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DEVELOPMENT OF ANAEMIA TREATMENT USING ETHANOL EXTRACT OF *MIRABILIS JALAPA* YELLOW FLOWERS

Pengembangan Terapi Anemia Menggunakan Ekstrak Etanol Bunga Mirabilis jalapa Kuning

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ABSTRAK

Mirabilis jalapa merupakan tumbuhan tropis dengan berbagai warna yang banyak ditemukan di Indonesia namun saat ini pemanfaatannya hanya sebatas tanaman hias dan pagar. Kombinasi M. jalapa warna kuning dan pink memiliki fitokimia yang berperan dalam metabolisme zat besi tetapi belum ada penelitian terkait dengan kandungan zat gizi. Penelitian ini bertujuan untuk mengidentifikasi kandungan zat gizi dan fitokimia ekstrak M. jalapa warna kuning dengan jenis penelitian deskriptif eksploratif. M. jalapa warna kuning terlebih dahulu digiling menjadi serbuk kemudian dilakukan maserasi dengan menggunakan larutan heksana dan etanol selama 24 jam di Laboratorium Fitokimia Fakultas Farmasi Universitas Setia Budi Surakarta pada bulan Januari 2024. Besi dan seng dianalisis dengan metode Atomic Absorption Spectrum (AAS) di Fakultas MIPA Universitas Gadjah Mada Yogyakarta. Kandungan zat gizi dianalisis dengan menggunakan uji proximate dan kandungan fitokimia dengan Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) pada mode ionisasi positif dan negatif di LPPT Universitas Gadjah Mada Yogyakarta. Penelitian dilaksanakan pada bulan Februari-April 2024. Ekstrak etanol M. jalapa kuning mengandung kadar air 18.71%, abu 12.08%, protein 25%, lemak 4.74%, karbohidrat 39.47%, besi 0.11%, seng 8.95% dan vitamin C 5.51%. Fitokimia family Betaxanthin terdeteksi pada mode ionisasi positif sedangkan Betacyanin ditemukan pada mode ionisasi negatif. Ekstrak etanol M. jalapa kuning mengandung besi dan Betaxanthin yang berpotensi untuk terapi alternatif anemia.

Kata kunci: *anemia, ekstrak M. jalapa kuning, fitokimia, kandungan gizi*

ABSTRACT

Mirabilis jalapa is a tropical plant that exhibits a wide range of colours and is particularly prevalent in Indonesia. However, its utilization currently is limited to ornamental plants and hedgerows. The combination of yellow and pink *M. jalapa* flowers have phytochemicals that play a role in iron metabolism. However, there is currently no research available on the nutrient content of these flowers. This research study aims to investigate the nutrient and phytochemical contents of yellow *M. jalapa* flower extract. We used explorative and descriptive research studies. The dried flowers were ground into powder and then macerated in hexane and ethanol solutions for 24 hours at the Phytochemistry Laboratory, Faculty of Pharmacy, Setia Budi University Surakarta, in January 2024. Fe and zinc were analysed using the Atomic Absorption Spectrum (AAS) method at the Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta. Nutrient contents were analysed using a proximate assay and phytochemicals using the Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method on positive and negative ionization modes at LPPT Gadjah Mada University Yogyakarta from February to April 2024. The ethanol extract of yellow *M. jalapa* flowers contained 18.71% water, 12.08% ash, 25% protein, 4.74% fat, and 39.47% carbohydrates. The results indicated the presence of iron (0.11%), zinc (8.95%),

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and vitamin C (5.51%). Additionally, the phytochemical family of *Betaxanthin* was detected in positive ionization mode, while betacyanin was identified in negative ionization mode. The ethanol extract of yellow *M. jalapa* flowers demonstrated the presence of iron and *Betaxanthin*, which have the potential to serve as an alternative therapy for anaemia.

