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ANALYSIS OF TOTAL FLAVONOID AND PHENOL CONTENT FROM THE COMBINATION OF RED SPINACH (AMARANTHUS TRICOLOR L.) ETHANOLIC EXTRACT AND CHRYSANTHEMUM FLOWER (CHRYSANTHEMUM MORIFOLIUM) ETHANOLIC EXTRACT AS A POTENTIAL ANTI-ANEMIC

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A B S T R A C T

Red spinach and chrysanthemum are two plants that contain various active compounds, including flavonoids and phenols which have good antioxidant activity. One of the benefits of antioxidants is as an anti-anemia. The combination of red spinach and chrysanthemum extracts is expected to be a potential alternative treatment for anemia by combining high Fe and antioxidants found in both herbal ingredients. Research related to test the levels of flavonoids and phenols in red spinach and chrysanthemum extracts (in combination) has not been carried out. This study aimed to measure the total flavonoid and phenol levels of the combination of red spinach ethanol extract (EEBM) and chrysanthemum flower ethanol extract (EEBK). The design of this research is descriptif quantitative. The method used for the extraction is maceration. The solvent used is 70% ethanol. Then the two extracts were combined by mixing the two extracts with a ratio of 5.6 ml EEBM and 1.4 ml EEBK. The process of mixing the extracts was carried out by the centrifugation method. The suspension of the combination of EEBM and EEBK was then measured for the total flavonoid and phenol content. Determination of total flavonoid content (TFC) was carried out using UV-Vis spectrophotometry method with AlCl3 reagent at 425 nm. TFC results are expressed in quercetin equivalent (QE). The TFC result from the combination of EEBM and EEBK was 85.33 mg QE/g. Determination of total phenol content (TPC) was carried out using UV-Vis spectrophotometry method with Folin-Ciocalteu LP reagent at 735 nm. TPC results are expressed in gallic acid equivalent (GAE). The TPC result from the combination of EEBM and EEBK is 25.22 GAE/g. Flavonoids and phenols contained in the combination of EEBM + EEBK extracts can be an alternative anti-anemia because they have the effect of helping the absorption of iron in the intestine.

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1. INTRODUCTION

Nowadays, research on herbal plants is growing rapidly because of the many health benefits with few side effects. Red spinach is known as a drought-resistant vegetable and has a complete nutritional content starting from carbohydrates, proteins, macro elements, micro elements, phytopigmentation, bioactive phytochemicals and potential antioxidant.(Sarker et al., 2022). Other studies report that red spinach contains abundant antioxidant pigments and phytochemicals such as beta carotene, chlorophyll, vitamin C, beta cyanins, carotenoids, betha xanthins, TAC, betalains, Total Phenol Content (TPC) and Total Flavonoids Content (TPC). (Sarker & Oba, 2020).

Red spinach is widely used as an alternative treatment of anemia to reduce the effects that occur with the use of synthetic Fe. Red spinach also has a combination of iron and antioxidants to enhance pharmacological effects in the management of anemia, especially anemia due to genetic disorders. In addition, there is a correlation between antioxidant capacity and iron deficiency anemia. Antioxidant capacity is decreased in patients with iron deficiency anemia. The low antioxidant capacity causes damage and changes to the erythrocyte membrane, increases membrane rigidity, decreases deformability and causes hemolysis as a result of oxidative damage to erythrocytes. (Chillab et al., 2019)

Chrysanthemum flowers, which are better known as ornamental flowers, also have a high antioxidant content. Chrysanthemum flowers that are dried in an oven at a temperature of 55-60°C produce optimal content in the form of flavonoids, chlorogenic acid, vitamin C and soluble sugar content (Shi et al., 2017). Research by Liang, Gong and Zhang (2020) explained that chrysanthemum extract has the main composition in the form of flavonoids, volatile oils, organic acids, poly-saccharides and other elements such as vitamin C and metal elements. Flavonoids group compounds found were quercetin, luteolin, linarin and apigenin. These components have pharmacological effects as antioxidants, anti-inflammatory, anti-tumor, anti-HIV-AIDS and have an effect on anemia.

Red spinach leaves and chrysanthemum flowers also contain relatively high levels of flavonoids and phenols. Flavonoids and phenols have good antioxidant activity. Antioxidant agents have been shown to have anti-anemia effects by correcting blood disorders (Ousaaid et al., 2022).

