



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2017; 6(5): 250-255  
Received: 22-07-2017  
Accepted: 24-08-2017

**Dipak Sharma**  
Department of Food  
Engineering, College of Food  
Technology, Vasantrao Naik  
Marathwada Krishi Vidyapeeth,  
Parbhani, Maharashtra, India

**AR Sawate**  
Department of Food  
Engineering, College of Food  
Technology, Vasantrao Naik  
Marathwada Krishi Vidyapeeth,  
Parbhani, Maharashtra, India

**BM Patil**  
Department of Food  
Engineering, College of Food  
Technology, Vasantrao Naik  
Marathwada Krishi Vidyapeeth,  
Parbhani, Maharashtra, India

**RB Kshirsagar**  
Department of Food  
Engineering, College of Food  
Technology, Vasantrao Naik  
Marathwada Krishi Vidyapeeth,  
Parbhani, Maharashtra, India

**Correspondence**  
**Dipak Sharma**  
Department of Food  
Engineering, College of Food  
Technology, Vasantrao Naik  
Marathwada Krishi Vidyapeeth,  
Parbhani, Maharashtra, India

## Studies on physico chemical characteristics of *Gymnema sylvestre* (Leaf, powder and extract)

**Dipak Sharma, AR Sawate, BM Patil and RB Kshirsagar**

### Abstract

*Gymnema sylvestre* is a reputed herb in the ayurvedic system of medicine. The herb exhibits a broad range of therapeutic effects as an effective natural remedy for diabetes. The mandate of current study was to explore the nutritional and anti diabetic worth of *Gymnema sylvestre* because of its easily availability and mostly use. The fresh *Gymnema sylvestre* leaves were green in colour and elliptical in shape. The average weight, length and width of leaves were 0.157, 4.46 and 2.56 respectively. The physico-chemical characteristics of *Gymnema sylvestre* powder like bulk density (g/ml), tapped density (g/ml), Carr's index, Hausner's ratio, pH 1% solution, acid insoluble ash (%), water soluble ash (%), water-soluble and alcohol soluble extractive values (%) were 0.43, 0.54, 20.37, 1.255, 6.93, 1.95, 2.60, 17.23 and 4.57 respectively. The proximate composition of *Gymnema sylvestre* powder like moisture, crude fat, crude protein, crude fiber, total ash and total carbohydrate contents were 7.38, 5.80, 10.94, 11.50, 9.49 and 54.89 per cent respectively. The minerals calcium, magnesium, chromium, zinc, copper and iron were analyzed from *Gymnema sylvestre* powder, the concentration of these minerals were 1542.63, 592.40, 2.70, 21.80, 12.71 and 36.91 (mg/100g) respectively. *Gymnema sylvestre* liquid extract had a brownish green colour, no specific odour and bitter in taste while dried extract was brown in colour and also bitter in taste. The physico-chemical characteristics of *Gymnema sylvestre* dried ethanolic extract like pH 1% solution, saponin test, foaming index, ash content (%), water soluble fraction (%), water-insoluble fraction (%) and gymnemic acid content (%) were 6.8, positive, less than 100, 1.90, 65, 35 and 8.00 respectively.

**Keywords:** *Gymnema sylvestre*, Physico-chemical characteristics, Proximate composition, Minerals, Gymnemic acid.

### 1. Introduction

In India diabetic patient are increasing day by day and according to world diabetic foundation it has the world's largest diabetes population, followed by China with 43.2 million and it has major concern among health experts and national and international healthcare. World health organization (WHO) has identified diabetes as an epidemic condition and one of the major killers of the decade. Estimation by WHO, there will be about 250 million cases of diabetes mellitus throughout the world by 2025 (Mishra, 2011)<sup>[24]</sup>.

