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ISOLATION AND CHARACTERIZATION OF GYMNEMIC ACID FROM *GYMNEMA SYLVESTRE* R. Br

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ABSTRACT

Gymnema sylvestre, a well-known medicinal plant belongs to family Asclepiadaceae is under threat. The plant is used in rural areas for the treatment of several ailments and also in the preparation of herbal drugs by pharmaceutical industries. Major bioactive constituents of *G. sylvestre* are a group of oleanane type triterpenoid saponins known as Gymnemic acids. Although, the composition of active bioactive compounds varies from region to region, the plant is used abundantly without looking at location of the plant. Therefore, to know the content of Gymnemic acid at Gulbarga region, the present study was carried out. Gymnemic acid was estimated using standard method**.** The leaves were extracted under continuous hot extraction in Soxhlet apparatus with 90% methanol and recorded yield of Gymnemic acid (0.17g). Gymnemic acid was purified by preparative chromatographic methods i.e. TLC and UV analysis, The presence of Gymnemic acid was further confirmed by various color tests.

KEYWORDS: Gymnema sylvestre, Gymnemic acid, Extraction, TLC.

INTRODUCTION

Nature has blessed India with immense variety of medicinal plants that grow in its different climate and region. Humans have used plant parts, products and metabolites since early historical times. Plants are the chemical factories of nature, producing many chemicals and bioactive compounds these are very use full Plant products.

Gymnema Sylvestre R. Br. is a valuable herb belonging to the family Asclepiadaceae, widely distributed in India, Malaysia, Srilanka, Australia, Indonesia, Japan, Vietnam, tropical Africa and the south-western region of China. The plant is commonly known as Periploca of the woods (English); Gurmar (Hindi); Meshashringi, madhunashini(Sanskrit); Kavali, kalikardori (Marathi); Dhuleti, mardashingi (Gujrathi); Adigam, cherukurinja (Tamil); Podapatri (Telgu) and Sannagerasehambu (Kannada).[1-4] The word "Gymnema" is derived from a Hindu word "Gurmar" meaning "destroyer of sugar" and it is believed that it might neutralize the excess of sugar present in the body in Diabetes mellitus.[5]

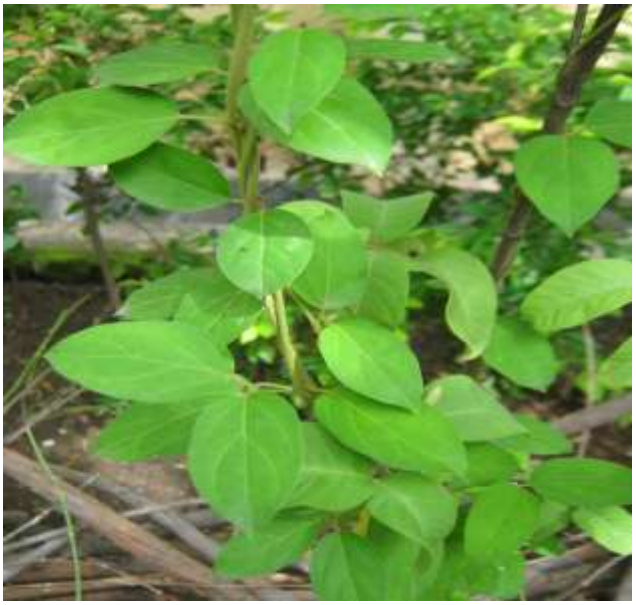

Fig. 1: *Gymnema sylvestre* plant.

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[6], anti-sweetner[7] and antihypercholesterolemia.[8] It also has stomachic, diuretic and cough suppressant property.[9,10] The plant has been reported to possess antimicrobial (11) and ethnoveterinary medicinal properties.[12] In addition, it possesses hepatoprotective, and anti-saccharine activities.[13,14] Hence, *Gymnema sylvestre* is an important plant for prospecting (fig. 1).

Lot of work has been done on this plant[15,16,17] around the world particularly in India on its anti-diabetic activity. As the secondary metabolite contents varies with region to region and no such work is carried out from Gulbarga region, the present work carried out to determine the Gymnemic acid content.

MATERIALS AND METHODS

G.sylvestre collected from Gulbarga district, Karnataka in July, 2014 and was authenticated with the help of flora of Gulbarga district.[18] A specimen is deposited in the herbarium, Dept of Botany, Gulbarga university kalaburagi (Voucher speciemen No HGUG-58).

Processing of Plant Material

About 3 Kg cleaned leaves dried under shade, powdered and stored in closed vessel for further use. The dried power material was subjected to Soxhelt extraction with petroleum ether, chloroform and methanol for continuous hot extraction.

EXTRACTION OF GYMNEMIC ACID BY HOOPERS'S METHOD

Extraction with petroleum ether

500g of dry leaf powder was packed into a clean Soxhlet extraction unit. Petroleum ether (60-80^0 C) was added and extracted for 24-36 h till all the components are dissolved in petroleum ether. Petroleum ether extract was collected and after distillation, the extracts was collected.

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 h till the total methanol dissolved. After distillation, the extract was collected.