There have been many studies related to testing the levels of flavonoids and phenols in red spinach extract and chrysanthemum flower extract separately, but there has been no research that has tested the levels of flavonoids and phenols in the combination of the two extracts. The combination of red spinach and chrysanthemum flower extracts is expected to be a potential alternative treatment for anemia by combining fe and high antioxidants found in the two herbal ingredients.

2. METHODS

Research Methods

The design in this research is descriptive quantitative. Ethical clearance have been carried out at LPPM Islamic University of Malang.

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Materials

The chrysanthemum flowers used in this study were obtained from Batu City, East Java. While the red spinach used in this study was obtained from Malang City, East Java. The solvent used in the extraction process for the two plants is 70% ethanol.

Materials used in the process of analyzing the levels of flavonoids and total phenols include: gallic acid (sigma-aldrich S7768449017), ethanol pro analysis (pa), quercetin (sigma Q4951), folin ciocalteu LP (7.5%), concentrated aluminum chloride 10 % (AlCl3), sodium acetate 1M (CH3COONa). The main tools used in the extraction process and analysis of TFC and TPC levels include: oven, blender, macerator, vacuum rotary evaporator, UV-Vis spectrophotometer, analytical balance, and vortex.

Reasearch Procedure

Combination of Red Spinach Ethanol Extract (EEBM) and Chrysanthemum Flower Ethanol Extract (EEBK) Combination

Red Spinach Ethanol Extract (EEBM) was prepared by maceration method. The red spinach used is the leaves and stalks. The process of drying red spinach uses the wind drying method, which is drying it in the sun without being exposed to direct sunlight for approximately 3 days. As much as 1 kg of red spinach produces 40 g of simplicia. Next, the simplicia is put into a bottle with a mouth (macerator) and 70% ethanol is added until it is completely submerged. Red spinach simplicia is soaked for 24 hours with occasional stirring after the first 6 hours.

After that, the macerate is separated with a cloth. The extraction process is repeated at least twice with the same type and amount of solvent. All macerate was collected, then evaporated with a vacuum rotary evaporator at a temperature of no more than 500C to obtain a thick extract. The amount of EEBM obtained was 10.8 ml.

The preparation of ethanol extract of chrysanthemum flowers (EEBK) is also the same as that of EEBM, namely by the maceration method. The difference is only in the method of drying the material. The drying process of the chrysanthemum flowers was carried out in an oven at 60°C for 20 hours. The parts of the plant used are the corolla, petals, pistils, stamens, ovaries and flower bases. A total of 1 kg of chrysanthemum flowers produces 100 g of simplicia. After going through the drying process, the chrysanthemum simplicia is extracted in the same way as red spinach. The solvent used in the extraction is also the same, namely 70% ethanol. The amount of EEBK obtained was 75 ml.

The combination of EEBM and EEBK was prepared by mixing the two extracts in a ratio of 5.6 ml EEBM and 1.4 ml EEBK. Then the extract mixture was centrifuged for five minutes at 300 rpm to obtain a homogeneous mixed suspension. The mixed suspension was finally used as the sample in this study.Measurement of Total Flavonoid Levels from the Combination of EEBM and EEBK

A total of 25 mg of the extract sample was put into a 25 ml flask and ethanol pa was added to the mark. The test solution was put into a 0.5 ml vial. Then added 1.5 ml of ethanol pa; 0.1ml AlCl3 10%; 0.1 ml of 1M sodium acetate (CH3COONa), and 2.8 ml of distilled water. After that it was incubated at room temperature for 30 minutes. Then the absorbance measurement was carried out at a maximum absorption wavelength of 425 nm.

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The same procedure was carried out on the quercetin standard solution (sigma Q4951). A standard solution of quercetin was prepared with a concentration series of 250; 125; 62.5; 31.25; 15.625 ppm for comparison. After going through the same procedure as the test solution, the absorbance of the quercetin standard solution was also measured at a maximum absorption wavelength of 425 nm.

