

ISOLATION AND CHARACTERIZATION OF GYMNEMIC ACID FROM GYMNEA SYLVESTRE R.BR. IN CONTROL OF DIABETES

*R. BALAMURALI KRISHNA¹, SUJITHA R. REDDY REDDY², HARIKA JAVANGULA³, D. SWAPNA⁴, K. JAGADEESWARA REDDY⁵

¹IUP Publications, Plot No. 53, Nagarjuna Hills, Punjagutta, Hyderabad-500039, India.

² Le-Sel Research (Pty) Ltd., No.1, New Road, Grand Central, Opposite Grand Central Airport, Midrand 1685, Johannesburg, Gauteng, South Africa.

^{3,4}JJ College of Pharmacy, Maheswaram, RR District, Hyderabad, India.

⁵Sugen Life Sciences Pvt. Ltd., 4/86 SV Nagar, Perumalla Palli (post), Tirupati-517505.

ABSTRACT

Diabetes is one of the oldest wide spread diseases of the world which is due to lack of insulin utilization or production. It is a gene-controlled disorder besides the expression is regulated by other environmental factors like food habits, life style, age etc. In this context, plant-based therapeutics are promising. The invaluable traditional anti-diabetic plants are sure to provide alternative therapeutic agents for diabetes. *Gymnema sylvestre* is a vine-like plant and is considered as herbal remedy for high blood sugar. The important active ingredient of *Gymnema sylvestre* is an organic acid called "Gymnemic acid". The recent studies have shown that the extract of *Gymnema sylvestre* is useful in controlling blood sugar to treat type-II diabetes. It increases the insulin producing b-cells of pancreas and significantly reduces the metabolic effects of sugar by preventing the intestine from absorbing the sugar molecules during the process of digestion. The objective of the present investigation was to isolate and characterize the Gymnemic acid, from seventeen ecotypes of *Gymnema sylvestre* leaves with different solvent systems like petroleum ether, benzene, and methanol. The defatted leaves were extracted under continuous hot extraction in Soxhlet apparatus with 90% methanol gave the maximum yield of gymnemic acid (42%) from the ecotype collected from Warangal-1. Gymnemic acid was purified by preparative chromatographic methods i.e., TLC and HPTLC. The analysis by HPTLC showed 30% purity of Gymnemic acid.

Key Words : *Gymnema sylvestre*, gymnemic acid, extraction, TLC, HPTLC

1. INTRODUCTION

Medicinal plants played an important role in Indian culture since Rigveda (5600 BC) where about 67 medicinal plants were recorded. It is estimated that 80% of about 4 billion population have to rely on traditional medicines due to high cost, lack of availability of required medicines and personal preferences. Out of 2,50,000 higher plants more

than 80,000 have medicinal value and India occupies unique position among world's 12 biodiversity centers. It is identified that about 20,000 plants have good medicinal value and 7500 species are used by traditional communities. It is estimated that Ayurveda and Unani system of medicine uses about 700 species each, Siddha and

Anchi uses 600 species each whereas modern medicine uses only 30 species of medicinal plants.

Herbal drugs, in India are also used as household remedy for common ailments since time immemorial. Our ancestors have a profound knowledge of these medicinal plants and they knew innumerable remedies, a fact indicated in the writings of *Siddhars* of Tamil Nadu. Their expertise if documented properly would help the modern man find more effective prophylactic use of these herbs. The relevance of pharmacognosy in standardization of herbal drugs has been long been stressed. Many monographs on pharmacognostic have emerged as an aid in the pharmacognostic investigations (Kalidass *et al.*, 2009a; Edward, 1956). The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material (Ozarkar, 2005).

