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ANALYSIS OF FLAVONOIDS AND ANTIOXIDANT ACTIVITY IN CAFFEINE-FREE COFFEE FROM DATE SEEDS (Phoenix dactylifera)

Arum Dwi Agustin[1)], Indra Kurnia Setyani[2)], Ratih Purwasih[*)], Hani Widya Safitri[4)]

Diploma III Study Program of Pharmaceutical and Food Analyst,
Health Polytechnic Ministry of Health Surakarta

E-mail:ratihpurwasih0509@gmail.com

Abstract: Date seed coffee is obtained by drying, roasting and grinding the date seeds. There are many benefits that can be obtained from the ingredients in date seeds, such as nourishing your stamina. The flavonoids contained therein also have antioxidant properties. Date seeds have also been developed into a coffee substitute. Therefore, researchers are interested in analyzing the content of flavonoids, caffeine and antioxidant activity in them. This research was conducted to determine the presence or absence of caffeine content, to determine the presence or absence and levels of flavonoids and strong antioxidant activity in dated coffee seeds. This type of research is quantitative with a descriptive research design. Date seeds are processed into coffee by drying the clean date seeds in the oven and then roasting them until the seeds are torn and quite burnt. Date seeds that have been roasted, crushed and sieve with a 60 mesh sieve. The date palm coffee powder was extracted by socletation using 80% ethanol as a solvent. The extracts of date seeds were analyzed qualitatively of flavonoids and caffeine with color testing, quantitative analysis of flavonoids with UV-Vis spectophotometry and antioxidant activity using the DPPH method. The qualitative test showed that positive date coffee seeds containing flavonoids was indicated by a red color change when reacted with Mg and HCl powder, and negative containing caffeine was indicated by a red color change when reacted with concentrated ammonia. In the quantitative analysis of flavonoids, the levels of flavonoids in dated seed coffee were 7523.03 ± 10.5078 mg QE / 100 g, and the antioxidant activity test showed that the level of IC50 in dated seed coffee was 26, 747 ppm. It shows that date seed coffee is free of caffeine, has a high content of flavonoids and has very strong antioxidants

Keywords:Flavonoids, Antioxidants, UV-Vis Spectrophotometry, Caffeine-free Coffee, Date Seeds

INTRODUCTION

The introduction includes the background of the problem, the formulation of the problem and research objectives, a summary of theoretical studies related to the problem under study, and sometimes the expectations of the results and benefits of the research are included. The introduction is about 2-3 pages long. Typed in 1 space, Times New Roman 11pt font.

Date seeds are flaked seeds one (monocot), odorless, tasteless and slightly bitter. According to Abdul et al. (2013), 11-18% of the weight of dates is in the seeds. The fat content in date seeds ranges from 5.7 to 12.7%. Date seeds also contain antioxidants whichcan reduce levels of free radicals.

Antioxidants on flavonoids due to the reducing properties of flavonoids which can donate hydrogen to free radicals (Silalahi, 2006). As antioxidants, flavonoids are able to inhibit cancer cells and the clumping of blood platelets. The relationship between flavonoids and antioxidants is known throughthe statement that the higher the total flavonoid content of an ingredient, the higher the antioxidant activity (Erukainure et al., 2011).

The role of antioxidants is to provide stability by deactivating free radicals before they attack body cells. Apart from that, antioxidants also play a role in maintaining cellular health and systemic health of thebody (Sapakal et al., 2008).

The use of date seeds can be saidto be inadequate. However, in Arabia, waste date seeds are used to make caffeine-free coffee drinks (Siregar et al., 2018). Making caffeine-free coffee from date seeds is the same as making regular coffee. Another benefit of date seeds is healing weak lust, cure diabetes, healthy body stamina, relieves fatigue, warms the body, relaxes tense nerves, cholesterol, strokes, gout and dizziness (Hamada et al., 2002). Analysis of date coffee seeds was carried out using qualitative tests of flavonoids and caffeine using color test, quantitative flavonoid test usemethodUV-Vis spectrophotometry and antioxidantsactivity test using the DPPH method.

METHODS

Contains an explanation of how the research was carried out, including research design, population and sample (research target), data collection techniques for instrument development, and data analysis techniques. References are given to lesser known methods. Typed in 1 space, Times New Roman 11pt.

The type of research used is quantitative research with a descriptive research design. The variable used is a single variable, namely the flavonoid and antioxidant content in date coffee.

Materials used were date coffee samples, GF254 silica gel plates, filter paper, 80% ethanol, ethyl acetate PA, methanol PA, quercetin, AlCl3 2%, AlCl 5%, H2O2, HCl 37%, NH3 6N, Mg powder, potassium acetate 120 mM, and DPPH.

