Trop J Nat Prod Res, January 2018; 2(1):12-17
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Pharmaceutical and Therapeutic Potential of Miraculin and Miracle Berry

Bharti Mangla* and Kanchan Kohli

Department of Pharmaceutics, Jamia Hamdard, Hamdard University, New Delhi, India-110062

ARTICLE INFO

Article history:
Received 05 December 2017
Revised 23 December 2017
Accepted 01 January 2018
Published online 07 January 2018

ABSTRACT

Richadella dulcifica or *Synespalum ducificum* also known as miracle fruit is a kind of cherry plant, which originates from West Africa. The glycoprotein, miraculin in miracle berry is responsible for its unique function (suppression of sour taste). It acts by binding to the sweet receptor cells of the tongue, thus suppressing the response to sour taste in the central nervous system and this effect can be sustained up to 50 minutes to 4 hours. It could be harnessed as a novel or alternative additive in the food, cosmetic and pharmaceutical industries and could be an abundant source of hydrophilic and lipophilic antioxidant-rich phytochemicals. In miracle berry, more than ten phenolics were identified. Also, contains oxidizable vitamins, essential and non-essential amino acids. Its application is interesting, particularly, it is known to exhibit anti hyperuricaemic activity, improves dysgeusia, and expressed in transgenic plant. This review is meant to enhance the fruits' acceptability and also promotes its utilization in reducing the risk of chronic diseases generally associated with lipid metabolism. This article provides an overview of *Synespalum ducificum,* commonly known as Miracle berry and its unique taste modifying properties by miraculin, a glycoprotein with particular reference to its history, the proposed mechanism of action, functions, limitations and potential applications. In addition, it focuses on the Indian varieties of miracle fruit and their formulations. The method of extraction of miraculin from the berry in laboratory scale is also discussed with emphasis on the storage condition(s).

Keywords: Miracle fruit, Miraculin, Taste modifier, *Richadella dulcifica, hyperuricaemia.*

Introduction

The Miracle berry *Richardella dulcifica* or *Synsepalum dulificum* also called miracle fruit[2,3] is indigenous to tropical West Africa and belongs to the family Sapotaceae. The Miracle Fruit plant was introduced by the U.S. Dept. of Agriculture at the Federal Experiment Station in Puerto Rico[4]. The fruits of miracle berry are red in colour where it derives its name red berry and their fruits are collected in the month of December to June. It has a big seed surrounded by a thin layer of berry flesh with a faint cherry-like flavour.[4,5.]

Miracle berry acts as a taste modifier. The glycoprotein miraculin in miracle berry is responsible for this unique function by binding to the sweet receptor cells of the tongue, thus suppressing the response of a sour taste in the central nervous system.[6] This effect would last until the miraculin is diluted and eliminated by saliva. With the taste modification function, miracle berry has a great potential in food application as an alternative sweetener to mask undesirable sour tastes in food products.[7-10]

*Corresponding author. E mail: bharti.mangla91@gmail.com, kanchankohli50@gmail.com
Tel: +91 11 26059688

Citation: Mangla B and Kohli K. Pharmaceutical and Therapeutic Potential of Miraculin and Miracle Berry. Trop J Nat Prod Res. 2018; 2(1):12-17. doi.org/10.26538/tjnpr/v2i1.3.

Methodology

The review was systematically conducted by searching the databases of MEDLINE, PubMed, the Web of Knowledge, Google Scholar, and Science Direct for original research articles and books using relevant search terms or their combinations: "Miraculin, miracle berry, miracle fruit, *Richadella dulcifica, Gymnema sylvestre, Synsepalum dulcificum*, and taste-modifier." Our search was not limited by date but to all relevant publications available in the English language.

Miracle berry phytochemicals

Besides Miracle Fruit's unique potential to make sour food taste sweet, the pigment from the red-coloured skin could be a natural colour food ingredient. Miracle fruit is rich in antioxidants. These phytochemical antioxidants have been confirmed to possess health-promoting functions in preventing various chronic diseases, such as cardiovascular diseases, obesity, diabetes and certain cancers. Miracle berry also contains hydrophilic and lipophilic phytochemicals.[11,12] Anthocyanin and flavanol pigments in miracle fruits[13] and leaves[14] are identified as cyanidin-3-monoglucoside, cyanidin-3-monogalactoside, delphinidin-3-monoarabinoside, cyanidin-3-monoarabinoside, and delphinidin-3-monogalactoside using paper chromatography and spectral analysis. Rutin, gallic acid, ferulic acid, quercetin, epicatechin, myricetin, kaempferol, syringic acid are phenolics, whereas also contain leutin, vitamin A, C and E with tocopherols and tocotrienols. [11]