The fresh leaves of *Gymnema sylvestre* when chewed have the remarkable property of paralyzing the sense of taste of sweet substance for some time (Gent, 1999)<sup>[13]</sup>. The atomic arrangement of gymnemic acid molecules is similar to that of glucose molecules. These molecules fill the receptor locations on the taste buds thereby preventing its activation by sugar molecules present in the food. This prevents craving for sugar. Similarly, Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the sugar molecules absorption, which results in low blood sugar level (Sahu *et al.*, 1996)<sup>[38]</sup>. Traditionally it was recommended for stomach problems, constipation, liver disease but the recent studies have shown that the extract of *Gymnema sylvestre* is useful in controlling blood sugar to treat type-II diabetes (NIDDM). When *Gymnema* leaf extract is administered to a diabetic patient it stimulates the pancreas to increase release of insulin (Persaud *et al.*, 1999)<sup>[30]</sup>.

Crude or low molecular weight *Gymnema sylvestre* extracts have been reported to have antidiabetic effect in alloxan or streptozotocin treated animals by raising plasma insulin levels and attenuating blood glucose responses during oral glucose/sucrose tolerance tests (Okabayashi *et al.*, 1990 and Terasawa *et al.*, 1994)<sup>[28] 41]</sup>. Similarly, crude or low molecular weight *Gymnema sylvestre* extracts have been reported to have hypoglycemic effects in patients with hyperglycemic diabetics (Khare *et al.*, 1983 and Baskaran *et al.*, 1990)<sup>[18, 6]</sup>. Clinical studies investigating antidiabetic effects have typically used 200 or 400 mg extract daily standardized to contain 25% gymnemic acids (Baskaran *et al.*, 1990; Joffe *et al.*, 2001 and Preuss *et al.*, 2004)<sup>[6, 16, 32]</sup>.

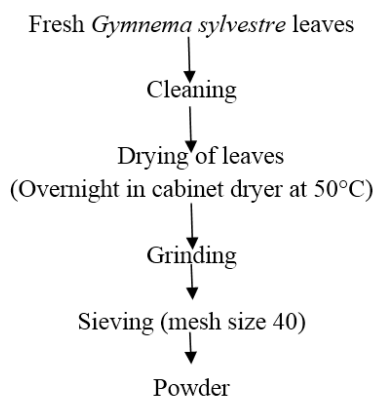
Though *Gymnema sylvest* has been used for several centuries and considered to be safe, there is a paucity of data on interaction between *Gymnema* and pharmaceuticals or minerals. Even US FDA also states some of the illness and injuries associated with the use of dietary supplements (USFDA, 1993)<sup>[44]</sup>.

### Materials and methods

The present investigation was carried out in Department of Food Engineering, College of Food Technology, VNMKV, Parbhani. Fresh *Gymnema sylvest* (gudmar) leaves were obtained from the CFT, Parbhani.

### Preparations of *Gymnema sylvest* (gudmar) leaf powder

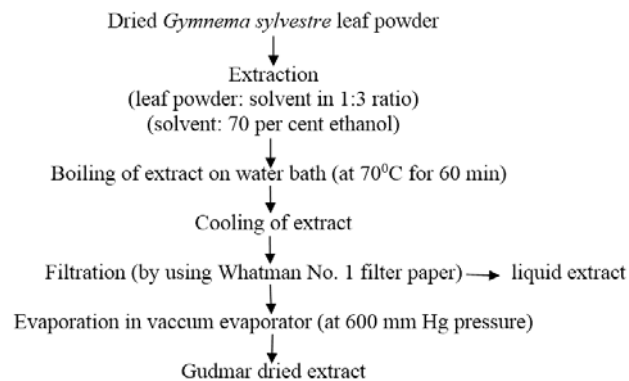
*Gymnema sylvest* (gudmar) leaf powder was prepared as per the method given by Farzana and Muhammad (2010)<sup>[11]</sup>.