The tools used were a stove, frying pan, oven, simplicia blender, 60 mesh sieve, UV-Vis spectrophotometer, cuvette, 23 glass beakers, measuring cup, measuring pipette, test tube, soxhletation tool, porcelain cup and water bath.

a) Preparation of Raw Materials for Date Seeds
   The raw material used is khalas type date seeds. The date seeds used are production waste from one of the MSMEs in the North Klaten area. A total of 500 grams of khalas type date seeds are cleaned until the remaining sticky date flesh is gone. Clean date seeds are dried in an oven at a temperature of 60 oC for 2 hours (Abdillah and Andriani, 2012).
b) Making Date Coffee
   The dried date seeds are then roasted until the date seeds are torn and quite charred, then crushed with a simplicia blender. The finely ground date seeds will be filtered using a 60 mesh sieve. Date seed coffee is ready to be served (Abdillah and Andriani, 2012).
c) Date Coffee Bean Extraction
   60 g of date coffee powder is wrapped in filter paper, tied with string and put into a soxhletizer. The solvent was 300 ml of 80% ethanol put into a round bottom flask. Soxhletation was carried out at a temperature of ±90 oC for 3 hours. The extract obtained was concentrated over a water bath (Ali et al., 2015).
d) Flavonoid Qualitative Test
   A total of 0.5 g of ethanol extract of date seed coffee was dissolved in 2 ml of heated methanol pa, marked as a sample solution. A total of 0.5 g of standard quercetin was dissolved in 2 ml of methanol pa, marked as a positive control. The test solution and positive control were then added with Mg powder and 5 drops of concentrated HCl. The results are declared positive if a red or orange solution is formed which indicates the presence of flavonoids from the flavonol and flavanone groups (Rahayu et al., 2015).
e) Qualitative Test of Caffeine
   A total of 10 mg of date seed coffee plus 1.5 ml of 3% H2O2. Then add 5 drops of 37% HCl and heat over a water bath until dry. The sample was added with 3 ml of NH3 6 N. A positive test was indicated by a red color change in the solution. (Irawati et al., 2018).
f) Flavonoid Quantitative Test
   1) Making a standard stock solution: 20 mg of quercetin is dissolved in sufficient methanol, then put into a 100 ml volumetric flask and add methanol to the limit mark to obtain quercetin with a concentration of 200 ppm (Azizah, 2014).
   2) Determination of the maximum wavelength by running a 60 ppm quercetin solution at a wavelength of 400-450 nm with a wavelength interval of 2.

The resulting maximum wavelength will be used to measure the absorption of a sample of date palm coffee ethanol extract (Aminah et al., 2017).

3) Preparation of a quercetin standard curve. A standard solution of 200 ppm quercetin was prepared in several concentrations, namely 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. 1 ml of each concentration was taken then 1 ml of 5% AlCl3 and 8 ml of 120 mM potassium acetate were added. The solution was incubated for 30 minutes at room temperature. Absorbance was determined using a UV-Vis spectrophotometer with the maximum wavelength obtained (Sari and Ayuchecaria, 2017).
4) Determination of total flavonoid content. 10 mg of extract was weighed, then dissolved in 10 ml of 80% ethanol, to obtain a concentration of 1000 ppm. From this solution, pipet 1 ml then add 1 ml of 5% AlCl3 solution and 8 ml of 120 mM potassium acetate. Samples were incubated for 30 minutes at room temperature. Absorbance was determined with a UV-Vis spectrophotometer at the maximum wavelength. Three replicate samples were made for each analysis and the average absorbance value was obtained (Desmiaty et al., 2009).

g) DPPH method Antioxidant test
1) Preparation of 100 ppm DPPH solution: 5 mg of DPPH was dissolved with methanol in a 50 ml volumetric flask to obtain a concentration of 100 ppm (Aritonang, 2019).
2) Determination of the maximum wavelength. 10 ml of 100 ppm DPPH solution was taken and placed in a 25 ml measuring flask, then methanol was added until the limit mark, so that a DPPH solution concentration of 40 ppm was obtained. The solution is stored in a place protected from light. The 40 ppm DPPH solution was put into a cuvette and run in the wavelength range 400-550 nm using a UV-Vis spectrophotometer at intervals of 2 (Aritonang, 2019).
3) Preparation of blank solution
2 ml of 40 ppm DPPH solution was taken and 2 ml of methanol was added, homogenized then incubated in a dark room for 30 minutes. This solution is marked as a blank solution and the absorbance is then calculated at the maximum wavelength that has been obtained (Aritonang, 2019).
4) Making a Comparative Solution: 10 mg of quercetin was dissolved in 10 ml of methanol pa to obtain a concentration of 1000 ppm. From the mother liquor, several concentration variations were made, namely 1 ppm, 2 ppm, 4 ppm, 6 ppm and 8 ppm for 10 ml. 2 ml of each series of quercetin solution was measured and 2 ml of 40 ppm DPPH solution was added, homogenized and incubated in the dark for 30 minutes. Analysis was carried out by measuring the absorbance using a UV-Vis spectrophotometer at the maximum wavelength obtained (Aritonang, 2019).
5) Antioxidant Activity Testing A total of 25 mg of sample was dissolved in ethanol and homogenized then the volume was increased to 25 ml, so that a concentration of 1000 ppm was obtained as a stock solution. The sample stock solution was made into a series of solution concentrations (10, 20, 40, 60 and 80 ppm). 2 ml of each concentration was taken and 2 ml of 40 ppm DPPH solution was added. The mixture was homogenized and left for 30 minutes in a dark place. Absorbance was measured using a UV-Vis Spectrophotometer at the maximum wavelength obtained (Aritonang, 2019).