3. RESULT

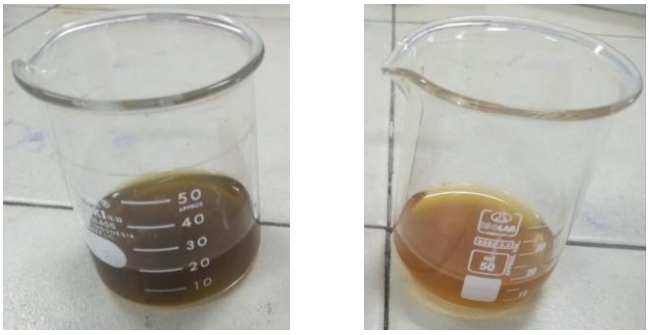
Drying Simplicia of red spinach leaves and chrysanthemum flowers

Simplisia is a natural substance that is ready to be extracted after going through several processes such as wet sorting, chopping, drying, dry sorting, packing and storage. The drying process of red spinach simplicia in this study used wind drying, namely drying in the sun without direct sunlight for about 3 days until the moisture content is below 10% or the leaves can be crushed easily. This drying is the most effective technique for obtaining optimal total phenol levels in red spinach leaves when compared to drying in direct sunlight, drying in an oven or in a greenhouse.

(a) **(b)** **(c)**

Figure 1. Red Spinach Leaves (a), Chrysanthemum (b), and Simplicia (c)

Simplicia Extraction of Red Spinach Leaves and Chrysanthemum Flowers



(a) **(b)**

Figure 2. (a) Ethanolic Extract of Red Spinach (Amaranthus tricolor L.), dan (b) Ethanolic Extract of Chrysanthemum Flower (Chrysanthemum morifolium)

Extraction is a technique for binding active compounds from a material or sample (Ibrahim et al., 2016). According to Rasul (2018), Extraction is a process that involves the separation of active compounds in plant or animal tissues from inactive or inert components using selective

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solvents in standard extraction procedures. The extraction technique used in this study was a maceration technique using 70% ethanol solvent for both red spinach leaf simplicia and chrysanthemum flowers. This study aims to measure the levels of total flavonoids and phenols. Phenolic compounds and flavonoids can be extracted from plant materials using polar solvents such as water, ethanol, methanol, acetone and ethyl acetate. (Katja, 2009).

Total Flavonoid Levels from the Combination of EEBM and EEBK

In this study, the total flavonoid content (TFC) test was carried out using the UV-Vis spectrophotometry method with AlCl3 reagent. The final TFC results are expressed in quercetin equivalent (QE). Quercetin equivalent, namely the number of equivalent milligrams of quercetin in 1 gram of extract. The maximum wavelength of quercetin in this study is at λ 425 nm. The maximum wavelength was used to determine the quercetin series curve and total flavonoid content in the EEBM and EEBK combination samples. Quercetin is made in concentration series of 500, 250, 125, 62.5 and 31.5 ppm. The absorbance of the quercetin obtained is then plotted against the concentration to obtain a calibration curve equation that can be used to calculate the percent content. The equation obtained is y=0.0012x-0.05 with a correlation coefficient (R2)= 0.9787. The TFC determination was carried out by entering the absorbance value of the sample into the quercetin standard curve equation. The results of the TFC combination of EEBM and EEBK are expressed in QE/gr extract. The TFC result of the combination of EEBM and EEBK was 85.33 mg QE/g. The quercetin standard curve can be seen in Figure 3.

Table 1. The results of measuring the absorbance of the quercetin standard solution at λ 425nm

Concentration (ppm)	Absorbance (y)
500	0,5655
250	0,1981
125	0,0777
62,5	0,0326
31,5	0,0200

Total Phenol Content of the Combination of EEBM and EEBK

Total phenol content (TPC) test was carried out using the UV-Vis spectrophotometry method with Folin-Ciocalteu LP reagent. The principle of TPC measurement with the Folin-Ciocalteu reagent is based on the reducing power of the hydroxy phenol group which is indicated by the formation of a blue complex compound (Pourmorad *et al.,* 2006). The standard used in the TPC test in this study was gallic acid. Gallic acid is used as a measurement standard because it is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid and is stable (Lee *et al*., 2003).

The final TPC result is expressed in gallic acid equivalent (GAE). Gallic acid equivalent is the equivalent number of milligrams of gallic acid in 1 gram of extract. The maximum wavelength of gallic acid in this study is at λ 735 nm. In this study, gallic acid was prepared in concentration series of 500, 250, 125, 62.5 and 31.5 ppm. The absorbance of the gallic acid obtained is then plotted against the concentration to obtain a calibration curve equation that can be used to calculate the percent content. The equation obtained is y=0.0018x-0.002 with a correlation coefficient (R2) = 0.999. Determination of TPC is done by entering the absorbance value of the sample into the standard curve equation of gallic acid. The TPC results of the combination of EEBM and EEBK

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are expressed in GAE/g extract. The TPC result of the combination of EEBM and EEBK was 25.22 mg GAE/g. Gallic acid standard curve can be seen in Figure 4.