Gymnema sylvestre R.Br. is one of the important anti-diabetic medicinal plant, there is a growing demand for *G. sylvestre* leaves in the pharmaceutical trade. Gymnemic acid, the active ingredient of this plant, is extracted from leaves and used widely as anti-diabetic (Shanmugasundaram *et*

al., 1983), anti-sweetner (Kurihara, 1992) and anti-hypercholesterolemia (Bishayee and Chatterjee, M 1994). It also has stomachic, diuretic and cough suppressant property (Kapoor, 1990, and Sastri 1956). The plant has been reported to possess antimicrobial (Sative *et al.*, 2003) and ethno-veterinary medicinal properties (Kalidass *et al.*, 2009b). In addition, it possesses antimicrobial, hepatoprotective, and anti-saccharine activities (Komalavalli, N and Rao, M.V. 2000, Nadkarni, A.K. and Nadkarni, K.M. 1976). Hence, because of these properties, *Gymnema sylvestre* is most important for plant prospecting.

The “destroyer of sugar” is a traditionally used term for *Gymnema sylvestre* because chewing the leaves will abolish the taste of sweetness. *Gymnema sylvestre* belongs to “Asclepiadaceae” family, *Laticiferous* “genus. *Gymnema sylvestre* is a slow growing, perennial medicinal woody climber found in central and peninsular India (Fig.1). It is a large or more pubescent shrub with young stems and branches, Leaves are opposite, 2.5-6 cm long, usually ovate or elliptical. Flowers are small, yellow, in umbellate cymes and follicles are terete, lanceolate, up to 3 inches in length (Kanetkar, P *et al.*, 2007).

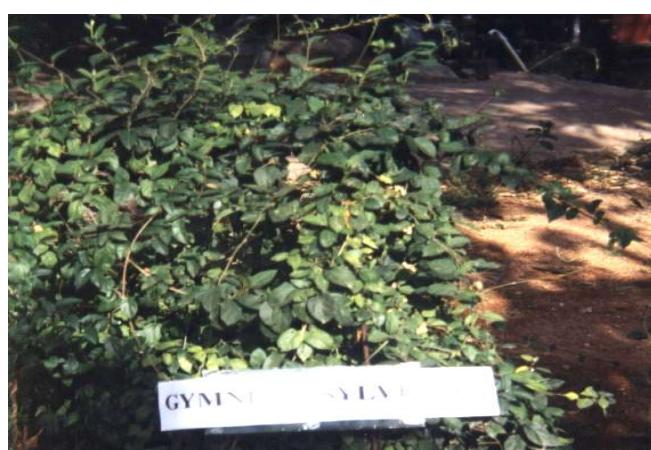


Figure 1. *Gymnema sylvestre*

G. sylvestre leaves contain triterpene saponins belonging to oleanane and dammarenane classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarenane saponins are

gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, I and J-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid,

butyric acid, lupeol, J-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives (Dateo G.P., and Long L 1973). Among these bioactive compounds of *Gymnema sylvestre*, gymnemic acids have anti-diabetic, anti-saccharine and anti-inflammatory activities (Liu, H.M, et al., 1992, Masayuki Y, et al., 1997). 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, thereby curbing the sugar craving. Similarly, Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the sugar molecules absorption by the intestine, which results in low blood sugar level (Sahu N et al, 1996). 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, there is stimulation of the pancreas by virtue of which there is an increase in insulin release (Persaud S.J., et al, 1999). These compounds have also been found to increase fecal excretion of Cholesterol (Nakamura Y et al., 1999), but further studies to prove clinical significance in treating hypercholesterolemia (high serum cholesterol) are required. Lot of work has been done on this plant (Sinsheimer J.E and Subbarao G, 1971, Sinsheimer J.E., et al., 1970, Yoshikawa K, et al., 1989) around the world particularly in India regarding to its anti-diabetic activity. However the present investigation is the first ever attempt for the isolation, purification and characterization of gymnemic acid from seventeen ecotypes of *Gymnema sylvestre* with the purpose to obtain its maximum yield using various techniques.

