ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

In Vitro Determination of Antioxidant Activity, Total Phenolics, Total Flavonoid, Anti-cholesterol of Extracts Saffron (Crocus sativus)

Muhammad Ryan Radix Rahardhian[1], Nunung Yuniarti[3], Lilies Wahyu Ariani[2], Ririn Suharsanti[1]*

1. *Department of Pharmaceutical Biology, Semarang College of Pharmaceutical Sciences (Stifar), Semarang, Indonesia, 50192.*
2. *Department of Pharmaceutical Technology, Semarang College of Pharmaceutical Sciences (Stifar), Semarang, Indonesia, 50192.*
3. *Department of Pharmacology and Clinical Pharmacy, Gajah Mada University, Yogyakarta Special Region, Indonesia 55281.*

***Corresponding Author: Ririn Suharsanti**

Abstract

Background: In Indonesia, saffron (Crocus sativus) drinks are now the latest healthy lifestyle trend. Saffron is used as a natural antioxidant, where antioxidants are related to cholesterol inhibition. The antioxidant activity of saffron is closely related to the content of flavonoids and phenolic compounds. This study aimed to determine Saffron 50% ethanol extract's effect on antioxidant activity, total phenolic content (TPC), total flavonoid content (TFC), and anti-cholesterol. Methods: Inhibition of antioxidant activity with 2-diphenyl-1-picrylhydrazyl DPPH, determining TPC using Folin-Ciocalteu and TFC reagent using AlCl3 reagent, and anti-cholesterol with Cholesterol Standard. Results: TFC 19.14 ± 0.9 mg QE / g, TPC 4.8 ± 0.9 mg GAE / g, antioxidant activity with extract IC 50 6939.02, Quercetin 9.57, Rutin 41.70, and decreased the greatest cholesterol occurred at a concentration of 400 ppm with a decrease of 13.22%. Conclusion: The 50% saffron ethanol extract can be an antioxidant and anti-cholesterol in vitro, but it needs further testing in vivo with the same extract.

Keywords: *Saffron; Flavonoids; Phenolic; Antioxidant; Anti-cholesterol.*

Introduction

WHO data for 2008, the global prevalence of elevated total cholesterol among adults (≥ 5.0 mmol / l) is 39% (37% for men and 40% for women) [1]. In Indonesia in 2013, it can be seen that there are 35.9% of Indonesia's population aged ≥ 15 years with abnormal cholesterol levels with cholesterol levels ≥ 200 mg/dl) where there are more women than men and more urban residents than rural residents [2].

Based on epidemiological studies showing an inverse relationship between foods rich in polyphenols and flavonoids reduces cholesterol disease risk. This association is mainly due to antioxidant activity [3]. The body needs cholesterol in average amounts, but it will harm the body [4]. Many studies have shown that cholesterol metabolism disorders have a higher risk of cardiovascular disease[5]. Excessive fatty acid oxidation will increase the amount of cholesterol in the blood. Antioxidants' presence will stabilize free radicals by complementing the lack of electrons that free radicals have and constraining the chain response of free radical creation [6]. Reactive oxygen species (ROS) are involved in cardiovascular disease etiology by introducing lipid peroxidation in cell membranes [7].

Apart from the body's natural antioxidant defenses (endogenous antioxidants) to eliminate ROS, the body also needs to supply exogenous antioxidants through dietary supplements [8]. Natural products that have long been used to prevent ROS formation are phenolic compounds such as flavonoids.

Phenolic compounds are natural antioxidants and are found in all plant organs.

Several studies have reported the antioxidant activity of phenolics extracted from various plant parts such as leaves, flowers, stems, roots, and fruit [9]. Saffron contains crocin, a colored compound, picrocrocin, which is the primary substance that causes Saffron bitter taste, and safranal, an essential oil responsible for the distinctive aroma of saffron. Crocin and picrocrocin are the main compounds in saffron.