**Fig 1:** Flowchart for the preparation of *Gymnema sylvest* (gudmar) leaf powder

### Preparations of *Gymnema sylvest* (gudmar) leaf extract

*Gymnema sylvest* leaf extract was prepared as per the method adopted by Killedar *et al.*, (2012)<sup>[19]</sup>. Leaf powder (500 g) was extracted by 70 per cent ethanol extraction method with boiling on water bath for 1 hour and cooled extract was filtered through vacuum filtrations unit, evaporated to dryness on rotary film vacuum evaporator. The dried extract was kept in refrigerator for future use.



**Fig 2:** Flowchart for the preparation of *Gymnema sylvest* (gudmar) leaf extract

### Physical characteristics of fresh *Gymnema sylvest* (gudmar) leaf

Colour and shape of fresh *Gymnema sylvest* leaf was inspected visually. Weight of gudmar leaf was determined by

using electronic weighing balance. Leaf length and width were determined by using vernier calliper.

### Physico-chemical characteristics of *Gymnema sylvest* (gudmar) powder

Colour of *Gymnema sylvest* powder was inspected visually. The values of bulk density (g/ml), tapped density (g/ml), Carr's index and Hausner's ratio were determined as per the method given by Lachman *et al.*, (1991)<sup>[23]</sup>. pH of 1 per cent solution of *Gymnema sylvest* powder was determined by method as per WHO (1998). Acid insoluble ash and water soluble ash content of *Gymnema sylvest* powder was determined by method as per A.O.A.C., (2000). Water soluble extractive value and alcohol soluble extractive value of powder was determined by the method reported by Tripathi *et al.*, (2013)<sup>[43]</sup>.

### Proximate analysis of *Gymnema sylvest* (gudmar) powder

Analysis of *Gymnema sylvest* powder for moisture, crude fat, crude protein, crude fiber, total ash and total carbohydrates content were carried out according to their respective methods (A.O.A.C., 2005). All the tests were executed in triplicates.

### Mineral Estimation of *Gymnema sylvest* (gudmar) powder

The determination of calcium, magnesium and iron were done as per the method described by Ranganna (1986). The Atomic Absorption Spectrophotometer was used to determine chromium, zinc and copper content in *Gymnema sylvest* powder as per the method given by Ramachandra *et al.*, (2012).

### Physical (organoleptic) characteristics of *Gymnema sylvest* (gudmar) extracts

Physical (organoleptic) characteristics of prepared extracts like color, taste, odour and texture were determined visually, sensing by tongue, smelling by nose and sensing by finger respectively.

### Physico-chemical characteristics of *Gymnema sylvest* (gudmar) dried extract

Ash content, foaming index and pH of gudmar dried extract was determined by method as per WHO (1998)<sup>[45]</sup>. The ethanolic extract of *Gymnema sylvest* was subfractionated into watersoluble (W-S) fraction and water-insoluble (W-INS) fractions by the method reported by Alam *et al.*, (2005)<sup>[3]</sup>. Screening of saponins in gudmar dried extract was done as per the method given by Evans (2002)<sup>[10]</sup>. Total gymnemic acid content estimated as per the gravimetric method given by Killedar *et al.*, (2012)<sup>[19]</sup>.

### Results and Discussion

**Table 1:** Physical properties of fresh *Gymnema sylvest* (gudmar) leaf

Physical parameters	Results
Color	Green
Shape	Elliptical
Average Weight (g)	0.157*
Average Length (cm)	4.46*
Average Width(cm)	2.56*

\*Each value is an average of three determinations

The average weight of fresh gudmar leaves was 0.157 g, length and width of gudmar was measured by using vernier calliper which gives idea about the wholesomeness of the leaves and the length and width was found to be 4.46 cm and 2.56 cm respectively. These results were in good agreement with the results reported by Pandey and Yadav (2010)<sup>[29]</sup>. The shape and color was observed visually and the shape of leaf was found to be elliptical and color was green. Similar results reported by Kumar *et al.*, (2015)<sup>[20, 21]</sup>.