2. MATERIALS AND METHODS

Seventeen ecotypes of *Gymnema sylvestre* R.Br. (Asclepiadaceae) were collected from various parts of the country and maintained in G.M.R. Research foundation for further studies. The plant material was properly identified and confirmed with help of

2.1. PROCESSING OF PLANT MATERIAL

About 3 kg cleaned leaves from each ecotype dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. The dried power material was subjected to soxhelt extraction with petroleum ether, chloroform, methanol for continuous hot extraction.

2.1.1. EXTRACTION OF GYMNEMIC ACID BY HOOPERS'S METHOD

Step1: Extraction with petroleum ether

1 kg of dry leaf powder was packed into a clean soxhlet extraction unit. Seven liters of petroleum

various floras (Gamble, 1991; Matthew, 1991). All the chemicals and reagents were used analytical grade purchased from Sigma Chemical Co. and Merck.

ether (60-80⁰C) was added and extracted for 24-36 hours till all the components are soluble in petroleum. Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 250 gm of petroleum ether extracts was obtained. Petroleum ether extraction was used for defatting dried leaf power.

Step2: Extraction with 90% methanol

The plant material is then extracted with 90% methanol. 90% methanol was added and the extraction was carried out for 24-36 hours till the total methanol soluble extract was obtained. The methanol soluble extract was distilled and finally 175gm of the thick paste were obtained.

Step3: Isolation of pure gymnemic acid from methanol extract 175gm thick paste of methanol soluble extract was dissolved in 1% aqueous KOH solution on continuously stirring for 45min to 1 hour. The solution is then filtered through filter paper to separate the un-dissolved particles. Diluted HCl was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained.

2.2. VARIOUS COLOR TESTS TO CONFIRM THE GYMNEMIC ACID

Gymnemic acid gave positive test for phenolics, steroids and glycoside Phenolic test: A pinch of gymnemic acid was taken into a clean test tube and dissolved 2ml of methanol. Then a few drops of 1% alcoholic ferric chloride were added.

Steroid test: A pinch of gymnemic acid was added to a solution of 2ml CHCl₃ and 1ml of acetic anhydride. A few drops of Conc. H₂SO₄ were added from the sides of the tubes.

Glycoside test: A pinch of gymnemci acid was taken in a dried test tube and dissolved in 2ml of methanol. 1ml of alpha naphtholalcoholic solution was added from the sides of the test tube.

2.3. THIN LAYER CHROMATOGRAPHY (TLC)

3. RESULTS AND DISCUSSION

The detailed and systematic pharmacognostical evaluation would give valuable information for the future studies. The work carried out on this plant was mainly on the methods of extraction of gymnemic acid in order to obtain its higher yields, separation, identification and purification of the gymnemic acid by TLC and HPTLC. The extractions were carried out with different solvent

The identification and separation of the components present in different extracts of *Gymnema sylvestre* was carried out by Thin Layer Chromatography. The TLC of gymnemic acid was performed using different solvent systems i.e., Chloroform:Aceton, Chloroform:Methanol, Toulene: Ethyl acetate: Diethylamine, Ethyl acetate:Petroleum ether. The chromatograms were dried to remove the solvent, cooled and sprayed with the detecting reagents. The plates were dried at 105°C for 5 minutes to enable the full color of the spots to develop.

2.4. HPTLC Screening of *Gymnema sylvestre*

High Performance Thin Layer Chromatography is a planar Chromatography where the separation of the sample components is achieved on high performance layers with detection and acquisition using an advanced workstation. CAMAG HPTLC System, equipped with a Linomat IV sample applicator, a twin chamber tank, a model III Thin Layer Chromatography (TLC)Scanner and wincats software (1.21version) was used in the study. TLC Aluminum sheets (20 x 10 cms) of silica gel GF254 were used. A 1 μ g/ μ l stock solution of gymnemic acid, reference standard of 92% purity was prepared in methanol and 20 μ l were applied to the TLC plate. 20 μ l extract of each sample was applied to TLC plate. Three identical plates were prepared for concurrent results. The plates were developed up to 80nm under chamber saturation conditions. After air drying the solvent, the plates were scanned using scanner III at 290 nm wavelength in absorbance mode (D2 and W lamp).

systems like petroleum ether, benzene and methanol and were extracted under continuous hot extraction in Soxhlet apparatus. All the three solvents tested, the extraction with 90% methanol gave the maximum yield of gymnemic acid. The yields of gymnemic acid from seventeen ecotypes were calculated and presented in Table. 1.