Miracle berry also contains essential amino acids (leucin, methionine), non-essential amino acid (Glutamic acid, glycine).[14]

Miracle berries leaves, and roots are rich in carbohydrate, fibres, tannins, cardiac glycosides, polyphenols, flavonoids and minerals.[15]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Figure 1: Miracle berry[1]

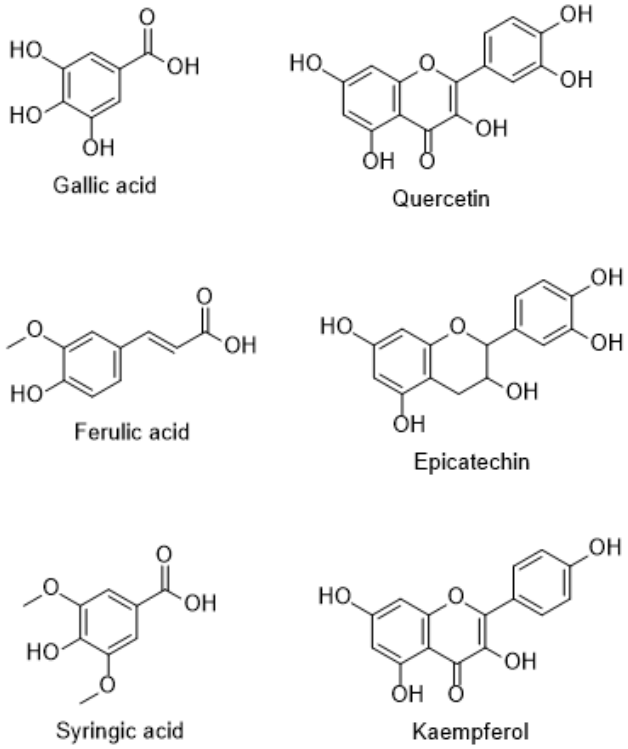


Figure 2: Molecular structures of phenolics present in Miracle berry

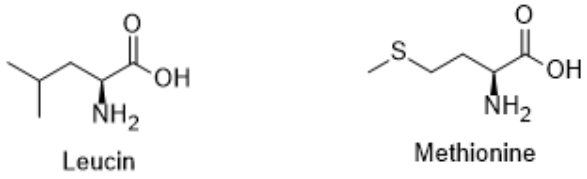


Figure 3: Molecular structures of essential amino acids present in Miracle berry

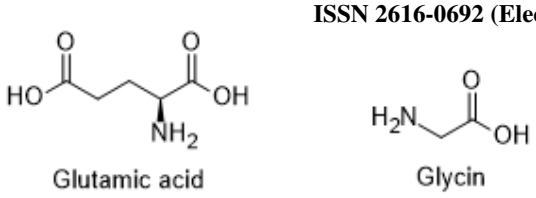


Figure 4: Molecular structures of non-essential amino acids present in Miracle berry

