia</i> methanol solvent	$y = 1.055x + 2.5847$	50	44.94
Fresh maceration methanol solvent	$y = 0.5922x + 19.214$	50	25.22
Maceration extract <i>simplicia</i> ethanol solvent	$y = 0.524x + 25.07$	50	47.54
Ascorbic acid (control)	$y = 0.702x + 13.94$	50	51.33

Table 3. Antioxidant activity based on IC50 value

IC50	Antioxidant Activity
< 50 ppm	Very strong
50 – 100 ppm	Strong
100 – 150 ppm	Medium
150 – 200 ppm	Weak

The relationship curve of % antioxidant activity in various combinations of pomegranate extract, figs, grapes, and olive leaves show in Figure 1. The linear regression equation shows the relationship of extract concentration gradually with absorbance using a UV-

Vis spectrophotometer at a wavelength of 517 nm.

The coefficient of determination (R²) indicated the value of 0.92% - 0.99%, which has a very close relationship. The higher the value on the X-axis means the higher the

amount of DPPH free radicals extracted by the compounds. Antioxidant properties based on IC50 values presented in Table 3. IC50 (Inhibition Concentration) indicated the number of antioxidant activity. If the IC50 value <50 ppm then the tested compound has potent antioxidant activity, on the contrary, if the IC50 value ranges from 150-200 ppm, the compound has a weak activity.

The combination of pomegranate, grape, tin and olive extract with the extraction of fresh methanol solvent showed the highest antioxidant activity with the IC50 value of 25.22. The extraction solution should be adjusted to the polarity of the extract. According to the principle dissolves likeness, a solvent will tend to dissolve a compound that has the same polarity. Polar solvents will dissolve po-

lar compounds and vice versa. Flavonoids are polyphenolic compounds that are widely distributed in plants in the form of glycosides that bind to sugar, therefore flavonoids are polar compounds. Polar solvents commonly used for flavonoid extraction are methanol, acetone, ethanol, water, and isopropanol. A similar result to this study has been reported that the Antioxidant activity test using DPPH on flavonoid extract of mangosteen peel (*Garcinia mangostana*) gave the highest antioxidant activity, through low IC50 value on methanol extract sample of 44.49 mg/L, followed by methanol extract fresh sample (54.95 mg/L), water extract dried sample (346.73 mg/L), and water extract fresh sample (346.74 mg/L) (Dungir et al., 2012).

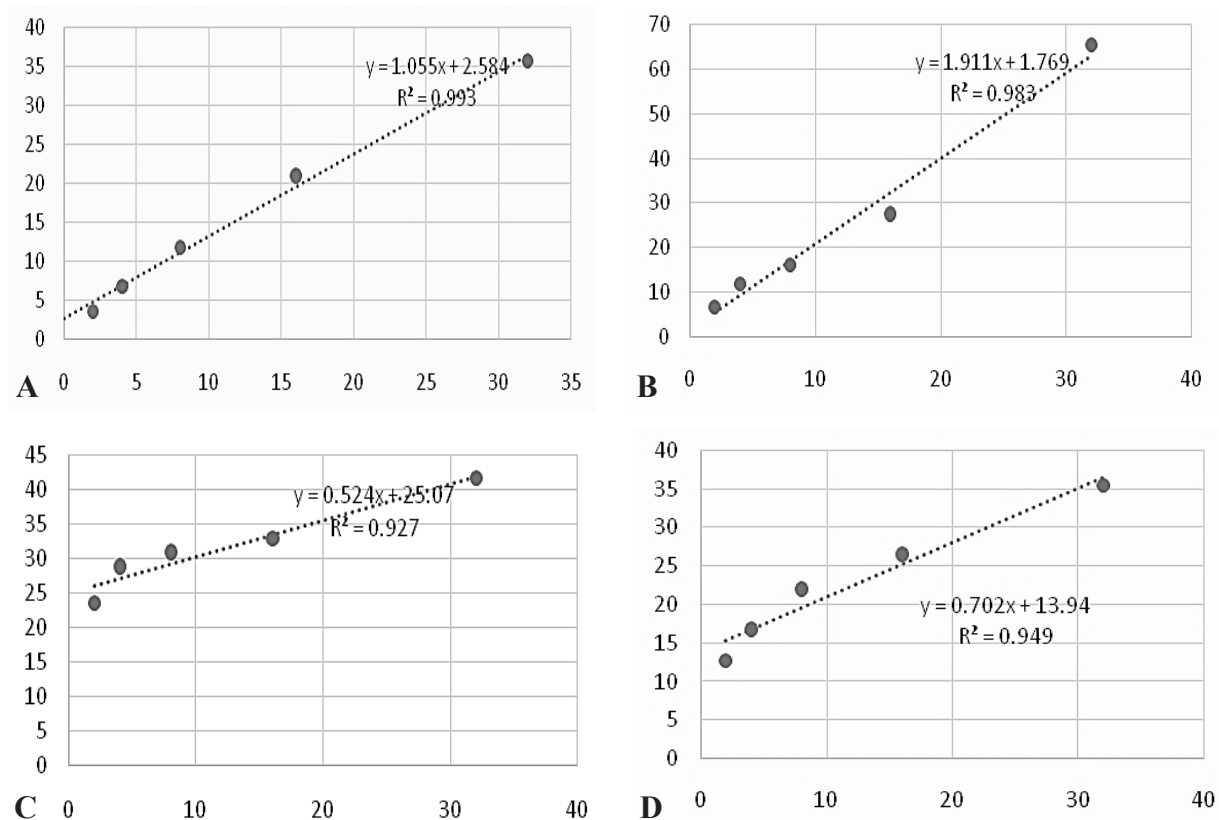


Figure 1. The relationship curve % of antioxidant activity in the various extract of pomegranate, figs, grapes, A. Maceration extract simplicia methanol solvent B. Fresh maceration methanol solvent C. Maceration extract Simplicia ethanol solvent D. Ascorbic acid (control)

CONCLUSION

The chemical content of methanol extracts combined with pomegranate, grapes, tin, and olives contains flavonoids, tannins, polyphenols, and steroids showed a higher antioxidant activity than ascorbic acid as a control. The combination extract with the extraction of fresh methanol solvent showed the highest antioxidant activity with the IC50 value of 25.22. This study used a balance composition extract by 1:1. For further research, it was necessary to examine the differences in the composition of each extract.

AUTHOR CONTRIBUTION

E.S.S designed the research and supervised all the process, R.S.S. and K.H. collected the data and wrote the manuscript.

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CONFLICT OF INTEREST

The all of authors whose names are list below have no conflict of interest to declare.

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