Strong or weak antioxidant activity is assessed by calculating IC50 (Inhibition Concentration 50%) which is obtained from linear regression obtained from graphs of sample concentration compared to percent inhibition.

RESULTS AND DISCUSSION

The research results presented are only the results of analysis and hypothesis testing results that need to be reported, not data analysis

processes such as statistical calculations and hypothesis testing processes do not need to be presented. Tables and graphs can be used to clarify the verbal presentation of research results, and should be commented on or discussed. For qualitative research, the results section contains detailed sections in the form of sub-topics that are directly related to the research focus and categories. Typed in 1 space, Times New Roman 11pt.

1. Sample Preparation Results

   Table 4.1 Caffeine Free Coffee Making Table

Sample weight (g)	Weight of date seed coffee powder (g)	Weight of extracted coffee (g)	Weight of thick extract (g)	% rendement (%)
500	428,3597	60	7,7951	12,992

   Based on Table 4.1, it can be seen that the sample used to make date coffee was 500 grams of clean date seeds, after processing it produced 428.3597 grams of date palm coffee powder. Extraction used 60 grams of date coffee powder and produced a thick extract weighing 7.7951 grams with a yield of 12.992%.

2. Results of Qualitative Analysis of Date Bean Coffee

   Table 4.2 Results of Qualitative Analysis of Date Bean Coffee

Test Parameter	Reagent	Result	Standart
Flavonoid Color Test	Concentrated Mg and HCl powder	+ (Positive) A red color forms in the solution	+ (Positive) A red color forms in the solution
Caffeine Color Test	H_2O_2 3%, HCl 37% and NH3 6N	- (Negative) No red color is formed in the solution	+ (Positive) A red color forms in the solution

   Based on Table 4.2 above, it can be seen that the sample coffee seeddates contains flavonoids.

3. Results of Quantitative Flavonoid Analysis

   a. Results of measuring the absorbance of the standard solution and the quercetin calibration curve

   Table 4.3 Absorbance results of standard flavonoid solutions

Concentration (ppm)	Average Absorbantion
20	0,313
40	0,427
60	0,548
80	0,654
100	0,748

   Based on the data in Table 4.3, a calibration curve will be created which shows a graph of the relationship between absorbance and concentration of the standard solution which is presented in Figure 4.1.

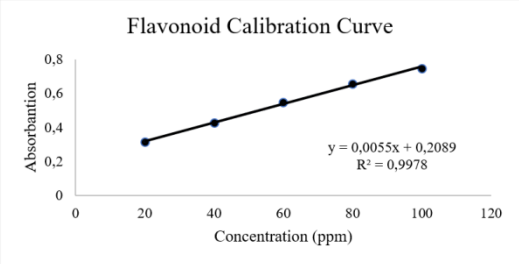

   Figure 4.1 Flavonoid Calibration Curve

   Based on Figure 4.1, it can be seen that the linear regression equation obtained is y = 0.0055x + 0.2089 with a correlation coefficient or R2 value of 0.9978.

   b. Results of determining flavonoid levels in date coffee

   Table 4.4 Flavonoid Content of Date Seed Coffee

Average absorbantion	Average Flavonoid Levels±SD (mg QE/100 g)
0,623	7523,03±10,5078

   Based on Table 4.4, it shows that the flavonoid content in date coffee is 7523.03 ± 10.5078 mg QE/100 g. The levels obtained are quite high, which proves that the levels of flavonoids in date coffee are high.

4. Antioxidant Activity Analysis Results
   a. Results of % inhibition and % inhibition curve of comparison solution

Table 4.5 Results of the absorbance of the comparison solution and % inhibition

Concentration (ppm)	Absorbantion	% inhibition
1	0,285	32,14
2	0,233	44,52
4	0,160	61,90
6	0,085	79,76
8	0,021	95,00

Based on Table 4.5, a % inhibition curve will be created which shows a graph of the relationship between % inhibition and the concentration of the reference solution which is presented in Figure 4.2.