Table 2. The results of the absorbance measurement of gallic acid standard solution at λ 735nm

Concentration (ppm)	Absorbance (y)
500	0,9264
250	0,4431
125	0,2383
62,5	0,1056
31,5	0,0641

4. DISCUSSION

Extracting Combination of EEBM + EEBK.

Extraction is a technique for binding active compounds from a material or sample (Ibrahim et al., 2016) after going through several processes such as wet sorting, chopping, drying, dry sorting, packing and storage. The process is expected to provide a long shelf life for the material without reducing the nutritional content much and minimizing chemical changes to the active compounds (Prasetyo, 2013). It can also remove putrefactive bacteria that can cause damage (Hartati and Maharani, 2021). Drying can be done by drying in an oven, drying in the wind (not in direct sunlight), drying in direct sunlight and drying in a greenhouse. The proper drying process will maintain the levels of nutrients and antioxidants contained in these natural ingredients(Dharma et al., 2020).

Research by Dharma *et al* (2020) found that the total phenol content in wedang uwuh with wind drying was 23.70 mg/L, oven drying was 20.89 mb/L, greenhouse drying was 19.74 and the lowest was with direct sunlight drying of 19.56 mg/L L. This is related to the drying temperature. The higher the drying temperature, the higher the possibility of changes in active chemical compounds in natural ingredients. The temperature with wind drying is lower than with other drying methods.

The process of drying the chrysanthemum simplicia is carried out using the oven drying method at a temperature of 50-60°C for approximately 20 hours until the moisture content is below 10% or the flowers can be crushed easily. This drying method is most suitable for chrysanthemum simplicia because according to Shi et al (2017), drying in an oven at 55-65°C, 680-850 W for 8-13 minutes is very suitable for maintaining the highest content of antioxidants and active compounds from chrysanthemum flower. There is a difference in drying time in this study which takes up to 20 hours. This difference in drying time can occur due to differences in the amount of material to be dried, the size of the oven used, and the part of the plant to be dried. In this study, 1 kg of chrysanthemum flowers were dried. Then the oven used is an oven with a size of 42.5 x 26.5 x 28 cm. The parts of the chrysanthemum flower used are the corolla, petals, pistils, stamens, ovule and flower base. The base of the flower requires a longer drying process because it is thicker than the other parts of the flower. So if the drying in the oven is only done for 8-13 minutes, the simplicia has not dried optimally and the moisture content in the simplicia is also still above 10%. This resulted in the simplicia not being sterile from spoilage bacteria and accelerating the decay of the simplicia within 2-3 days.

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According to Rasul (2018), The best solvents that can bind flavonoid compounds are ethanol, chloroform, and acetone. While the best solvents that can bind polyphenolic compounds are ethanol and methanol. So it can be concluded that ethanol is the best solvent for binding flavonoids and polyphenols together in an extraction process. In addition, ethanol can also bind other active compounds such as tannins, terpenoids, and alkaloids (Rasul, 2018). Ethanol is a solvent that can dissolve compounds from less polar to polar. Ethanol can dissolve phenolic compounds by degrading the cell wall so that the bioactive compounds are more easily excreted from plant cells. Ethanol has a hydroxyl group that can bind to the hydroxyl group of phenolic compounds. This causes an increase in the solubility of phenolic compounds in ethanol (Suhendra *et al.*, 2019)

Differences in ethanol concentration can affect the increase in the solubility of phenolic compounds in ethanol. The higher the ethanol concentration, the lower the polarity of the solvent. A substance will be dissolved and extracted properly if the solvent used has the same polarity level (Suhendra *et al.*, 2019) proved that 70% ethanol produced the highest antioxidants, namely 52.72% with total flavonoids of 7.14 mgQE/gram extract when compared to 70% methanol and water. This is supported by research Suhendra *et al* (2019) who concluded that extraction with 70% ethanol produced the highest antioxidants with total phenol 129.57 mg GAE/gr extract and total flavonoids 90.91 mg QE/gr extract when compared to ethanol concentrations of 40%, 50%, 60%, 80% and 90%. different polarity depending on the number and position of its hydroxyl groups. So that it will affect the solubility of flavonoids in solvents. The sample used for this study was a combination of red spinach ethanol extract (EEBM) and chrysanthemum flower ethanol extract (EEBK). The two extracts are both bipolar so they can dissolve in water and other bipolar compounds. Thus, the two extracts can be combined.