Apart from that, saffron also contains anthocyanins, flavonoids, vitamins (riboflavin and thiamine), amino acids, protein, starch, and mineral substances [10]. In another study, saffron increased diabetes mellitus in vitro and in vivo through the GLUT4 / AMPK mechanism [11]. Saffron has been reported to lower cholesterol and keep cholesterol levels healthy. In vivo, research shows that saffron can lower cholesterol by as much as 50%. Saffron has antioxidant properties, which can help maintain healthy arteries and blood vessels [12].

The flavonoids and phenolic content act as antioxidants in saffron [8]. The concentration of solvent in the extraction process is one factor that affects the content of the active compound in the extract, which is related to the physical and chemical properties of the compounds found in plants.

Based on this description, there have been many studies on saffron. However, there has been no research on saffron with 50% ethanol solvent. With increasing polarity, it is expected that antioxidant activity will also increase the content of flavonoids and phenolics, which are bound to polyhydroxy, which will affect the increase in antioxidant and anti-cholesterol activity in vitro.

Materials and Methods

Materials

Saffron, $AlCl_3$, methanol, chloroform, anisaldehyde-sulfuric acid, n-hexane, gallic acid (Sigma Aldrich), Folin-Ciocalteu (Sigma Aldrich), $NaCO_3$ (Merck), Rutin (Sigma Aldrich) Quercetin ≥95% (Sigma Aldrich), 2, 2-diphenyl-1-picrylhydrazyl DPPH (Sigma-Aldrich), Cholesterol (Sigma-Aldrich).

Methods

Sample Preparation

Saffron powder extraction by maceration method using ethanol 50% with a ratio of 1:10 for 6 x 24 hours. Then proceed with ultrasonic, then macerated, and then filtered. The liquid extract from each extraction method was evaporated on a rotary evaporator (Heidoplh®, Germany) until a viscous extract was obtained [13].

Total Phenolic Contents (TPC)

The procedure for determining Saffron extract's total phenolic content referred to a modified procedure [14]. TPC is expressed as milligrams (mg) of gallic acid equivalent (GAE) per gram (g) of extract. The stages are as follows:

Preparation of gallic acid Standard Solution

50.0 mg gallic acid was dissolved with distilled water to a volume of 50.0 ml. The obtained 1000 µg/mL stock liquor was then diluted to make a series concentration of 20-100 µg/mL at ten µg/mL intervals.

Determination of Total Phenolic Content

The standard solution or sample was taken 0.5 ml into a test tube added 2.5 ml of 10% Folin Ciocalteau, incubated (4-8 minutes), added 2 ml of 7.5% Na2CO3, homogenized. Incubated for 90 minutes, read at λ 752 nm using UV-Vis Spectrophotometry (Shimadzu®) type 1240.

Total Flavonoid Contents (TFC)

The procedure for determining Saffron extract's total flavonoid content referred to a modified procedure [15]. TFC is expressed as mg quercetin equivalent (QE)/g extract. The stages are as follows

Preparation of Quercetin Standard Solution

A total of 50.0 mg of quercetin was dissolved with distilled water to a volume of 50.0 ml. The obtained 1000 µg/mL stock liquor was then diluted to make a series of concentrations of 20–100 µg/mL at ten µg/mL intervals.

Determination of Total Flavonoid Content

The standard solution or sample was taken

0.5 ml into a test tube added 1.5 ml methanol added 0.1 ml 10% AlCl3, added 0.1 ml 1 M CH3COONa, added 2.8 ml distilled water, homogenized. It was incubated for 30 minutes at λ 413 nm using UV-Vis Spectrophotometry (Shimadzu®) type 1240.

Determination of Antioxidant activity

The procedure for determining Saffron extract's antioxidant activity is referred to as a procedure [16] with slight modifications. 1.0 mL of this solution was added to 1 mL of 0.4 mM DPPH and methanol to 5 mL in a volumetric flask. The contents were mixed and incubated for 30 minutes as the operating time.

