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EFFECT OF DIFFERENT SOLVENT ON THE ANTIOXIDANT CAPACITY OF BIDARA LEAVES EXTRACT (*ZIZIPHUS SPINA-CHRISTI*)

Azzahra Putri Rialdi, Endang Prangdimurti*, Saraswati
Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, Indonesia
Email: prangdimurti@gmail.com*, rialdiazzahra@gmail.com, saraswati-sa@apps.ipb.ac.id

KEYWORDS
Antioxidant; bidara leaves; drying method; maceration

ABSTRACT

Bidara leaves (*Ziziphus spina-christi*) contains secondary metabolites such as flavonoids, polyphenols, saponins, triterpenoids and tannins which can be a source of antioxidants. In Indonesia, bidara leaves are used as herbal medicine by boiling fresh leaves. The products that developed from bidara leaves are health supplements and herbal teas from bidara leaves, but scientific studies about the antioxidant compounds of bidara leaves are still limited. There are antioxidant components that are non-polar, semi-polar or polar. This study aims to examine the polarity of antioxidant compounds in bidara leaves. The study used quantitative method by observe how much antioxidant compounds in bidara leaves. In this study, bidara leaves dried with a fluidized bed dryer had better chemical and physical characteristics. The results of the extraction by using various solvents (water, ethanol, ethyl acetate, and hexane) with maceration method showed that the highest antioxidant capacity was in the ethanol fraction ($p<0.05$). These results were measured based on the antioxidant activity parameters that DPPH scavenging, FRAP values, total phenolic, and total flavonoids. These results indicate that most of antioxidant compounds in bidara leaves have high polarity.

INTRODUCTION

Bidara Arab is a deciduous shrub plant from the Rhamnaceae family which grows in almost all parts of the Middle East, in Indonesia it grows a lot in the regions of Java and Bali. Various previous scientific studies have found that The main compounds contained in the leaves of this bidara are flavonoids, alkaloids, polyphenols, saponins, triterpenoids, and tannins (Asgarpanah & Haghighat, 2012; Darusman & Fakih, 2020). Other studies also explain that bidara extract exhibits hypoglycemic capacity, treats papulopustular rashes, inhibits sepsis-induced liver and spleen injury, and has strong antioxidant effects and potential as a hepatoprotective, antidiabetic, anti-allergic, anti-inflammatory, antibacterial, and immunomodulator (Al-Ghamdi & Shahat, 2017; Pambudi et al., 2020). This research focused on the potential of bidara leaves, namely as an antioxidant.

The processing technology for bidara leaves that are commonly applied by the community is simple extraction in the form of boiling fresh bidara leaves. Processing bidara leaves into dry products has encouraged the use of bidara leaves and increased added value. The antioxidant content in dry products is also more stable than in the fresh form (Xu et al., 2019). The dry form also has a longer shelf life so that the range of its utilization becomes wider (Wulandari et al., 2013).

The antioxidant capacity of bidara leaves needs to be considered because the amount of active compounds extracted is influenced by the type of solvent used in the extraction process and various conditions at the processing stage such as the type of drying method, drying time, drying temperature, extraction process time, and solvent pH (Rachmawati et al., 2020; Yura et al., 2016). However, studies and scientific information related to the polarity of the antioxidant

compounds in Bidara leaves grown in Indonesia are still very limited. Scientific studies on the polarity of the antioxidant compounds of Bidara leaves are needed to optimize the utilization of the properties of Bidara leaves. This study aims to obtain information on the antioxidant capacity of antioxidant compounds in bidara leaves in various types of solvents, namely non-polar, semi-polar, and polar.

RESEARCH METHOD

Material

The main material used in this study was fresh leaves obtained from a private garden in Dramaga District, Bogor Regency, West Java. The materials used for extraction were distilled water, 70% ethanol, ethyl acetate, n-hexane, DPPH, ethanol pa, ascorbic acid, distilled water, TPTZ, acetate buffer pH 3.6, FeCl3.6H2O, HCl, FeSO4.7H2O, Na2CO3 5 %, 50% Folin Ciocalteau, 95% ethanol, gallic acid, distilled water. 2% AlCl3, methanol, and quercetin.

The tools used in this study included tray dryers, fluidized bed dryers, thermometers, blenders, 18 mesh sieve. The analytical equipment used includes UV-Vis Spectrophotometer (Thermo Scientific Genesys 20), Chromameter (Minolta CR-300), Rotary Evaporator (Buchi R-300) and other auxiliary equipment.

