



WATER EXTRACT OF *GYMNEMA SYLVESTRE* ANALYTICAL STUDY BY HPLC AND ITS ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS

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ABSTRACT

The purpose of this study was investigating experimentally the possible Antibacterial Activity of Water Extract and Active constituents were Isolated of Butanol from a *Gymnema Sylvestre* and Analytical Study by HPLC Method. The Antibacterial activity of the Water Extract of *Gymnema Sylvestre* was evaluated at two different concentrations by the diffusion method. The water Extract of the *Gymnema Sylvestre* shows antibacterial activity at varies levels in The *E. coli*, *S. aureus*, *B. cereus* Bacteria. The Bacteria *B. cereus* was found to be more active and *E. coli*, *S. aureus* was found to be less active in inhibition zone. Antibacterial activity of water extract of the plant was performed. Results exhibited that *Gymnema Sylvestre* contain good Antibacterial action and Active constituents were Isolated of Butanol.

Keywords: *Gymnema Sylvestre*, HPLC Chromatography, Analytical study, Antibacterial activity.

INTRODUCTION

Gymnema Sylvestre is placed important role in ancient therapy. Various parts of the plant used therapeutically are fruits, flowers, leaves, barks and root. Root is tonic styptic, galactogogue, aphrodisiac, cooling, useful in dysentery and as diuretic. Pounded roots are given in the treatment of Intestinal ulcer. Leaves, Flowers and Fruits are mucilaginous demulcent, astringent, and also used in gonorrhoea and against leprosy.¹⁻⁴

There are numerous Traditional Medicines are Complementary and Alternative Medicines (CAM) of Herbal Origin, used all over India. According to WHO survey up to 70% of the population of Developing Countries utilize TM/CAM for their primary health care needs, with advantages such as Affordability, Low technology solutions, Few reported adverse effects, Cultural acceptance and Availability.

These medicines are practiced in Rural and Adivasi Communities of Maharashtra for centuries together. These Medicines are free from side-effects and cheap compared to Allopathic Medicines. But still common people /educated people do not use them, as they are prepared and practiced by the Traditional Medical Practioners (Vaidyas, Babas and Maharajas), who are not trained by recognized medical Authorities and are not STANDARDIZED.

There are many Institutions abroad, taking keen interest in Herbal Medicines of India origin. If the Traditional Medicines are not properly taken care of, it is cock sure that these valuable medicines will vanish along with their practitioners and the foreign investigators will have the Patents for the same in near future. In order to get rid of above mentioned problems, the present work is undertaken. *Gymnema Sylvestre* plant is native of Western Ghats.

G. sylvestre is a slow growing, perennial, woody climber, distributed throughout the India, in dry forests upto 600 m height. It is mainly present in the tropical forest of Central and Southern India. It is also found in Banda, konkan, Western Ghats, Deccan extending to the parts of western and northern India⁶⁻⁸. The plant is a large, more or less pubescent, woody climber. The leaves are opposite, usually elliptic or ovate (1.25 – 2.0 inch x 0.5-1.25 inch).

Flowers are small, yellow, in axillary and lateral umbel in cymes; Follicles are terete and lanceolate upto 3 inches in length. The Calyx-lobes are long, ovate, obtuse and pubescent. Corolla is pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes; Anther connective produced into a memberanous tip, pollinia 2, erect, carpels 2, unilocular; locules many ovuled⁵⁻¹⁰.



Gymnema Sylvestre

The *Gymnema Sylvestre* for extractive values, ash values, P.H, refractive Index and separation of total extractive into acids and neutrals. The Antibacterial activity of water extract of the plant was performed. Results exhibited that

Gymnema Sylvestre contain good antibacterial action and Active constituents were Isolated of Butanol.

MATERIALS AND METHODS

Collection of plant material

Fresh green plant of *Gymnema Sylvestre*. were obtained from the plants grown in Sangamner College Dist. Ahmednagar, Maharashtra, India and the fresh green plant of *Triumfetta rhomboidea*. were obtained from the fields maintained by Department of Drug Chemistry S.M.B.S.T College Arts, Science and Commerce Sangamner Affiliated to university of Pune Sangamner Dist. Ahmednagar, Maharashtra, India Pin-422605 The plant were shade dried at room temperature and finely pulverized.

Preparation of extract

The powder prepared from shade dried plant was extracted directly with water using Soxhlet extractor as per the procedure standardized Methanol was used in the present study. The extracts were stored in desiccators until further use.