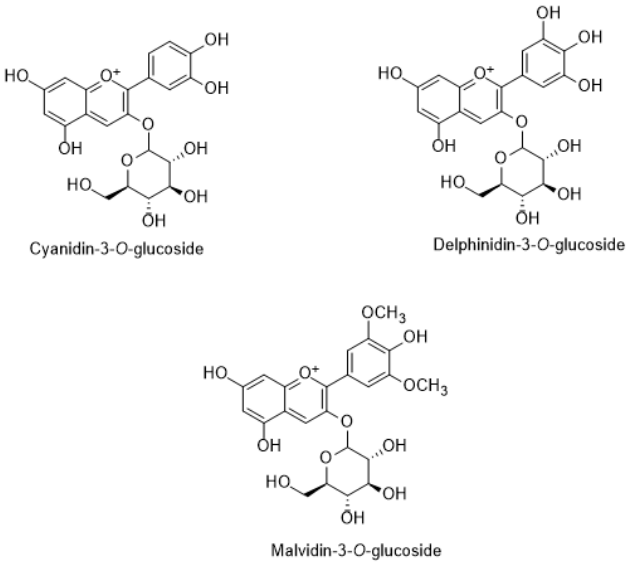


Figure 5: Molecular structures of anthocyanins present in *Synespalum ducificum*

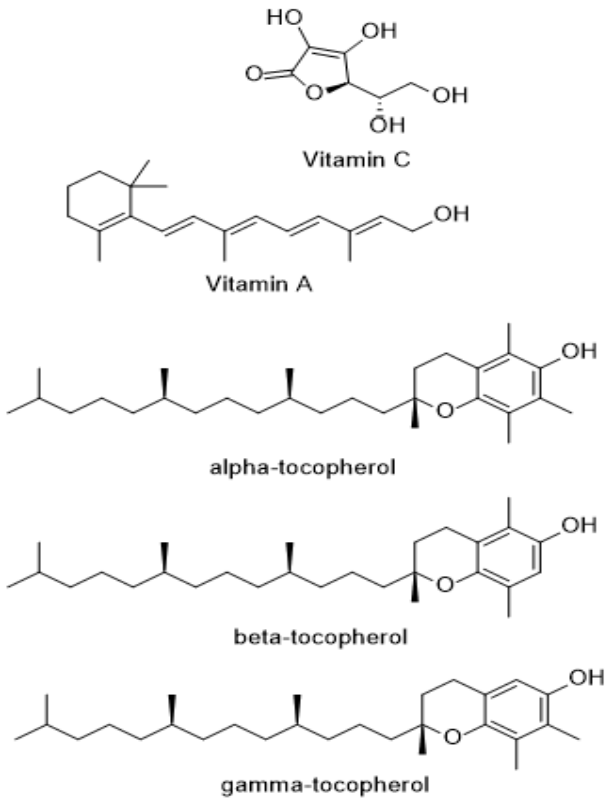


Figure 6: Molecular structures of vitamins present in *Synespalum ducificum*

Health benefits of Miracle berry

The value of the fruit as a taste modifier has possible benefits for human health. Various studies have proven that the fruit and leaf extracts of miracle berry help in reducing the blood glucose level in alloxan-induced albino rats.[16,17] Shi *et al*.,[18] described that the butanol extracts of miracle fruit may be an effective treatment for acute gouty arthritis.[18] This extract attenuated oxonic acid potassium salt-induced hyperuricaemia in mice by lowering serum uric acid levels and activating hepatic xanthine oxidase. Miracle fruits have tremendous therapeutic properties as shown in Figure 7.

Extraction and purification of miraculin

Inglett *et al*.,[5] modified the extraction procedure of Adom and Liu.,[19] which described two-extraction methods for the pulp and seeds of Miracle Fruit. The first method was Sequential extraction which includes two steps i.e. double extraction for free compounds and Alkaline extraction for bound compounds. The second method was direct extraction method. Before analysis dried pulp-seeds were separated into pulp and seeds after freeze–drying. The skin, pulp and seeds were ground individually to fine powder.[5,19]

Dioso et al.,[17] performed the extraction method of fruits and leaves of this plant. They macerate leaves and fruits of miracle plant with ethanol and then evaporate at room temperature.[17]

He, et al.,[30] modified the extraction method for miraculin from miracle berry as described by Theerasilp and Kurihara.,[22] In this method, lyophilized pulp powder was suspended with water and homogenized. The homogenate was centrifuged and the sediment was homogenized in 0.5 M sodium chloride solution. Then again centrifuged and the colourless supernatant was freeze-dried.[22,30]

Miraculin has been purified by using various methods like solvent extraction method from polar to non-polar and from non-polar to polar in succession,[9] ion-exchange chromatography technique,[10] immobilized-metal affinity chromatography (IMAC),[31] and reverse micelle system.[30]

The solvent extraction method is tedious and the purity of miraculin is relatively low. High purity miraculin was obtained by ion-exchange chromatography but this is time-consuming, inefficient and costly[10]. IMAC is a simple and efficient method, but this method is difficult to scale-up beyond laboratory scale and considered to be expensive.[32]. It has been proved that reverse micelle extraction method is an efficient method to extract miraculin from miracle berry due to its low energy requirements and also shows great potential for large-scale application and use in continuous separation of biological substances.[32]