Determination of Anti-cholesterol activity

The procedure for determining the anti-cholesterol activity of Saffron extract refers to the procedure [4].The saffron ethanol extract comprises various 400 ppm, 500 ppm, 600 ppm, 700 ppm, and 800 ppm. From each concentration, 2.0 ml were put into a test tube, then 5.0 ml of 140 ppm normal cholesterol was added. It was responded with 2.0 ml of anhydrous acetic acid and 0.1 ml of intense sulfuric acid. A solution in a dark place for 15 minutes till a green discoloration form. The color results obtained were read with a visible spectrophotometer at a wavelength of 673 nm.

Data analysis

Measurement of total flavonoids and total phenolics was carried out by first analyzing the data using a standard curve, linear regression y = b x + a, where y is the absorbance, and x is the concentration of the standard solution. The total phenolic content (mg GAE / g extract) and total flavonoids (mg QE / g extract) were calculated using equation 1.

$$\text{TPC or TFC} = c \times \frac{V}{m} \quad \text{..... Equation 1}$$

Where *c* is the extract concentration (mg/L), *v* is the sample volume (L), and *m* is the sample weight (g).

Calculation of the percentage of decreased levels of antioxidants and cholesterol using the following formula:

Inhibition Antioxidant or anti-cholesterol

$$= \frac{a-b}{b} x100\%. \text{ …….. Equation 2}$$

A is the absorbance of the DPPH or cholesterol standard; *b* is the sample's absorbance after treatment (ethanol extract is added to the standard).

Results and Discussion

This research was conducted by measuring the optimum saffron extract levels in reducing cholesterol levels in vitro and measuring total flavonoids and antioxidants in Saffron extract. Saffron is sourced from the International Gold Star supplier for quality-controlled Quality in the Saffron Quality Control Lab Department of the Republic of Afghanistan. Researchers obtained LoA from supplier Dr. Safron.

The flavonoids in saffron and other compounds are present in Saffron from Kozani Greece and have antioxidant activity [17]. According to [18], saffron contains flavonoids, carotenoids, glycosides, anthocyanins, picrocrocin, aldehydes, monoterpenes, thiamine, riboflavin, starch, protein, gums, amino acids, and minerals.

Based on the review journal [19], saffron's main ingredients are crocin, crocetin, picrocrocin, and safranal. Its pharmacological activities include anticancer, antidepressant, antihypertensive, anti-anxiety, insomnia, and Pre-Menstrual Syndrome (PMS) [20]. For this aim, it is necessary to research to reduce cholesterol levels of saffron extract and determine the content of flavonoids and antioxidants so that saffron as a treatment can be maximized.

The process of withdrawing the compounds is carried out by the re-maceration method for six days, as shown in Figure 1. The re-maceration method is an extraction method with the principle of soaking Saffron Dry using a suitable irradiation solution with several stirring times.

Times at room temperature. They are conducted periodically to accelerate the process of withdrawing active compounds by solvent and homogenizing active compounds insolvent. The solvent used in this extraction method is 50% ethanol because of its ability to dissolve polar compounds from saffron. 2 grams of saffron produces 1.1 grams of extract so that the extract yield is 55%.

Figure 1: Saffron extraction process

In other studies, it is stated that ethanol can extract the active components of plants, including tannins, polyphenols, polyacetylene, flavonols, terpenoids, steroids, and alkaloids [21]. Many hydroxyl groups in phenol and flavonoid compounds tend to bind more easily with ethanol, a hydroxyl group [22]. So that ethanol solvent is appropriate to use for extracting saffron, this contains these compounds. Extraction saffron was also found that ultrasonic waves were more efficient than the classic extraction method [23].

Determination of Total Flavonoid Content (TFC) was measured using the AlCl3 colorimetric method with quercetin as standard. AlCl3 is used as a complex compound because it can form complexes with quercetin, an –OH group adjacent to the carbonyl group, and 2 –OH groups in the ortho position. This method believes that AlCl3 forms a stable acid complex with C-4 keto groups, then with C-3 or C-5 hydroxyl groups from flavones and flavonols. Besides, AlCl3 will also form a stable acid complex with ortho-dihydroxyl groups on the A or B rings of flavonoids [15].As for the addition of sodium acetate to maintain the wavelength in the visible area. The flavonoid compounds found in Saffron ethanol extract include quercetin, where quercetin is a flavonoid in the flavonols group. Quercetin is a flavonoid aglycone, which means it is not a glycoside like Rutin flavonoids consisting of Rutinose glycones and quercetin aglycones that the solubility of quercetin is more easily dissolved in organic solvents. The determination of TPC was analyzed by the colorimetric method using the Folin Ciocalteu reagent.