**Table 2:** Physico-chemical properties of *Gymnema sylvestre* (gudmar) powder

Physico-chemical parameters	Results*
Color	Green
Bulk Density(g/ml)	0.43
Tapped Density(g/ml)	0.54
Carr's Index	20.37
Hausner's Ratio	1.255
pH 1% solution	6.93
Acid insoluble ash (%)	1.95
Water soluble ash (%)	2.60
Water soluble extractive values (%)	17.23
Alcohol soluble extractive value (%)	4.57

\*Each value is an average of three determinations

The color of gudmar powder was observed visually and found to be green in colour. The pH 1 per cent solution of gudmar powder was 6.93. The values of bulk density (g/ml) and tapped density (g/ml) were 0.43 and 0.54 respectively. Tapped density gives information on consolidation of a powder. A consolidated powder is likely to have a greater arch strength than a less consolidated one, and may therefore be more resistant to powder flow. *Gymnema sylvestre* had values 20.37 and 1.255 respectively for Carr's index and Hausner's ratio indicating fair compressibility. Similar results with respect to bulk density, tapped density, Carr's index and Hausner's ratio were reported by Tripathi *et al.*, (2013)<sup>[43]</sup>.

The values of acid insoluble ash and water soluble ash were 1.95 and 2.60 per cent respectively. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparation of powder. These values were found to be reasonably low indicating low contamination. Water-soluble ash is the part of the total ash content, which is soluble in water. It is a good indicator of extraction of water-soluble salts during preparation of extract. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. These values were in good agreement with the results reported by Kalidass and Mohan (2010)<sup>[17]</sup> and Dwivedi *et al.*, (2012)<sup>[9]</sup>.

Water-soluble and alcohol soluble extractive values play an important role in evaluation of properties of powder. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The values of Water soluble extractive and alcohol soluble extractive were 17.23 and 4.57 per cent respectively. It was observed that the water-soluble extractive values were higher than alcohol-soluble extractives. The results for the water-soluble and alcohol soluble extractive values were supported by finding of Chiranjeevi *et al.*, (2013)<sup>[7]</sup>. The results of proximate composition revealed that gudmar powder is good source of carbohydrate, crude fiber, protein and ash content. Table 3 revealed that moisture content in gudmar powder was found to be 7.38 per cent and the fat content was low in concentration i.e. 5.80 per cent. Gudmar

powder contained higher amount of carbohydrate (54.89 per cent) than other parameters. Crude fiber, protein and ash content of gudmar powder were found to be 11.50, 10.94 and 9.49 per cent respectively. These all chemical parameters are similar with results found in Goel and Tarvinderjeet (2013)<sup>[14]</sup>.

**Table 3:** Proximate composition of *Gymnema sylvestre* (gudmar) powder

Proximate composition	Results (%)
Moisture	7.38
Crude Fat	5.80
Crude Protein	10.94
Crude fiber	11.50
Total Ash	9.49
Total Carbohydrates	54.89

\*Each value is an average of three determinations

**Table 4:** Mineral composition of *Gymnema sylvestre* (gudmar) powder

Mineral composition	Results (mg/100g)
Calcium	1542.63
Magnesium	592.40
Chromium	2.70
Zinc	21.80
Copper	12.71
Iron	36.91

\*Each value is an average of three determinations

Minerals are inorganic elements needed by the body as structural component and regulators of body processes. The macronutrients (Ca, Mg, Cr, Zn and Cu) and Micronutrient (Fe) were analyzed from gudmar powder, the concentration of these minerals were 1542.63, 592.40, 2.70, 21.80, 12.71 and 36.91 (mg/100g) respectively. The values of calcium, magnesium, zinc and copper were similar with the results reported by Dey and Khaled (2015)<sup>[8]</sup> while results of chromium and iron are in good agreement with Goel and Tarvinderjeet (2013)<sup>[14]</sup>. The concentration of Ca and Mg were found much higher than the other inorganic minerals. Chromium was found very low as compared to other minerals.