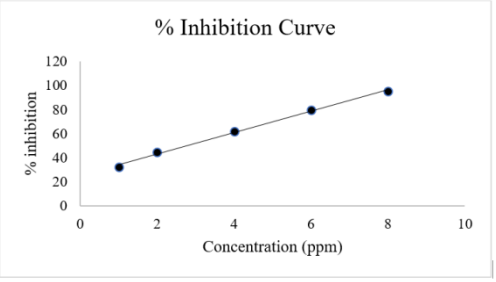

Figure 4.2 % Inhibition Curve

Based on Figure 4.2, the linear regression equation y = 8.884x + 25.351 is obtained with a correlation coefficient or R2 value of 0.9959.

   b. Results of % inhibition and % inhibition curve of sample solution

Table 4.6 Results of sample solution absorbance and % inhibition

Concentration (ppm)	Absorbantion	% inhibition
10	0,399	5,000
20	0,287	31,667
40	0,179	57,381
60	0,043	89,682

Based on Table 4.6 above, a % inhibition curve will be created which shows a graph of the relationship between % inhibition and the concentration of the standard solution which is presented in Figure 4.3.

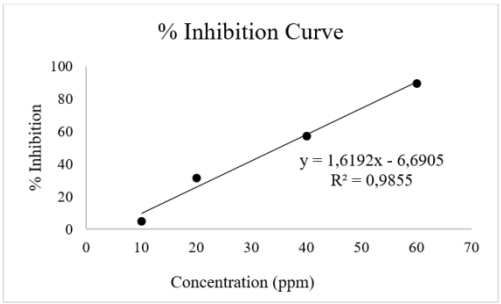

Figure 4.3 % Imhibition Curve

Based on Figure 4.3, the linear regression equation y = 1.6192x + 6.6905 is obtained with a correlation coefficient or R2 value of 0.9855.

   c. IC50 concentration

Table 4.7 IC50 Concentration

	Concentration IC_{50} (ppm)
Comparative Solution	2,775
Sample Solution	26,747

Based on Table 4.7, it shows the IC50 concentration in date coffee and quercetin as a comparison. The IC50 concentration of the comparison solution and the date coffee sample obtained was 2,775 ppm and 26,747 ppm. The concentration of the comparison solution and this sample is classified as a very strong antioxidant.

In this study, an analysis of caffeine-free coffee from khalas date palm seeds (Phoenix dactylifera) was carried out. Making date coffee goes through the stages of drying, roasting, grinding and filtering. Next, make coffee extract from date palm seeds by soxhletation. Soxhletation extraction produces more extract than maceration because soxhletation does not require filtering, and the time used is faster (Warnasih et al., 2019). The soxhletation temperature used is 90 oC because ethanol has a high boiling point, making it difficult for the

solvent to evaporate and condense. The solvent used is ethanol, because ethanol has a smaller structure so it can penetrate the cell walls easily to dissolve the compound components (Siregar et al., 2018).

Analysis of date bean coffee begins with a flavonoid color test. The color test was carried out using the Wilstater reaction with Mg powder and concentrated HCl solution. The addition of concentrated HCl is intended to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. Glycosyl will be replaced by H + from the acid because of its electrophilic nature. Reduction with Mg and concentrated HCl will produce complex compounds that are red or orange (Mariana et al., 2013). Based on Table 4.1, the flavonoid color test for date coffee beans is positive, indicated by the formation of a purplish red color in the solution. These results are in line with the results reported by Warnasih et al. (2019) that date seeds contain flavonoids in them.

Test the color of caffeine with the murexid reaction. The reagents in this test are H2O2 and HCl. The reaction that occurs in this test can be seen in Figure 4.5.

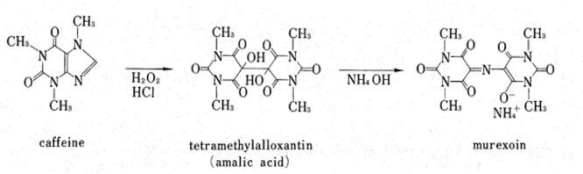

Figure 4.5 Reaction Murexide Caffein (Kozuka *et al.*, 1981)

The caffeine color test result was negative because no red color was formed in the solution. These results are in accordance with research by Venkatachalam and Sengottian (2016) which states that date seeds can be an alternative drink to replace coffee that does not contain caffeine.

Quantitative analysis of flavonoids using the UV-Vis spectrophotometric method. UV-Vis. The UV-Vis spectrophotometer method was used because flavonoids have a conjugated aromatic system so they show strong absorption bands in the UV and visible light spectrum (Aminah et al., 2017). The maximum wavelength obtained was 434 nm with an absorbance of 0.775. The maximum wavelength obtained was then used to analyze flavonoid levels.

