

ISOLATION AND CHARACTERIZATION OF GYMNEMIC ACID FROM GURMAR (*Gymnema sylvestre* R. BR.) FOR CONTROL OF DIABETES IN JHARKHAND

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ABSTRACT

Gymnema sylvestre R. Br. (Asclepiadaceae) is selected for the present study because of its medicinal values. It is used in traditional systems of medicine as stomachic, diuretic and as a remedy to control diabetes. Diabetes is one of the oldest wide spread diseases of the world which develops due to lack of insulin utilization or production. It is a gene-controlled disorder which is also regulated by other environmental factors like food habits, life style, age etc. In this context, plant-based therapeutics is 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 five 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%) in the ecotype collected from Ranchi. Gymnemic acid was purified by preparative chromatographic methods i.e., TLC

INTRODUCTION

The use of plants as source of remedies for the

treatment of many diseases dated back to prehistory and people of all continents have

this old tradition. Nowadays, medicinal plants receive attention of research centers because of their special importance in safety of communities. Plants have potent biochemical and have components of phyto-medicine. *Gymnema sylvestre* is an important medicinal plant native to Deccan Peninsula, and also found in different parts of Chhotanagpur plateau region. *Gymnema sylvestre* R. Br. (Asclepiadaceae) is commonly known as “Gurmar”, the destroyer of sugar. Leaves of this plant have been used for centuries in the traditional Indian system of Ayurvedic medicine. The term "destroyer of sugar"¹ is traditionally used for *Gymnema* because chewing the leaves abolish the taste of sweetness. The medicinally active parts of the plant are the leaves and the roots. Recent clinical trials conducted in India have shown that an extract of *Gymnema sylvestre* is useful for controlling Blood Sugar. Today, *Gymnema sylvestre* has become increasingly popular in the United States as a supportive treatment for diabetes. Use of gymnema was well-known to the Indian people since ancient days (“Meshashring”) as a source of anti diabetic drugs. In recent years, it became one of the better known names in the world of herbal medicine. It is rich source of many bioactive

compounds such as gymnemic acid (GA-I-X), quercitol, lupeol, stigmasterol, gymnemin, gymnemagenin, gurmarin, etc. which are mainly effective in lowering of blood sugar. 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 1994). It also has stomachic, diuretic and cough suppressant property (Kapoor 1990). The plant has been reported to possess antimicrobial (Sative *et al.* 2003) and ethno-veterinary medicinal properties (Kalidass *et al.* 2009). In addition, it possesses antimicrobial, hepatoprotective, and anti-saccharine activities (Komalavalli and Rao 2000, Nadkarni and Nadkarni 1976). Hence, because of these properties, *Gymnema sylvestre* is most important for plant prospecting. The leaves of gurmar are used medicinally, for its unique property to directly mask the tongue’s ability to taste sweet foods; at the same time suppresses glucose absorption from the intestine. This is the reason, it is known as "destroyer of sugar". The fresh leaves when chewed have the remarkable property of paralyzing the sense of taste of sweet substance for some time (Gent

1999, Persaud et al. 1999). 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). 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). These compounds have also been found to increase fecal excretion of Cholesterol (Nakamura et al. 1999), but further studies to prove clinical significance in treating hypercholesterolemia (high serum cholesterol) are required. However, the present investigation is the first ever attempt for the isolation, purification and characterization of gymnemic acid from five

ecotypes of *Gymnema sylvestre* with the purpose to obtain its maximum yield using various techniques.

MATERIALS AND METHODS

Five ecotypes of *Gymnema sylvestre* R. Br. (Asclepiadaceae) were collected from various parts of the Jharkhand and maintained in Jamshedpur Women's College, Jamshedpur Green house for further studies. The plant material was properly identified and confirmed with help of various floras. All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. and Merck.

PROCESSING OF PLANT MATERIAL

Leaves of each ecotype (*Gymnema sylvestre*) was washed thoroughly under running tap water, dried under shade and finally crushed to fine powder in mixture grinder and stored in closed vessel for further use. The dried powder of the leaves was allowed to Soxhlet for sequential extraction with petroleum ether, chloroform, and methanol. The liquid extract was collected, filtered and evaporated to find dry precipitate using Rotary evaporator.

EXTRACTION OF GYMNEMIC ACID BY HOOPERS'S METHOD

Step1: Extraction with petroleum ether

250gm of dry leaf powder was packed into a clean Soxhlet extraction unit. One liters of petroleum ether was added and extracted for 6-8 hours till all the components are dissolved in petroleum. Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 25 gm of petroleum ether extracts was obtained.

Step2: Extraction with 90% methanol

The plant material is then extracted with 90% methanol. The extraction was carried out for 6-8 hours till the total methanol soluble extract was obtained. The methanol soluble extract was distilled and finally 20 gm of the thick paste were obtained.

Step3: Isolation of pure gymnemic acid from methanol extract

20 gm thick paste of methanol soluble extract was dissolved in 1% aqueous KOH solution on continuously stirring for 30 min to 50 min. 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 to obtain pure gymnemic acid.

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 in 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. 1 ml of alpha naphtholalcoholic solution was added from the sides of the test tube.

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:Acetone, Chloroform:Methanol, Toulene:Ethylacetate: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.

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. The extractions were carried out with different solvent systems like petroleum ether, benzene and methanol and were extracted under continuous hot extraction in Soxhlet apparatus. Out of all the three solvents tested, the extraction with 90% methanol gave the maximum yield of gymnemic acid. The yields of gymnemic acid from five ecotypes were calculated and presented in Table 1.

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 2 ml CHCl₃.

Table 1. Acquisition of 5 ecotypes of *Gymnema sylvestre* from different parts of Jharkhand and the percentage of gymnemic acid

S.No	Name of the ecotype	Place of collection	% of Gymnemic acid
1	JRP	Palandu	42
2	JRM	Mandar	35.4
3	JRK	Kanke	23.68
4	JEG	Ghatshila	31.39
5	JEC	Chakulia	28.4

This is the positive test for steroids presence in the gymnemic acid. 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. 1 ml of alpha naphthol-alcoholic 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. 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 Rf 0.71

Table 2. Different Rf values of Gymnemic acid

S.No	Name of the ecotype	Rf value
1	JRP	0.79
2	JRM	0.77
3	JRK	0.66
4	JEG	0.72
5	JEC	0.74

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. 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.

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