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EFFECT OF METHANOL CONCENTRATION AS A SOLVENT ON TOTAL PHENOLIC AND FLAVONOID CONTENT OF BELUNTAS LEAF EXTRACT (*Pulchea indica* L.)

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ABSTRACT

Background: Beluntas (*Pluchea indica* L.) is an Indonesian plant that grows wild and is used as traditional medicine. Beluntas leaves are reported to contain phenolic, and flavonoid is a part of phenolic compounds. Phenolic compounds other than flavonoids include 1,3,4,5-tetra-O-caffeoylquinic acid, 3,4,5-tri-O-caffeoylquinic acid, chlorogenic acid, and ferulic acid. Flavonoid compounds in beluntas leaves are quercetin, apigenin, luteolin and chrysoeriol. Methanol solvents are reported to be able to extract higher polyphenol and flavonoid contents than other solvents. **Objective:** The purpose of this study is to determine the effect of 20%, 50% and 75% solvent concentration of methanol on total phenol and total flavonoid levels of beluntas leaf extract. **Methods:** In this study, the extraction process was carried out by maceration, the determination of total phenol content was carried out by the Follin-Ciocalteu method. Determination of total flavonoid levels was carried out by the Colorimetric method. Data on phenol and flavonoid levels were analyzed statistically. **Results:** The yield of beluntas leaf extract in this study was respectively from the lowest methanol concentration of 24.094% w/w, 31.126% w/w, 24.838% w/w. The value of total phenol levels increased with increasing methanol concentration, namely 124.84 mg GAE/g, 138.3 mg GAE/g, and 147.91 mg GAE/g. The highest total flavonoid value in 75% methanol extract is 69.72 mg QE/g, followed by 20% methanol extract at 46.29 mg QE/g and the lowest is found in 50% methanol extract at 32.80 mg QE/g. The results of statistical analysis using the Kruskal-Wallis test showed that there were significant differences ($p<0.05$) in the value of total phenol levels and total flavonoids of the three extracts. **Conclusion:** The difference in the concentration of methanol solvents affects the value of total phenol levels and total flavonoids with the highest value produced by a 75% methanol solvent.

Keywords: Beluntas Leaves (*Pluchea indica* L.), Total Phenol Content, Total Flavonoid Content, Gallic Acid, Quercetin.

INTRODUCTION

Indonesia is a country rich in biodiversity. Some plants are made as alternative drugs for treating diseases. One of Indonesia's native plants is widely spread and potentially to develop namely the beluntas plant (Pluchea indica L.) of the family Asteraceae[1]. Beluntas leaves are often used as a traditional medicine to eliminate body odour, increase appetite, joint pain, back pain, glandular tuberculosis, rheumatism[2], reduce fever, overcome vaginal discharge, overcome irregular menstruation[3], overcome diarrhea, and help digestion[4].

The compounds contained in beluntas leaves are tannins, sterols, flavonoids, and phenol hydroquinone[5]. One of the phenol

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compounds present on the beluntas leaves and is the identity compound of beluntas leaves, namely quercetin[6]. Quercetin is categorized as flavonols, one of six subclasses of flavonoid compounds, which has biological activities Flavonoids are polar compounds, so they will dissolve in other polar solvents such as ethanol, methanol, butanol, acetone, dimethylformamide and others. Quercetin compounds in beluntas leaves have good solubility in solvent mixtures that have polarity over water because they are in the form of glycosides[7].

Based on previous research[8,9], the greatest total phenol and total flavonoid levels in beluntas leaves are by using methanol as a solvent. The use of a combination of solvent types in one mixture allows more polyphenol compounds to dissolve. The effect of using methanol-water composition gives different levels of acquisition because water is a polar compound that has a high polarity index and dielectric constant so that the mixture of water with solvents has a higher polarity than pure solvents[10].

This study the determination of levels of phenols and flavonoids with variations in the concentration of methanol solvents. Research on total phenol and flavonoid levels in the extract of beluntas leaf obtained from methanol solvents with various variations of methanol solvents had never been done. So research is needed to see the effect of different concentrations of extraction solvents, namely methanol 20%, 50%, and 75% on the total levels of phenols and total flavonoid levels from the leaves of beluntas *Pulchea indica* (L.). Determination of total phenol content can be expressed in mg of Gallic acid equivalent /g of sample and total flavonoid content expressed in mg of Quercetin equivalent/g of the sample[11,12].