Mechanism of taste modifying activity of miraculin

The flesh of the berry contains aglycoprotein, miraculin, with a molecular weight of about 43,000 D. Miraculin is actually not a sweetener, but has the property of making sour products taste sweet[20-23]. It is a glycoprotein consisting of 191 amino acid residues with two glycosylated polypeptides i.e. Asn-186 and Asn-42, cross-linked by a disulphide bond.[24-26] It consists of approx. 14 % sugars, including glucosamine, mannose, fucose, galactose, and xylose. The taste-modifying effect of miraculin begins a few seconds after consumption, though several minutes of chewing the berry's pulp may be necessary to sufficiently coat the taste buds. The duration of the taste-modifying effect typically lasts 30 minutes to 2 hours.[27-28]

Miraculin binds tightly to the lingual epithelium's microvilli plasma membrane of sweet-taste receptors (hT1R2-hT1R3) without activating them. Miraculin does not activate these receptors until subjected to an acidic pH, generally, between pH 3.0 and 6.0.[29] The possible mechanism of miraculin in the modification of taste is shown in Figure 9.

Limitations and Preservation of miracle berry

Miracle berry or *Richadella dulcifica* is highly perishable. It is thermolabile and inactivated below pH 3 and above pH 12. Not used in cooking and in processed foods because it degrades at high temperature. These limitations can be overcome by preservation techniques using a polysaccharide chitosan coating.[33] Also, the miracle berry can be freeze-dried at -20° F for approximately 3 months before use without significant degradation.[34]

The miraculin protein itself very stable and can be stored in pure form or in solution for 6 months without loss of function[35] and long-time storage can be done in Ziploc bags.

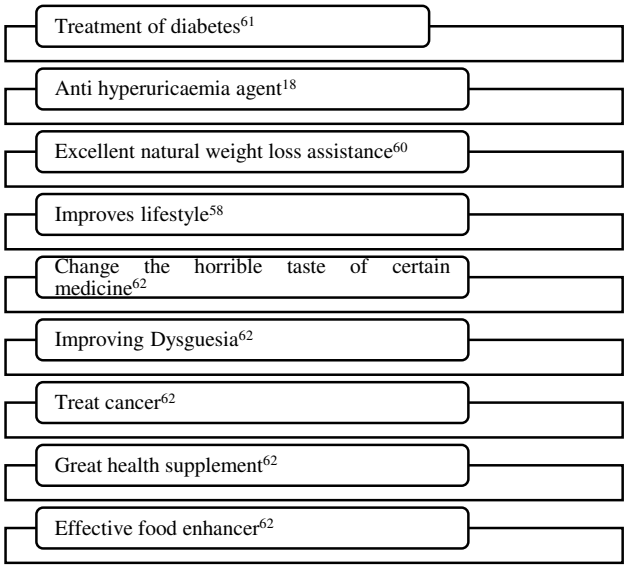


Figure 7: Health benefits of *Synespalum ducificum*

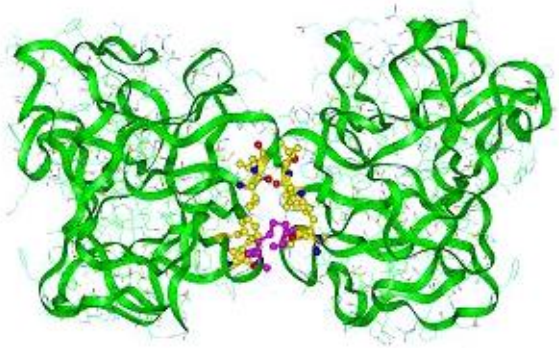


Figure 8: Structure model of Miraculin (Homodimer formed by Disulphide bond)[63]

1. Regulatory Issue

FDA, the U.S. Department of Agriculture (USDA) does not maintain restrictions on the miracle berry. Growing, selling, and eating miracle berries in the United States is legal.[35]

2. Miracle fruit: A Promising product

Miracle berry is a promising natural product and miraculin in the fruit enhances its application in the food industry as well as in pharmaceutical industry. In the market, various formulations are available in the form of granules, and tablets.[36-41] Various marketed formulations of miracle fruit tablet are in Figure 10.