Material preparation

Bidara leaves were all the leaves on the 3rd, 4th, 5th and 6th row leaf branches from the tip of the stalk (shoot) with the specifications of the leaves not being too young nor too old, not damaged, and not diseased. Fresh leaves that have been washed are sorted and washed with running water then drained for further drying.

Drying of bidara leaves

The drying used is drying with a tray dryer at a temperature of 50-52oC and a fluidized bed dryer at a temperature of 43-44oC. The drying time is when the leaf moisture content is obtained according to SNI 2014 concerning tea products (<8%). The best drying results were determined based on the parameters of antioxidant capacity and powder color analysis.

Characterization of the polarity of antioxidant compounds by multilevel maceration

Extraction was carried out using a multilevel maceration method referring to research by Sulmartiwi et al. (2018). The solvents used were n-hexane, ethyl acetate and 70% ethanol each of 100 mL with an extraction ratio of 1:10 (w/v). A total of 10 grams of sample was extracted by immersing the sample in Erlenmeyer using hexane for 24 hours with stirring (every 8 hours). The filtrate was then separated from the dregs using a vacuum filter and hereinafter referred to as hexane extract, while the dregs were macerated again using ethyl acetate followed by 70% ethanol with the same treatment to obtain ethyl acetate and ethanol extracts. All liquid extracts were then concentrated using a rotary evaporator. Extraction with water was carried out separately with the ratio of water solvent (w/v) and the same treatment.

Total Phenolic Analysis Folin-Ciocalteau Method (Javanmardi et al., 2003)

The 0.5 mL (500 ppm) extract sample was added with 0.5 mL 95% ethanol, 2.5 mL distilled water and 2.5 mL FolinCiocalteau 0.2 N. The mixture was allowed to stand for 5 minutes then 0.5 mL Na2CO3 5% was added and vortexed and then incubated for 60 minutes. The absorbance of the solution was measured at a wavelength of 725 nm. Gallic acid standard solutions at concentrations of 0, 20, 40, 60, 80, and 100 mg/L were used as standard curves to determine the total phenolic value of the samples.

Analysis of Total Flavonoids Aluminum Chloride (AlCl3) Method (Meda et al., 2005)

A total of 5 mL of bidara leaves extract at a concentration of 100 ppm was mixed with 5 mL of 2% AlCl3 in methanol pa. The mixture was homogenized with a vortex and left for 10

minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm. The total flavonoid content of the samples was determined using a standard quercetin curve with various concentrations of 0, 10, 20, 30, and 40 mg/L.

DPPH Method Antioxidant Capacity Analysis (Shim & Lim, 2009)

Extract 0.1 mL concentration of 500 ppm was added to 2.9 mL of 0.05 mM DPPH solution then homogenized using a vortex. The mixture was then stored in a dark room for 30 minutes. The absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Ascorbic acid standard solutions were prepared at concentrations of 0, 20, 40, 60, 80, and 100 mg/L and expressed in mg AEAC per gram of leaf dry basis.

Antioxidant Capacity Analysis of FRAP Method (Halvorsen, 2002)

The FRAP reagent was prepared using 25 mL of acetate buffer (pH 3.6), 2.5 mL of 10mM TPTZ solution in 40 mM HCl, and 2.5 mL of 20 mM FeCl3.6H2O solution, then added distilled water to exactly 100 mL in a measuring flask. 3 ml of FRAP reagent was then mixed with 0.1 ml of sample extract (500 ppm) and then stored in a water bath at 37°C for 30 minutes and the absorbance was measured at a wavelength of 595 nm. The standard curve was prepared by dissolving FeSO4.7H2O in distilled water with the final concentration of FeSO4.7H2O being 200, 400, 600, 800, and 1000 µmol/L respectively.

RESULTS AND DISCUSSION

Drying of Bidara Leaves

Bidara leaf powder is dried using two drying methods to determine the best bidara leaf powder used in the extraction process. The drying method used is tray dryer and fluidized bed dryer. The simplicia powder of bidara leaves produced in the tray dryer drying method has a moisture content of 7.55 ± 0.07 g/100 g (bb), whereas in the drying method with a fluidized bed dryer it has a moisture content of 7.82 ± 0.16 g/100 g (bb). The two drying methods were not significantly different ($p<0.05$) and produced leaf simplicia powder that complied with the requirements of SNI 01-4453-1998 with a maximum moisture content of 8%. The simplicia powder from Bidara leaves used in this study can be seen in Figure 1.