METHODS

1. Instrument and Materials

Beluntas leaves (Pluchea indica L.) obtained from the Manoko Balitro Gardens, West Bandung Regency, West Java, Methanol p.a (Merck®), Aquadest (Bratachem®), Gallic acid (Sigma®), Quercetin (Merck®), Folin-Ciocalteu (Sigma®), $AlCl_3$ (Merck®), rotary evaporator, UV-Vis spectrophotometer

2. Extraction

Add 100 grams of dried beluntas leaf powder to the macerator with 1000 ml of 20% v / v methanol solvent added (1:10). Making extract using 50% and 75% concentration methanol solvents uses the same comparison that is 1:10. Maceration was performed for 3 days, with stirring twice a day, every 10 times stirring. The macerate was filtered with filter paper and concentrated with a vacuum rotary evaporator at 40 ° C until a viscous oven at 40°C until got a constant weight and determined the extract yield by the formula in equation 1.

$$\% \text{ Yield} = \frac{extract\ weights}{sample\ weights} \times 100\% \quad \text{.....(1)}$$

3. Phytochemical Screening of Flavonoids and Phenols

Phytochemical screening was carried out on each extract to determine the presence of flavonoid and phenol compounds. The test solution was prepared by dissolving 0.5-gram thick extract with 10 mL methanol. The screening test method was adapted from the Indonesian Ministry of Health.

4. Determination of Phenol Levels

Phenol content testing refers to the procedure Chun et al (2003); Depkes RI et al. (2008); Malik et al. (2015) with some modifications. The total test phenol of beluntas leaf extract was carried out by the Folin-Ciocalteu method with gallic acid standard (GAE). Making a gallic acid calibration curve with the concentration used 3; 4,5; 6; 7.5; 9; 10.5; 12 µg/mL. Measurement of total phenol levels was carried out by weighing 10 mg of methanol extract 20%, 50% and 75%, then dissolved to 10 mL with methanol p.a., so get the concentration of 1000 µg/ml. Sample with a concentration of 1000 µg/ml was diluted until

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getting 50 µg/ml extract solution. Then, take 3 mL extract solution of 50 µg/ml concentration and added 0.4 mL of the Folin-Ciocalteu LP reagent. Next, added 4 mL of 1% NaOH solution and 4.6 mL aqua dest, shake till homogeneous, incubated for 2 hours at room temperature. Absorption is measured at a maximum absorption wavelength of 730 nm. Preparation of the sample solution was repeated six times. The phenol levels were obtained as mg GAE/g dried sample.

5. Determination of Flavonoid Levels

Determination of total flavonoid levels which refers to Chang et al. (2002); Ahmad et al. (2014) with some modifications. Determination of total flavonoid levels by a colorimetric method with quercetin standard (QE). Quercetin calibration curve was made by concentration of 0.5; 1,5; 2.5; 3.5; 4,5; 5.5 µg/mL. Methanol extracts of 20%, 50%, and 75% were weighed 10 mg each and dissolved in 10 mL methanol p.a. Add 1 mL of extract solution with 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, 0.2 mL of sodium acetate 1M and aqua dest until 10 mL. After that, incubated for 30 minutes at room temperature. Absorption is measured at a wavelength of 415 nm. The sample solution was made in 6 replications. Flavonoid levels were obtained as mg QE/g of dried samples.

6. Data Analysis

The statistical data is the result of total phenol, and flavonoid levels from each methanol extract beluntas (*Pluche indica* L.) leave. The statistical method used is Kruskal-Wallis if there is a significant difference in the Kruskal Wallis Test ($p < 0.05$) then the test is continued with the Mann-Whitney test. Statistical analysis using the SPSS 22 application for Windows.

RESULTS AND DISCUSSION

The extraction process of beluntas leaves (*Pluchea indica*) by maceration method. Maceration is a simplicia extraction process that uses certain solvents with several stirring at room temperature[19]. Remaceration is a process of repeating the addition of solvents after previous macerate filtering[20]. The purpose of re-maceration was to filter the compounds that are still left behind or are not founded. Regular stirring is also carried out to homogenize the compound to contact with the liquid solution to get the maximum extraction results. The extracts yield is a comparison between the extract weight obtained with the simplicia weight[20]. The amount of yield from an extract indicates the amount of active ingredient that can be extracted based on the solvent and the method. The percentage of extract yield from the extraction obtained can be seen in Table 1.

The extract yield results in the table showed that the methanol extract 50% higher than methanol extract 20% and 75%. This is because the extract component does not only consist of flavonoid compounds, but there are other compounds[21]. Based on the nature of polarity, water has a high polarity, so that it can attract polar compounds and methanol is a universal solvent that can attract polarly, semi-polar, and non-polar compounds. So that the yield of the extract at 50% is able to attract other compounds contained in the extract so that the yield obtained is high.