Folin Ciocalteu reagent is used because phenolic compounds can react with Folin Ciocalteu to form a colored solution whose absorbance can be measured. The principle is forming a complex blue compound, which can be measured at the maximum wavelength of the research result is 752 nm. The greater the attention of phenolic compounds, the more phenolic ions will reduce the heteropoly acid (phosphomolybdate-phosphotungstate) to the molybdenum-tungsten complex blue color is getting darker.

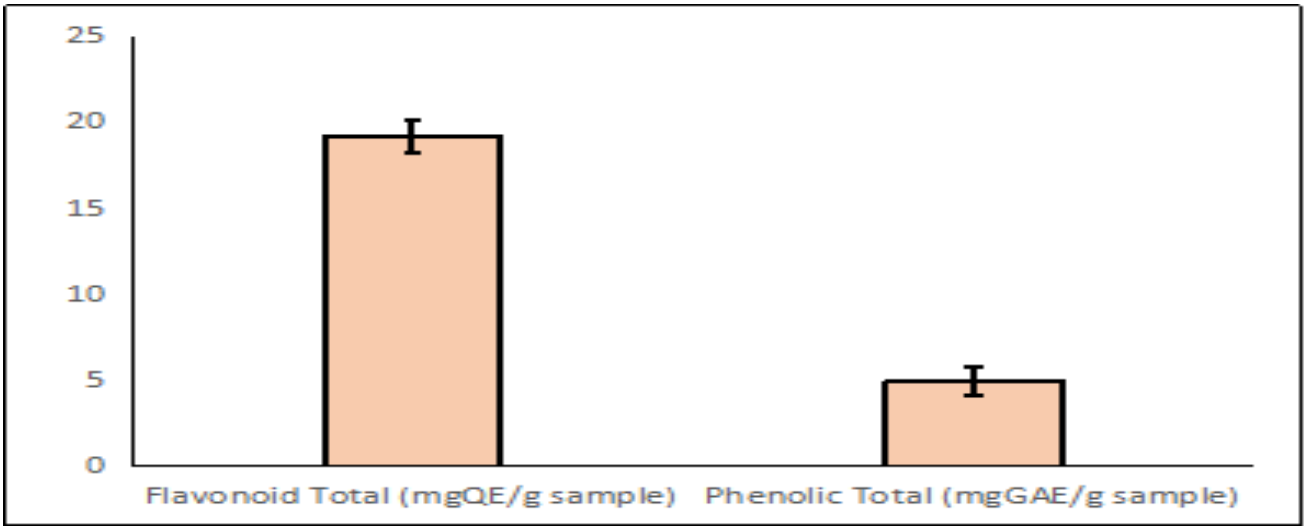

Figure 2: Results of total flavonoids and phenolic total extract saffron

Based on Figure 2, the total flavonoids were 19, 14 ± 0.9 mg QE/g of the sample when calculated with quercetin standards. In another study, the saffron extract in ethanol had a total flavonoid level of 2.9 ± 0.02 mg RE/g of the sample using Rutin as the standard [24]. The results of the study were more significant than in the study [8] with ethanol solvent (2.9 ± 0.02), water (3.8 ± 0.09), and methanol (5.8 ± 0.12) mg rutin equivalent (RE)/g.

