Available online on 15.10.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access

Review Article

A Review: Analysis of Betacyanin Levels in Various Natural Products

Larra Halimfanezi, Rusdi, Ridho Asra*

School of Pharmaceutical Science (STIFARM) Padang, Indonesia 25147

A B S T R A C T

Objectives: Betacyanin is a pigment that can be used as a natural pigment for food and as an alternative to synthetic dyes because it has an attractive color. Other than that, it also dissolves easily in water, and has high antioxidant activity so it is safer for the body when consumed. The purpose of this article is to discuss the levels of betacyanin in various natural products that can be analyzed using methods UV-Vis Spectrotometer, HPLC.

Data Sources Study Selection: The review method used is by studying the literature relevant to the research. Data sources of this article were selectively taken from Google Schoolar, Pubmed, Science Direct, Researchgate and Mandeley.

Summary of contents of the article: Betacyanin is one of the most widely used natural dyes in food. Betacyanin is a pigment that functions to provide a red color and has the potential to be a natural dye for food that is safer for health than synthetic dyes. Betacyanin is found in plants such as in red dragon fruit (*Hylocereus polyrhiyus*), beets (*Beta vulgaris L.*), cactus fruit (*Opuntia elatior Mill.*), and in Inflorescence *Celosia*. The level analysis was carried out in various types of plants that were known to contain betacyanin, the most common method used in analyzing the levels of betacyanin was using a UV-Vis spectrofotometer and HPLC with the results of the study showing that the observed betacyanin had a maximum wavelength of 538 nm.

Conclusion: Betacyanin is of betalain pigments which can be used as a natural dye alternative to synthetic dyes. The analysis of betacyanin levels was tested in various plants. Betacyanin has a sligtly different levels in various natural products that has been widely analyzing using the UV-Vis spectrofotometer and HPLC methods.

Keywords: Keywords: Betacyanin, *Hylocereus polyrhiyus*, *Beta vulgaris L.*, *Opuntia elatior Mill.*, *inflorescence Celosia* , Level analysis.

A R T I C L E I N F O: Received 10 July 2020; Review Completed 05 Oct. 2020; Accepted 09 Oct. 2020; Available online 15 Oct. 2020

Cite this article as:

Halimfanezi L, Rusdi, Asra R, A Review: Analysis of Betacyanin Levels in Various Natural Products, Asian Journal of Pharmaceutical Research and Development. 2020; 8(5):89-95. **DOI:** http://dx.doi.org/10.22270/ajprd.v8i5.846

***Address for Correspondence:**

Ridho Asra, School of Pharmaceutical Science (STIFARM) Padang, Indonesia 25147

INTRODUCTION

Betacyanin is a red or red-violet pigment from the betalain pigment group. Betalain pigments can only be found in plants of several family members of the Caryophyllales order, including Amaranthaceae, which are mutually exclusive with anthocyanin pigments[1]. Betacyanin pigment is classified into the betalain group[2]. Soluble in water and organic solvents that are not free of water while Betacyanin is not soluble in pure organic solvents[3].

Betacyanin is a pigment that can be used as a natural pigment for food and as an alternative to synthetic dyes because it has an attractive color. Other than that, it also dissolves easily in water, and has high antioxidant activity so it is safer for the body when consumed[4]. Betacyanin gives a red color and has the potential to be a natural pigment for food which is safer for health compared to synthetic dyes[5].

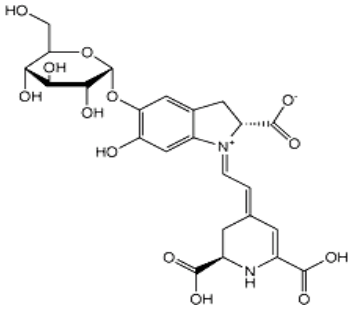

Figure: 1 Structure of betacyanin[17].

All betacyanins are glycosylated. It originates from the main basic structural units, namely the aglycon betanidine and isobetanidine(C-15epimers). Betacyanin has 4 sub-classes, namely amaranthin, betanin, gompgrenim and 2- descarboxy betanin[6].