The high yield obtained is due to the main component extracted in the form of polyphenols which have polar and nonpolar groups. The yield of extract in this study is much higher than the yield of methanol extract yield reported by Safitri et al. (2018), which is 23% w/w.

Table 1. The yield of Beluntas (*Pluchea indica* L.) Leaf Extract

Sample	Powder Weight (gram)	Extract Weight (gram)	Yield (%)b/b
20% methanol extract	100	24.094	24.094
50% methanol extract	100	31.126	31.126
75% methanol extract	100	24.838	24.838

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Table 2. Phytochemical Screening Results of Phenol and Flavonoids Beluntas (*Pluchea indica* L.) Leaf Extract

Sample	Phytochemical Screening	References	The Results	Information
20%	Flavonoid	Intensive yellow fluorescence solution, showing the presence of flavonoid (Depkes RI, 1995)	Yellow fluorescence on UV light 366 nm	(+)flavonoid
50%			Yellow fluorescence on UV light 366 nm	(+)flavonoid
75%			Yellow fluorescence on UV light 366 nm	(+)flavonoid
20%	Phenol	The formation of dark blue or blackish green (Vijayalakshmi dan Ravindhran, 2012).	blackish green color	(+) Phenol
50%			blackish green color	(+) Phenol
75%			blackish green color	(+) Phenol

On the dissolved process using a mixture of organic-water solvents, it is said that the polar group will be dissolved in a polar water solvent[22]. While the compounds with nonpolar groups will be dissolved in organic solvents which have semi-polar then water.

Phytochemical screening is one of the methods used to determine active ingredients which are secondary metabolites in the sample[23]. Phytochemical screening results from each extract can be seen in table 2. The result of phytochemical screening of phenols and flavonoids in this study showed that the sample of beluntas leaves methanol extract with a concentration of 20%, 50%, and 75% contains phenol and flavonoid compounds. This is the same with the study of Widyawati et al. (2012), which showed that the methanol extract of beluntas leaves positively contained phenol and flavonoid compounds. Besides that, in Indonesian Herbal Formalurium (FHI) which showed that the identity compound of beluntas leaf is quercetin compound which is a flavonoid group[6].

In determining phenol and flavonoid levels, first, make a standard calibration curve to get a linear regression equation to know the correlation between concentration and absorbance through spectroscopic measurements. The results of the calibration curve can be seen in Figures 1 and 2. The linear regression equation is shown in the figure. On the standard acid, gallic curve the value of the correlation coefficient (r) is 0,9929. While, the standard curve of quercetin, the value of r is 0,9974. The value of r close to 1 indicates a linear calibration curve, and there is a correlation between the concentration of the solution and absorption value[25].

The results of the determination of total phenol in beluntas leaf extract (*Pluchea indica* L.) at figure 3, show that the value of total phenol increases with the increasing concentration of the methanol solvent. Methanol solvent 75% concentration was able to dissolved phenol with the highest levels of 147.91 ± 1.13 mg GAE/g. The highest level of flavonoid in figure 4 in this study was the use of 75% methanol solvent in the amount of 69.72 ± 0.15 mg QE/g. In previous study Safitri et al. (2018), stated that phenol content in the methanol extract of beluntas leaves was obtained at 84.11 mg GAE/g sample while the flavonoid level was 51.59 mg QE/g sample. Based on these result, phenol and flavonoid levels by using variations in the concentration of methanol solvent are greater than pure methanol solvent. Methanol solvent 75% concentration can dissolve phenols and flavonoids better than solvents with lower concentrations. This is consistent with the statement of Salim et al. (2019), that methanol-water mixture which has more water composition than methanol will give lower levels of yield. This can be caused by the carbohydrate content in the extract will be dissolved in the water solvent, while the complex formation of some phenolic