Table 1. Acquisition of 17 ecotypes of *Gymnema sylvestre* from different parts of the country and the percentage of gymnemic acid

S.No	Name of the ecotype	Place of collection	% of Gymnemic acid
1	Panchagani	Pune-Westernghats	31.39
2	Kandala	Pune-Westernghats	35.4
3	Ambavale	Pune-Westernghats	38.34
4	Housaryghat – I	Pune-Westernghats	26.35
5	Housaryghat – II	Pune-Westernghats	23.68
6	Bhubaneswar	Bhubaneswar-Orissa	26.4
7	Warangal – I	Warangal	42
8	Warangal – II	Warangal	38.55
9	Mulugu – I	Mulugu village	40.4
10	Mulugu – II	Mulugu villages	39.33
11	Mulugu – III	Mulugu village	36.8
12	Rajahmundry – I	Rajahmundry	33.4
13	Rajahmundry – II	Rajahmundry	32
14	Osmania University – I	OU, Hyderabad	28.4
15	Osmania University – II	OU, Hyderabad	26.55
16	O.U – Bridge	OU, Hyderabad	23
17	O.U – Botanical garden	OU, Hyderabad	31.39

The results obtained on conducting the phenolic test a dark blue color was developed which is the positive test indicating the presence of OH group in the molecule. A pink/red color ring was formed when few drops of Conc. H₂SO₄ were added from the sides of the tube containing a pinch of gymnemic acid in a solution of 2ml CHCl₃. This is the positive test for steroids presence in the gymnemic acid. The glycosidic nature of gymnemic acid was a disputed question when it was first isolated. Hooper (1987) isolated it and proved it to be a glycoside. To confirm the glycosidic nature in the present study, a small pinch of gymnemic acid was taken in a dried test tube and dissolved in 2ml of methanol. 1ml of alpha naphtholalcoholic solution

was added from the sides of the test tube. A bluish red ring was developed at the junction of the two layers indicating the presence of glycoside.

Thin layer Chromatography studies were carried with different solvent systems i.e., Chloroform : Acetone, Chloroform : Methanol, Toulene : Ethyl acetate : Diethylamine and Ethyl acetate : Petroleum ether. All the samples of gymnemic acid gave 9 spots with different R_f values (Table. 2). The solvent system Chloroform : Methanol (6 : 5) gave better results when compared with the other solvent systems. TLC studies revealed that the profiles are similar when compared with the standard gymnemic acid having R_f 0.71.

Table 2. Different R_f values of Gymnemic acid

S.No	Name of the ecotype	R _f value
1	Panchagani	0.77
2	Kandala	0.79
3	Ambavale	0.67

4	Housaryghat – I	0.74
5	Housaryghat – II	0.66
6	Bhubaneswar	0.70
7	Warangal – I	0.79
8	Warangal – II	0.80
9	Mulugu – I	0.82
10	Mulugu – II	0.78
11	Mulugu – III	0.76
12	Rajahmundry – I	0.72
13	Rajahmundry – II	0.75
14	Osmania University – I	0.74
15	Osmania University – II	0.80
16	O.U – Bridge	0.68
17	O.U – Botanical garden	0.66

HPTLC has been used previously for the quantification of the chemical constituents of *Gymnema sylvestre*, viz., gymnestrogenin and gymnemagenin (Puratchimani and Jha, 2004a and 2004b). In the proposed study the separation of components is achieved on High Performance Thin Layer Chromatography with detection and acquisition giving sharper and more compact bands

with shorter distances of migration. Three ecotypes showed highest percentage of gymnemic acid, Warangal – I had high gymnemic acid percentage of 42% (Figure 2), Mulugu – I had 40.4% (Figure 2a) and mulugu – II had 34.33% (Figure 2b). HPTLC studies with all the ecotypes have shown that gymnemic acid varies from 23-42%.