HPLC chromatography analysis

HPLC was applied for testing the presence of number of organic compounds available of Water extract of *Gymnema Sylvestre* 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 may have detected.

Method

Column : YMC ODS 150 mm × 4.6 mm × 5 μ, ID: E-AC-1/06/COL/013
 Mobile Phase:
 A: 0.05 % TFA in Water /B: 0.05 % TFA in Acetonitrile
 Inj. Vol: 10 μL, Col. Temp.: 30°C, Flow rate: 1.4 mL/min
 Gradient:
 5% to 95 % B in 8 min, Hold for 1.5 min, 9.51-12 min 05 % B

Antibacterial Activity

In the present research work, the antibacterial activity spectrum of water extract of *Gymnema Sylvestre* was analyzed. (Table-1) Two Gram-positive bacteria, *Staphylococcus aureus* and One Gram negative bacteria *Escherichia coli* were used. Inoculum size was adjusted to 1 to 2 × 10⁷ CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of nonfastidious bacteria. Stock solution of 10000μg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 6000μg/ml, 4000μg/ml, 2000μg/ml and 1500μg/ml solutions were prepared from which 100 μl solution was taken for assay. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar Well Diffusion Method.¹⁻³ After 16 to 18 hours of incubation, each plate is examined.

RESULTS AND DISCUSSION

The results of preliminary evaluation showed that the Antibacterial activity of the water Extract of *Gymnema Sylvestre* was evaluated at two different concentrations by the diffusion method. The water Extract of the *Gymnema Sylvestre* shows antibacterial activity at varied levels in The *E. coli*, *S. aureus*. The Bacteria *S. aureus* was found to be more active and *E. coli*, was found to be less active in inhibition zone. Antibacterial activity of water extract of the plant was performed.

Table 1: Zone of inhibition of different concentration of Water extract of *Gymnema Sylvestre* by the diffusion method

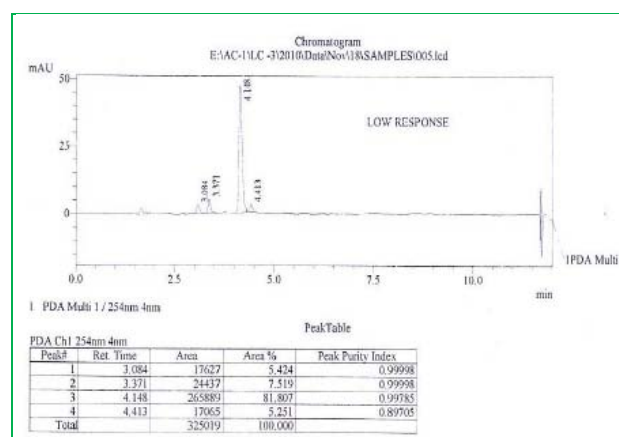
Bacteria	Inhibition Zone		
	Reference substance	Water extract	
		150μg/well	200μg/well
<i>E. coli</i>	35.60 ± 0.53	0.00 ± 0.10	0.00 ± 0.10
<i>S. aureus</i>	39.10 ± 0.95	8.98 ± 0.85	17.80 ± 0.63

Table 2: The water extract of *Gymnema Sylvestre* for extractive values, ash values, Ph, refractive index and separation of total extractive into acids and neutrals.

S. No	Parameter	Standard Value
1	Total Ash	0.25+0.05%
2	Total dissolved solids	27+2%
3	Total Acids	87+1%
4	Total neutrals	8+1%
5	Refractive Index	1.395
6	pH (Extract)	6.9

HPLC chromatography analysis spectrum of Active constituents were Isolated of Butanol from Water extract of *Gymnema Sylvestre*

HPLC was applied for testing the presence of number of organic compounds available of Water extract of *Gymnema Sylvestre* and this water extract Active constituents were Isolated of Butanol from Water extract. One of the major organic components with 81.807 % and 4.148 retention time may have detected.



CONCLUSION

Analytical study suggests that water extract contain various constituents which are given in the table 2. Preparative HPLC study revealed presence only one constituents were Isolated of Butanol from water extract of *Gymnema Sylvestre* and further investigations are in progress in the laboratory to identify the active structure and synthesis for application of this compound The results concluded showed that the water extract of *Gymnema Sylvestre* posses good antibacterial activity and only one constituents were Isolated of Butanol from water extract of *Gymnema Sylvestre*.

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