Betacyanin type betanine which is a major or minor component in some betacyanin-producing plants has a hydrosil group that allows the formation of glycosides, especially as 5-O-glycosides, which are often found in amaranthin type betacyanins. Betacyanin has four subclasses, namely amaranthin, betanine, gomphrenin and 2-descarboxy betanin[35]. Several members of the Amaranthaceae family are known to have non-acylated Betacyanin, namely amaranthine, isoamaranthine, betanine and isobetanin or acylated Betacyanin, including celosyanin I, isocelocyanin I, celosianin II and isocelocyanin II[8].

Betacyanin pigments are hydrophilic so that in the extraction process organic solvents such as ethanol are used[12,13]. The use of water solvent in the heat concentration process can cause damage because the boiling point of water is quite high (100^0C), while the stability of betacyanin decreases at heating temperatures of 70^0C and 80^0C[7,8]. The synthesis of betacyanin is influenced by temperature, place of growth, and light[8]. Betacyanin has a high level of stability at pH5[7]. The breakdown of betacyanin increases sharply below pH 4 and at neutral pH values it causes the breakdown of betacyanin and turns brown[8]. Betacyanin identification is mostly done by comparison of spectrosmetry, chromatography, electrophoretic properties with authentic standards or secondary data and using traditional and modern analytical techniques, such as paper chromatography, thin layer chromatography, High Performance Liquid Chromatography (HPLC), Liquid Chromatography - Mass Spectrometry (LC-MS)[9,10,11]. Based on comparison with the results of previous studies on the same UV-vis spectra conditions from DAD HPLC, it was shown that the observed betacyanin has a maximum wavelength of 538 nm[35].

METHODS

Data Sources Study Selection:

The review method used is by studying the literature relevant to the research. Data collection in this review article was done by collecting journals, articles and a portion of the internet resources that were taken from the previous 10 years (2010-2020).

The journals used in making this review article are from international and national journals. Data sources of this article were selectively taken from Google Schoolar, Pubmed, Science Direct Researchgate and Mandeley. Articles review were carried out in ournals related to the analysis of betacyanins in various natural products.

The keywords used are “betacyanins”,” betacyanin levels analysis”,”betasianin levels Hylocereus polyrhiyus”,”betasianin levels *Beta vulgaris L*”,”betasianin levels *Opuntia elatior Mill*.”,”betasianin levels Celosia ”. This review article has been carried out in the research journal by looking at the levels of betacyanin ound in various natural products.

DISCUSSION

Red dragon fruit (*Hylocereus polyrhiyus*)

Dragon fruit is a cactus, namely the *Hylocereus* and *Selenicereus* genus from the *Cactaceae* family. The dragon fruit classification is as follows:[37]

Division	: *Spermatohyta*
Subdivision	: *Angiosperms*
Class	: *Dicotyledonae*
Order	: *Cactales*
Family	: *Cactaceae*
Subfamily	: *Hylocereanea*
Genus	: *Hylocereus*
Species	: *Hylocereus polyrhizus*

Figure: 2 Red dragon fruit (Hylocereus polyrhiyus)[49].

Dragon fruit has many benefits, consumption of dragon fruit - can be eaten directly in the form of fresh fruit - can help reduce cholesterol levels in the body as an alternative therapy for people with high cholesterol levels[39], dragon fruit contains various important substances such as niacin, fiber, antioxidants, and protein that are thought to reduce cholesterol levels in the body[38]. So far, what is often used is the pulp of the fruit, even though the peel of the dragon fruit has many benefits. The ingredients found in dragon fruit peel are flavonoids, dietary fiber, phenolic, and the betacyanin pigment[12]. The percentage of red dragon fruit peel is 22% of the total fruit weight[13]. So far, research on dragon fruit peel extraction has been carried out several times, including extraction of betacyanin with a maximum yield at a temperature of 50-55° C for 25 minutes[14], betacyanin extraction with the best results heating to 100° C for 5 minutes[15], and extraction of dragon fruit peel with the best results at 60°C for 4 hours[16].Analysis of betacyanin levels was carried out by taking 1 mL of sample, then diluting it with citrate buffer pH 5 and measuring the absorbance at 537 and 500. The absorbance value was calculated as A = 1.095 (537-500). Total betacyanin can be calculated using the following formula:[21]

$$\frac{\Delta Absorbance \times Dilution\ Factor \times Molecular\ Weight}{\sum x\ 1}$$