(a) (b)

Figure 1. Bidara leaf powder which is dried using the tray dryer (a) and fluidized bed dryer (b) drying methods

Chemical and Physical Characterization of Bidara Leaf Powder

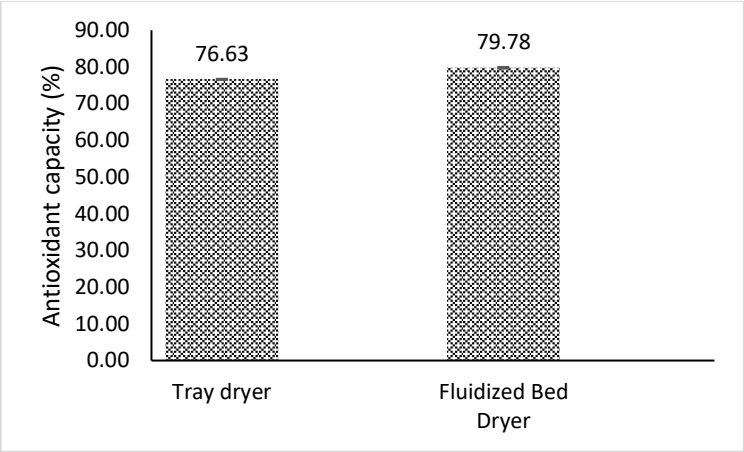

Figure 2. Antioxidant capacity (%) of bidara leaf powder

Rapid measurement of antioxidant capacity in bidara leaf powder using the DPPH and total phenol methods. Tests for antioxidant capacity and total phenol in bidara leaf powder were carried out with the help of methanol solvent because methanol is a universal solvent so that it can attract most of the compounds that are polar and also non-polar (Salamah & Widyasari, 2015). In the DPPH method, the absorbance measured is the absorbance of the remaining DPPH solution which does not react with antioxidant compounds at a maximum wavelength of 517 nm (Shim and Lim 2009) . The antioxidant capacity of the powder using the tray dryer drying method was 76.63 ± 0.15 %, this result was smaller than that of the fluidized bed dryer method which was 79.78 ± 0.23 % (Figure 2).

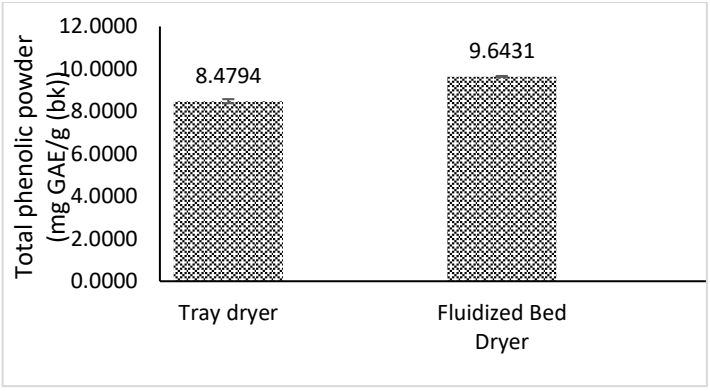

Figure 3. Total phenolic (mg GAE/g (bk)) bidara leaf powder

The total phenolic component of the powder using the tray dryer drying method was 8.4794 mg GAE/g ± 0.0096 powder, this result was smaller than that of the fluidized bed dryer method which was 9.6431 ± 0.0136 mg GAE/g powder (Figure 3). Drying with a fluidized bed dryer is better at maintaining antioxidant capacity presumably because during the drying process the bidara leaf samples receive more even heat. This is supported by Mardiah et al. (2012) that a strong hot wind from the blower will scatter the sample in the container so that the sample will get an even heat effect on each surface and dry quickly.

In addition to chemical changes, changes in physical properties, namely the color after drying, were also observed. Besides being associated with quality factors, color changes in

materials are also associated as indicators of freshness and purity (Winarno, 2004). In simplicia powder, the more the color does not change from the original material, the better. The color change of simplicia powder is related to the number of chemical reactions during drying (Purwanti et al. 2018) . The results of the chromameter observations of the resulting leaf powder were interpreted based on the L*, a* and b* values. The greener the color of the leaves, it is assumed that the better the quality of bidara leaf powder will be.