Calcium is reported to play an important role in glucose tolerance factor (GTF), which decreases the blood glucose level by utilising insulin (Gurson and Saner, 1971). Calcium content of *Gymnema sylvestre* was higher than the value reported by Goel and Tarvinderjeet (2013)<sup>[14]</sup>. Chromium is a critical cofactor in the action of insulin (Anderson, 1997)<sup>[5]</sup> and an active component of the glucose tolerance factor (GTF). Deficiency of chromium has been implicated as one of the causes of diabetes mellitus and risk factor in atherosclerotic disease (Anderson, 1995)<sup>[4]</sup>. Chromium content in *Gymnema sylvestre* analysed in the present study was higher than the value reported by Ray *et al.* (2004)<sup>[37]</sup> but lower than that reported by Naga Raju *et al.* (2006)<sup>[26]</sup>. Copper possesses insulin-like activity and it has been found that its deficiency leads to glucose intolerance, decreased insulin response and increased glucose response (Mooradian and Morely, 1987)<sup>[25]</sup>. Zinc plays an important role in production, storage, and regulation of insulin. Zinc levels tend to be low in diabetic patients (Garg *et al.*, 2005)<sup>[12]</sup>. Naga Raju *et al.* (2006)<sup>[26]</sup> have determined zinc content of *Gymnema sylvestre* as 2.89 mg/100 g dry weight. Iron is a strong pro-oxidant that catalyses several cellular reactions that result in the production of reactive oxygen species (ROS),

with a consequent increase in the level of oxidative stress (Puntarulo, 2005) [33]. This contributes to tissue damage that may potentially elevate the risk of type 2 diabetes. Serum ferritin levels (marker of body iron stores) positively correlate with levels of circulating insulin, glucose and also with dyslipidemia (Ramakrishnan *et al.*, 2002 and Tilbrook, 2004) [32], [42]. The concentration of iron in *Gymnema sylvestre* was higher than that found by Ray *et al.*, (2004) [37] and lower than the value reported by Naga Raju *et al.*, (2006) [26].

**Table 5:** Physical characteristics of *Gymnema sylvestre* (gudmar) leaf extract

Parameter	Liquid Extract	Dried Extract
Color	Brownish green	Brown
Taste	Bitter	Bitter
Odour	No specific	No specific
Touch	Soft	Soft

Results presented in Table 5 with respect to physical characteristics of extract revealed that colour and odour of liquid extract was brownish green and no specific smell was observed. Similar results were reported by Chiranjeevi *et al.*, (2013) [7]. Liquid extract had a brownish green colour, it had no specific odour and bitter in taste while dried extract was brown in colour and also bitter in taste. Both liquid and dried extract were smooth in touch. Gudmar liquid and dried extract were bitter in taste may be due to the presence of gymnemic acid. Gymnemenin (group of gymnemic acids) is bitter in taste. Studies show that gurmarin-a peptide from *Gymnema sylvestre*, block the ability to sweet taste or bitter flavors and thus reduces sweet cravings (Ninomiya and Imoto, 1995; Pierce, 1999 and Saneja *et al.*, 2010) [27] [31] [39]. Bitter taste of *Gymnema sylvestre* was reported by Syedy and Nama (2014) [40].

**Table 6:** Physico-chemical properties of *Gymnema sylvestre* (gudmar) dried extract

Properties	Dried extract
pH (1% solution)	6.8
Ash (%)	1.90
Water soluble (W-S) fraction (%)	65
Water-insoluble (W-INS) fraction (%)	35
Foaming Index	less than 100
Saponin test	Positive
Gymnemic acid content (%)	8.00