Total Flavonoid Levels from the Combination of EEBM and EEBK

In this study, the total flavonoid content (TFC) test was carried out using the UV-Vis spectrophotometry method with AlCl3 reagent. The standard used is quercetin. Quercetin is used as a standard solution because quercetin is a flavonol group of flavonoids that has a keto group at C-4 and has a hydroxyl group (OH) at C-3 or C-5 atoms. (Azizah *et al.*, 2014). AlCl3 will react with the keto group on C-4 and the OH group on C-3 or C-5 in flavones or flavonols to form a stable complex compound (Anwar, 2016). Thus, this method can be used to determine the amount of flavonoids belonging to the flavones and flavonols.

The TFC determination was carried out by entering the absorbance value of the sample into the quercetin standard curve equation. The results of the TFC combination of EEBM and EEBK are expressed in QE/gr extract. The TFC result of the combination of EEBM and EEBK was 85.33 mg QE/gr. Analysis regarding the total flavonoid content of the combination of EEBM and EEBK has never been done before. Most of the studies conducted TFC analysis of red spinach and chrysanthemum flower extracts in a non-combination manner. Khanam and Oba (2013) conducted a TFC analysis of four types of Amaranthus tricolor extract cultivars. The highest TFC of the four cultivars came from the cultivar rocto ranga, which was 70.4 μg QE/g or 0.0704 mg QE/g.

In addition, based on research conducted by Guntarti and Ruliyani (2020), the TFC level of the Amaranthus tricolor extract of the red Giti and green Giti varieties is 186.198 μg QE/g or 0.186 mg QE/g. Other research also found that the TFC of red spinach extract was 16.75 mg QE/gr

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(Jahan et al., 2022), 12,63 mg QE/gr (Sarker & Oba, 2018) dan 53,6 – 70,4 mg QE/gr (Khanam & Oba, 2013).

While previous research on levels of flavonoids in chrysanthemum flowers was 0.068 mg QE/gr (Zheng et al., 2019), 6,71-13,52 mg QE/gr (Hodaei et al., 2021) and 0,0276 mg QE/gr. Based on these results, the combination of EEBM and EEBK in this study was classified as having relatively high levels of flavonoids when compared to red spinach and chrysanthemum flower extracts (non-combination). However, the TFC results in this study (a combination of EEBM and EEBK) were lower when compared to the TFC results of chrysanthemum flower extract (non-combination) with ethyl acetate solvent in the study.Hyun *et al* (2011).

In this study, TFC analysis of chrysanthemum flower extract was carried out with several solvents using the high performance liquid chromatography (HPLC) method. This study showed that the highest TFC was found in ethyl acetate (EtOAc) chrysanthemum flower extract, which was 143 mg QE/g. The 2nd highest TFC yield was in n-BuOH solvent (60 mg QE/g), which was lower than the TFC combination of EEBM and EEBK in this study.

Total Phenol Content of the Combination of EEBM and EEBK

Total phenol content (TPC) test was carried out using the UV-Vis spectrophotometry method with Folin-Ciocalteu LP reagent. The principle of TPC measurement with the Folin-Ciocalteu reagent is based on the reducing power of the hydroxy phenol group which is indicated by the formation of a blue complex compound (Pourmorad *et al.,* 2006). The standard used in the TPC test in this study was gallic acid. Gallic acid is used as a measurement standard because it is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid and is stable (Lee *et al*., 2003).

Determination of TPC is done by entering the absorbance value of the sample into the standard curve equation of gallic acid. The TPC results of the combination of EEBM and EEBK are expressed in GAE/g extract. The TPC result of the combination of EEBM and EEBK was 25.22 mg GAE/g. These results were lower when compared to TPC results of red spinach leaf extract and chrysanthemum flowers (non-combination) in previous studies. Research on TPC analysis of red spinach and chrysanthemum flower extracts in combination has never been done. Previous studies regarding the TPC of red spinach extract showed results of 59.23 mg GAE/g (Sarker & Oba, 2018), 133,4-146,1 mg GAE/g (Khanam & Oba, 2013) dan 30,27 ng GAE/g (Jahan et al., 2022). While the TPC results of chrysanthemum flower extract in previous studies showed results of 34.8 mg GAE/g (Hanganu et al., 2016), 20,1-40,7 mg GAE/g (Suhendra et al., 2019)(Cai et al., 2004) dan 216,18 mg GAE/g (Kennouche et al., 2016).

