



<https://ijrps.com>

ISSN: 0975-7538

Review Article

Review article on HPLC-RP HPLC technique on the development and validation of *Gymnema sylvestrate*

Swetha M*, Saritha N, Devanna N

JNTUACEK, Kalikiri, Chittoor district, Andhra Pradesh, India

ABSTRACT

A new, simple, fast, precise and efficient reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the validation of herbal drugs. *Gymnema sylvestrate* is a medicinal plants. The objective of the present *Gymnema sylvestrate* investigation was to isolate and characterize the Gymnemic acid, from marker compound of leaf powder with different solvent systems like petroleum ether, benzene, and methanol. The leaf powder 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 Tirupathi. Gymnemic acid was purified by preparative chromatographic methods i.e., RP-HPLC and HPLC. The analysis by HPLC showed 30% purity of Gymnemic acid

Keywords: *Gymnema sylvestrate*; Gymnemic acid; HPLC; RP-HPLC; method development; validation.

INTRODUCTION

Gymnema sylvestrate R. Br. is a medicinal plant belonging to the family Asclepiadaceae. *G. Sylvestrate* is regarded as one of the plants with potent antidiabetic properties. It has been used as a natural treatment for diabetes for nearly two millennia and commonly known as gudmar. The plant has been reported to possess antimicrobial, anti eurodonic and antiviral effects. It is also used for controlling obesity in the form of *Gymnema* tea. The active compound of the plant is a group of acids termed as gymnemic acid. Secondary metabolites like alkaloids, terpenoids phenolic, steroids and flavonoids play an important role in interaction of the plant with its environment. The amount and type of phytochemical compounds vary from plant to plant. In *Gymnema* species a number of phytochemical constituents have been reported. Phytochemicals were exhibited possess hypocholesterolemic and antidiabetic properties. The terpenoids saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects. Saponins have also been shown to decrease blood sugar level in animal studies. The steroids and saponins are responsible for central nervous system activities. More recently, drug discovery techniques have been applied to the standardization of herbal medicines to elucidate analytical marker compounds.

The plants of *G. sylvestrate* R.Br. (Asclepiadaceae) were collected from commercial nursery in Pune, India. Leaves were obtained from well grown healthy plants. Shade dried leaves were Powdered using mixer grinder. Filtered leaf extracts were concentrated and were subjected to Qualitative tests for the identification of various phytochemical constituents like alkaloids, Flavonoids, saponins, cardiac glycosides, sterols, steroids and anthraquinone glycosides as per the standard protocol. Methanolic and aqueous extracts of the leaves were subjected to Phytochemical analysis. Separation of the secondary metabolites such as alkaloids, flavonoids, Saponins, cardiac glycosides and sterols from the leaves of *G. sylvestrate* was carried out by thin layer chromatography. For antimicrobial studies extraction of plant material was prepared by cold percolation method. The air-dried and powered plant material, 5 g of each, was soaked in 50 ml methanol and kept for 48 hrs. with intermittent shaking. Filtered and dried plant extracts were dissolved in 1 ml dimethyl sulfoxide (DMSO) and 50ml and 100ml of each sample was taken for experiment.



Figure 1: *Gymnema sylvestrate* R. Br

* Corresponding Author

Email: swetha.shivaprasad@yahoo.com

Contact: +91-

Received on: 15-01-2016

Revised on: 05-04-2016

Accepted on: 09-02-2016

Various color tests to confirm the gymnemic acid

Gymnemic acid gave positive test for phenolic, 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 CHCl_3 and 1ml of acetic Anhydride. A few drops of Conc. H_2SO_4 were added from the sides of the tubes.

Glycoside test: A 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.

HPLC chromatography analysis: HPLC was applied for testing the presence of number of organic compounds available in Water extract of *Gymnema Sylvestrae* and this water extract active constituents were Isolated of Butanol from Water extract. One of the major organic components with 100 % and 4.428 retention time had been detected.

Antimicrobial Screening

Antimicrobial activities of the extracts were determined by the disk diffusion method as described by Taylor, R.S.L., 1995. Each purified extract and gymnemic acid were dissolved in DMSO with (100 $\mu\text{g}/\text{ml}$), sterilized by filtration using a sintered glass filter, and stored at 4°C. PDA and NAM plates were inoculated with each fungal and bacterial culture by pouring plate method. The filter paper discs (5mm in diameter) impregnated with concentrations of the extracts was placed on test organism seeded plates. DMSO was used to dissolve the extract and was completely evaporated.

Antibacterial activity

The antibacterial test was performed using the agar well diffusion method [12]. The test organisms were inoculated on nutrient agar plates and spread uniformly with the help of sterile glass spreader. On the nutrient agar wells of 5mm diameter were made using a sterile cork borer. The cut agar was carefully removed by the use of sterile forceps. To each well different concentration of plant extracts were added. Control experiment with DMSO was done on the same agar plate. The petri plates were incubated overnight at 37°C. The antibacterial spectrum of the extract was determined in terms of Zone sizes (inhibition zone diameters) around each well. Pure solvents were used as control for each Bacterial strain. The experiment was repeated thrice and the average values were recorded for Antibacterial activity.

RESULTS AND DISCUSSION

The results obtained in the present study relieved that the tested *Gymnema Sylvestre* leaf extract from differ-

ent solvent and gymnemic acid possesses potent antimicrobial activity against on different solvent (Nonpolar polar) extract dissolve in DMSO.