\*Each value is an average of three determinations

The results of physico-chemical properties of dried ethanolic extract revealed that the pH of gudmar dried extract was 6.8 and ash content 1.90 per cent respectively. The ethanolic extract of *Gymnema sylvestre* was further sub fractionated into water soluble (W-S) fraction and water-insoluble (W-INS) fractions by the method reported by Alam *et al.*, (2005) [3]. The ethanolic extract was stirred in distilled water at room temperature and filtered to give water-soluble (W-S) fraction and water-insoluble (WINS) fractions. The yield obtained of W-S and W-INS were 65 per cent and 35 per cent respectively in terms of total ethanolic extract. The foaming index was determined on the basis of method given by WHO (1998) [45] and value obtained was less than 100. The values of all physico-chemical parameters were similar with results found in Kumar *et al.*, (2014) [22].

The result of saponin test was found positive, it indicated the presence of saponins in dried gudmar extract. Similar results of saponin test were reported by Kumar and Husain (2015) [20, 21]. The gymnemic acid content was found 8.00 per cent in 70

% ethanolic extracted dried gudmar extract. The result expressed was in good agreement with Killedar *et al.*, (2012) [19]

## Conclusion

*Gymnema sylvestre* had values 20.37 and 1.255 respectively for Carr's index and Hausner's ratio indicating fair compressibility of powder. *Gymnema sylvestre* found rich source of nutrients. *Gymnema sylvestre* powder had good amount of carbohydrate (54.89 per cent), protein (10.94 per cent) and crude fiber (11.50 per cent). *Gymnema sylvestre* powder was found to be higher in calcium (1542.63 mg/100g), magnesium (592.40 mg/100g) and iron content (36.91 mg/100g). Calcium is reported to play an important role in glucose tolerance factor (GTF), which decreases the blood glucose level by utilising insulin. *Gymnema sylvestre* dried extract had 65 per cent water soluble (w-s) fraction that make it suitable to utilize in liquid food products also. *Gymnema sylvestre* (leaf, powder and extract) is beneficial to the diabetic patients with regards to its antidiabetic activity (8 per cent gymnemic acid content in dried extract) and nutritional status specially mineral contents.

## References

1. A.O.A.C. Official Methods of Analysis. Association of Official Analytical chemists, EUA, 2000.
2. A.O.A.C. Official Methods of Analysis, Official Methods of Analysis. Association of Official Analytical chemists, 16th ed. Washington DC, U.S.A, 2005.
3. Alam MM, Javed K, Jafri MA. Effect of *Rheum emodi* (*Revand Hindi*) on renal functions in rats. Journal of Ethnopharmacology, 2005; 96(1-2):121-125.
4. Anderson RA. Chromium glucose tolerance, diabetes and lipid metabolism. Journal of Advancement in Medicine, 1995; 8:37-49.
5. Anderson RA. Nutritional factors influencing the glucose/insulin system: chromium. Journal of the American College of Nutrition, 1997; 16:404-410.
6. Baskaran K, Ahamath B, Shanmugasundaram K, Shanmugasundaram ER, Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin-dependent diabetes mellitus patients. Journal of Ethnopharmacology, 1990; 30:295-300.
7. Chiranjeevi T, Koteswara rao, Srinija K, Rama rao P, Sajjad SK, lavanya P *et al.* Phytochemical Evaluation Of *Calotropis Procera Gymnema Sylvestre Hemidesmus Indicus*. International Research Journal of Pharmaceutical and Applied Sciences. 2013; 3(6):31-34.
8. Dey P, Khaled KL, Quantitative Estimation of Some Essential Minerals of *Gymnema sylvestre* as a Potential Herb in Counteracting Complications of Diabetes. International Journal of Research Studies in Biosciences. 2015; 3(1):71-74.
9. Dwivedi D, Gharia AK, Thanwar M. An Investigation Studies of *Gymnema Sylvestre* R.Br. with Special Reference to Pharmacognostical and Phytochemical Aspects. Journal of Chemistry and Chemical Sciences. 2012; 2(2 and 3):124-127.
10. Evans WC, Trease and Evans, *Pharmacognosy*, W.B. Saunders. An imprint of Elsevier Limited, 15th edition, 2002; 471.
11. Farzana C, Muhammad HR. Isolation and characterization of gymnemic acid from Indigenous *gymnema sylvestr*. Journal of Applied Pharmaceutical science, 2010; 3(2):60-65.