(A: absorbance, FP: dilution factor, BM: 550g /mol,: 60000 L / mol cm, l:1 cm thick cuvette).

Data measurement done using a UV-Vis spectrophotometer obtained an absorbance of 1.18 and 1.10, respectively;at that point the absorbance was 0.08. Then, after obtaining the absorbance, it can be calculated using the formula above, the betacyanin content was 36.67 mg /100g[17]. The stability of betacyanin extract of red dragon fruit peel was influenced by the type of solvent, pH, and temperature. The level of betacyanin obtained from dragon fruit peel extracted using methanol at pH 5 was 515.20µg /100g. This value was higher than the level of betacyanin obtained using a water solvent at the same pH, namely 491.16 µg / 100g[18].

Beetroot (*Beta vulgaris L.*)

Beetroot (*Beta vulgaris L.*) is a root-shaped plant that looks like a tuber; it is classified to the Amaranthaceae family.

Figure: 3 Beetroot (*Beta vulgaris L.*)[50].

Taxonomy: [40]

Division	: *Magnoliophyta*
Subdivision	: *Spermatophyta*
Class	: *Magnoliopsida*
Order	: *Caryopyllales*
Family	: *Chenopodiaceae*
Genus	: *Beta*
Species	: *Beta vulgaris L.*

Red beet is a plant that can be found in Europe and parts of Asia and America. The physical characteristics of the red beet are that the tubers are round like potatoes with a dark purple red color, only about 1-3 meters high, and when the fruit is cut it will show white lines with a pink color[19]. Apart from antioxidants, beets also have a major component, namely the betacyanin pigment which gives a red-purple color[20]. Strong purple color with high beta carotene content and antioxidant properties. Some active compounds such as carotenoids, glycine betaine, saponins, betacyanin, betanin, polyphenols and falvonoids. Beets are rich in carbohydrates which are easy to become energy and iron which helps the blood to carry oxygen to the brain[41]. Beets are red, this color is caused by the combination of the purple betacyanin pigment and the yellow betaxantin pigment. Betacyanin present in beetroot is known to have anti-radical effects and high antioxidant activity[21].The extraction process using a solvent type chloroform results in low extract yields, because chloroform is non-polar. Therefore, beets extraction is mostly done using ethanol as a solvent. The process of extracting beetroot using ethanol solvent aims to separate the active ingredient components from fresh ingredients using ethanol solvent[22].

Analysis of the ethanol extract of red beet fruit by HPLC showed that the ethanol extract of red beet fruit contains betacyanin, this is indicated by the peak that appears at the retention time of 2.739 minutes with the largest percentage area of 16.45% and according to previous studies the standardized pigment of betacyanin appeared at the time retention of 2.857 minutes with a percentage area of 41.82%. Cai[34] noted that the maximum absorption value of betacyanin extract from beetroot (*Beta vulgaris L.*) had a maximum absorption value at a wavelength of 537 nm. The results of betacyanin identification in the red beet extract sample when compared to other studies were not much different or almost similar. So it can be concluded that the ethanol extract sample of red beet fruit contains betacyanin compounds that appear at a retention time of 2.739 minutes with a percent area of 16.45%[23] .

Cactus fruit (*Opuntia elatior Mill.*)

Cactus (*Opuntia elatior Mill*.) is a flowering plant, it lives wild in upland and dry areas. The fruit as well as the stems can be used to prepare value-added products, such as body lotions, shampoos, creams, and jams. Cactus is a plant in the Cartaceae family that can be used as a natural dye because the fruit contains the natural betacyanin pigment[24].