3. Indian Variety of miracle berry

Indian variety of miracle berry named as *Gymnema sylvestre* with botanical synonyms *Asclepias geminate, Periploca sylvestris, and Marsdenia sylvestris*. It is native to India and also found in tropical Africa, Australia, Malaysia and Srilanka. It is also known as GUDMAR and Sugar Destroyer. Flowers of *Gymnema sylvestre* are collected in the month of April and November whereas fruits in winter season.[42]

Gymnema sylvestre, miracle plant having some tremendous activities like anti-diabetic, anti-obesity, anti-inflammatory, anti-microbial, hypolipidemic and anti-arthritic acitivity[46]. It is also used in the treatment of urinary complaints, stomach problems, piles, chronic cough, breathing troubles, asthma, eye complaints, cardiopathy, constipation, jaundice, and bronchitis. These activities are due to the presence of the bioactive compounds Gymnemic acid, Gymnemagenin and Gurmarin.[43]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

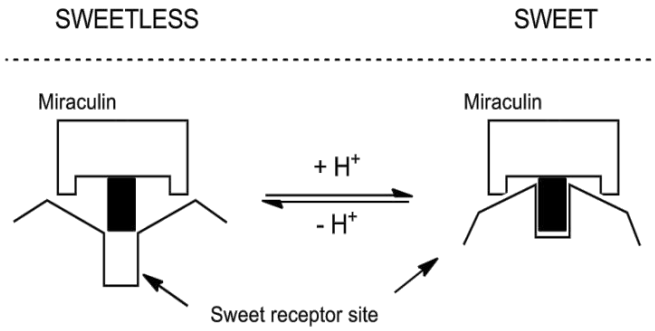
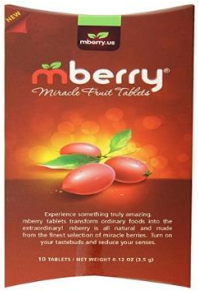


Figure 9: Mechanism of taste modifying activity of miraculin[27]

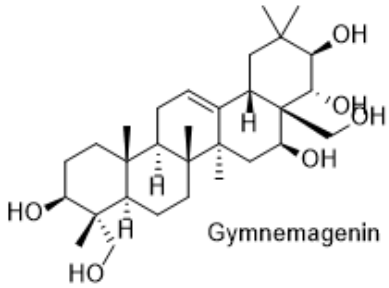


Miracle berry tablets[37]

Mouth dissolving tablets.[38]

Miracle fruit powder[41]

Figure 10: Marketed formulation of miracle berry



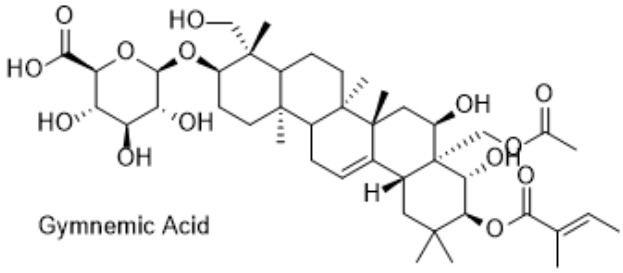


Figure 11: Molecular structures of bioactive compounds present in *Gymnema sylvestre*

Patents on Miracle berry and its variety

*Table 1 illustrates an updated report on pate*nts on therapeutic application of miracle berry and miraculin along with its Indian variety i.e. *Gymnema sylvestre.*

Table 1: Updated list on Patents of miracle berry and *Gymnema sylvestre.*

Patent No.	Title	Publication date	Ref.
US 20160317430 A1	Cosmetic use of an extract of *Gymnema sylvestre*	Nov 3, 2016	44
CA 2919487 A1	Method and kit for bowel preparation	Feb 6, 2014	45
246537 (Indian Patent)	A process for preparation of a novel compound gymnemic triacetate from *Gymnema sylvestre* R. Br. leaves with antidiabetic activity	Mar 11,2011	46
US 20110144218 A1	Taste modified consumable Products and methods of preparation	Jun 16, 2011	47
EP 1750736 B1	Uses for the extract of *Gymnema sylvestris*	Apr 21, 2010	48
US 20040071801 A1	Herbal formulation of *Gymnema sylvestre* as a dietary aid.	Apr 15, 2004	49
US 5886155 A	Purification of miraculin glycoprotein using tandem hydrophobic interaction chromatography	Mar 23, 1999	50
EP 0570591 A1	Process for producing sweetness inducer miraculin	Nov 24, 1993	51
EP 0406516 A1	A process for extracting the concentrated gymnemate from *Gymnema sylvestre* and an equipment for using in the process	Jan 9, 1991	52
CA 1162094 A	Chewing gum containing sugar substitute	Feb 14, 1984	53
US 3995031 A	Method of controlling obesity with purified active principle of fruit of *Synsepalum dulcificum.*	Nov 30, 1976	54
US 3925547 A	Isolation and purification of active principle of fruit of *Synsepalum dulcificum*	Dec 9, 1975	55
US 3849555 A	Method for modifying sour and bitter taste	Nov 19, 1974	56
US 3682880 A	Protein flavouring agents prepared from *Synsepalum dulcificum*.	Aug 8, 1972	57
US 3620770 A	Coated straw for modifying sour taste	Nov 16, 1971	58