Keywords: anaemia, nutrition values, phytochemicals, yellow *M. jalapa* flower extract

INTRODUCTION

Anaemia is one of the most common health problems around the world, especially in Indonesia and other developing countries [1]. Adolescent girls are one of the more susceptible age groups to suffering anaemia, resulting in less concentration and achievement in their studies compared to their counterparts [2]. The proportion of anaemia in Indonesia was 15.5% in 15-24 years old [3] but the anaemia prevalence in female adolescents adults is higher than in male adolescents adults [4]. The Indonesian government has implemented iron supplementation to alleviate anaemia in adolescent girls, but some adolescent girls have adverse effects such as nausea, vomiting, and stomach ache [4], prompting the exploration of alternative anaemia therapies using medicinal plants.

M. jalapa flower is a seasonal plant that grows and lives in lowlands. This flower only blooms around four in the afternoon [5] and sheath displays a wide range of hues, including white, pink, yellow, red, orange, and various combinations belong to *Nyctaginaceae* [6]. These flowers have some active compounds, i.e., flavonoids, saponins, alkaloids, phenones, steroids, triterpenes, glycosides, flavonoids, politenol, and tannins [7], [8]. *M. jalapa* flowers are usually utilized exclusively as ornamental plants [9].

Yellow flowers *M. jalapa* contain *Betaxanthin* groups, i.e., *Indicaxanthin, Miraxanthin-III, and 3-Methoxytyramine-Betaxanthin* [5]. A similar in vitro study was observed using a combination of yellow and pink *M. jalapa* flowers containing *Indicaxanthin, Miraxanthin-V, and Boeravinone-F*. This study used an alkaloid fraction from *M. jalapa* flowers to treat the iron deficiency HepG2 cell model. The alkaloid fraction increases intracellular iron absorption and iron levels in the iron deficiency HepG2 cell model [10]. However, the nutritional values of yellow *M. jalapa* flowers which consist of carbohydrates, proteins, fats, zinc, vitamin C, iron and their bioactive compounds, such as *Betaxanthin* have not been evaluated. Therefore, this study aimed to identify nutrient and phytochemical contents in yellow *M. jalapa* flowers using proximate and LC-MS/MS methods.

METHODS

Research Design

This research was an explorative and descriptive study conducted at the Phytochemistry Laboratory, Faculty of Pharmacy, Setia Budi University, Surakarta, from February to April 2024. The inclusion criteria for this study were *M. jalapa* plants at two months of age and yellow flowers. This research study does not need any sample size and statistical analysis because we explored something new from yellow *M. jalapa* flower extract, which has never been known before [11].

Materials

Dried yellow *M. jalapa* flowers were obtained from the Merapi Farma Herbal medicinal plant nursery in Sleman Yogyakarta. N hexane and ethanol solution were bought from Sigma Aldrich with Cat: 1.04367 and 1.00983, respectively.

Extraction of Yellow *M. jalapa* Flowers

Ten kilograms of fresh yellow *M. jalapa* flowers were washed under running water 1-2 times, dried under the sunlight for 3 weeks, and then followed by oven at 60°C for

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1 hour. After that, dried flowers were crushed, ground, and sieved with an 80-mesh size to obtain powder. The powder was then extracted using a maceration method adopted from [10]. In brief, the powder was soaked with a 1:5 ratio of n-hexane solution for 24 hours and filtered with filter paper to obtain residues, from which it dissolved using 96% (v/v) ethanol solvent with a 1:10 ratio for 24 hours at room temperature. The filtered solution was evaporated using a rotary vacuum evaporator (IKA RV 8 series, USA) at 45±2ºC and 70 rpm speed and then put into an oven blower (Binder WTB FD 56, Indonesia) to get a thick extract. The ethanol extract was stored in a glass bottle and kept at 2-4°C before further analysis [10].