Table 1. Results of color analysis of bidara leaves on two drying methods using a chromameter

Drying Method	L*	a*	b*
Tray dryer	43.03 ± 0.72 [a]	-6.59 ± 0.14 [a]	20.74 ± 1.02 [a]
Fluidized bed dryer	44.52 ± 0.44 [b]	-7.72 ± 0.73 [b]	20.33 ± 1.01 [a]

The results of the research in Table 1 show that higher L* values and lower a* values were found in the fluidized bed dryer sample, meaning that the simplicia powder produced was brighter and had a better green color. The b values are both positive and not significantly different, meaning they have a yellow color which is objectively similar in intensity. Overall, the powder produced from the drying of the fluidized bed dryer is better than the tray dryer. These results are supported by Amanto et al. (2015) who stated that the drying temperature could affect the color of the leaf simplicia powder. The drying temperature of the tray dryer is higher than that of the fluidized bed dryer, so it is thought to make the leaf color darker. Another cause is thought to be due to the uneven drying conditions in the drying process.

Polarity Characterization of Antioxidant Compounds

Characterization of the polarity of antioxidant compounds was carried out using bidara leaf powder as a result of drying with a fluidized bed dryer. The existence of intensive solvent contact with the material will cause the active compounds in the material to move into the solvent and can be analyzed. The degree of polarity of the solvent used greatly determines the amount of active substance extracted. This is because of the principle "like dissolves like" in the extraction process that a substance will only dissolve well in solvents that have the same level of polarity (Sudarmadji et al., 1989).

Table 2. Yield of bidara leaf extract from various types of solvents

Solvent Type	Extract Yield (%)
N-hexane	1.19
Ethyl acetate	4.96
Ethanol 70%	19.09
Water	39,56

In this study, four types of solvents with different polarities were used, namely hexane (nonpolar), ethyl acetate (semipolar), ethanol (polar), and water (polar). The yield values of bidara leaf extract to hexane, ethyl acetate, methanol and water are shown in Table 2. The lowest yield was macerated extract with hexane solvent, while the highest yield was found in water extract. The difference in the yield value is caused by the different types of solvents used in accordance with the "like dissolves like" principle where the substance will be dissolved and extracted in solvents that have the same polarity level. Therefore, the amount of extract produced from a material can be different depending on the type of solvent used.

Based on the yield calculation results, the polar components contained in the leaves of the bidara are quite high, when compared to the very low nonpolar components. Previous

research by Khaleel (2018) found that the largest yield of bidara leaf extract was water extract (46.2%), then ethanol (20.3%), then ethyl acetate (15.0%) and n-hexane (0.81%). The experimental results on water, ethanol, and ethyl acetate were different, presumably due to the difference in time and extraction method used, causing the resulting crude yield to also be different. According to Khaleel (2018) , the high yield in water and ethanol can also be caused by the high solubility of proteins and carbohydrates in polar solvents. A higher extraction yield does not mean that the extract will have a high concentration of bioactive compounds. Therefore, the yield values generated in Table 2 above do not represent the polarity of the antioxidant compounds in bidara leaves.