Conclusion

Owing to its numerous attributes, miracle berry exploitation has aroused researchers interest in discovering an efficient taste modifier via miraculin. Though miracle berry has interesting and promising uses in the food, pharmaceutical, as well as in cosmetic industry, the fruit wide spread usage is however limited by its availability and perishability. To overcome this limitation, researchers are producing recombinant miraculin in transgenic plants, notably tomatoes. The various phytochemicals in the miracle fruit have been confirmed to possess health promoting functions in reducing various chronic diseases such as diabetes, cancers, acute gouty arthritis, hypertension, cardiovascular diseases and in weight loss. This information enhances the fruits acceptability by more people and thus, generally promotes its utilization and appreciation in human diets.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to thank Department of Science and Technology (DST) for providing INSPIRE fellowship award and granting financial assistance.

References

1. https://www.ebay.com/itm/MIRACLE-FRUIT-Synsepalum-dulcificum-rare-tropical-exotic-berry-edible-4-SEEDS-/151116932149 . Last accessed on 28th September, 2017.
2. Daniell WF. Miraculous berry of Western Africa. Pharm J. 1852; 1:445-448.
3. Brouwer JN, Van DerWel H, Francke A, Henning GJ. Miraculin, the sweetness-inducing protein from miracle fruit. Nature. 1968; 220:371–373.
4. Inglett GE and May JF. Tropical plant with unusual taste properties. Econ bot. 1968; 22:326–331.
5. Inglett GE and Chen D. Contents of phenolics and flavonoids and antioxidant activities in skin, pulp, and seeds of miracle fruit. J Food Sci. 2011; 76:79-82.
6. Yamamoto C, Nagai H, Takahashi K, Nakagawa S, Yamaguchi M, Tonoike M, Yamamoto T. Cortical representation of taste-modifying action of miracle fruit in humans. NeuroImage. 2006; 33:1145–1151.
7. Wong JM and Kern M. Miracle fruit improves sweetness of a low-calorie dessert without promoting subsequent energy compensation. Appetite. 2011; 56:163–166.
8. Bartoshuk LM, Gentile RL, Moskowitz HR, Meiselman HL. Sweet taste induced by miracle fruit (*Synsepalum dulcificum*). Physiol Behav. 1974; 12:449–456.
9. Inglett GE, Dowling B, Albrecht JJ, Hoglan FA. Taste-modifying properties of miracle fruit (*Synsepalum dulcificum*). Agric Food Chem. 1965; 13:284–287.
10. Kurihara K and Beidler LM. Taste-modifying protein from miracle fruit. Sci. 1968; 161:1241–1243.
11. Du L, Shen Y, Zhang X, Prinyawiwatkul W, Xu Z. Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts. Food Chem. 2014; 153:279-284.
12. Xu Z. Important antioxidant phytochemicals in agricultural food products. In Z. Xu, & L.R. Howard, (Eds.), Analysis of antioxidant-rich phytochemicals. Iowa: John Wiley& Sons. Inc. 2012:1-16.
13. Buckmire RE and Francis FJ. Anthocyanins and flavonols of miracle fruit. J Food Sci. 1976; 41:1363–1365.
14. Njoku NE, Ubbaonu CN, Alagbaoso SO, Eluchie CN, Umelo MC. Amino acid profile and oxidizable vitamin content of *Synsepalum dulcificum* berry (miracle fruit) pulp. Food Sci Nutr. 2015; 3:252–256.