Figure: 4 Cactus fruit (*Opuntia elatior Mill.*)[51]

Taxonomi: [42]

Division	: *Magnoliophyta (Angiosperms)*
Class	: *Magnoliopsida (Dicotyledons)*
Subclass	: *Archichlamydeae*
Order	: *Caryophyllales (Cactales)*
Family	: *Cactaceae*
Subfamily	: *Cereoideae, Opuntioideae, Pereskioideae*
Genus	: *Opuntia*
Species	: *Opuntia elatior Mill.*

Opuntia elatior is traditionally used to treat abscesses, wounds, asthma, cough, inflammation, diabetes, gonorrhea, diphtheria, burning sensation in the stomach and body aches and bronchitis in children[47]. Pharmacological activities were found to have hematinic activity[43], anti-ulcerogenic[44], anti-leukemic activity[45], anti-inflammatory activity and analgesic activity[46].

Cactus fruit (*Opuntia elatior Mill.*) was found to contain 47.10 mg / 100ml of Betacyanin. The analysis was carried out using a UV-Vis spectrophotometer and the maximum absorption value was obtained at a wavelength of 531nm[30]. The total Betacyanin content of cactus fruit from Mantikulore District was 0.02217mg/100 g and the total Betacyanin content of cactus fruit from East Palu District was 0.02121mg/100g[26]. According to the results of research conducted by Chauhan[33], betacyanin from the same plant has a maximum absorption value at a wavelength of 538 nm, the levels of betacyanin obtained using ethanol solvent, a mixture of ethanol: water and water solvent were 4 respectively. 94mg/100g; 11.95 mg/100g; and 15.42mg /100g[36].

Inflorescence *Celosia*

Celosia is a member of the Amaranthaceae family which has 60 species. It originates from subtropical areas in Africa, South America and Southeast Asia. Celosia is widely cultivated for ornamental plants because Celosia plants have various colored flowers which are compound flowers, this plant is a member of the Amaranthaceae family which contains a lot of Betacyanin pigment in its flower. In Malang and its surrounding areas, you can find Celosia plants with various flower colors[35].

Taxonomi :[48]

Division	: *Tracheopyta*
Class	: *Angiosperms*
Subclass	: *Mesangiosperms*
Order	: *Caryophyllales (Cactales)*
Family	: *Amaranthaceae*
Subfamily	: *Amaranthoideae*
Genus	: *Celosia*
Species	: *Celosia argentea var.cristata*

Figure: 5 Samples of Celosia argentea var.cristata[52].

In Indonesia, Celosia is better known as Jengger Ayam (Chicken's Comb). In many areas in Indonesia, inflorescence Celosia has been widely used as a traditional medicine to cure bleeding such as nosebleeds (epistaxis), coughing up blood (hemoptysis), vomiting blood (hematemesis), bloody urine (hematuria), bleeding hemorrhoids, uterine bleeding, dysentery, diarrhea, blurred vision, red eyes, urinary tract infection[49].The HPLC profile of methanol extract from several Celosia species detected at a wavelength of 538 nm generally showed 4-5 distinct peaks. The individual content of Betacyanin in the inflorescence sample can be detected from the area of each peak on the HPLC profile. Some samples have amaranthin content of more than 60% compared to the total peak area. And the other samples that were used had an average peak area of amaranthin<30% containing compounds which were thought to be classified in the celocyanin groups, namely 46.03%, 40.1%, 97.7% and 94.9%, respectively. Although the profile of the main betacyanin pigment in several observed inflorescences Celosia can be said to be the same, the color variations are thought to be due to variations in the content of each of these pigments [49].

