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Original Contribution

STUDIES ON *GYMNEMA SYLVESTRE* – A MEDICINALLY IMPORTANT PLANT OF THE FAMILY ASCLEPIADACEAE

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ABSTRACT

Gymnema sylvestre (Retc.) R. Br. belongs to the family Asclepiadaceae, having medicinal values selected for the present study, as it is very common in Western Ghats of Maharashtra (India). It has a reputation in traditional systems of medicine as a stomachic, diuretic and as a remedy to control diabetes. Present investigation includes morphology, histology, quantitative microscopy and phytochemistry of *G. sylvestre* collected from various ecological conditions. Results indicated that Plants growing under various ecological conditions showed histological similarities, but size and thickness of leaves, quantity of gymnemic acids, alkaloids and proteins content were different. Results of root zone soil analysis indicated that, there was positive correlation between available k and gymnemic acids content of *G. sylvestre*. The results of this investigation will be helpful for the correct botanical identification of plants and also different sources of medicine and Pharmaceutical industries.

Key words: Gymnemic acids, Marsdenieae, Ecology

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 to research centers because of their special importance in safety of communities. Plants have potent biochemical and have components of phytomedicine. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (1, 2). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Every detail of the composition and development of vegetation is dependent on the environmental factors. For detailed ecological study *Gymnema sylvestre* (Retc.) R. Br. is selected because it is very common in Western Ghats of Maharashtra (India). *G. sylvestre* is a medicinal member belongs to the tribe Marsdenieae of the family Asclepiadaceae. In ancient Indian texts, *Gymnema* is referred to as Gurmar, which means, "sugar killer" in Sanskrit. *G. sylvestre* leaves contain gymnemic acids, which are known to suppress transport of glucose from the intestine into the blood stream, therefore, it is useful in lowering blood sugar, balancing insulin levels,lowering blood cholesterol levels and also for promoting weight loss (3, 4).

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MATERIALS AND METHODS

The plant material was collected from Mulashi, Dapoli, Amboli, Khambataki Ghat and Botanical garden of the Department of Botany University of Pune. **Mulshi** is a small village lies in Mulshi Tahsil of Poona district of Maharashtra state. It is located about 40 km West of Pune city. The soil is roughly classified as red, gray and black. The pH of soil is 5.5 to 7. The maximum temperature is 38^0c and minimum 10^0c. The total annual rainfall is about 5000ml – 6500ml. The plant

material was collected from Tamhini and Dongarwadi; 30 km West of Mulshi and 70 km West from Pune city. **Dapoli** is situated very near to the Arabian sea. National highway rainfall 250mm to 3500mm, Temperature is 22^0c to 28^0c. **Amboli** is a quiet pleasant hill station in the range of Sahyadri hills about 118 kms away of Konkan and it is situated at 26 km away from Sawantwadi. The rainfall usually varies from 3000mm - 6000mm. Mean annual temp. is 16^0c, mean max. tempt. 22^0c and mean min. temp. is 10^0c. Soil is prominently lateritic formed by laterite rocks which is rich in Bauxite. As it is rich in Fe, compounds in the upper layers, it is red in colour. Climate is moist, warm, humid, pleasant. Annual rainfall is 7446 mm. The annual relative humidity is 83% in three different seasons i.e. Monsoon, winter and summer. The vegetation is evergreen and semi evergreen type. **Khambataki Ghat** is a part of Khandala Tahsil of Satara District. It is situated 50 km North-West of Satara 6o km East-West of Pune. It is an extremely dry, because it false under rain shadow region. The average annual rainfall is 30 - 40 mm. The soil colour is gray to off-white. Temperature is 40^0c Max. and 20^0c Min. (Annual Average). Height from Mean sea level is about 1200 mtrs. **Botanical Garden of Department of Botany University of Pune** is situated in Ganeshkhind 4.5 miles North-West of Pune city proper and lies between 18^0 34' North latitude and 73^0 53' East longitude at an elevation of about 1880 above mean sea level. The soil formation in this area is comparatively poor. The soils are reddish brown at high level while darker brown on flat areas. The soils are alkaline and are of pedocal type. The average rainfall, climate and other environmental conditions affecting the vegetation are more or less similar to that of Pune. During the hot season the diurnal maximum temperature of at Poona often rises above 40^0c and temperature as high as 43^0c have been occasionally recorded. The average maximum annual rainfall is recoded as 186.8 mms and minimum annual rainfall is recorded as 100 mms.