Based on Table 4.2, it can be seen that there is a significant increase in absorbance values from low concentration to high concentration, namely from 0.313 at 20 ppm to 0.748 at 100 ppm. This data produces a calibration curve so that a linear equation is obtained as in Figure 4.1, namely y = 0.0055x + 0.2089 with a correlation coefficient (r) of 0.9989 or R2 = 0.9978. The large correlation or r value which is almost close to 1 proves that a calibration curve produces a linear line because all points are on a straight line and is a good analysis method (Chandra et al., 2019).

In measuring flavonoid compounds, AlCl3 and potassium acetate were added to the sample solution and quercetin. AlCl3 functions to create complex compounds, resulting in a wave shift towards the visible which can be seen by changing the color of the solution to yellow, while potassium acetate is intended to maintain the wavelength in the visible region. The solution was incubated for 30 minutes with the aim that the reaction could run perfectly and the resulting color intensity would be maximized (Azizah et al., 2014).

This study resulted in an average flavonoid level of 7523.03 ± 10.5078 mg QE/100 g. These results are not in line with the results in the research of Warnasih et al. (2019) namely 282.84 mg QE/100 g. The difference in results obtained could be caused by the different samples used, in this study using khalas dates while in the research of Warnasih et al. (2019) used Shiva dates. In the research of Warnasih et al. (2019) explained that the flavonoid content of each date seed varies depending on the variety, supported by research by Riyadi (2018) which explained that the content of date seeds is influenced by the environmental conditions where they are grown, the time of collection and post-harvest processing.

The antioxidant activity of coffee date seed extract was carried out using the DPPH method. This method was chosen because it is simple, fast, and does not require quite a lot of reagents (Sayuti and Yenrina, 2015). Antioxidant activity was measured based on the IC50 value. The IC50 value is the concentration of antioxidant compounds that can reduce DPPH radicals by 50%. The smaller the IC50 value, the

more active the extract is as an antioxidant compound (Warnasih et al., 2019). The IC50 value is calculated using a linear equation obtained from the % inhibition curve.

Based on Figure 4.2, it can be seen that there was a significant increase in the % inhibition value of the reference solution from low concentration to high concentration, namely from 32.14% at 1 ppm to 95% at 8 ppm. The % inhibition curve for the comparison solution produces a linear regression equation, namely y = 8.884x + 25.351 with a correlation coefficient or R2 value of 0.9959.

In Figure 4.3, it can be seen that there was a significant increase in the % inhibition value of the sample from low concentration to high concentration, namely from 5% at 10 ppm to 89.682% at 60 ppm. The sample inhibition % curve produces a linear regression equation, namely y = 1.6192x + 6.6905 with a correlation coefficient or R2 value of 0.9855.

This linear equation is then used to determine the IC50 level. The results of this research show a quercetin value of 2.76 ppm. These results do not show a big difference with the results in the study by Muthia et al. (2019) which states that the IC50 value of quercetin is 2.04 ppm. Based on Table 4.6, the IC50 value for the date palm coffee sample extract is 26,747 ppm. These results are not in accordance with those stated in the research of Siregar et al. (2018), namely 2.27 ppm. Siregar et al.'s research. (2018) used date seed extract samples, while this study used date seed coffee which went through the oven and roasting stages. According to Rompas (2012), flavonoids are a class of compounds that are not heat resistant and are easily oxidized at high temperatures, so it is very likely that during the sample making process there are flavonoid compounds that are lost. The difference in IC50 value results between quercetin and date palm coffee ethanol extract could be because the date palm coffee ethanol extract contains other ingredients that can interfere with the reduction concentration of DPPH free radicals (Muthia et al., 2019). Apart from other disturbing ingredients, antioxidant stability can also be influenced by light, oxygen and pH, while DPPH radicals are sensitive to light, oxygen and pH (Giuliama et al., 2015). Based on the results of the IC50 value, it shows that quercetin and date coffee have very strong antioxidant activity. This classification is based on the classification of antioxidant activity in the journal Molyneux (2004) which is presented in Table 4.8.

Table 4.8 Classification of Antioxidant Activity

Concentration IC_{50}	Classification
$IC_{50} \leq 50$ µg/mL	Very Strong
50 µg/mL < $IC_{50} \leq 100$ µg/mL	Strong
100 µg/mL < $IC_{50} \leq 150$ µg/mL	Currently
150 µg/mL < $IC_{50} \leq 200$ µg/mL	Weak
$IC_{50} > 200$ µg/mL	Very Weak

CONCLUSIONS

Based on results The research and discussion described previously can be concluded as follows:

a. Date palm coffee (Phoenix dactylifera) does not contain caffeine.
b. Qualitative testing of flavonoids showed that there was flavonoid content in date palm coffee (Phoenix dactylifera) and the flavonoid content in date palm coffee (Phoenix dactylifera) was 7523.03 ± 10.5078 mg QE/100 g.
c. The antioxidant activity of date palm coffee (Phoenix dactylifera) is classified as very strong, seen from the calculated IC50 value of 26,705 ppm.