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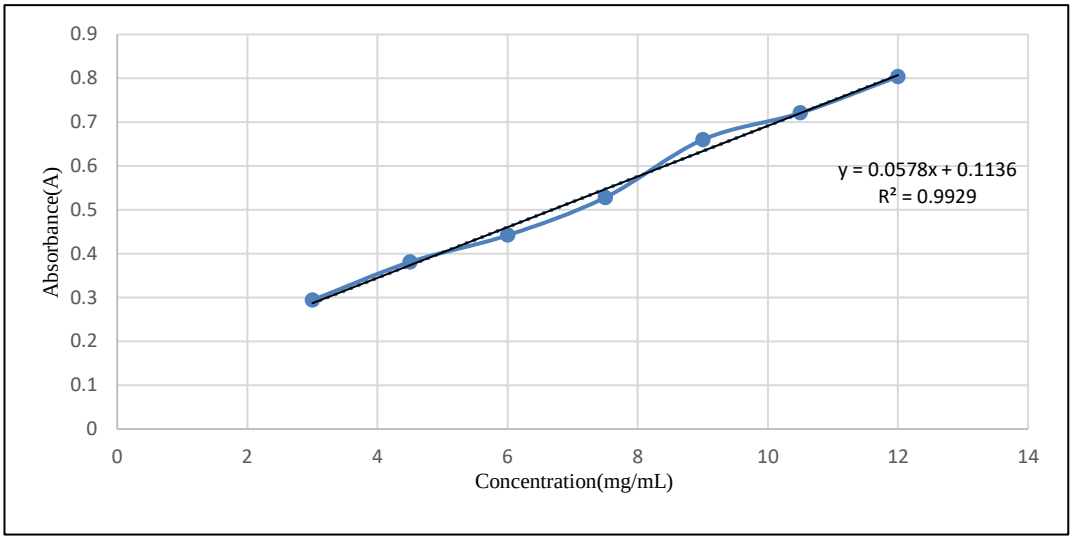

Figure 1. Standard curve for gallic acid

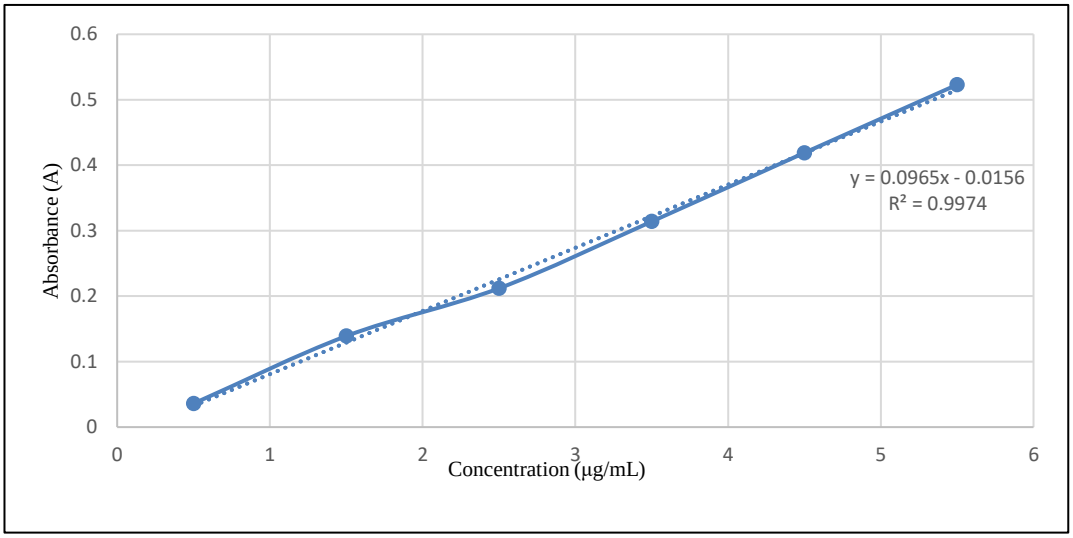

Figure 2. Quercetin standard curve

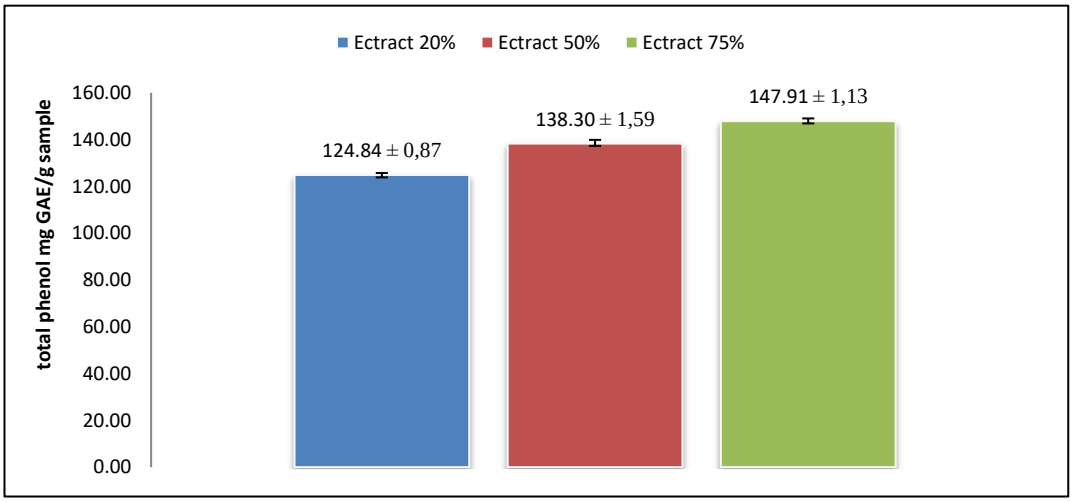

Figure 3. Graph of total phenol content extract of beluntas (*Pluchea indica* L.) leaf