FV

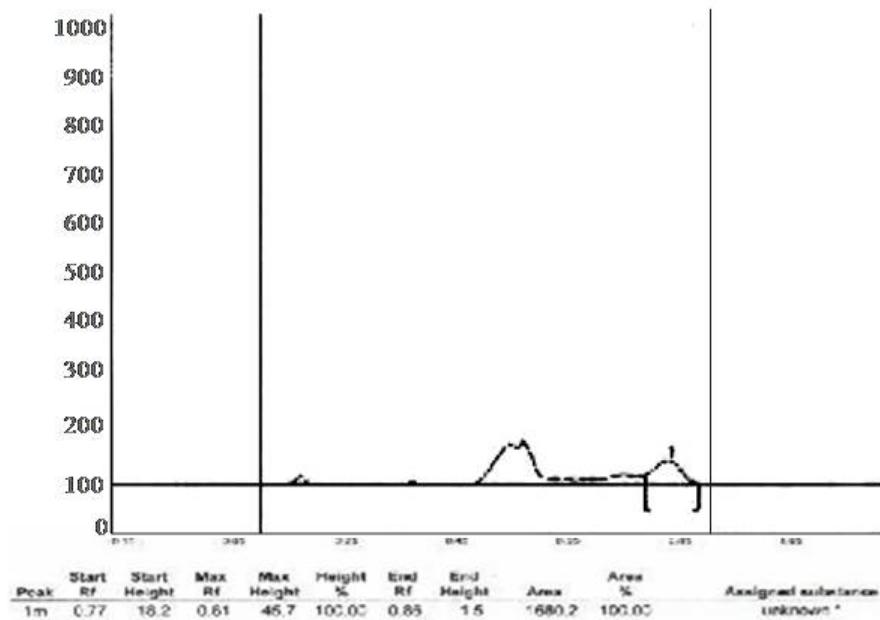


Figure 2. HPTLC chromatograms of gymnemic acid, the sample Warangal – I showed high gymnemic acid content of 42%