15. Osabor VN, Etiuma RA, and Ntinya MU. Chemical Profile of Leaves and Roots of Miracle Fruit (*Synsepalum dulcificum*). ACSJ. 2016; 12:1-8.
16. Chen CC, Liu IM, Cheng JT. Improvement of insulin resistance by miracle fruit (*Synsepalum dulcificum*) in fructose-rich chow-fed rats. Phytotherapy Res. 2006; 20:987–992.
17. Dioso MKM, Satsatin DCA, Ching JA. Hypoglycemic effects of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell (Miracle Berry) fruit and leaf extracts on the blood glucose level of albino rats. Der Pharmacia Lettre. 2016; 8:104-108.
18. Shi YC, Lin KS, Jhai YF, Lee BH, Han Y, Cui Z, Hsu WH, Wu SC. Miracle Fruit (*Synsepalum dulcificum*) Exhibits as a Novel Anti-Hyperuricaemia Agent. Molecules. 2016; 21:140.
19. Adom KK, Liu, RH. Antioxidant activity of grains. J Agric Food Chem. 2002; 50:6182–6187.
20. Hiwasa-Tanasa K, Hirai T, Kato K, Duhita N, Ezura H. From miracle fruit to transgenic tomato: mass production of the taste-modifying protein miraculin in transgenic plants. Plant Cell Rep. 2012; 31:513-525.
21. Theerasilp S, Hitotsuya H, Nakajo S, Nakaya K, Nakamura Y, Kurihara Y. Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. J Biol Chem. 1989; 264:6655-6659.
22. Theerasilp S and Kurihara Y. Complete purification and characterization of the taste-modifying protein, miraculin, from miracle fruit. J Biol Chem. 1988; 263:11536-11539.
23. Ito K, Asakura T, Morita Y, Nakajima K, Koizumi A, Shimizu-Ibuka A, Masuda K, Ishiguro M, Terada T, Maruyama J, Kitamoto K, Misaka T, Abe K. Microbial production of sensory-active miraculin. Biochem Biophys Res Commun. 2007; 360:407-411.
24. Matsuyama T, Satoh M, Nakata R, Aoyama T, Inoue H. Functional expression of miraculin, a taste-modifying protein in escherichia coli. J Biochem. 2009; 145:445-450.
25. Paladino A, Costantini S, Colonna G, Facchiano AM. Molecular modeling of miraculin: structural analyses and functional hypotheses. Biochem Biophys Res Commun. 2008; 367:26-32.
26. Temussi PA. Natural sweet macromolecules: how sweet proteins work. Cell Mol Life Sci. 2006; 63:1876-1888.
27. Kurihara Y. Characteristics of antisweet substances, sweet proteins, and sweetness-inducing proteins. Crit Rev Food Sci Nutr. 1992; 32:231-252.
28. Asakura T, Miyano M, Yamashita H, Sakurai T, Nakajima KI, Ito K, Misaka T, Ishimaru Y, Abe K. Analysis of the interaction of food components with model lingual epithelial cells: the case of sweet proteins. Flavour Fragr J. 2011; 26:274-278.
29. Swamy KB, Hadi SA, Sekaran M, Pichika MR. The Clinical Effects of *Synsepalum dulcificum:* A Review. J Med Food. 2014; 17:1165–1169.
30. He Z, Tan JS, Abbasiliasi S, Lai OM,Tam YJ, Halim M, Ariff AB. Primary recovery of miraculin from miracle fruit, *Synsepalum dulcificum* by AOT reverse micellar system. Food Sci Tech. 2015; 64:1243-1250.
31. Duhita, N, Hiwasa-Tanase K, Yoshida S, Ezura H. Single-step purification of native miraculin using immobilized metal-