Potential anti-anemic of flavonoid Phenol Content of the Combination of EEBM and EEBK

The combination of red spinach and chrysanthemum has high levels of flavonoids. Flavonoids have a role to increase the effectiveness of vitamin C, while vitamin C plays an important role in the prevention and management of anemia. Vitamin C plays a role in iron metabolism, namely reducing Ferri (Fe3+) to Ferro (Fe2+) so that it is more easily absorbed in the intestine. and the hemoglobin level will increase. (Siregar & Adelina, 2012)

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Flavonoids and polyphenolic compounds have at least two iron-binding properties. Flavonoids have 2 categories namely lipophilic and hydrophilic chelator. Lipophilic chelators have the role of increasing iron absorption, reducing iron excretion, and increasing the deposition of excess iron in tissues. Therefore it is very suitable for treatment for iron deficiency anemia. (Cotoraci et al., 2021)

The flavonol found in red spinach and chrysanthemum flowers is quercetin which is known for its antioxidant and anti-inflammatory activity. Quercetin increases the expression of hepcidin, one of the main hormones involved in intestinal absorption of iron, which can involve the Nrf2 pathway. Quercetin can activate the Nrf2 pathway by supporting its nuclear translocation and transcriptional activity. Given that ferroportin (FPN) and ferritin levels are expressed transcriptionally by the Nrf2 pathway, quercetin can affect iron homeostasis and help cells to fight oxidative stress. Exposure to quercetin results in increased hepatic iron deposits and induces hepcidin overexpression in adults. (Cotoraci et al., 2021)

Rutin (quercetin-3-rhamnosyl glucoside) is a flavone intensively studied for its antioxidant properties. Rutin has antiplatelet effects and protection of the vascular endothelium against oxidative stress in sickle cell anemia. In addition, it restores the integrity of the erythrocyte membrane, prevents and reverses lipid peroxidation, induces increased levels of GSH and CAT, and decreases SOD activity. The beneficial effects of rutin in sickle cell anemia can be attributed to deoxy-hemoglobin modulation and changes in redox homeostasis. Similar results were obtained for chrysin. (Cotoraci et al., 2021)

5. CONCLUSION

Based on the research results, it can be concluded that the combination of ethanol extract of red spinach (EEBM) and ethanol extract of chrysanthemum flowers (EEBK) contains active compounds of flavonoids and phenols through UV-Vis spectrophotometry test. The results of the total flavonoid content (TFC) from the combination of EEBM and EEBK were 85.33 mg QE/g. Meanwhile, the total phenol content (TPC) of the combination of EEBM and EEBK was 25.22 mg GAE/g. Flavonoids and phenols contained in the combination of EEBM + EEBK extracts can be an alternative anti-anemia because they have the effect of helping the absorption of iron in the intestine.

6. CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

7. REFERENCES

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, *74*(17), 2157–2184.

Chillab, E. D., Talib, R. A., & Al-Awsi, G. R. L. (2019). Genetics of sickle cell anemia disorders in Baghdad City, Iraq. *Indian Journal of Public Health Research and Development*, *10*(2), 817–822.

Cotoraci, C., Ciceu, A., Sasu, A., & Hermenean, A. (2021). Natural antioxidants in Anemia

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e-ISSN 2477-3743 | p-ISSN 2541-0024

treatment. *International Journal of Molecular Sciences*, *22*(4), 1–29.

Hanganu, D., Benedec, D., Vlase, L., Popica, I., Bele, C., Raita, O., Gheldiu, A. M., Mihali, C. V., & Ţărmure, V. (2016). Polyphenolic content and antioxidant activity of Chrysanthemum Parthenium extract. *Farmacia*, *64*(4), 498–501.

Hodaei, M., Rahimmalek, M., & Arzani, A. (2021). Variation in bioactive compounds, antioxidant and antibacterial activity of Iranian Chrysanthemum morifolium cultivars and determination of major polyphenolic compounds based on HPLC analysis. *Journal of Food Science and Technology*, *58*(4), 1538–1548.