While the total phenolic of the saffron extract in this study was 4.8 ± 0.9 mg GAE / g sample smaller than in the study [24], amounting to 6.3 ± 0.9 mg QE / g sample. The results were smaller than the study [8] with solvents of ethanol (6.3 ± 0.01), water (5.7 ± 0.04), and methanol (6.5 ± 0.02) mg (GAE) / g. The method used in testing the antioxidant activity is the radical absorption method 1,1-diphenyl-2-picrylhydrazyl (DPPH) because it has the compensations of being simple, fast, easy, and using small amounts of samples in a short time [25]. The amount of antioxidant activity was carried out using UV-Vis spectrophotometry at a wavelength of 517 nm, the maximum wavelength for DPPH. DPPH compound is a molecule containing unstable nitrogen free radical compounds that can bind hydrogen ions, so it is used for testing antioxidant activity.

The presence of antioxidant compounds from the sample resulted in a color change in the DPPH solution in methanol from dark violet to pale yellow [26]. The results of the saffron extract's antioxidant activity, when compared with the Rutin standard and quercetin, can be seen in Table 1. The IC 50 value obtained from the study results was smaller than the study conducted by [17]. The saffron methanol extract from Greece had an IC50 of 2482 ppm. in another study [8], extract methanol (210.79), water (255.44), and ethanol 299.44 µg/mL.

Table 1: Results of saffron antioxidant activity

Sample/standard	Antioxidant IC 50 (µg / mL)
Extract	6939.02
Quercetin	9,57
Rutin	41.70

Free radicals are extremely reactive molecular kind with an unpaired electron. They can react with and modify fatty acids, nucleic acids, and proteins in plasma lipoproteins and cell membranes [27]. The antioxidant properties of saffron due to its phenolic content and active ingredients such as safranal, crocetin, crocin, and carotene have all been reported to have antioxidant properties [7]. In a study [28], saffron significantly ($p < 0.05$) increased superoxide dismutase and decreased lipid peroxidation activity in the kidney, liver, lungs, and heart tissues compared to control.

In a study [29], saffron containing crocin was able to be hypolipidemic and antioxidant by decreasing the elevated levels of triglyceride, total cholesterol, alkaline phosphatase, aspartate transaminase, alanine aminotransferase, malondialdehyde, glutathione peroxidase enzyme activity, total glutathione, and oxidized glutathione in serum and increasing superoxide dismutase, catalase, ferric reducing / antioxidant power, and total sulfhydryl values in liver tissue with a reduction in thiobarbituric acid reactive species. Test for lowering cholesterol levels using the Liebermann-Burchard method. The choice of the Liebermann-Burchard method is because the process is simple, and this method is precise for measuring compounds in the steroid class, one of which is cholesterol [30]. This method requires the addition of anhydrous acetic acid and concentrated sulfuric acid. The purpose of adding anhydrous acetic acid is to extract cholesterol, ensure water-free media, and form acetyl derivatives. Focused sulfuric acid performances as a catalyst and accelerates the formation of color complexes in cholesterol, changes color to blue-violet, and subsequently becomes blue-green [30].

This study requires a standard cholesterol solution to control cholesterol. The cholesterol standard is dissolved in chloroform. First, this is because the cholesterol standard is non-polar so that it will dissolve more easily in non-polar solvents such as chloroform. The absorbance with UV-VIS spectrophotometry at λ max was 673.6 nm. Based on Figure 3, the percentage of cholesterol reduction shows that the ethanol extract of saffron can reduce cholesterol by 500 ppm at the highest concentration of 400 ppm, with a decrease of 13.22%.