affinity chromatography. J Agric Food Chem. 2009; 57:5148-5151.
32. Zainuddin AM, Mohamed MN, Siti HMS. Advances in separation processes. Johor, Malaysia: Universiti Teknologi Malaysia. 2007.
33. Liu CL, He CL, Xie TP, Yang YE, Liang TX. Research on preservation of *Synsepalum dulcificum* by coatings. Adv Mat Res. 2011; 239-242:2158-2162.
34. Hellekant G, Segerstad CH, Roberts T, van der Wel H, Brouwer JN, Glaser D, Haynes R, Eichberg JW. Effects of gymnemic acid on the chorda tympani proper nerve responses to sweet, sour, salty and bitter taste stimuli in the chimpanzee. Acta Physiol Scand. 1985; 124:399-408.
35. Cudnik DR. Miraculin and The Miracle Berry: An Option for Dysguesia?. Oncol Nutr Conn. 2016; 23:7-12.
36. Chacon JRL. Miracle Fruit - Miracle Berry (*Synsepalum dulcificum* Daniell)-Basic Aspects for Contextualization. J Invest Manag. 2015; 4:330-333.
37. https://www.amazon.com/mberry-Miracle-Fruit-Tablets-10-Count/dp/B001LXYA5Q/ref=pd_lpo_vtph_325_bs_img_1?_encoding=UTF8&psc=1&refRID=ESA6X8ZR0P0EZAJMA0Q3. Last accessed on 28th September, 2017.
38. https://www.amazon.com/Miracle-Frooties-Fruit-Tablets/dp/B004SOMH3M/ref=pd_sim_325_5?_encoding=UTF8&psc=1&refRID=MX3J9WGGNCCCV9GJTYAS. Last accessed on 15th November, 2017.
39. https://static1.squarespace.com/static/515b7cbbe4b054dae3f9c548/t/5965398f20099e106f309ff6/1499806120283/ortaste_1_1.jpg?format=750w . Last accessed on 28th September, 2017.
40. https://www.amazon.com/Sen-Yuh-Farm-Science-Co/dp/B001JH93BU, Last accessed on 13th October, 2017.
41. http://miraclefruitfarm.com/shop/commercial-sample-of-miracle-fruit-powder-50-g. Last accessed on 28th September, 2017.
42. https://www.healthbenefitstimes.com/miracle-fruit/. Last accessed on 30th September,2017.
43. Syedy M and Nama KS. *Gymnema sylvestre* : A miracle fruit for Diabetes cure. Int J Pure App Biosci. 2014; 2:318-325.
44. Courtin O. Cosmetic use of an extract of G*ymnema sylvestre*. US 20160317430 A1. (2016).
45. Gorelick S, Schiffman M, Olmstead M, Gorelick A. Method and kit for bowel preparation. CA 2919487 A1. (2014).
46. Ignacimuthu S, Daisy P, Farook M. A process for preparation of a novel compound gymnemic triacetate from *Gymnema sylvestre* R. Br. leaves with antidiabetic activity. 246537. (2011).
47. Posner D, Abelson N. Taste modified consumable Products and methods of preparation. US 20110144218 A1. (2011).
48. Danoux L, Freis O, Moser P, Pauly G. Uses for the extract of G*gymnema sylvestris*. EP 1750736 B1. (2010).
49. Edell D, Handel R. Herbal formulation of *Gymnema sylvestre* as a dietary aid. US 20040071801 A1. (2004).
50. Armah GE, Achel DG, Acquaah RA, Belew M. Purification of miraculin glycoprotein using tandem hydrophobic interaction chromatography. US 5886155 A. (1999).
51. Kamimura N, Makino TM, Hirano A, Kurihara Y. Process for producing sweetness inducer miraculin**.** EP 0570591 A1. (1993)
52. Hwang BY, Choi SY. A process for extracting the concentrated gymnemate from *Gymnema sylvestre* and an equipment for using in the process. EP 0406516 A1. (1991).
53. Hopkins W, Friello DR,Cherukuri SR, Roy RL, Mackay DAM. Chewing gum containing sugar substitute. CA 1162094 A. (1984).
54. Henkin RI and Giroux EL. Method of controlling obesity with purified active principle of fruit of *Synsepalum dulcificum*. US 3995031 A. (1976).
55. Henkin RI and Giroux EL. Isolation and purification of active principle of fruit of *Synsepalum dulcificum*. US 3925547 A. (1975).
56. Harvey R. Method for modifying sour and bitter taste. US 3849555 A. (1974).
57. Nicolaas BJ, Johannes HG, Van Der WH. Protein flavouring agents prepared from *Synsepalum dulcificum*. US 3682880 A. (1972).
58. Harvey RJ. Coated straw for modifying sour taste. US 3620770 A. (1971).
59. Miracle Fruit: Health Benefits & Uses. Organic facts. Available at https://www.organicfacts.net/miracle-fruit.html. Last accessed on 30th September, 2017.
60. HealthBenefitsTimes.com, "Miracle Fruit Facts and Health Benefits". Available at https://www.healthbenefitstimes.com/miracle-fruit/. Last accessed on 21st November, 2017.
61. Chen CC, Liu IM, Cheng JT. Improvement of insulin resistance by miracle fruit (*Synsepalum dulcificum*) in fructose-rich chow-fed rats. Phyother Res. 2006; 20:987-92.
62. 14 Scientific Health Benefits of Miracle Fruit. Available at https://drhealthbenefits.com/food-bevarages/fruits/health-benefits-of-miracle-fruit. Last accessed on 24th September, 2017
63. http://houston.eventful.com/events/flavor-tripping-houston-party-/E0-001-032057903-0. Last accessed on 30th September, 2017.