CONCLUTIONS

Betacyanin is a red or red-violet pigment from the group of betalain pigments which can be used as a natural dye

alternative to synthetic dyes. The analysis of betacyanin levels was tested in various plants. UV-Vis and HPLC spectrotometers were the methods used in the analysis of Betacyanin levels. In the red dragon fruit (*Hylocereus polyrhiyus*) the betacyanin level was 36.67 mg / 100g, in beets (*Beta vulgaris L.*), it was found to have 16.45% betacyanin content, cactus fruit (*Opuntia elatior Mill.*) contains 47.10mg/100ml betacyanin and in *Celosia* inflorescences the content of amaranthin-type betacyanin was more than 60%. And the other samples used had an average peak area of amaranthin<30% containing compounds which were thought to be more of the celocyanin group, namely 46.03%, 40.1%, 97.7% and 94.9%, respectively.

REFERENCES

1. Grotewold, E. The genetics and biochemistry of floral pigments. Ann. Rev. Biol Plant. 2006; 57(1):761-780.
2. Wybraniec, S., Yosef, M. Fruit flesh betacyanin pigments in hylocereus cacti, *Journal of Agricultural and Food Chemistry.* 2012; 50(1):6086-6089
3. Wybraniec, S., Barbara, NW, Katarzyna M., Piotr K., Yosef M. Minor betalains in fruits of Hylocereus species, *Phytochemistry.* 2007; 68(2):251-259
4. Kirsten, M., Herbach, Florian, C., Stintzing, Reinhold C. Betalain Stability and Structural Degradation and Chromatic Aspects, *Journal of Food Science.* 2006; 10(1):1750-3841
5. Novatama, BC, Kusumo, E., & Supartono. Betacyanin Identification and Antioxidant Test of Red Beetroot Extract (*Beta vulgaris L.*). *Indonesian Journal Of Chemical Science.* 2016; 5(3):217-220
6. Khalida Y. A comparative study on the extraction of betacyanin in the peel and flesh of dragon fruit. Faculty of Chemical and Natural Resources Engineering Universiti Malaysia Pahang. Malasyia. 2010.
7. Castellar, R., Obon, JM, Alacid, M., Lopes, JAF. Color Properties And Stability Of Betacyanin From Opuntia Fruits. *J. Agric.Food Chem.* 2003; 5(1):2772-2776
8. Coultate, TP. Food The Chemistry Of Its Components. 3rd Edition. The Royal Society And Chemistry Company. Cambridge. 1996
9. Schleimann, W., Y. Cai., T. Degenkolb, J., Schmidt, and H. Corke. Betalains of Celosia argentea. *Phytochemistry.* 2001; 58(2):159-165
10. Stintzing, FC, J. Conrad, I. Klaiber, U. Beifuss, R. Carle. Structural Investigation on Betacyanin Pigments by LC NMR and 2D Spectroscopy. *Phytochemistry.* 2004; 65(2):415-422
11. Sari, NMI, Hudha, AM, Prihanta, W. Test the levels of betacyanin in beets (*Beta vulgaris L.*) with ethanol solvent and its development as a source of learning biology. A*biology education jurnal* Indonesia. 2016; 2(1):72-77
12. Sornyatha, K., Anprung, P. (*Hylocereus polyrhizus* (Weber) Britton & Rose) Bioactive Compounds and Stability of Betacyanins from Skin and Flesh of Red Dragon Fruit (*Hylocereus polyrhizus* (Weber) Britton & Rose), *Agricultural Science Journal*. 2009; 40 (1):15-18
13. Jamilah, B., Shu, CE, Kharidah, M., Dzulkifly, MA, Noranizan, A. Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. *Int. Food Res. J.* 2011; (18):279–286
14. Mai, DS, VT Tong and NL Hong. Survey of the Betacyanin Extraction from the Skin of Vietnamese Dragon Fruit. *BITEC ASEAN Food Conference.* 2011; (12):695-698.
15. Harivaindaran, KV Rebecca, OPS and Chandran, S.Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*) peel for use as potential natural pigment. *Pakistan Journal of Biological Sciences.* 2008*;* 11 (18):2259-2263
16. Chet, NW. Total Phenolic and Total Flavonoids Content of Pitaya Peels by Water Extraction. 2013; (12):949-971
17. Romdona, FS, Kusumo, E., & Supartono. Identification of Betacyanin and Antioxidant Test of Red Dragon Fruit Skin Extract (*Hylocereus polyrhiyus*). *Indo. J. Chem .Sci.* 2017; 6(1):1-4.
18. Priatni, S., Pradita, A. Stability study of betacyanin extract from red dragon fruit (*Hylocereus polyrhizus*) peels. *Procedia Chem.* 2015; (16):438–444.
19. Nanda, RW. Production of Red Beetroot (Natural Dye Powder*Beta vulgaris L.*) by Oven Drying Method. Semarang: Faculty of Food Technology. 2014
20. Ahmad. Extract and Stability of Blood Leaf (*BetacyaninAlternanthera dentata*) (*Comparative Study of Water Solvent*: Ethanol and Extraction Temperature). Malang: Faculty of Agricultural Technology, Brawijaya University. 2015