Antioxidant Capacity of DPPH and FRAP Methods

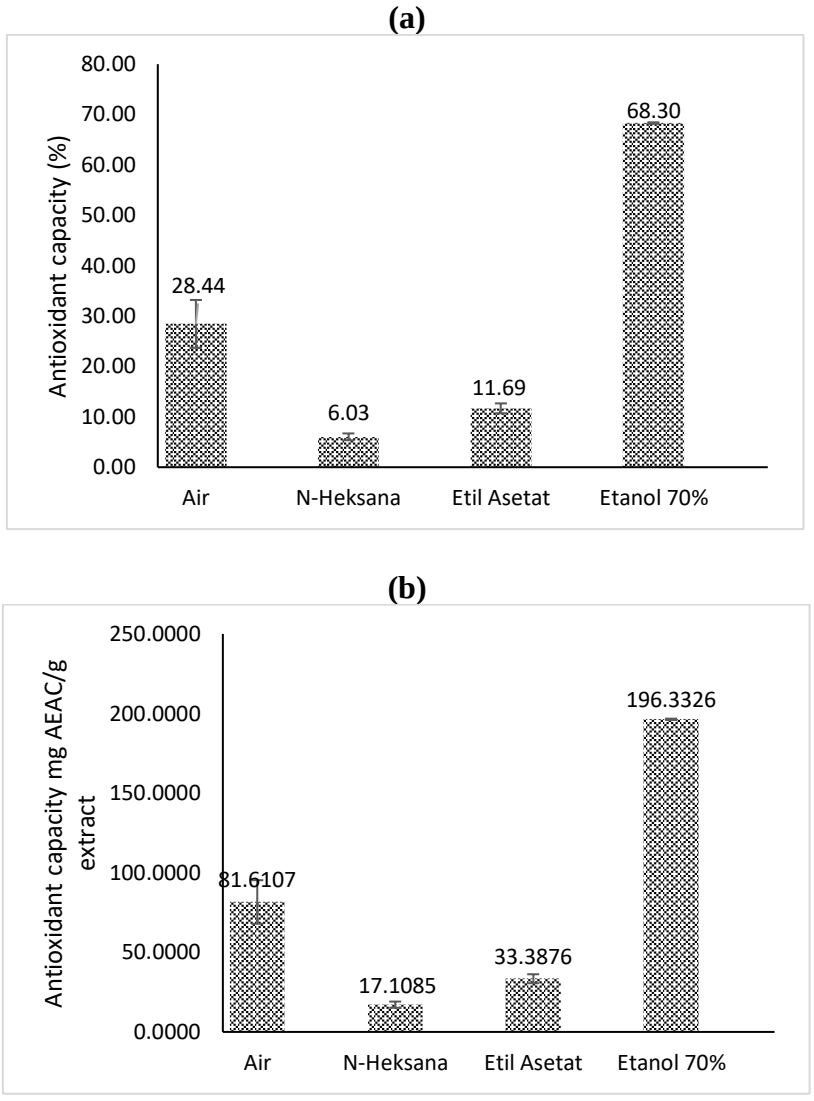

Figure 4. The antioxidant capacity of bidara leaf extract in various solvents is expressed in percent inhibition (a) and mg ascorbic acid eq./g of leaves (b).

Note: Values followed by different letters show highly significant differences ($p < 0.05$) using Duncan's advanced test.

The value of the antioxidant capacity of the Arab bidara leaf extract is expressed in percent antioxidant capacity and the mg AEAC/g extract value is presented in Figure 4. The antioxidant capacity value (%) of the bidara leaf extract ranges from 6.03 ± 0.68% to 68.30 ± 0.15 % . The antioxidant capacity of bidara arab leaves was greater in ethanol solvent, namely 196.3326 ± 0.4344 mg AEAC/g extract or a percent inhibition of 68.30 ± 0.15%. The results of the analysis of antioxidant capacity show that there are many antioxidant components that are active in reducing DPPH free radicals, but their values vary between solvents and are mostly in the polar fraction.

This result is due to differences in the antioxidant components present in each extract, thus providing different antioxidant capacities (Anokwuru et al., 2011). The antioxidant capacity of the extract was greater in ethanol solvent, indicating that the antioxidant compounds in the bidara leaves were more dominant in the polar fraction. Water solvents are polar solvents with the highest yields of extracts, but the capacity of antioxidant compounds is thought to be low because in very polar and universal solvents such as water, it is possible that other compounds such as carbohydrates and proteins will also dissolve (Pambudi et al., 2020). In addition, it is possible that the large amount of solvent that has not yet evaporated in the yield is thought to have contributed to the small antioxidant capacity per weight of the extract.

Different from the DPPH method, the FRAP method measures the capacity of antioxidant compounds based on the reduction reaction of the complex compound Fe3+ and the compound 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) into a form Ferro (Fe2+) at low pH (Kusmiati et al. 2018) . The antioxidant measurement of the FRAP method uses iron II sulfate heptahydrate (FeSO4.7H2O) as a standard so that FRAP values are expressed in units of µmol FeSO4 equivalent/g extract.