Antimicrobial activity was observed by filter Paper Disc Diffusion method and compare to fluoro quinoloneciprofloxacin as a Standard Drug as a control sample. Remarkable antimicrobial activities were recorded with *S. aureus* (9.25mm) on gymnemic acid and on aqueous extract (8.50mm) (Plate. G), it shows good result compared to standard drug. On the other side aqueous extract present maximum zone of inhibition on *E. coli* (9.00mm) (Plate. H) and *C. albicans* (8.76mm) (Plate. I) showed comparatively best inhibition than standard drug and gymnemic acid (8.65mm and 8.60mm), (Plate B and C).

As per the present investigation petroleum benzene extract showed significantly negative result on all microorganisms. On the other hand ethanol leaf extract shows positive result in comparing to petroleum benzene extract against organisms. Comparatively the aqueous leaf extract showed significantly maximum zone of inhibition result against both types of pathogen fungus species than gymnemic acid and sometimes as a control drug but gymnemic acid present large zone of inhibition on bacterial strains.

Same as hydro alcohol large scale isolation of secondary metabolites from *G. sylvestre* can be predicted to remain an essential component in the search for new secondary metabolites and its pharmacological activities. Its leaf extracts exhibit broad spectra of antimicrobial activity. However, further studies are needed to isolate and identify of compounds responsible for antimicrobial activity.

The result of present investigation clearly indicates the antifungal activity, antimicrobial activity of leaves and ascertains the value of this plant used in Ayurveda, which could be of considerable interest to the development of new drugs. The result of this study supports the use of plants as therapeutic agents for the treatment of several diseases caused by the pathogenic bacterial and fungal populations.

CONCLUSION

The revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine and the herbal drug industries is now a very fast growing sector in the international market. But sometimes, lack of scientific input in herbal drugs leads to ineffectiveness of the same. With these objectives, we identified the efficient extraction technique, stability assessment, optimum storage conditions and changes occurring during annual cycle to get a high yield of marker compound and maintain the quality assurance of herbal drugs that would open floodgate for the development of market potential all around the world.

REFERENCES

- An easy and simple method of isolation and purification of genomic dna from the leaves of *Gymnema sylvestre*-An anti-diabetic plant* Vol 2/Issue 1/Jan-Mar 2012
- Antimicrobial activity of Gymnemic acid on pathogens - *Gymnema sylvestrae* (2014) 3(5): 40-45
- B. N.: Sastri, The Wealth Of India: Raw Materials. Vol Iv, Csir, Delhi, Pp 27 (1956).
- Bhuva neswari CH, Kiranmayee Rao, Archana Giri Evaluation of *Gymnema sylvestre* Antimicrobial Activity in Methanol 2011, 3(8): 73-75
- Cohen J.H. Et Al "Fruits And Vegetable Intakes And Prostate Cancer Risk" Journal Of National Cancer Institute, 92, 2002, 61-68.
- D. Ayfer Atefi1, Özlem Turgay Erdo.Rul2, Antimicrobial Activities Of Various Medicinal And Commercial Plants Extracts, Turk J Boil, 2003, 27,
- Dr. Shobha S. Borhade*, Water Extract Of *Gymnema Sylvestre* Analytical Study By Hplc And Its Antibacterial Activity Of Various Extracts. Volume 8, Issue 1, May – June 2011;
- Figgin Nt, Cayci Y.T., Coban A.Y., Ozatli D, Tanyel E, Durupinar B, Tulek N, Antimicrobial Activity Of Plant Extract Ankaferd Blood Stopper, Fitoterapia 80, 2009, 48–50.
- Isolation And Characterization Of Gymnemic Acid From *Gymnema Sylvestre* R.Br. In Control Of Diabetes.
- Lawless, *The Illustrated Encyclopedia Of Essential Oils*, The Bridgewater Book Company Ltd., Shaftesbury 1999, Pp. 44–45.
- Pascual C. Gonzalez R. Torricella R.G. Scavenging action Of Propolis Extract Against Oxygen Radicals. J.Ethnopharmacol, 41, 1994, 9-13
- Puratchimani V And Jha S (2004a),
- Qualitative phytochemical analysis and antimicrobial activity studies of *Gymnema sylvestre* 2012, 1(1):121-124
- R. Balamurali Krishna, Sujitha R. Reddy Reddy, Harika, Javangula, D. Swapna, K. Jagadeeswara Reddy.
- R. Balamurali Krishna1, Sujitha R. Reddy Reddy2, Harika Javangula3, D. Swapna4, K. Jagadeeswara Reddy5
- R. Br. Minal Wani, Faizan Ali Sarvar, Juhi Agrawal, Jyoti Deshpande, Siji Mathew, Madhukar Khetmalas Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune.
- R.Br. Pratibha Gupta and Pratibha Singh Original Research Article
- S.P. Kinjawadkar, WHO Guidelines For Promoting Traditional Medicines. J. Nima 44(10), 2002, P-3.
- Sinsheimer J.E., Subba R.G., Mc Ilhennyh.M. Constituents From *Gymnema Sylvestre* leaves V: Isolation And Preliminary characterization Of Gymnemic Acids. J. Pharm.Sci. 1970;59:622–628.
- 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
- Vol 2/Issue 1/Jan-Mar 2012
- Yoshikawa K., Amimoto K., Arihara S., Matsuura K. Structure Studies Of New Antisweet Constituents From *Gymnema Sylvestrae*. Tetr. Lett. 1989;30: 1103–1106