21. Mastuti, Yizhong, C., Harold, C. Betacyanin Pigment Identification in Several Types of Celosia Inflorescence, *UGM Biology Journal*. 2010; 66 (06):664-672
22. Wibawanto, NR, Victoria, KA, & Rika, P. Production of Red Beetroot (Natural Dye Powder *Beta vulgaris L.*) by Oven Drying Method. Semarang: Soegija Pranata Catholic University.2014
23. Noatama, SM, Kusumo, E., & Supartono. Betacyanin Identification and Antioxidant Test of Red Beetroot Extract (*Beta vulgaris L.*).*Indo. J. Chem .Sci*, 2017; 6(1):1-4.
24. PM Rengku, A. Ridhay, and P. Prismawiryanti. Extraction and Stability Test of Betacyanin in Cactus Fruit Extract (*Opuntia elatior Mill.*). KOVALEN. 2017; 3(2):1-9
25. Woo, KK, Ngou, FH, Ngo, LS, Soong, WK, Tang, PY. Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *Am. J. Food Technol*. 2011; 6:140–148.
26. Sirham. Analysis of Total Betacyanin Levels of Cactus Fruit (*Opuntia elatior Mill*.) From Palu City by UV-Vis Spectrophotometry. 2014
27. Havlikova, LK, Mikova, K. Fatstability of betacyanins. Lebens Unters Forsch. 1983 (177: 247-250)
28. Azeredo, HMC. Betalains Properties, Source, Applications, and Stability-aReview, *International Journal of Food Science and Technology*. 2006; 44:2365-2376
29. Khuluk AD, Widjarmoko SB, Murtini ES. Extraction and Stability of Betacyanin Leaf Blood (Althernathera dentate) Study of Ethanol Solvent: Water and Extraction Temperature, *Journal of Agricultural Technology*. 2007; 8 (3):1-9
30. F. Gandía Herrero, J. Escribano, and F. García-Carmona, Biological Activities of Plant Pigments Betalains, *Crit.Rev.Food Sci. Nutr*. 2016; 10:1080-1086
31. Stintzing, FC and R. Carle. Betalains emerging prospects for food scientists. *Tends Food Sci. Technol*. 2007; 18: 514 - 525
32. Raveh, E., Weiss, J., Nerd, A., Mizrahi, Y. Pitayas (*genus Hylocereus*): a new fruit crop for the Negev Desert of Israel.In: Janick, J., Simon, JE (Eds.), New Crops. Wiley, New York, pp. 2013; 491–495.
33. Chauhan, SP, ShethN.R., RathodI.S., SuhagiaB.N., MaradiaR.B. Phytochemial Screening of Fruits of *Opuntia elatior Mill*. *American Journal of Pharmatech Research*, 2013; 3(2):2249-3387
34. CaiY., Sun M., Corke H.Colourant Properties and Stability of Amaranthus betacyanin piments, *Journal of Food Aglicultural and Food Chemistry*. 2001; 46:4491-4495
35. Rengku, P., M., Ridhay, A., Prismawiryanti. Extraction and Stability Test of Betacyanin in Cactus Fruit Extract, *KOVALEN Chemical Research Journal*, 2017; 3(2):142-149
36. Arifin, AA, Bakar, J., Tan, CP, Rahman, Abdul, R. Essential fatty acids of pitaya (dragon fruit) seed oil. *Food Chemistry, 2012;* (2):561-564
37. Budi, A. The effect of giving red dragon fruit juice (*hylrocereus polyrhizus*) on the total cholesterol levels of hypercholesterolemic men. 2014; (6):1–52.
38. Sigarlaki, ED, Agustyas, T. Effect of Giving Red Dragon Fruit (*Hylocereus polyrhizus*) on Total Cholesterol Levels, 2016; 5(5):14-17
39. https://en.m.wikipedia.org/wiki/Beta_vulgaris
40. Singh, Bhupinder., Hathan, BS Optimization of Osmotic Dehydration Process of Beetroot (*Beta Vulgaris*) in Sugar Solution Using Rsm ', *International Journal of Food, Agriculture and Veterinary Science*, 2013; 3(3):1–10.
41. http: //www.theplantlist.orgqtplqrecordqkew2391929
42. Sanjay P Chauhan, NR Sheth, Haematinic evaluation of fruit of *Opuntia elatior Mill*., On mercuric chloride induced anemia in rats. *International Journal of Research in Ayurveda and Pharmacy*. 2014; 5(1):115-122
43. Sivasubramanian, Saravanan VS, Royal Frank P, Sengottuvel T, Anti-ulcer activity of ethanolic extract of stem of *Opuntia elatior Mill*. *International Journal of Universal Pharmacy and Bio Sciences.2013;* 2(5):614-620
44. Prakash Itankar, Dr, Sonali Acharya, Sumit K Arora, Priya T. Thakre, Phytochemical study and evaluation of anti-leukemic activity of ripe fruit of *Opuntia elatior Mill*. *Ancient Science of Life*. 2012; 32(1):S47
45. Oryza Sativa, _uliet, Evi Sulastri, Study on Antiinflamatory Activity of Cactus Fruits (*Opuntia elatior Mill*.) Extract Gel in Rats (*Rattus norvegicus L.*) At Induced Lamda Carragenan. *Journal of Natural Science*. 2014; 3(2):79-94
46. Chhavi Sharma, Sangeeta Rani, Bijander Kumar, Arvind Kumar and Vinit Raj, Plant Opuntia dillenii: A Review on Its Traditional Uses,