12. Garg AN, Kumar A, Maheshwari G, Sharma S. Isotope dilution analysis for the determination of zinc in blood samples of diabetic patients. *Journal of Radioanalytical and Nuclear Chemistry*. 2005; 263:39-43.
13. Gent JF, Hettlinger TP, Frank ME, Marks LE, Taste confusions following gymnemic acid rinse. *Chemical Senses*. 1999; 24(4):393-403.
14. Goel S, Tarvinderjeet K. Nutritional Composition of Medicinal Plants Commonly Grown in the Kurukshetra District, Haryana, India. *Malaysian Journal of Nutrition*. 2013; 19(2):261-270.
15. Gurson CT, Saner G, Effect of chromium glucose utilisation in marasmic protein-caloric malnutrition. *American Journal of Clinical Nutrition*. 1971; 24(1):313-319.
16. Joffe DJ, Freed SH, Effect of extended release *Gymnema sylvestre* leaf extract ( Beta Fast G-XR ) alone or in combination with oral hypoglycemics or insulin regimens for type 1 and type 2 diabetes. *Diabetes in Control Newsletter*, 2001; 76:1-4.
17. Kalidass C, Mohan VR. Pharmacognostical and phytochemical investigation studies on *Gymnema sylvestre* R.Br. *International Journal of Biological Technology*. 2010; 1(1):08-11.
18. Khare AK, Tondon RN, Tewari JP, Hypoglycaemic activity of an indigenous drug (*Gymnema sylvestre*, "Gurmar") in normal and diabetic persons. *Indian Journal of Physiology and Pharmacology*. 1983; 27:257258.
19. Killedar SG, Rathod AM, Salunkhe RM, Bhore NV, Mahamuni SS, Parameters studied for development of *Gymnema sylvestre* leaf extracts: As injectable anti-diabetic. *International Journal of Research in Ayurveda and Pharmacy*. 2012; 3(2):283-285.
20. Kumar MS, Astalakshmi N, Arshida PT, Deepthi K, Nidhin DM, Shafna PM *et al.* A concise review on gurmar -*Gymnema sylvestre* R.Br. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4(10):430-448.
21. Kumar PS, Husain MK thnobotanical, preliminary phytochemical, extraction assessment study of gurmar buti leaves (*Gymnema sylvestre* R.Br.) and their immense traditional therapeutic values. *European Journal of Biomedical and Pharmaceutical Sciences*. 2015; 2(4):275-294.
22. Kumar V, Bhandari U, Tripathi CD, Khanna G. Protective Effect of *Gymnema sylvestre* Ethanol Extract on High Fat Diet-induced Obese Diabetic Wistar Rats. *Indian Journal of Pharmaceutical Sciences*. 2014; 76(4):315-322.
23. Lachman L, Liberman HA, Kanig JL. *The Theory and Practice of Industrial Pharmacy*. Mumbai: Varghese Publishing House. 1991, 3:67.
24. Mishra N. An analysis of antidiabetic activity of stevia rebaudiana extract on diabetic patient. *Journal of Natural Sciences Research*, 2011; 1(3):1-10.
25. Mooradian AD, Morley JE, Micronutrient status in diabetes mellitus. *American Journal of Clinical Nutrition*. 1987; 45:877-895.
26. Naga Raju GJ, Sarita P, Ramana Murty GAV, Ravi Kumar M, Seetharami Reddy B, John Charles M *et al.* Estimation of trace elements in some anti-diabetic medicinal plants using PIXE technique. *Applied Radiation and Isotopes*, 2006; 64:893-900.
27. Ninomiya Y, Imoto T. Gurmarin inhibition of sweet taste response in mice. *American Journal of Physiology*, 1995; 268(4):1019-1025.