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REFERENCES

Abdillah, L. A. Andriani, M., 2012. Friendly Alternative Healthy Drinks Through The Use Of Date Seeds As Coffee Powder. *Proceeding of ICEBM-UntarJakarta-ISBN; 978-602-18994-0-3*

Abdul, A. M. J. Abdul, R. Che Man, Y. B. Al-Kahtani, H. A. and Mansor, T. S. T. 2013. Date Seed and Date Seed Oil. *International Food Research Journal*. 20(5): 2036-2037

Ali; M. A. Al-Hattab, A. T. and Al-Hydary I. A. 2015. Extraction of Date Palm Seed Ooil (*Phoenix dactylifera*) by Soxhlet Apparatus. *International Journal of Advances in Engineering & Technology*. 8(3) : 261-271

Aminah, Tomayahu, N. dan Abidin, Z., 2017. Penetapan Kadar Flavonoid Total Ekstrak Etanol Buah Alpukat (*Persea americana* Mill.) dengan metode Spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia*. 4(20 : 226-230

Aritonang, D. 2019. *Uji Aktivitas Antioksidan pada Minuman Kemasan dengan Metode DPPH*. [Skripsi]. Medan : Fakultas Farmasi dan Kesehatan Institut Kesehatan Helvetia

Azizah, B. dan Salamah, N. 2013. Standarisasi Parameter Non Spesifik dan Perbandingan Kadar Kurkumin Ekstrak Etanol dan Ekstrak Terpurifikasi Rimpang Kunyit. *Jurnal Ilmiah Kefarmasian.* 3(1) : 21-30

Azizah, D.N., Kumolowati, E. dan Faramayuda, F. 2014. Penetapan Kadar Flavonoid Metode AlCl3 pada Ekstrak Metanol Kulit Buah Kakao (*Theobrama cacao* L.). *Kartika Jurnal Ilmiah Farmasi*. 2(2): 45-49

Chaira, N., Ferchichi, A., Mrabet, A., & Sghairoun, M. 2007. Chemical Composition of the Flesh and the Pit of Date Palm Fruit and Radical Scavenging Activity of Their Extracts. *Pakistan Journal of Biological Sciences.* 10(13) : 2202-2207

Chandra, B., Zulharmita, dan Putri, W.D. 2019. Penetapan Kadar vitamin C dan B1 Pada Buah Naga Merah (*Hylocereus lemairel* (Hook.) Britton & Rose) dengan Metode Spektrofotometri UV-Vis. *Jurnal Farmasi Higea*. 11(1): 62-74.

Deinstrop, E. H. 2007. *Applied Thin-layer Chromatography: Best Practice and Avoidance of Mistakes (2nd Ed)*. Penerjemah: R.G Leach. Germany: Wiley-vch Verlag GmbH & Co

Departemen Kesehatan RI. 2000. *Acuan Sediaan Herbal*. Jakarta : Diktorat Jendral POM-Depkes RI

Desmiaty, Y., Ratnawati, J., dan Andini, P. 2009. *Penentuan Jumlah Flavonoid Ekstrak Etanol Daun Buah Merah (Pandanus conoideus Lamk.) secara Kolorimetri Komplementer*. Presentasi Seminar Nasional POKJANAS TOI XXXVI Universitas Sanata Dharma: Yogyakarta

Erukainure, O. L., Oke O. V., dan Ajiboye A. J. 2011. Nutritional Qualities and Phytochemical Constituents of *Clerodendrum volubile*, a Tropical Non-conventional Vegetable. *International Food Research Journal.* 18(4) : 1393-1399

Farah, A. 2012. *Coffee Constituens in Coffee : Emerging Health Effects and Disease revention. First Edition.* United Kingdom : Blackwell Publishing Ltd.

Firmansyah M., Widyandana, dan Rahayu, G. R. 2015. Studi Kualitatif Dampak Uji Kompetensi Dokter Indonesia Terhadap Pembelajaran Pada Mahasiswa Kedokteran. Indonesia: *Jurnal Pendidikan Kedokteran Indonesia*. 4(3) : 129-139

Giuliama, F.E, Ardana, M., dan Rusli, R. 2015. *Pengaruh pH terhadap Aktivitas Antioksidan Ekstrak Daun Miana (Coleus atropurpureus* L. Benth*)*. Universitas Mulawarman: Samarinda

Hamada, J. S., Hashim, I. B., and Sharif, F. A. 2002. Preliminary Analysis and Potensial Uses of Date Pits in Foods. *Journal Food Chemistry*. 76 (2002) : 135-137

Irawati, D. Styawan, A. A. dan Nurhaini, R., 2018. Penetapan Kadar Kafein pada The Oolong (*Camellia sinensis*) dengan Metode Titrasi Bebas Air. *The 7th University Research Colloqium STIKES PKU Muhammadiyah Surakarta*