Proximate Analysis

Proximate analysis was carried out to detect water and ash levels using the enzymatic gravimetric method. Protein and fat levels were measured using the Kjeldahl and Soxhlet method. Meanwhile, carbohydrate levels were calculated by subtraction of (100– (water+ash+protein+fat levels)) [12]. Vitamin C levels were measured using a High-Performance Liquid Chromatography (HPLC). All described analyses were conducted at the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada, Yogyakarta. Iron and zinc levels were detected using the atomic absorption spectrum (AAS) method at the Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta.

Phytochemicals Analysis

The phytochemical analysis used the LC-MS/MS, provided by the Waters Xevo TQD Acquity UPLC BEH with C18 1.7 µm 2.1x50 mm column PN. 186002350. Sample solvent used 10mMol of CH3CN, H2O, and ammonium acetate to provide 50-1200 mass positive and negative ions. Mobile phases consisted of A: Water Formic Acid 0.1% and B: CH3CN Formic Acid 0.1%. Chromatogram peaks and retention time of detected phytochemicals were fitted with the phytochemical database from PubChem (https://pubchem.ncbi.nlm.nih.gov) and then were adjusted with similar compounds in relevant published articles [10], [13], [5], [14], [15].

RESULTS

Nutrient Levels in Ethanol Extract of Yellow *M. jalapa* Flowers

One thousand g of yellow *M. jalapa* flower powder was extracted using 96% ethanol solution to produce the yellow *M. jalapa* flower extract with 23.3% (w/w) yield and paste consistency. We used a proximate analysis to measure nutrient levels in the ethanol extract of yellow *M. jalapa* flowers (Table 1). The high nutrient levels were 39.47% (w/w) carbohydrates, 25.00% protein, and 18.71% water. Meanwhile, mineral and vitamin levels were less than 10%, and ash levels were detected at 12.08%.

Table 1. Proximate Analysis of Yellow *M. jalapa* Flowers Extract

Macro and Micro Nutrients	Concentration (%)
Water	18.71
Ash	12.08
Protein	25.00
Fat	4.74
Carbohydrate	39.47
Iron	0.11
Zinc	8.95
Vitamin C	5.51

Phytochemical Contents in Ethanol Extract of Yellow *M. jalapa* Flowers

Detection of possible phytochemicals in yellow *M. jalapa* flower extract ethanol extract was done using LC-MS/MS analysis in positive and negative ionization modes, respectively (Figures 1 and 2). As shown in Figure 1a, 12 peaks were detected in the

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chromatogram with positive ionization mode. The highest peak was found at 0.388-minute retention time and peak number 1. There were six similar peaks with 0.708, 4.349, 4.753, 8.108, 13.957, and 18.947-minute retention times, respectively. From negative ionization mode, Figure 1b indicated that the highest peak was detected at 14.09-minute retention time, followed by the next peaks at 0.405 and 18.104-minute

