Alkaloid Fraction of *Mirabilis Jalapa* Leaves has Higher *Betaxanthin* Levels than Ethanol Extract and is Potentially Developed for Anemia Treatment

Yuliana Heri Suselo[1,2,4(✉)], Dono Indarto[2,4], Brian Wasita[3], and Hartono[2]

[1] Doctoral Program of Medical Sciences, University of Sebelas Maret, Surakarta, Indonesia
yulianaheri@staff.uns.ac.id
[2] Department of Physiology, Faculty of Medicine, University of Sebelas Maret, Surakarta, Indonesia
[3] Department of Anatomical Pathology, Faculty of Medicine, University of Sebelas Maret, Surakarta, Indonesia
[4] Biomedical Laboratory, Faculty of Medicine, University of Sebelas Maret, Surakarta, Indonesia

Abstract. *Mirabilis jalapa* is a medicinal plant, which is widely distributed in some tropical countries including in Indonesia. *Indicaxanthin, Miraxanthin* and *Boeravinone F* active compounds, which are the family member of *Betaxanthins* in the *M. jalapa* plant, are able to inhibit hepcidin and to activate matriptase-2 for increase of iron absorption *in silico*. Meanwhile, *Betalains* compound belongs to alkaloid compound, which is the precursor for *Betaxanthin* and *Betacyanin* synthesis. Therefore, this study aimed to identify *Betaxanthin, Betacyanin* and *Betalain* compounds in extract and fraction of *M. jalapa* leaves. Dried *M. jalapa* leaves were purchased from Merapi Farma, Yogyakarta. Extraction and fractionation of *M. jalapa* leaves were carried out using maceration and physical methods respectively. Alkaloid compounds in ethanol extract and alkaloid fraction were identified using a thin layer chromatography with *piperine* as the standard compound. *Betaxanthin, Betacyanin* and *Betalain* levels were measured using the UV-Vis spectrophotometry. From *M. jalapa* simplicia, it yielded only 7.7% ethanol extract while we got 41.33% alkaloid fraction. Alkaloid compounds were only detected in *M. jalapa* leaves fraction instead of the ethanol extract. *Betaxanthin* levels in alkaloid fraction were 23 times higher than that of the ethanol extract. Higher *Betaxanthin* levels were also observed in ethanol extract, compared to *Betacyanin* and *Betalain* levels. The alkaloid fraction of *M. jalapa* leaves is a better source of *Betaxanthin* compound and its derivatives than the ethanol extract. Further investigation is needed to determine *phytochemicals* compounds in the alkaloid fraction of *M. jalapa* leaves and their role in iron metabolism.

Keywords: Mirabilis jalapa leaves · Betaxanthin · Extraction and fractionation · Iron metabolism

A. Lelono et al. (Eds.): ICOLIB 2021, ABSR 27, pp. 370–377, 2023.
https://doi.org/10.2991/978-94-6463-062-6_37

1 Introduction

Iron deficiency anemia (IDA) is maternal and child health problems that need pay attention due to the high prevalence in the last 5 years [1]. A previous study found that oral iron supplementation in female adolescents with IDA had a minor effect to increase Hemoglobin (Hb) levels, associated with higher hepcidin and lower matriptase-2 levels [2]. Hepcidin and matriptase-2 are important proteins regulator in iron metabolism [3].

Based on an insilico study, *Indicaxanthin, Miraxanthin V* and *Boeravinone F* (members of *Betaxanthin* compounds) become potential candidates for treatment of IDA patients with higher hepcidin and lower matriptase-2 levels. *Indicaxanthin* play a role as an erythropoietin agonist which will increase the expression of matriptase-2. *Miraxanthin V* and *Boeravinone F* can neutralize hepcidin and inhibit hepcidin-ferroportin binding [4–6]. In silico, *M. jalapa* has also been developed for cancer and stroke therapy [7, 8].