Jayaprakasha, G.K., Rao, L.J., and Sakariah, K.K., 2002. Improved HPLC Method for Determination of Curcumin, Dimethoxy Curcumin, and Bisdemethoxy Curcumin. *J. Agric. Food Chem.* 50(13) : 3668- 3672

Kozuka, H., Koyama, M., and Okitsu, T. 1981. The Murexide Reaction of Caffeine Using Nitric Acid. *Pharmaceutical Society of Japan*. 30(3): 941-946

Maramis, R. K., Citraningtyas, G., dan Wehantouw, F., 2013. Analisis Kafein Dalam Kopi Bubuk Di Kota Manado Menggunakan Spektrofotometri UV-Vis. *Jurnal Ilmiah Farmasi*. 2(4) : 122-128

Mariana, L., Andayani, Y., dan Gunawan, E.R.

2013. Analisis Senyawa Flavonoid Hasil Fraksinasi Ekstrak Diklorometana Daun Keluwih (*Artocarpus camansi*). *Chem. Prog.* 6(2): 50-55

Molyneux, P., 2004. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin J. Sci. Technol.* 26(2) : 212-219

Mukhriani. 2014. Ekstraksi, Pemisahan Senyawa dan Identifikasi Senyawa Aktif. *Jurnal Kesehatan.* 7(2): 361-367

Mustikaningrum, M. 2015. *Aplikasi Metode Spektrofotometri Visibel Genesys-20 untuk Mengukur Kadar Curcuminoid pada Temulawak (Curcuma Xanthorrhiza)*. Tugas Akhir, Program Studi Diploma III Teknik Kimia Fakultas Teknik Universitas Diponegoro. Semarang

Muthia, R.; Saputri, R.; Verawati, S. A. 2019. Uji Aktivitas Antioksidan Ekstrak Etanol Kulit Buah Mundar (*Garcinia forbesii* King.) menggunakan Metode DPPH (*2,2-Diphenyl-1-Picrylhydrazil*). *Jurnal Pharmascience*. 6(1) : 74-82

Notoatmodjo, S., 2010. *Metodologi Penelitian Kesehatan*. Jakarta: Rineka Cipta

Ogah, C. O. and Obebe, T. O. 2012. Caffeine Content of Cocoa and Coffee Beverages in Lagos, Nigeria. *Global Research Publishing*. 3(1) : 404-405

Oktaria, S., 2019. Hubungan antara Konsumsi Minuman Berkafein dengan Pola Tidur Pada Mahasiswa Teknik. *Jurnal Kesehatan Masyarakat dan Gizi.* 1(2) : 10-15

Olavia, L., 2019. 2020, Konsumsi Kopi Diproyeksi Naik 13,9%. https://www.beritasatu.com/ekonomi/591071/2020-konsumsi-kopi-diproyeksikan-naik-139. Diakses pada 30 November 2020

Palupi, A.D., dan Purnama, H. 2020. *Pengaruh Ukuran Partikel dan Metode Hidrolisis pada Pembuatan Bioetanol dari Limbah Kulit Kopi Arabika*. The 11th University Research Colloquium 2020. Yogyakarta : Universitas 'Aisyiyah Yogyakarta

Purnamayanti, N. P. A. Gunadnya, I. B. P. dan Arda, G. 2017. Pengaruh Suhu dan Lama Penyangraian terhadap Karakteristik Fisik dan Mutu Sensori Kopi Arabika (*Coffea arabica L*). *Jurnal Biosistem dan Teknik Pertanian*. 5(2) : 39-48

Rahayu, S., Kurniasih, N., dan Amalia, V. 2015. Ekstraksi dan Identifikasi Senyawa Flavonoid dari Limbah Kulit Bawang Merah sebagai Antioksidan Alami. *Al Kimiya*. 2(1): 1-8

Ramadhani, R. A. Kusrini, D. dan Fachriyah, E., 2013. Isolasi, Identifikasi dan Uji Antioksidan Senyawa Flavonoid dari Ekstrak Etil Asetat Daun Tempuyung (*Sonchus arvensis* L.). *Chem info.* 1(1) : 247-255

Ridwansyah. 2002. *Pengolahan Kopi, 2nd ed*. Medan : Usulibrary

Ritna, A.; Anam, S. dan Khumaidi, A., 2016. Identifikasi Senyawa Flavonoid pada Fraksi Etil Asetat Benalu Batu (*Begonia sp.*) Asal Kabupaten Morowali Utara. *Journal of Pharmacy.* 2(2) : 83-89

Riyadi, W. 2018. *Ekstraksi Biji Kurma (Phoenix dactylifera L.) dengan Metode Sokletasi dan Karakterisasinya*. [Skipsi] Jakarta: Fakultas Sains dan Teknologi Universitas Islam Negeri Syarif Hidayatullah