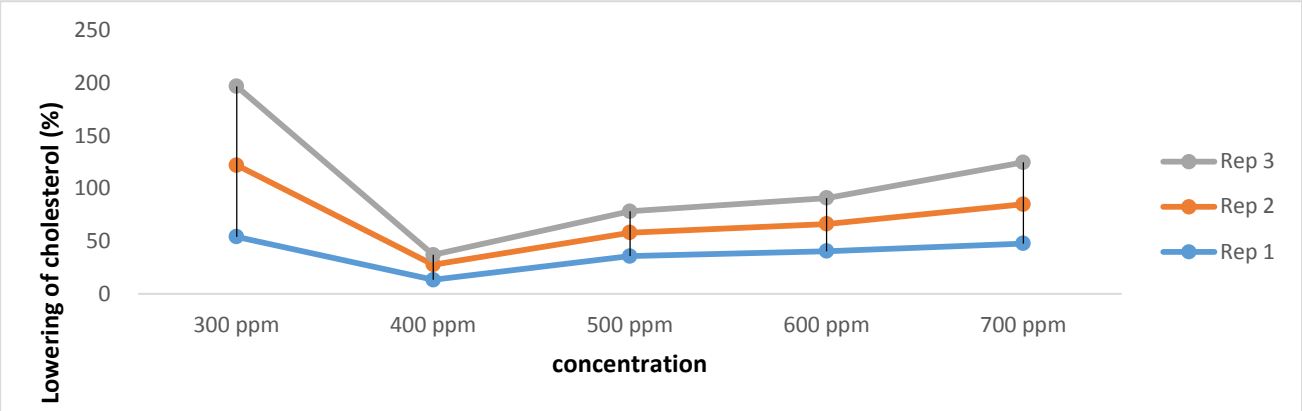

Figure 3: Graph of reduction in cholesterol *In vitro*

High-dose Saffron has more excellent anti-hyperlipidemic activity than high-dose crocin. Proves that apart from crocin in saffron, other components are responsible for anti-hyperlipidemic properties. The vigorous hyperlipidemic activity can be directly attributed to the presence of flavonoids in saffron since it is known that flavonoids have strong hypolipidemic properties [29].

In the review [31] in known that saffron was containing phytosterols and carotenoids shown to reduce low-density lipoprotein (LDL) cholesterol, plasma triglyceride (TG), and cholesterol concentrations.

Saffron containing crocin has Hypolipidemic Effect. Crocin decreased the amount of cholesterol. Crocetin could reduce serum, total cholesterol, and malondialdehyde levels and prevent a reduction of nitric oxide in the serum of hyperlipidemia [10]. Studies [11] Saffron with resistance exercise improves diabetic parameters through the GLUT4 / AMPK pathway in-vitro and in-vivo. Natural phenolic compounds (phenolic, flavonoids) have antioxidant activity (ABTS, DPPH, FRAP, ORAC).

Ordinary phenolic compounds existing in the human diet can constrain low-density lipoprotein (LDL) oxidation, retard / prevent foam cell formation, and further minimize the possible damage caused by vessels oxidized LDL [32]. However, in human clinical trials, the dosage of antioxidants supplements does not always show lipid peroxidation inhibition convincingly. In other words, clinical results are not always consistent with the findings from in vitro studies [32]. Several kinds of literature have shown that flavonoids have potential as anti-cholesterol, including flavonoid compounds (quercetin, kaempferol, myricetin, Rutin, naringenin, catechin, fisetin, and gossypetin) also exhibit lipid-lowering effects and anti-inflammatory and antiatherogenic properties [33]. According to a review article [34], Flavonoids (flavanols, flavonols, flavones, flavanones, isoflavones, and anthocyanidins) show the potential to improve HDL function. In other research, [35] phenolic compounds have a lipid-lowering effect.

References

1. World Health Organization (2008) "Raised cholesterol," https://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/ (accessed Oct. 23, 2020).
2. P M Arsana, R Rosandi, A Manaf, A Budhiarta, H Permana (2019) "Pedoman Pengelolaan Dislipidemi di Indonesia 2019," *Pb. Perkeni*, 9.
3. W Widowati, T Herlina, H Ratnawati, T Mozef, V Immanuel (2011) "Potency Of Antioxidant, Anti-cholesterol And Platelet Antiaggregating Of Black Tea (Camellia Sinensis)," *Bul. Littro*, 22(1): 74-83.
4. D Ingrid, L Fathrah (2018) "Activity Test of Suji Leaf Extract (Dracaena angustifolia Roxb .) on in vitro cholesterol-lowering," *J. Kim. Sains dan Apl.*, 21 (2): 54-58.
5. W H El-Tantawy, A Temraz (2019) "Natural products for controlling hyperlipidemia: review," *Arch. Physiol. Biochem.*, 125 (2): 128-135, 2019, DOI: 10.1080/13813455.2018.1441315.
6. N Yunarto, N Aini, I S Oktoberia, I Sulistyowati, A A Kurniatri (2019) "Aktivitas Antioksidan serta Penghambatan HMG CoA dan Lipase dari Kombinasi Ekstrak Daun Binahong-Rimpang Temu Lawak," *J. Kefarmasian Indones.*, 9 (2): 89-96. doi:

10.22435/jki.v9i2.1930.