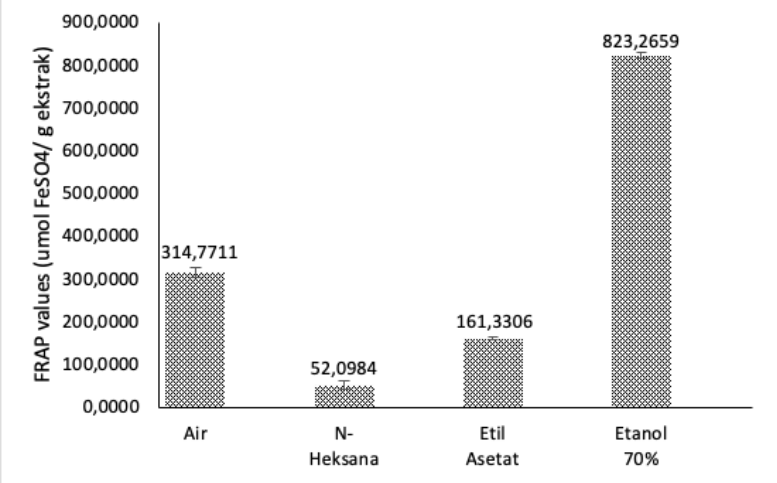

Figure 5. FRAP values (µmol FeSO4/g) of bidara leaf extract in various solvents

Note: Values followed by different letters show highly significant differences ($p < 0.05$) using Duncan's advanced test.

The FRAP value obtained indicates the potential of the compound in Bidara leaves as an antioxidant with its mechanism of preventing oxidation initiated by metal ions such as Fe3+ and Cu2+. Therefore, the more reduced Fe3+ ions, the better the antioxidant capacity of the sample. The results of the test for the antioxidant capacity of FRAP values are presented in Figure 5. The antioxidant capacity of bidara arab leaves was greater in 70% ethanol solvent, namely 823.2659 ± 6.7286 µmol FeSO4/g extract. In this study, the polar ethanol fraction had the highest antioxidant capacity from both the DPPH and FRAP test results, meaning that the

antioxidant mechanism of the antioxidant components in the ethanol fraction works with a hydrogen atom donor and electron donor mechanism.

Total Phenolic Analysis

One of the natural and main sources of antioxidants from plants is the class of phenolic compounds. Phenolic compounds have a rich spectrum with different solubility properties so that the extraction process using various solvents will produce different extracted phenolic components.

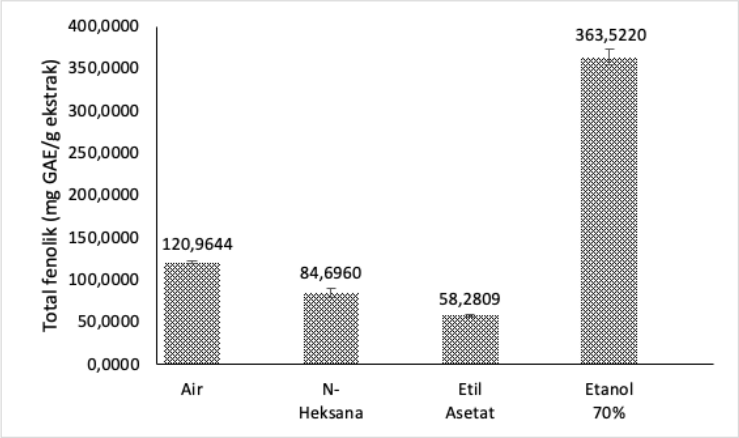

Figure 6. Total phenolic (mg GAE/g) of bidara leaf extract in various solvents

Note: Values followed by different letters show highly significant differences ($p < 0.05$) using Duncan's advanced test.

Based on the results of measuring the total amount of phenolic, the ethanol extract of bidara arab leaves was able to extract the highest phenolic compounds compared to other solvents, namely 363.5220 mg ± 8.8944 GAE/g extract (Figure 6). This result probably occurred because the ethanol solvent has hydroxyl groups which can form bonds with existing phenolic groups thereby increasing its solubility. This is also supported by research conducted by Suhendra et al. (2019) who stated that ethanol solvent was the best solvent used to extract and identify phenolic compounds. The experimental results were higher when compared to research by Al-Ghamdi and Shahat (2017) on the total phenolic methanol extract of the leaves of Bidara Arab grown in Jeddah and research by Khaleel et al. (2016) on bidara growing in Jordan. These results are also thought to be influenced by differences in the types of Arabian bidara and several other factors including geographical factors, genetics, plant seed sources, climatic conditions and soil fertility which can affect the content of plants. This is also supported by research by Utomo et al. (2020) which stated that the total phenolic content of horsetail leaves grown in hot areas has a significantly different value compared to those grown in cold areas, while specific research regarding the total phenolic content of bidara arabia grown in Indonesia is unknown.