a. Positive Ionization Mode **b. Negative Ionization Mode**

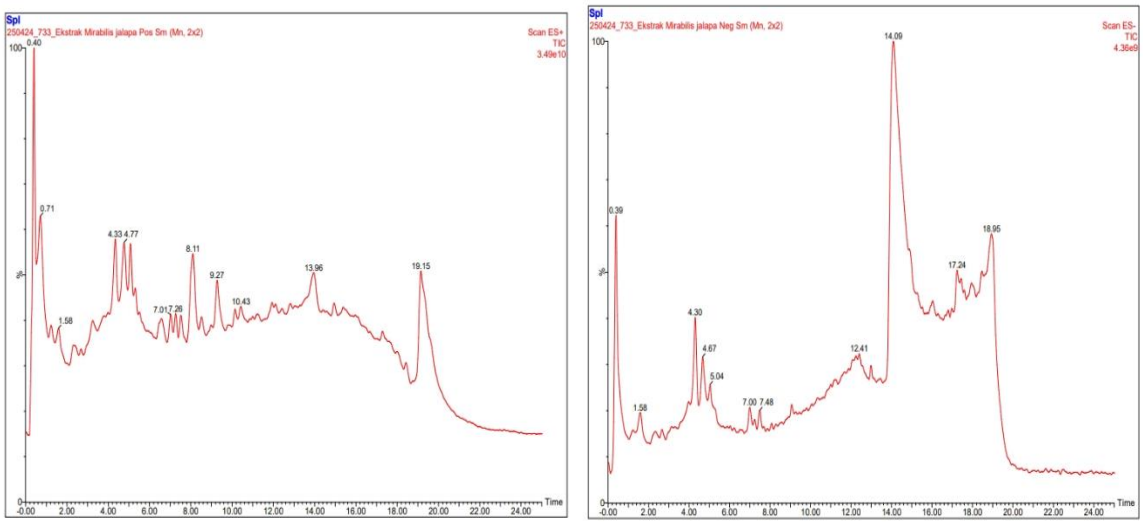

Figure 1. Chromatograms of yellow *M. jalapa* flowers extract with positive and negative ionization modes

Figure 1. Chromatograms of yellow *M. jalapa* flowers extract using the LC-MS/MS with positive (1a) and negative (1b) ionization modes. Five µL samples were injected into the column and separated using A: Water Formic Acid 0.1% and B: CH3CN Formic Acid 0.1% solutions. Chromatogram peaks and retention time of detected phytochemicals were fitted with the phytochemical database from PubChem.

Tables 2 and 3 showed the chemical structures and phytochemicals of yellow *M. jalapa* flower extract using the LC-MS/MS found in the positive and negative ionization modes.

Table 2. Identification of Phytochemicals in the Yellow *M. jalapa* Flowers Extract Using the LC-MS/MS with Positive Ionization Mode

Peak number	Retention time	m/z	Molecule formula	Possible phytochemicals
1	0.388	542.69	$C_{34}H_{67}NO_3$	*Arginine-betaxanthin*
2	0.708	382.65	$C_{18}H_{12}N_2O_8$	*Betanidin quinone*
3	1.584	542.95	$C_{24}H_{24}N_2O_{13}$	*Betanidin 5-O-beta-glucoside*
4	4.349	815.59	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*
5	4.753	927.9	$C_{52}H_{72}O_{14}$	*Astaxanthin glucoside*
7	7.501	353.79	$C_{14}H_{18}N_2O_7S$	*Miraxanthin I*
8	8.108	405.88	$C_{27}H_{47}NO_5$	*Mirabilene D isonitrile*
9	9.254	389.93	$C_{10}H_{15}N_3$	*Betanidin*
10	10.434	392.7	$C_{20}H_{19}N_3O_6$	*Tryptophan-Betaxanthin*
11	13.957	688.46	$C_{44}H_{56}O_6$	*Astaxanthin diacetate*
12	18.947	826	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*

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Table 3. Identification of Phytochemicals in the Yellow *M. jalapa* Flowers Extract Using the LC-MS/MS with Negative Ionization Mode

Peak number	Retention time	m/z	Molecule formula	Possible phytochemicals
1	0.405	754	$C_{46}H_{62}O_9$	*Astaxanthin-beta-D-glucoside*
2	1.618	754	$C_{46}H_{62}O_9$	*Astaxanthin-beta-D-glucoside*
3	4.298	462	$C_{27}H_{47}NO_5$	*Mirabilene F isonitrile*
4	4.686	756	$C_{46}H_{62}O_9$	*Astaxanthin glucoside*
5	5.023	837	$C_{48}H_{58}Na_2O_{10}$	*Disodium salt di succinate diester of astaxanthin*
6	6.979	561	$C_{24}H_{26}N_2O_{13}$	*Betalains*
7	7.467	412	$C_{27}H_{47}NO_5$	*Mirabilene F isonitrile*
8	12.423	826	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*
9	14.109	826	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*
10	17.227	826	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*
11	18.104	826	$C_{50}H_{64}O_{10}$	*Astaxanthin dimethyl succinate*