Mirabilis jalapa, is a member of *Caryophyllales order* and recognized as a four o'clock flower plant that is widely used for ornament, food coloring, and medicine [9]. *M. jalapa* has beautiful flowers, which usually blooms around 4 pm, has white, yellow, pink or varied colors and a fragrant smell [9, 10]. In general, more than 200,000 different types of compounds in plants are classified into three major groups, alkaloids, phenolics and terpenoids [11]. *M. jalapa* contents of phytochemicals such as alkaloids, flavonoids, tri-terpenes, proteins, steroids, campesterol, daucosterol, alanine, alpha-amyrin, arabinose, beta-amyrin, and dopamine. The *M. jalapa* plant contain some phytochemicals, which belong to alkaloid such as *Betalain.* The *Betalain* compound has *Betaxanthin* and Betacyanin derivatives. In recent years, the *M. jalapa* plant has been widely studied *in vitro* and *in vivo*, which has antioxidant, anti-microbial activities, reduce inflammation and pain [10, 12–15]. *Indicaxanthin* in the *M. jalapa* plant is the largest Betaxanthin derivative that has been studied for a supportive therapy of anemia and beta thalassemia but the mechanism of action is not established yet [16, 17]. Moreover, *Miraxanthin V* and *Boeravinone F* are other *Betaxanthin* derivatives, which are also found in the *M. jalapa* plant [18]. Therefore, it is necessary to isolate the chromoalkaloids (*Betalain* derivatives) from *M. jalapa* plant.

From author knowledge, isolation of alkaloids from *M. jalapa* has not been reported. For example, to isolate terpenoid from *M. jalapa,* a previous study used hexane, ethyl acetate and methanol mixture. Meanwhile, chloroform, ethyl acetate and formic acid mixture are used by other researchers to isolate flavonoids from the same plant [10]. Another study extracted *Opica indicus* using ethanol and water (60%:40% ratio) to obtain *Betaxanthin* [19] while extraction of *M. jalapa* using the maceration method using 96% ethanol obtained 30 active compounds including the present of *Indicaxanthin, Miraxanthin V* and *Boeravinone F* [15]. Therefore, this study aimed to identify *Betaxanthin, Betacyanin* and *Betalain* compounds in extract and fraction of *M. jalapa* leaves.

2 Materials and Methods

2.1 Sample Collection

M. jalapa leaves were harvested from a medicinal plant nursery center "Merapi Farma", Yogyakarta, Indonesia. *M. jalapa* leaves were selected based on genetic similarity and harvesting time. For simplicia production, *M. jalapa* leaves were washed and dried under the sun for 6 days and an oven at 60 °C for 24 h. The dried leaves were grounded to make powder.

2.2 Extraction and Fractionation

Powder of *M. jalapa* leaves were extracted using maceration method with ethanol solvent (1:10 ratio) for 24 h. The filtrate of *M. jalapa* was separated using *Whatmann* paper and the filtrate was re-filtered following adding a half volume of the same solvent to make a clear filtrate. Collected filtrate was evaporated using a vacuum evaporator at 45 °C for 4 h and an oven at 45 °C for 24 h [20]. A Fractionation method using the acid-base method, which was modified from dos Santos and co-workers [21], to obtain ethyl acetate fraction was conducted. In brief, the ethanol extract was dissolved in ethyl acetate (1:10 ratio) and was adjusted with 2 M HCl to reach pH 3. The acid layer was then added with NH_4OH to reach pH 9. Finally, the ethyl acetate layers 1 and 2 were concentrated using vacuum evaporator 70 rpm at 45 °C for 4 h continued by an oven at 45 °C for 24 h.

2.3 Thin Layer Chromatography (TLC)

To detect alkaloid compounds in the ethyl acetate fraction, it used a TLC with silica gel 60 F254 plate (Merck, US) and using *piperine* (Sigma, US) and *caffeine* (Sigma, US) as the alkaloid compounds standard. The mobile phase used chloroform and methanol solvents with 9:1 ratio. The plate was dried and observed under visible and ultraviolet light at 366 nm wavelength. The retention factor (Rf) value was calculated based on the formula:

$$\mathrm{Rf} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}} \tag{1}$$

2.4 UV-Vis Spectrophotometry

Alkaloid compounds, *Betaxanthin, Betacyanin* and *Betalain* in the *M. jalapa* extract and fraction were detected using a UV-Vis spectrophotometer (U-2900 Shimadzu®) at 480, 535, 600 nm wavelength respectively. The quantification of *Betaxanthin, Betacyanin* and *Betalain* used the formula [22]:

$$\mathrm{Rf} = \frac{\text{Abs xDF x MW x 1000}}{\varepsilon \text{ x1}} \tag{2}$$

Note: Abs = absorbance, DF = dilution factor, MW = molecular weight, ε = molar extinction coefficient, l = cuvette length.