Analysis of Total Flavonoids

Flavonoids are plant secondary metabolites with various phenolic structures and are found in almost all parts of the plant. Flavonoids have many health benefits including being antiviral, hypoallergenic, antimicrobial, antioxidant, hydrolyzing enzyme inhibitors, and anti-inflammatory (Aminah et al. 2017) . Aluminum chloride as a test reagent will form stable acid

complexes with C-4 keto groups and C-3 or C-5 hydroxyl groups of flavones and flavonols and form labile acid complexes with orthodihydroxyl groups in the A and B rings of flavonoids causing a color change. observable. Quercetin is used in preparing the standard curve so that the total flavonoids are expressed in quercetin eq. per extract weight.

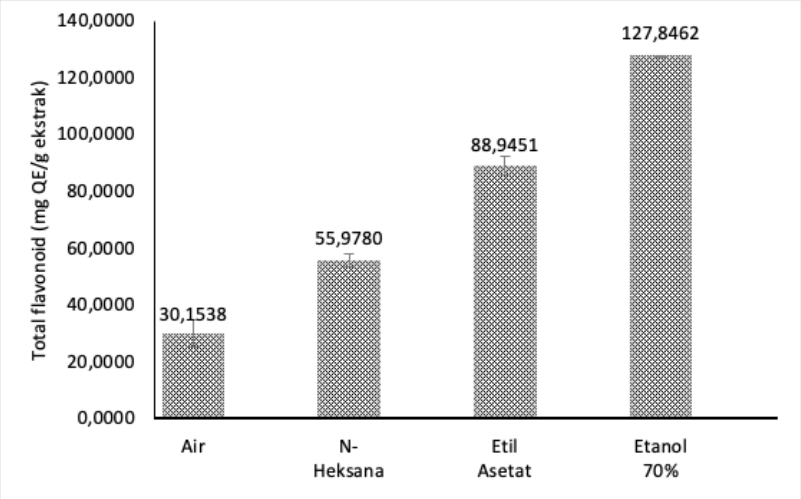

Figure 7. Total flavonoids (mg QE/g) of bidara leaf extract in various solvents

Note: Values followed by different letters show highly significant differences ($p < 0.05$) using Duncan's advanced test.

Figure 7 shows that the highest total flavonoids were obtained in ethanol extract, namely 127.8462 ± 0.1554 mg QE/g and the lowest total flavonoids were obtained using water as a solvent, namely 30.1538 ± 4.6622 mg QE/g. The highest total flavonoids were found in the ethanol extract which indicated that the flavonoid compounds of bidara arab leaves could be extracted well in polar solvents. However, on the other hand, this can also be caused by the structure of the flavonoid compounds found in Bidara leaves which bind to sugars, which tends to cause the flavonoids to dissolve easily in polar solvents. This assumption is reinforced by the research of Hanin and Pratiwi (2017) which states that flavonoids can bind to sugar (glycosides) or in their free form (aglycones), but most of them are found in the form of glycosides. Flavonoid compounds as well as other phenolic compounds consist of several types, each type of flavonoid has a different polarity depending on the number and position of the hydroxyl groups of each type of flavonoid so that this will affect the solubility of flavonoids in solvents (Aguilera et al., 2010). Total flavonoids in water extract of bidara arab leaves were found to be significantly smaller than hexane extracts, this shows that the solubility of flavonoids is not only always present in the polar fraction, but still depends on the structure of the flavonoids themselves.

CONCLUSION

The results showed that the drying process of bidara leaves with a fluidized bed dryer produced bidara leaf powder which was better than the tray dryer method based on the parameters of antioxidant capacity, total phenolics, and color analysis. The results of the extract polarity test showed that there was a significant effect ($p<0.05$) of the 4 types of solvents on the antioxidant capacity observed based on the DPPH, FRAP, total phenolic and total flavonoid tests. The ethanol extract of bidara arab leaves significantly ($p<0.05$) resulted in the highest antioxidant capacity, total phenolic and total flavonoids so that the utilization and development

of bidara leaf products such as herbal teas are considered appropriate because they have been shown to contain bioactive components in the polar fraction which act as antioxidants.

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