Table 2 described the possible phytochemical in the positive ionization modes compounds of *Betaxanthin* family were *Arginine-betaxanthin* (0.388-minute retention time, $C_{34}H_{67}NO_3$ molecule formula, 542.69 m/z), *Miraxanthin I* (7.501-minute retention time, $C_{14}H_{18}N_2O_7S$ molecule formula, 353.79 m/z) and *Tryptophan-Betaxanthin* (392.7-minute retention time, $C_{20}H_{19}N_3O_6$ molecule formula, 392.7 m/z). The remaining phytochemicals (8) belonged to the *Betacyanin* family.

In negative ionization mode, the *Betacyanin* family was detected in yellow *M. jalapa* flower extracts (Table 3). Interestingly, *Astaxanthin dimethyl succinate* was detected in both positive (4.349- and 18.947-minute retention time) and negative (14.109- and 18.104-minute retention time) with $C_{50}H_{64}O_{10}$ molecular formula. *Astaxanthin-beta-D-glucoside* (0.405-minute retention time, $C_{46}H_{62}O_9$ molecular formula, 754 m/z). Meanwhile, we found other *Betacyanin* family members, *Mirabilene F* and *Betalains.*

DISCUSSION

We evaluated nutrient values and bioactive compounds in ethanol extract of yellow *M. jalapa* flowers, which had a 23.3% yield. The extract contained macronutrient levels (39.47% carbohydrates, 25% proteins, and 4.74% fat) and micronutrient levels (8.95% zinc, 5.51% vitamin C, and 0.11% iron). Eleven phytochemicals were detected in the positive and negative ionization modes with various retention times. The highest peaks in the positive ionization mode were *Arginine-betaxanthin, Miraxanthin I*, and *Tryptophan-Betaxanthin*, while *Astaxanthin* derivates were in the negative ionization mode. It suggests that the ethanol extract of yellow *M. jalapa* flowers potentially become an alternative treatment for iron deficiency anaemia.

The yield of our ethanol extract of yellow *M. jalapa* flowers differs from another previous study in a combination of pink and yellow *M. jalapa* flowers and leaves was extracted using the same method, yielding 17.05% and 7.7% extract [10][16]. Research on the leaves of *M. jalapa* was extracted using ethanol, and 96% produced yield (2.05%) [8]. Differences in particle size, time and storage conditions, drying temperature and time, extraction method and time, stirring process during maceration, a ratio of sample to solvent, and solubility of bioactive components [17][18][8] affect the amount of yield produced. A small sample size will increase the surface area of contact between water as a solvent and solids [19]. The maceration of leaves of *M. jalapa* in Nigeria with n-hexane, ethyl acetate, and methanol produced a 6.05%, 6.03%, and 20.02% yields [20]. Our extraction used the maceration method with n-hexane and 96% ethanol. Non-polar n-hexane and 96% ethanol, which has polar properties [21] can bind compounds that have the same properties in *M. jalapa* flower powder. In addition to binding polar

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compounds, 96% ethanol is a solvent that maximally attracts flavonoid and phenolic compounds [22][23], non-toxic, has good absorption, and has high solubilization ability that can extract non-polar, semi-polar, and polar compounds [18]. Extraction of *M. jalapa* using 96% ethanol maceration method obtained 30 active compounds [14].

The yellow *M. jalapa* flower extract in this study contained 39.47% carbohydrates, which differed from a previous study that used a combination of white, yellow, and red *M. jalapa* flower seeds. They reported that the Egypt *M. jalapa* flower leaves and seeds contained 75.29% and 75.20% [24]. Theoretically, carbohydrates are an essential macronutrient that provides intracellular energy for the human body, including haemoglobin formation and iron metabolism [25],[26]. When energy food intake is inadequate, the human body cells will break down protein into energy, which results in low haemoglobin production (anaemia) [27].