Romadhani, H., 2016. *Validasi Metode Penetapan Kadar Tablet Floating Metformin Hidroklorida dengan Spektrofotometri*. [Skripsi] Purwokerto : Fakultas Farmasi Universitas Muhammadiyah Purwokerto

Sapakal, V.D., Shikalgar T.S., Ghadge R.V., Adnaik R.S., Naikwade N.S., dan Magdum C.S., 2008. In Vivo Screening Antioxidant Profile : a review. *Journal of Herbal Medicine and Toxicology*. 1(1) : 1-8

Saputra, E. 2008. *Kopi.* Yogyakarta : Harmoni

Sari, A.K., dan Ayuchecaria, N. 2017. Penetapan Kadar Fenolik Total dan Flavonoid Total Ekstrak Beras Hitam (*Oryza sativa* L.) dari Kalimantan Selatan. *Jurnal Ibnu Sina*. 2(2): 327-335

Satuhu, S., 2010. *Kurma, Kasiat dan Olahannya*. Jakarta : Penebar Swadaya

Sayuti, K., dan Yenrina, R., 2015. *Antioksidan, Alami dan Sintetik*. Padang : Andalas University Press

Silalahi, J., 2006. *Makanan Fungsional*. Yogyakarta : Kanisius

Simanjuntak, K., 2012. Peran Antioksidan

Flavonoid dalam Meningkatkan Kesehatan. *Jurnal Bina Widya.* 23(3) : 135-140

Siregar, Y. D. I., Rudiana, T., Riyadi, W., 2018. Identifikasi Komposisi Kimia dan Uji Aktivitas Antioksidan dari Biji Kurma (*Phoenix dactylifera*). *Jurnal Penelitian dan Pengembangan Ilmu Kimia*. 4(2) : 182-189

Sugiyono. 2013. *Metode Penelitian Pendidikan Pendekatan Kuantitatif, Kualitatif, dan R&D*. Bandung: Alfabeta.

Suhartati, T., 2017. *Dasar-Dasar Spektrofotometri UV-Vis dan Spektrometri Massa untuk Penentuan Struktur Senywa Organik*. Lampung: CV. Anugrah Utama Raharja

Sukmadinata, N. S., 2013. *Metode Penelitian Pendidikan*. Bandung : PT Remaja Rosdakarya

Syah, H., Yusmanizar, dan Maulana, O. 2013. Karakteristik Fisik Bubuk Kopi Arabika Hasil Penggilingan Mekanis dengan Penambahan Jagung dan Beras Ketan. *Jurnal Teknologi dan Industri Pertanian Indonesia*. 5(1): 32-37

USDA, 2012. National Nutrient Database for Standard Reference, Release 25. Nutrient data for 09231, Date-palm (Phoenix dactylifera) Purple. http://www.nal.usda.gov. diakses 2 Desember 2020

Venkatachalam, C.D., and Sengottian, M. 2016. Study on Roasted Date Seed Non Caffeinated Coffee Powder as a Promising Alternative. *Asian Journal of Research in Social Sciences and Humanities*. 6(6): 1387-1394

Verawati, H.S. dan Satria, B. 2014. Penetapan Kadar Konsumsi Kafein dalam Minuman Teh Seduhan yang Beredar di Pasaran secara KLT-Densitometri. *SCIENTIA ISSN : 2087-5045*. 4(1) : 43-45

Warnasih, S. Widiastuti, D. Hasanah, U. Ambarsari, L. dan Sugita, P., 2019. Aktivitas Antioksidan dan Flavonoid Ekstrak Biji Kurma. *Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup.* 19(1) : 34-38

Widyowati, C.H. 2015. *Identifikasi Senyawa Aktif Antioksidan Ekstrak Biji Kurma (Phoenix dactylifera).* [Skripsi]. Jakarta: Fakultas Sains dan Teknologi Universitas Islam Negeri Syarif Hidayatullah

Winarsih, H., 2007. *Antioksidan Alami dan Radikal Bebas : Potensi dan Aplikasinya dalam Kesehatan*. Yogyakarta : Kanisius

Yasin, B. R., El-Fawal, H. A. N., Mousa, S. A., 2015. Date (*Phoenix Dactylifera*) Polyphenolics and Other Bioactive Compounds: a Traditional Islamic Remedy's Potential in Prevention of Cell Damage, Cancer Therapeutics and Beyond. *International Journal of Molecular Sciences.* 16(12) : 30075–30090

Yazid, E. 2015. *Fakta Tentang Kopi*. Artikel Ilmiah. AAK Delima Husada Gresik. Volume VI

Yuslianti, E. R., 2018. *Pengantar Radikal Bebad dan Antioksidan Edisi I.* Yogyakarta: Deepublish

Yusuf, A. M., 2017. *Metode Penelitian: Kuantitatif, Kualitatif, Dan Penelitian Gabungan*. Jakarta: Kencana