28. Okabayashi Y, Tani S, Fujisawa T, Koide M, Hasegawa H, Nakamura T *et al.* Effect of *Gymnema sylvestre* R. Br. on glucose homeostasis in rats. *Diabetes Research and Clinical Practice*, 1990; 9:143-148.
29. Pandey AK, Yadav S. Variation in gymnemic acid content and non-destructive harvesting of *Gymnema sylvestre* (Gudmar). *Pharmacognosy Research*. 2010; 2(5):309-312.
30. Persaud SJ, Al-Majed H, Raman A, Jones PM. *Gymnema sylvestre* stimulates insulin release in vitro by increased membrane permeability. *Journal of Endocrinology*. 1999; 163:207-212.
31. Pierce A. *Gymnema Monograph: Practical guide to natural medicine*, Stonesong Press Book, New York. 1999, 324-326.
32. Preuss HG, Bagchi D, Bagchi M, Rao CV, Dey DK, Satyanarayana S *et al.* Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract on weight loss. *Diabetes, Obesity and Metabolism*. 2004; 6(3):171-180.
33. Puntarulo S. Iron, oxidative stress and human health. *Molecular Aspects of Medicine*, 2005; 26:299-312.
34. Ramachandra YL, Ashajyothi C, Padmalatha SR, Analysis of various metal ions in some medicinal plants using atomic absorption spectrophotometer. *International Journal of Pharmaceutical Sciences and Research*, 2012; 3(7):2208-2212.
35. Ramakrishnan U, Kuklina E, Stein AD. Iron stores and cardiovascular disease risk factors in women of reproductive age in the United States. *American Journal of Clinical Nutrition*. 2002; 76: 1256 - 1260.
36. Ranganna S. *Handbook of analysis and quality control for fruit and vegetable products*. 2<sup>nd</sup>. *Tata Mc Graw- Hill*, New Delhi, 1986, 1-201.
37. Ray DK, Nayak PK, Rautray TR, Vijayan V, Jena S. Elemental analysis of antidiabetic medicinal plants using energy dispersive X-ray fluorescence technique. *Indian Journal of Physics*, 2004; 78(2):103-105.
38. Sahu N, Mahato SB, Sarkar SK, Poddar G, Triterpenoid saponin from *Gymnema Sylvestre*. *Phytochemistry*, 1996; 41:1181-1185.
39. Saneja A, Sharma C, Aneja KR, Pahwa R. *Gymnema sylvestre* (Gurmar): A Review. *Der Pharmacia Lettre*, 2010; 2(1):275-284.
40. Syedy M, Nama KS, *Gymnema sylvestre*: A miracle fruit for Diabetes cure. *International Journal of Pure and Applied Bioscience*, 2014 2 (6):318-325.
41. Terasawa H, Miyoshi M, Imoto T. Effects of long-term administration of *Gymnema sylvestre* watery-extract on variations of body weight, plasma glucose, serum triglyceride, total cholesterol and insulin in Wistar fatty rats. *Yonago Acta Medica*, 1994; 37:117-127.
42. Tilbrook L. Cross talk between iron metabolism and diabetes. *Annals of Clinical Biochemistry*, 2004; 41(3):255-262.
43. Tripathi R, Verma S, Easwari TS, Shah H. Standardization of some herbal antidiabetic drugs in polyherbal formulation and their comparative study. *International Journal of Pharmaceutical Sciences and Research*, 2013; 4(8):3256-3265.
44. USFDA. *Illnesses and Injuries Associated With the Use of Selected Dietary Supplements*, Center for Food Safety

and Applied Nutrition. United State Food and Drug Administration, 1993.

45. WHO. Quality control methods for medicinal plant material, World Health Organization, Geneva, 1998; 28-36.