Protein is another macronutrient that plays important roles in iron metabolism, and it includes hepcidin, ferroportin, transferrin, and ferritin. Therefore, the lack of protein intake will result in iron deficiency [27], reduction of haemoglobin synthesis [28], and reduction of many enzymes for haemoglobin formation [29]. The protein levels in our ethanol extract of *M. jalapa* flowers are 25%, higher than the 80% ethanol extract of *M. jalapa* flower leaves (6.86%) and seeds (10.4%) [24]. Another study in Nigeria using the Soxhlet extraction method on *M. jalapa* leaves showed that the protein was detected (12.8%) [20].

Micronutrients are also required for iron metabolism other than macronutrients. Zinc is a micronutrient that the body needs to maintain health, helping the formation of haemoglobin. Iron metabolism is influenced by zinc, which helps carbonic anhydrase stimulate the production of gastric HCL, which can increase haemoglobin levels and influence the work of the immune system optimally [30]. The zinc levels in the ethanol extract of yellow *M. jalapa* flowers (8.95%) are higher when compared to that in the root extract of *M. jalapa* flowers, which is 40.25 mg/L [31]. Besides helping in the digestive system and absorption of nutrients, it also helps iron to form haemoglobin [32].

Vitamin C is a nutrient that helps increase the absorption and metabolism of iron to form haemoglobin [33]. Vitamin C content in ethanol extract 5.51% is higher when in *Hibiscus Sabdariffa Linn* flower petals 0.244% and 0.336% in the extract [29]. Iron consumption, together with vitamin C, is more effective in increasing haemoglobin levels [40], and can increase iron absorption by converting ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}). Ferrous iron (Fe^{2+}) will be transported in the form of transferrin and then stored in the liver, lymph, and spinal cord [38] the food consumed will provide an acidic atmosphere that facilitates the reduction of ferric iron to ferrous, which is more easily absorbed by the small intestine. It is one of the water-soluble vitamins that functions to help increase or enhance iron absorption, which forms iron ascorbate groups that remain soluble at higher pH in the duodenum [34]. Effective and efficient iron absorption requires an acidic atmosphere and the presence of reductants; the absorption of iron in the non-heme form can be increased. Vitamin C deficiency can inhibit the iron absorption process, making anaemia easier to occur [27].

Iron, as one of the haemoglobin formers, is a micronutrient that the body needs in small amounts compared to macronutrients [35]. The iron levels in the ethanol extract of yellow *M. jalapa* flowers were 0.11% higher than that found in *M. jalapa* flower root extract, which was 0.026 mg/L, but they did not explain the used solvent and amount of material [31]. Iron can affect energy metabolism by replacing energy expended in activity. Optimal iron intake requires 100 mg or more of vitamin C per day with 80-100% absorption [33]. The iron content contained in *M. jalapa* flowers is sufficient to meet the daily needs of adolescent girls, which is 15 mg by the Regulation of the Indonesian Minister of Health regarding the recommended nutritional adequacy rate (AKG) in 2019 [36].

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The possibility of 12 phytochemical compounds identified in the positive ionization mode in this study from the *Betaxanthin* group (*Arginine-betaxanthin,* which is the highest peak, *Miraxanthin I* and *Tryptophan-Betaxanthin*) and *Betacyanin* (*Betanidin derivates, Astaxanthin derivates, Mirabilene D isonitrile*). The negative ionization mode only identified the possibility of *betacyanin groups, namely Astaxanthin derivates, Betalains, and Mirabilene F isonitrile, which had the highest peak of Astaxanthin dimethyl succinate*. Previous research [10] that the alkaloid fraction of *M. jalapa* flowers using a combination of pink and yellow flowers with LC-MS/MS analysis identified 15 phytochemical compounds namely *Indicaxanthin, Portulaxanthin III, Lampranthin II, Isolampranthin II, Tryptophan-betaxanthin, Boeravinone F, Miraxanthin V, Betanin, Isobetanin, Vulgaxanthin I, Miraxanthin I, II, Betanidin and Gomphrenin I, II*. *Betaxanthin* and *Betacyanin* are two groups of *Betalains*. *Betaxanthin* has a yellow pigment colour, and *Betacyanin* has a purplish-red pigment colour [37]. *Betalains*, which is a nitrogen pigment from plants [38] is a thermolabile compound that necessitates a longer extraction process. Consequently, the extraction method employs maceration techniques [10], which are typically observed in pigmented plants, such as *Bougainvillea* and *Caryophyllales.* However, the levels of *Betaxanthin* and *Betacyanin* vary between different plant species.