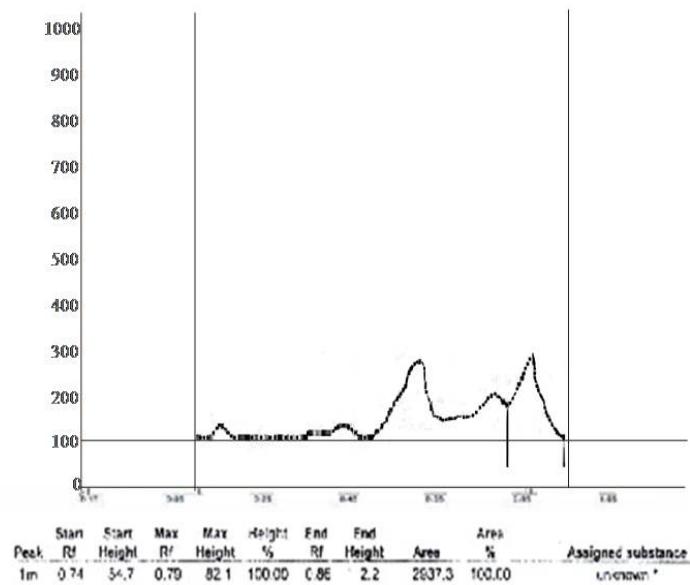


Figure 2a. HPTLC chromatogram of sample Mulugu – I had 40% gymnemic acid

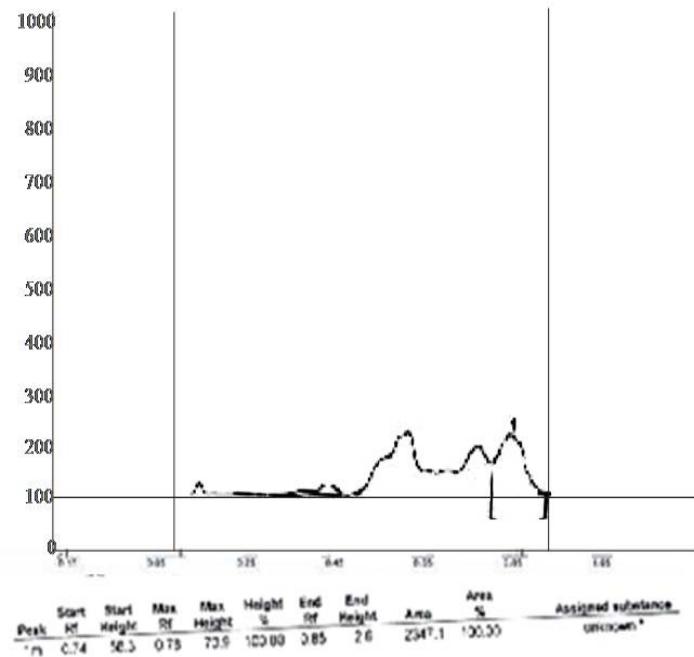


Figure 2b. HPTLC chromatogram of sample Mulugu – II had 34.33% gymnemic acid

4. CONCLUSION

On the basis of the results of the present study, it was concluded that the extraction with 90% methanol under continuous hot extraction in Soxhlet apparatus gave the maximum yield of gymnemic acid. The gymnemic acid thus obtained can be further identified, purified and characterized using TLC, and HPTLC techniques. HPTLC method is

found to be accurate, precise, and less time consuming and hence, it can be used for analysis of gymnemic acid and for standardization of herbal drugs in general laboratory conditions. These parameters could be useful in preparation of Herbal drugs.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the authorities of the project "Molecular and chemical

characterization of potential anti-diabetic plants" funded by DBT for providing experimental materials and necessary facilities to conduct the present study.

REFERENCES

1. Bishayee, A. and Chatterjee, M., 1994. Hypolipidaemic and antiatherosclerotic effects of oral *Gymnema sylvestre* R. Br. Leaf extract in albino rats fed on a high fat diet. *Phytothera. Res.*, 1994, 8, 118-120.
2. Dateo G.P., Long L. Gymnemic acid, the antisaccharine principle of *Gymnema sylvestre*. Studies on isolation and heterogeneity of gymnemic acid A1. *J. Agric. Food Chem.* 1973;21:899–903.
3. Edward, P.C. 1956. Leaf and Febiger Philadelphia, 731.
4. Gamble J.S. 1991. Flora of the Presidency of Madras. Reprinted Edition, Vol I – III, Bishen singh Mahendra Pal Singh, Dehra Dun.
5. Hooper, D. (1887). Isolation and antiviral activity of gymnemic acid. *Pharm. J. Trans.* 17:867-868.
7. Kalidass, C., Shanmugasundaram, R. and Mohan, V.R. 2009a. Pharmacognostic studies of *Capparis separia* (L.) R.Br. *Pharmacognosy Journal.* 1(2): 121 - 125.
8. Kalidass, C., Muthukumar, K., Mohan, V.R. and Manickam, V.S. 2009b. Ethnoveterinary medicinal uses of plants from Agasthiamalai Biosphere Reserve (KMTR), Tirunelveli Tamil Nadu, India. *My Forest*, 45(1): 7 – 14
9. Kapoor, L.D. 1990. *CRC Handbook of Ayurvedic medicinal plants*: Boca Raton, Fl. pp: 200 - 201.
10. Kanetkar, P.; Singhal, R.; Kamat, M. (2007). *Gymnema sylvestre*: A Memoir. *J. Clin. Biochem. Nutr.* 41(2): 77-81.
11. Komalavalli, N.; Rao, M.V. (2000). *In vitro* micrpropagation of *Gymnema sylvestre*: multipurpose medicinal plant. *Cell tissue organ culture.* 61:97-105.
12. Kurihara, Y. 1992. Characteristics of anti-sweet substances, sweet proteins and sweetness inducing protein. *Crit. Rev. Food Sci. Nutr.*, 32: 231-252.
13. Liu, H.M.; Kiuchi, F.; Tsuda, Y. (1992). Isolation and structure elucidation of Gymnemic acids,
14. Anti-sweet principles of *Gymnema sylvestre*. *Chem. Pharm. Bull.* 40:1366-1375.
15. Masayuki, Y.; Toshiyuki, M.; Hisashi, M. (1997). Structures of new triterpene glycosides, gymnemasides C, D, E and F from the leaves of *Gymnema sylvestre* R Br.: Influence of Gymnema glycosides on glucose uptake in rat small intestinal fragments. *Chem. Pharm. Bull.* 45:2034-2038.
16. Matthew, K.M. 1991. *An Excursion Flora of Central Tamil Nadu, India*. Oxford and IBH