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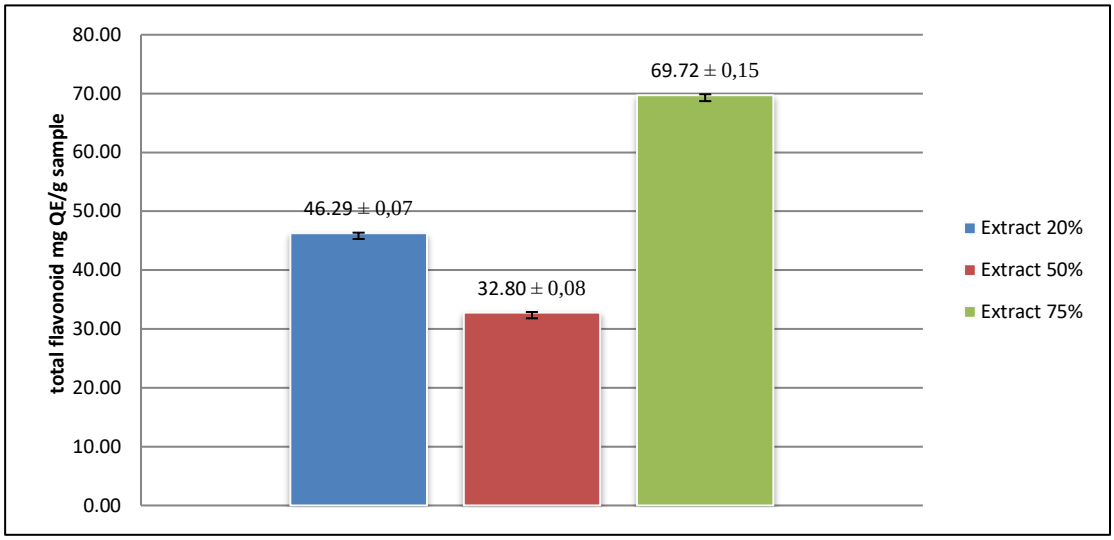

Figure 4. Graph of total flavonoid content extract of beluntas (*Pluchea indica* L.) leaf

compounds in the extract will dissolve in the methanol solvent. The combination of solvents in one mixture are allows more polyphenol compounds to dissolve[10]. This is also caused by the higher levels of methanol used, the more phenols are dissolve.

The results of the phenol and flavonoid levels in this study were much higher than the results reported by Safitri et al (2018), that showed the phenol levels in the methanol extract of beluntas leaves were obtained at 84.11 mg GAE/g sample, while the flavonoid levels obtained were 51.59 mg QE/g sample. The effect of using methanol-water composition gives different levels of phenol and flavonoid because water is a polar compound that has a high polarity index and dielectric constant so that the mixture of water with a solvent has a higher polarity than pure solvents[10].

The highest total phenol and flavonoid is 75% methanol solvent was inversely proportional to the highest extract yield in 50% methanol solvent. This can be caused because the components of beluntas leaf extract not only consist of flavonoid compounds, but there are other compounds, whose solubility varies in solvents, including compounds that are soluble in water.

The results of statistical analysis using the Kruskal-Wallis non-parametric test showed that variations in the concentration of methanol had an effect on the total phenol and flavonoid total of each extract. The results showed a significant difference in the value of total phenol and total flavonoid with a significance value of 0,000 (P <0.005).

CONCLUSION

The highest yield in the methanol extract of beluntas leaves (*Pluchea indica* L.) in the 50% methanol extract with the yield of 31.126% w/w. The increase in the concentration of methanol solvents affects of total phenol and flavonoid total, which is the highest results are obtained from 75% methanol solvent with a total phenol content of 147.91 ± 1.13 mg GAE/g of sample and the total flavonoid totals of 69.72 ± 0, 15 mg QE/g of sample.

CONFLICTS OF INTEREST

This paper was written independently. All authors do not disclose financial or personal relationships with others that can affect work.

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