Table 1. Extraction-fractionation yield of simplicia of *M. jalapa* leaves

Powder (g)	Ethanol Extract (g)	Yield Ethanol Extract (%)	Alkaloid fraction (g)	Yield Alkaloid fraction (%)
200	15.00 10.09	7.70	- 4.17	- 41.33

3 Results and Discussion

3.1 Yield of Extract and Fraction of M. Jalapa Leaves

Ethanol extract from maceration with ethanol solvent was obtained only 7.7% yield while the alkaloid fraction was obtained 41.33% yield (Table 1).

Maceration was selected extraction technique for thermolabile compounds although it will produce less yield and take a long time [23]. Maceration is an extraction technique that is quite effective to obtain phenolics, alkaloids, flavonoids and polyphenols [24, 25]. Extraction efficiency increases with increasing extraction duration within a certain time [23]. Maceration was chosen method for *Betalain* group because they are thermolabile phytochemicals and it takes time for 6 days. The choice of 96% ethanol as solvent to obtain alkaloid-enriched extract (alkaloid fraction) was in accordance with research on *Murraya paniculata* leaves. The highest yield (33.4%) was obtained by maceration with ethanol solvent with a concentration of 96% [26].

3.2 Alkaloid Compounds in the Extract and Fraction of M. Jalapa Leaves

Detection of alkaloids in the extract and fraction of *M. jalapa* leaves using TLC showed that the extract did not detect any alkaloid compounds such as *piperine* while the fraction showed the presence of spots corresponding to *piperine* with different colour of light. Rf value of the fraction and standard was almost the same as the distance moved of the mobile phase. Distance moved extract faster than fraction and *piperine*, while *caffeine* was not showed any movement (Fig. 1).

To determine the polarity of the compound in TLC, the Rf of each sample and standard was measured. The Rf value of the extract was higher than the fraction and standard (Table 2). This indicates that the polarity of the extract was the lowest. If the polarity of the compound was higher, the Rf value was lower [27].

However, all of them were non-polar compounds because the Rf value was similar to the mobile phase Rf. There are several spots in the alkaloid fraction which indicate that there is a mixture of alkaloid compounds other than *piperine*, while there is only 1 spot in *piperine* which indicates a pure compound (Table 2). Alkaloids generally emit fluorescence violet-blue, green-yellow, violet, and blue under UV-Light 366 nm [28].

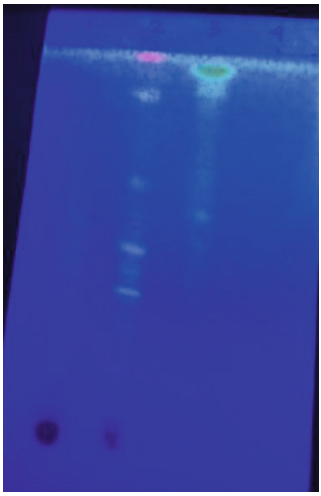

Fig. 1. Chromatogram samples and standards by TLC. (1) ethanol extract, (2) alkaloid fraction, (3) *piperine,* (4) *caffeine.*

Table 2. Chromatographic profile of extract ethanol and alkaloid fraction compared than *piperine*

Chromatographic profile	Ethanol Extract	Alkaloid Fraction	Piperine
Rf	0.96	0.86	0.88
Spot color	No light	Light violet- blue	Light green-yellow

Table 3. Levels of *Betalain* group in extract and fraction of *M. jalapa* leaves

Compounds	*Betalain* (mg/mL)	*Betacyanin* (mg/mL)	*Betaxanthin* (mg/mL)
Ethanol Extract	0.30	0.44	0.54
Alkaloid Fraction	8.39	11.23	12.45

3.3 Levels of Betaxanthin, Betacyanin and Betalain in Extract and Fraction of M. Jalapa Leaves

Betaxanthin levels in the alkaloid fraction of *M. jalapa* leaves increased 23 times compared to the ethanol extract. *Betaxanthin* levels are also the highest phytochemical in the *Betalain* group although it is almost the same as *Betacyanin* (Table 3).