7. S Rahaiee, S Moini, M Hashemi, S A Shojaosadati (2015) "Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (Crocus sativus L.): a review," *J. Food Sci. Technol.*, 52(4): 1881-1888. doi: 10.1007/s13197-013-1238-x.
8. E Karimi, E Oskoueian, R Hendra, H Z E Jaafar (2010) "Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity," *Molecules*, 15 (9): 6244-6256. doi: 10.3390/molecules15096244.
9. A Chandrasekara, F Shahidi (2012) "Antioxidant phenolics of millet control lipid peroxidation in human LDL cholesterol and food systems," *JAOCS, J. Am. Oil Chem. Soc.*, 89(2): 275-285. doi: 10.1007/s11746-011-1918-5.
10. M Mashmoul, A Azlan, H Khaza'Ai, B N M Yusof, S M Noor (2013) "Saffron: A natural potent antioxidant as a promising anti-obesity drug," *Antioxidants*, 2 (4): 293-308. doi: 10.3390/antiox2040293.
11. F Dehghan *et al* (2015) "Saffron with resistance exercise improves diabetic parameters through the GLUT4/AMPK pathway in-vitro and in-vivo," *Sci. Rep.*, 6: 1-12. 2016, doi: 10.1038/srep25139.
12. M Kamalipour, S Akhondzadeh (2011) "Cardiovascular effects of saffron: An evidence-based," *J. Tehran Univ. Hear. Cent.*, 6 (2): 59-61.
13. R Suharsanti, N Sugihartini, E Lukitaningsih, M R R Rahardhian (2019) "Effect of Different Solvent on Total Phenolic, Total Flavonoid, and Sun Protection Factor of Belimbing Wuluh (Averrhoa Bilimbi linn.) Fruits Fraction," *J. Glob. Pharma Technol.*, 11: 154-162.
14. M R R Rahardhian, R Suharsanti, N Sugihartini, E Lukitaningsih (2019) "In Vitro Assessment of Total Phenolic , Total Flavonoid and Sunscreen Activities of Crude Ethanolic Extract of Belimbing wuluh (Averrhoa bilimbi) Fruits and Leaves," *J. Glob. Pharma Technol.*, 11(04): 308-313.
15. C C Chang, M H Yang, H M Wen, J C Chern (2002) *Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods*, 10.
16. P Molyneux (2003) "The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity," *Songklanakarin J. Sci. Technol.*, 26: 211-219. doi: 10.1287/isre.6.2.144.
17. A N Assimopoulou, Z Sinakos, V P Papageorgiou (2005) "Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents," *Phyther. Res.*, 19 (11): 997-1000. doi: 10.1002/ptr.1749.
18. J A Fernandez (2006) "Anticancer properties of saffron , Crocus sativus Linn.," *Lead Mol. from Nat. Prod.*, 313-330.
19. M N Afifah, A N Hasanah (2020) "Saffron (Crocus sativus L): Kandungan dan Aktivitas Farmakologinya," *Maj. Farmasetika*, 5 (3): 116-123. doi: 10.24198/mfarmasetika.v5i3.26291.
20. J T Dipiro, G C Yee, L M Posey, S T Haines, T D Nolin, V Ellingrod (2020) *Pharmacotherapy: A Pathophysiologic Approach. 11th Edition*, 11 (1) McGraw Hill.
21. P Tiwari, B Kumar, M Kaur, G Kaur, H Kaur (2011) "Phytochemical screening and Extraction: A Review," *Int. Pharm. Sci.*, 1 (1): 98-106. doi: 10.1002/hep.29375.
22. Hasim S Falah, L K Dewi (2016) "Effect of Boiled Cassava Leaves (Manihot esculenta Crantz) on Total Phenolic, Flavonoid and its Antioxidant Activity," *Curr. Biochem.*, 3 (3): 116-127. doi: 10.29244/1-12.
23. S Gazeran, F Tajalli, A Mohamadi Sani (2016) "Effect of Ultrasonic Extraction on Qualitative Parameters of Saffron Edible Extract," *J. Essent. Oil-Bearing Plants*, 19 (5): 1286-1291. doi: 10.1080/0972060X.2015.1004126.
24. E Karimi, E Oskoueian, R Hendra, H Z E Jaafar (2010) "Evaluation of Crocus sativus L. Stigma Phenolic and Flavonoid Compounds and Its Antioxidant ActivityKarimi, E., Oskoueian, E., Hendra, R., & Jaafar, H. Z. E. (2010). Evaluation of Crocus sativus L. Stigma Phenolic and Flavonoid Compounds and Its Antioxida," *Molecules*, 15 (9): 6244-6256. 2010, doi: 10.3390/molecules15096244.
25. S Soradech (2016) "Radical scavenging, antioxidant and melanogenesis stimulating activities of diiferent species of rice (Oryza sativa L.) extracts," *Thai J.*