17. Publishing Co., New Delhi.
18. Nadkarni, A.K.; Nadkarni, K.M. (1976). *Indian Materia Medica*, Vol.1. Popular Prakashan: Bombay, pp.569.
19. Nakamura Y., Tsumura Y., Tonogai Y., Shibata T. Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in *Gymnema sylvestre* leaves. *J. Nutr.* 1999; 129:1214–1222
20. Ozarkar, K.R. 2005. Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* L. and *Valeriana wallichii* DC. Using mouse model, *Ph.D.* thesis, University of Mumbai, Mumbai.
21. Persaud S.J., Al-Majed H., Raman A., Jones P.M. *Gymnema sylvestre* stimulates insulin release in vitro by increased membrane permeability. *J. Endocrinol.* 1999;163:207–212.
22. Puratchimani V and Jha S (2004a), Standardization of *Gymnema sylvestre* R. Br. With reference to gymnemagin by High-Performance Thin Layer Chromatography, *Phytochem. Anal.*, Vol. 15, No. 3, pp. 164-166
23. Puratchimani V and Jha S (2004b), HPTLC Standardization of *Gymnema sylvestre* R. Br. Using gymnestrogenin as reference, *Ind. J. Pharma. Sci.*, Vol. 66, pp. 164-166
24. Sahu N., Mahato S.B., Sarkar S.K., Poddar G. Triterpenoid Saponins from *Gymnema sylvestre*. *Phytochem.* 1996;41:1181–1185.
25. Sative, R.K., Abhilash, P. and Fulzele, D.D.2003. Antimicrobial activity of *Gymnema sylvestre* leaf extract. *Fitoterapia*. 74: 699-701.
26. Sastri, B. N.: The Wealth of India: Raw materials. Vol IV, CSIR, Delhi, pp 275 (1956).
27. Shanmugasundaram, K.R, Panneerselvam, C., Samudram, P. and Shanmugasundaram, E.R. 1983. Enzyme changes and glucose utilisation in diabetic rabbits: the effect of *Gymnema sylvestre*, R.Br. *J. Ethnopharmacol.*,7:205-234
28. Sinsheimer J.E., Subbarao G. Constituents from *Gymnema sylvestre* leaves VIII: Isolation, chemistry and derivatives of gymnemagenin and gymnestrogenin. *J. Pharm. Sci.* 1971;60:190–193.
29. Sinsheimer J.E., Subba R.G., Mc Ilhenny H.M. Constituents from *Gymnema sylvestre* Leaves V: Isolation and preliminary characterization of Gymnemic acids. *J. Pharm. Sci.* 1970;59:622–628.
30. Yoshikawa K., Amimoto K., Arihara S., Matsuura K. Structure studies of new antisweet constituents from *Gymnema sylvestre*. *Tetr. Lett.* 1989;30:1103–1106.