Betalains are generally found in pigmented plants such as *Bougainvillea*, *Carryophylales*, *Amaranthin*, *Beets* and other pigmented plants [18, 29]. The levels of *Betalain* groups including *Betaxanthin* and *Betacyanin* vary among plants [19, 30]. In general, *Betacyanin* levels are higher than *Betaxanthin*, such as in *Pear cactus* and *Bougainvillea*

[11]. Betaxanthin levels in *M. jalapa* leaves are higher than *Betacyanin*, indicating that *M. jalapa* is the best source to obtain *Betaxanthin*-rich extract.

The alkaloid fraction of *M. jalapa* leaves is a better source of *Betaxanthin* compound and its derivatives than the ethanol extract. Further investigation is needed to determine *phytochemicals* compounds in the alkaloid fraction of *M. jalapa* leaves and their role in iron metabolism.

Acknowledgments. The authors acknowledged the financial support from Institute of Research and Community Service, Universitas Sebelas Maret through Non-APBN grant in 2021.

Authors' Contributions. Conceptualization, DI; Data curation, YHS, DI, and BW; Formal analysis, YHS, DI, and HH; Investigation, YHS; Methodology, YHS, DI, BW, and HH; Supervision, DI and HH; Validation, BW; Writing-original draft, YHS; Writing-review & editing, DI, BW, and HH. All authors read and approved the final manuscript.

References

1. Kemenkes RI. Hasil Riset Kesehatan Dasar Tahun 2018. Kementrian Kesehat RI. 2018;53(9):1689–99.
2. Suselo YH, Wulandari S, Ayusari AA, Indarto D. Hepcidin and Matriptase-2 as a potential biomarker for responsiveness to oral iron supplementation in adolescents female with iron deficiency anemia. Bali Med J. 2017;6(3):61.
3. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260–72.
4. Suselo YH, Alhaqq A, Indarto D. Cathafiline from Cassytha filiformis and BR-Xanthone A from Garcinia mangostana as potential bone morphogenetic protein receptor type i (BMPR-I) inhibitor for iron-refractory iron deficiency anemia in silico. IOP Conf Ser Mater Sci Eng. 2020;858(1).
5. Suselo YH, Kamil K, Wulandari S, Indarto D. Indicaxanthin, Miraxanthin-V, and Hexahydrocurcumin as Potential Erythropoietin Agonist in Silico to Treat Anemia in Chronic Kidney Disease. 2017;2(Hsic).
6. Yotriana S, Suselo YH, Muthmainah, Indarto D. Miraxanthin-V, Liriodenin and Chitranone are Hepcidin Antagonist in silico for Iron Deficiency Anemia. In: IOP Conference Series: Materials Science and Engineering. 2018.
7. Kusumawati R, Nasrullah AH, Pesik RN, Muthmainah, Indarto D. Secondary metabolites of Mirabilis jalapa structurally inhibit Lactate Dehydrogenase A in silico: A potential cancer treatment. IOP Conf Ser Mater Sci Eng. 2018;333(1).
8. Pangestu AP, Indarto D, Balgis B. Neuroglobin activator in Silico found from Mirabilis Jalapa for stroke treatment. AIP Conf Proc. 2018;2021(October).
9. Rozina R. Pharmacological and biological activities of Mirabilis jalapa L. Int J Pharmacol Res. 2016;06(05):160–8.
10. Gogoi J, Nakhuru KS, Policegoudra RS, Chattopadhyay P, Rai AK, Veer V. Isolation and characterization of bioactive components from Mirabilis jalapa L. radix. J Tradit Complement Med. 2016;6(1):41–7.
11. Coy-Barrera E. Analysis of betalains (betacyanins and betaxanthins). Recent Adv Nat Prod Anal. 2020;593–619.