Isolation of crude gymnemic acid

Thick paste of methanol crude extract was dissolved in 1% aqueous KOH solution with continuous stirring for 45 min to 1 h. The extract 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 was precipitated. Precipitated solution was filtered and dried at room temperature. The crude gymnemic acid was obtained.

Various Color Tests to Confirm The Gymnemic Acid

Gymnemic acid gave positive test for phenolics, steroids and glycoside.

Phenolic test: A pinch of crude gymnemic acid was taken into a clean test tube and dissolved in 2ml of methanol. Then a few drops of 1% alcoholic ferric chloride was added,a dark blue color appears it shows the positive result for Phenolic test.

Steroid test: A pinch of crude 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. A pink/red color ring is formed it shows the positive test for steroids presence in the gymnemic acid

Glycoside test: A pinch of crude gymnemci acid was taken in a dried test tube and dissolved in 2ml of methanol. 1ml of alpha naptholalcoholic solution was added from the sides of the test tube. A bluish red ring is developed at the junction of the two layers indicating the presence of glycoside.

THIN LAYER CHROMATOGRAPHY (TLC)

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 105^0C for 5 min to enable the full color of the spots to develop.

UV Analysis

After chromatography, the area with silica gel on the plates containing putative gymnemic acid was scrapped at the appropriate relative front (Rf) and eluted with methanol. The purified gymnemic acid was analyzed by UV spectrometer, between 200 and 300 nm.

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 to know the content of Gymnemic acid at Gulbarga region. The extractions were carried out with different solvent systems like, petroleum ether, chloroform and methanol and were extracted under continuous hot extraction using Soxhlet apparatus. Among the solvents tested, methanol at 90% recorded yield of Gymnemic acid (0.17g).

Color Tests To Confirm The Gymnemic Acid

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[19], later it was proved 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 naptholalcoholic 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 (TLC)

Thin layer Chromatography studies were carried out with different solvent systems i.e., Chloroform: Acetone, Chloroform: Methanol, Toulene: Ethyl acetate: Diethylamine and Ethyl acetate: Petroleum ether. The gymnemic acid shown bands with Rf value. The solvent system Chloroform: Methanol (6: 5) given better results when compared with the other solvent systems. TLC studies revealed that the profiles are similar when compared to the standard gymnemic acid with Rf value 0.71([20], Fig 2).

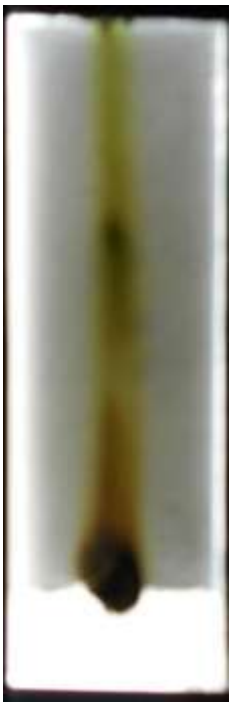

Fig. 2: Thin layer Chromatography.

UV Analysis

The crude gymnemic acid extracted from *G. sylvestre* showed 0.17g content and recorded UV spectrum value similar to standard value(276.6 nm, 21)(Fig.3).

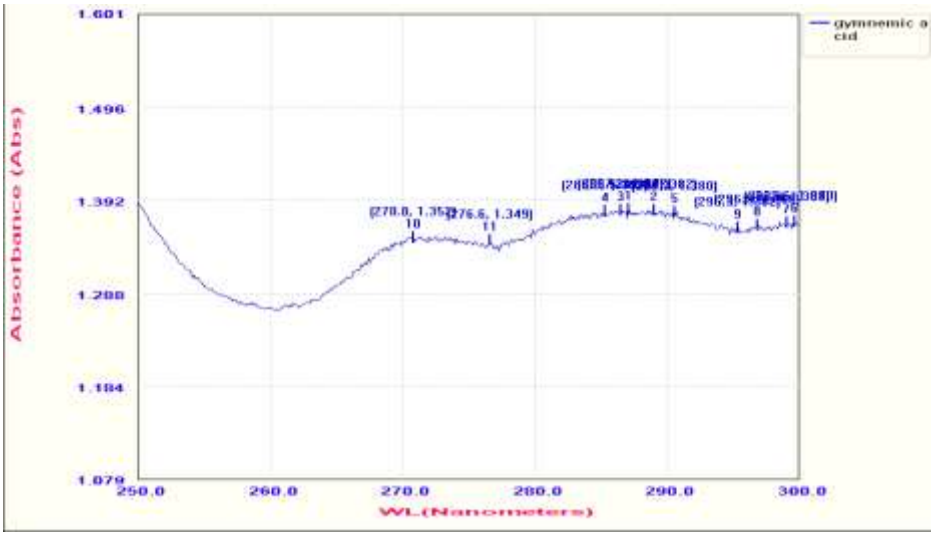

Fig. 3: UV Analysis.

CONCLUSION

On the basis of the results the present study, it is concluded that the extraction with 90% methanol under continuous hot extraction using Soxhlet apparatus yields gymnemic acid. The presence of Gymnemic acid was further confirmed by various color tests. The gymnemic acid thus obtained can be further characterized using TLC and UV analysis. The method used 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.

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