Phytochemical and Pharmacological Properties. *Ecronicon Pharmaceutical Science*. 2015; 1(1):29-43.
47. https://en.m.wikipedia.org/wiki/Celosia_argentea_var._cristata
48. Retno, M. Identification of betacyanin pigment in several types of inflorescence celosia. Department of Biology, Faculty of Mathematics and Natural Sciences, Gajah Mada University. 2010; 60(1)664-672.
49. Faridah, A., Holinesti,R., Syukri,D. Identifikasi Pigmen Betasianin dari Kulit Buah Naga Merah *(Hylocereus polyrhizus)*. *Jurnal Rekapangan.* 2016; 11(2):1-11.
50. Fatmasari, D., Mustofa, S., Santoso, B. Efektifitas buah bit (*Beta vulgaris*) sebagai disclosing solution (*Bahan identiikasi plak*). ODONTO *Dental Journal*. 2014; 1(2):1-9
51. Patel,Krihna N., Ishnava,Kalpeshkumar B. Evaluation of nutritional and medicinal properties of *Opuntia elatior Mill*. *Phytochemicals*. 2018; 10:121-138
52. Kitti, B., Sompoch, N., Intira, Y., Waranya B, David W.M. Comparison of In Vitro And In Vivo Inflorescence of Common Cockscomb (Celosia argentea var. cristata) *Science Asia.* 2010; 36:68–71.