Pharm. Sci., 40: 0.

26. S B Kedare, R P Singh (2011) "Genesis and development of DPPH method of antioxidant assay.," *J. Food Sci. Technol.*, 48 (4): 412-22, Aug. 2011, doi: 10.1007/s13197-011-0251-1.
27. R K Murray, D A Bender, K M Botham, P J Kennelly, V W Rodwell, P A Weil (2009) *Harper's biochemistry 24th edition*, 28: 1.
28. H Makhlouf, M Saksouk, J Habib, R Chahine (2011) "Determination of antioxidant activity of saffron taken from the flower of Crocus sativus grown in Lebanon," *African J. Biotechnol.*, 10(41): 8093-8100. doi: 10.5897/ajb11.406.
29. S M B Asdaq, M N Inamdar (2010) "Potential of Crocus sativus (saffron) and its Constituent, Crocin, as Hypolipidemic and Antioxidant in Rats," *Appl. Biochem. Biotechnol.*, 162 (2):358-372. doi: 10.1007/s12010-009-8740-7.
30. K H Schunack, W Mayer (1990) *Senyawa Obat, Buku Pelajaran Kimia Farmasi. Edisi kedua. (diterjemahkan oleh Joke R. Wattimena dan Sriwoelan Soebito).* GMU-Press, Yogyakarta.
31. J Rahmani *et al* (2019) "The effect of saffron on weight and lipid profile: A systematic review, meta-analysis, and dose–response of randomized clinical trials," *Phyther. Res.*, 33(9): 2244-2255. doi: 10.1002/ptr.6420.
32. R Amarowicz (2016) "Editorial Natural phenolic compounds protect LDL against oxidation," *Eur. J. Lipid Sci. Techno*, 677-679. doi: 10.1002/ejlt.201600077.
33. S Salvamani, B Gunasekaran, N A Shaharuddin, S A Ahmad, M Y Shukor (2014) "Antiartherosclerotic Effects of Plant Flavonoids," *Biomed Res. Int.*, 1-11. doi: 10.1155/2014/480258.
34. C L Millar, Q Duclos, C N Blesso (2017) "Effects of Dietary Flavonoids on Reverse Cholesterol Transport , HDL Metabolism , and HDL Function," *Am. Soc. Nutr. Adv. Nutr.*, 8: 226-239. doi: 10.3945/an.116.014050.226.
35. A N A L B Zeni *et al* (2017) "Evaluation of phenolic compounds and lipid-lowering effect of Morus nigra leaves extract," *An. Acad. Bras. Cienc.*, 89 (4): 2805-2815. doi: 10.1590/0001-3765201720160660.