12. Hajji M, Jarraya R, Lassoued I, Masmoudi O, Damak M, Nasri M. GC/MS and LC/MS analysis, and antioxidant and antimicrobial activities of various solvent extracts from Mirabilis jalapa tubers. Process Biochem [Internet]. 2010;45(9):1486–93. Available from: https://doi.org/10.1016/j.procbio.2010.05.027
13. Walker CIB, Trevisan G, Rossato MF, Silva CR, Pinheiro F V., Franciscato C, et al. Antinociceptive effect of Mirabilis jalapa on acute and chronic pain models in mice. J Ethnopharmacol [Internet]. 2013;149(3):685–93. Available from: https://doi.org/10.1016/j.jep.2013.07.027
14. Chandrasekhar J, Sonika G, Madhusudhan MC, Raghavarao KSMS. Differential partitioning of betacyanins and betaxanthins employing aqueous two phase extraction. J Food Eng. 2015;144:156–63.
15. Maulina D, Sumitro SB, Amin M, Lestari SR. Identification of bioactive compounds from Mirabilis jalapa L. (Caryophyllales: Nyctaginaceae) extracts as biopesticides and their activity against the immune response of Spodoptera litura F. (Lepidoptera: Noctuidae). J Biopestic. 2018;11(2):89–97.
16. Tesoriere L, Gentile C, Angileri F, Attanzio A, Tutone M, Allegra M, et al. Trans-epithelial transport of the betalain pigments indicaxanthin and betanin across Caco-2 cell monolayers and influence of food matrix. Eur J Nutr. 2013;52(3):1077–87.
17. Gheith I, El-Mahmoudy A. Laboratory evidence for the hematopoietic potential of beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. Brazilian J Med Biol Res. 2018;51(11):1–8.
18. Naqvi SFH, Husnain M. Betalains: Potential drugs with versatile phytochemistry. Crit Rev Eukaryot Gene Expr. 2020;30(2):169–89.
19. Fernández-López JA, Giménez PJ, Angosto JM, Moreno JI. A process of recovery of a natural yellow colourant from Opuntia fruits. Food Technol Biotechnol. 2012;50(2):246–51.
20. Kementerian Kesehatan Republik Indonesia. Farmakope Herbal Indonesia. 2nd ed. Jakarta; 2017. 529 p.
21. dos Santos ET, Pereira MLA, da Silva CFPG, Souza-Neta LC, Geris R, Martins D, et al. Antibacterial activity of the alkaloid-enriched extract from Prosopis juliflora pods and its influence on in Vitro ruminaldigestion. Int J Mol Sci. 2013;14(4):8496–516.
22. Mocanu GD, Nistor OV, Andronoiu DG, Ceclu L, Gheonea ID, Mihalcea L, et al. Effects of drying methods on quality parameters of potato and red beetroot purée with Lactobacillus delbrueckii. J Food Nutr Res. 2020;59(1):23–34.
23. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med (United Kingdom) [Internet]. 2018;13(1):1–26. Available from: https://doi.org/10.1186/s13020-018-0177-x
24. Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G, Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chem. 2016;194:135–42.
25. Salamah N, Ningsih DS. Total alkaloid content in various fractions of Tabernaemonata sphaerocarpa Bl. (Jembirit) leaves. IOP Conf Ser Mater Sci Eng. 2017;259(1).
26. Wardani E, Harahap Y, Mun'im A, Bahtiar A. Influence of extraction on the yield, phytochemical, and LCMS profile from standardized kemuning leaf (Murraya paniculata (L.) Jack). Pharmacogn J. 2019;11(6):1455–62.
27. Issaq HJ, Klose JR, Cutchin W. The Effect of Binary Solvent Composition and Polarity on Separations in Reversed Phase Thin Layer and High Performance Liquid Chromatography. J Liq Chromatogr. 1982;5(4):625–41.
28. Svendsen AB, Verpoorte R. TLC Separation and identification of alkaloids in general. J Chromatogr Libr. 1983;23(PA):19–49.
29. Khan MI, Giridhar P. Plant betalains: Chemistry and biochemistry. Phytochemistry [Internet]. 2015;117:267–95. Available from: https://doi.org/10.1016/j.phytochem.2015.06.008

30. Lavanya V, Thamaraiselvi SP, Uma D. Studies on Extraction of Betalain Pigments by Different Solvents and Assessing Antioxidant Activity of Bougainvillea spectabilis and Celosia argentea Flowers. Madras Agric J. 2019;106(1–3):104–8.