Betaxanthin groups identified in positive ionization mode in our study are similar to previous research, such as *Arginine-Betaxanthin* in the *Beta vulgaris L.* extracts [13], *Miraxanthin I* in the *M. jalapa* flowers and *Tryptophan-Betaxanthin* in the alkaloid fraction of pink and yellow *M. jalapa* flowers [10] and *Caenorhabditis elegans* [39]. The previous research identified *Betaxanthin* groups such as *Indicaxanthin, Vulgaxanthin-I, Miraxanthin-I, II, III, IV, V*, and *VI* in the *M. jalapa* flowers. Based on their petal, yellow *M. jalapa* flowers contain *Indicaxanthin, Miraxanthin-III, and 3-methoxy tyramine-betaxanthin* [5]. A previous study revealed that the alkaloid fraction of pink and yellow *M. jalapa* flowers contained Indicaxanthin, Miraxanthin-V, and Boeravinone F [10]. In contrast, a different study reported that 30 phytochemicals were identified in *M. jalapa* leaf extract, including Indicaxanthin, Miraxanthin II, III, Vulgaxanthin I, Boeravinone F, and some Betacyanin family members [14]. *Indicaxanthin* and *Miraxanthin-V* have less than 500 Daltons molecular weight, so they easily penetrate into the cell membrane of the human body, which become a natural erythropoietin agonist and thalassemia treatment [40]. *In vitro*, research of the alkaloid fraction of the combination of yellow and pink *M. jalapa* flowers, which contained *Indicaxanthin, Miraxanthin-V,* and *Boeravinone F*, demonstrated the capacity to enhance the regulation of erythropoietin, transmembrane serine proteases activity, and matriptase-2 expression. Additionally, it exhibited the ability to elevate intracellular iron levels in an iron deficiency HepG2 cell model, thereby increasing intracellular iron levels [10]. However, our study has some limitations during extraction and nutrient and phytochemical analysis. At first, *M. jalapa* plants had a seasonal time to make flowers, which required 3-4 months [9]. Therefore, we did not do replication for nutrient and phytochemical analysis because we had to wait longer to produce sufficient *M. jalapa* flower extract. Secondly, LC-MS/MS analysis was used to identify phytochemicals in *M. jalapa* flower extract, but the bioactive compound library is not available. We used the PubChem database, which was fitted to similar bioactive compounds in relevant published articles.

CONCLUSION

Based on the results obtained, it can be concluded that the extract of the yellow *M. jalapa* flower contains 25% proteins, 0.11% iron, 8.95% zinc, 5.51% vitamin C, and bioactive compounds: *Arginine-Betaxanthin* (0.388 minute retention time, $C_{34}H_{67}NO_3$ molecule formula, 542.69 m/z), *Miraxanthin I* (7.501 minute retention time, $C_{14}H_{18}N_2O_7S$ molecule formula, 353.79 m/z) and *Tryptophan-Betaxanthin* (10.434 minute retention

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time, $C_{20}H_{19}N_3O_6$ molecule formula, 392.7 m/z) in positive ionization mode that belong to *Betaxanthin* family with antianemia properties. Further investigation is needed to verify its nutrition and phytochemical effects on female rats with anaemia.

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