Ibrahim, W., Mutia, R., Nurhayati, N., Nelwida, N., & Berliana, B. (2016). Penggunaan Kulit Nanas Fermentasi dalam Ransum yang Mengandung Gulma Berkhasiat Obat Terhadap Konsumsi Nutrient Ayam Broiler. *Jurnal Agripet*, *16*(2), 76-82.

Jahan, F., Huda, N., Islam, J., Ahmed, S., Hasan, S., Al, M., Nahar, A., Islam, B., Kanti, B., & Prosad, S. (2022). Amaranthus tricolor (red amaranth), an indigenous source of nutrients , minerals , amino acids , phytochemicals , and assessment of its antibacterial activity. *Journal of Agriculture and Food Research*, *10*(October), 100419. 1-7.

Katja, D. G. (2009). Efek Penstabil Oksigen Singlet Ekstrak Pewarna Dari Daun Bayam Terhadap Fotooksidasi Asam Linoleat, Protein Dan Vitamin C. *Chemistry Progress*, *2*(2), 79–86.

Kennouche, S., Bicha, S., Bentamene, A., Crèche, J., Benayache, F., & Benayache, S. (2016). In vitro antioxidant activity, phenolic and flavonoid contents of different polarity extracts from Chrysanthemum segetum L. growing in Algeria. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(9), 1522–1525.

Khanam, U. K. S., & Oba, S. (2013). Bioactive substances in leaves of two amaranth species, Amaranthus tricolor and A. hypochondriacus. *Canadian Journal of Plant Science*, *93*(1), 47–58.

Liang, W.-L., Gong, D., & Zhang, W. K. (2020). The Composition of chrysanthemum extracts and their pharmacological functions. *STEMedicine*, *2*(5), e69-e69.

Ousaaid, D., El Ghouizi, A., Laaroussi, H., Bakour, M., Mechchate, H., Es-Safi, I., Al Kamaly, O., Saleh, A., Conte, R., Lyoussi, B., & Arabi, I. El. (2022). Anti-Anemic Effect of Antioxidant-Rich Apple Vinegar against Phenylhydrazine-Induced Hemolytic Anemia in Rats. *Life*, *12*(2), 1–14.

Sarker, U., & Oba, S. (2018). Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. *Scientific Reports*, *8*(1), 1–9.

Sarker, U., & Oba, S. (2020). Nutritional and bioactive constituents and scavenging capacity of radicals in Amaranthus hypochondriacus. *Scientific Reports*, *10*(1), 1–10.

Sarker, U., Oba, S., Ercisli, S., Assouguem, A., Alotaibi, A., & Ullah, R. (2022). Bioactive Phytochemicals and Quenching Activity of Radicals in Selected Drought-Resistant Amaranthus tricolor Vegetable Amaranth. *Antioxidants*, *11*(3). 1-16.

Shi, X. F., Chu, J. Z., Zhang, Y. F., Liu, C. Q., & Yao, X. Q. (2017). Nutritional and active ingredients of medicinal chrysanthemum flower heads affected by different drying methods. *Industrial Crops and Products*, *104*(April), 45–51.

Siregar, Y. I., & Adelina, A. (2012). Pengaruh Vitamin C terhadap Peningkatan Hemoglobin (Hb)

DOI: http://dx.doi.org/10.17509/jpki.v9i1.58209
e-ISSN 2477-3743 | p-ISSN 2541-0024

Darah dan Kelulushidupan Benih Ikan Kerapu Bebek (Cromileptes altivelis). *Jurnal Natur Indonesia*, *12*(1), 75-81.

Suhendra, C. P., Widarta, I. W. R., & Wiadnyani, A. A. I. S. (2019). Pengaruh Konsentrasi Etanol Terhadap Aktivitas Antioksidan Ekstrak Rimpang Ilalang (Imperata Cylindrica (L) Beauv.) Pada Ekstraksi Menggunakan Gelombang Ultrasonik. *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, *8*(1), 27-35.

Widarti, W., Hartati, I., Harianingsih, H., & Maharani, F. (2021). Pembuatan Bubuk Bayam dengan Metode Foam Mat Dying. Jurnal Inovasi Teknik Kimia, 6(1).1-10

Zheng, J., Meenu, M., & Xu, B. (2019). A systematic investigation on free phenolic acids and flavonoids profiles of commonly consumed edible flowers in China. *Journal of Pharmaceutical and Biomedical Analysis*, *172*, 268–277.

DOI: http://dx.doi.org/10.17509/jpki.v9i1.58209
e-ISSN 2477-3743 | p-ISSN 2541-0024