The plant material was collected and brought to the laboratory and identified with the help of flora of Maharashtra State (5) and Fasicals of flora of India (6). For microscopic studies, uniform and thin free hand section were taken from the fresh leaves and stems, dehydrated, double stained and finally mounted in Canada balsam by following the standard microtechniques method (7). Macro and microscopic characters were studied as per Wallis and Trease and Evanse (8, 9). For Phytochemical investigation, mature and healthy leaves were separated and dried in shade, so as to prevent the decomposition of chemical constituents, powdered in blender and analysed qualitatively and quantitatively for different chemical parameters as per standard methods (3, 10, 11). In the present investigation qualitative test and quantity estimation of gymnemic acid from the stem and leaf of *G. sylvestre* were carried out (12). The soil samples of the root of *G. sylvestre* were collected from Mulashi, Dapoli, Amboli, Khambataki Ghat and Botanical garden of Pune University. These soil samples were air-dried, processed and analysed for various physical and chemical properties. All the soil samples collected beneath the roots of this medicinal plant and were analyses for the confirmation of pH (13), EC (13, 14), Organic Carbon (14), available nitrogen (15), available phosphorous (16) , available potassium (13), free calcium carbonate (14, 17) and exchangeable Sodium (13).

OBSERVATION

A) Macroscopic characters of *Gymnema sylvestre* growing under various ecological conditions:

Gymnema sylvestre is a large climber running over the tops of trees. The root system is of tap root type. Stem is cylindrical, internodes terete, 0.7-17.2 cm long and 2 -10 mm in diameter, young stems and branches densely pubescent. Leaves are opposite, lamina 1 - 9.5 cm long and 0.6 - 5.5 cm broad, ovate or elliptic-oblong, acute at apex, rounded at base, ciliate along margin, pubescent above, sometimes densely so beneath, especially on the nerves, petioles terete, pubescent, 6 -12 mm long and 1 -1.5 mm in diameter. Flowers are born in extra-axillary position in spirally arranged corymbose cymes. Calyx is pubescent, five lobed, ovate, obtuse and ciliate. Corolla is yellow, campanulate, 3.5 mm long, corolla tube 1.5 mm long, about equaling the lobes; lobes five, ovate-deltoid, spreading and glabrous. Corona is corolline, of 5, fleshy processes inserted on the corolla tube, alternate with its lobes, free at the short deltoid sub-acute tip, which are protruding out of the mouth of corolla tube. Stamens are five. Gynostegium is 2 mm long. Style apex is thick, sub hemispherical, much exerted beyond the

anthers and follicles are paired in all plants collected from different localities.

B) Microscopic characters of *Gymnema sylvestre* growing under various ecological conditions:

In transverse section stem shows circular outline with entire or somewhat wavy margin. Epidermis forms the single outermost layer consisting of barrel to rectangular cells. A thick cuticle covers the epidermis. Uniseriate multicellular hairs are present. Cortex consists of parenchymatous cells. A distinct endodermis is absent. Pericycle is represented by scattered groups of thick walled stone cells, forms a large and complete ring. The vascular tissues occur in the following sequence – primary phloem, secondary phloem, cambium, secondary xylem, primary xylem, interaxylary phloem and pith. Different types of crystals of calcium oxalate and stone cells are present. Laticiferous tubes are also found in this region in all plants collected from different localities.

In transverse section leaf shows a typical dorsiventral structure. The epidermis of both the surfaces are single layered, cells are rectangular and covered externally with cuticle. The upper epidermal cells are slightly bigger in dimension. A number of cells of upper as well as lower epidermis are longate and are covered by mostly confined on bi-cellular and multicellular, uniseriate hairs. Stomata are ranunculaceous type and are present only on the lower epidermis Mesophyll is differentiated in two layers viz. palisade tissue and armpalisade tissue. The vascular bundle is surrounded by a parenchymatous bundle sheath. Laticiferous tubes are present in the midrib. The xylem is characterized by the presence of small vessels, tracheids and fibers. Xylem lies toward upper epidermis and phloem lies below the xylem toward lower epidermis in all plants collected from different localities.

RESULTS

To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way (18). The detailed of phytochemical screening in the two forms of extract is given in the **table 1**. Analysis of tannins, saponins, proteins, anthraquinones, alkaloids and flavanoids was positive while starch, reducing sugars and glycosides showed negative results in the stem extract. leaf extract showed positive results for starch, saponins, proteins, anthraquinones, reducing sugars, alkaloids, glycosides and flavanoids while negative results for tannins. The results of phytochemical investigation indicated that the quantity of gymnemic acid, alkaloids and proteins shows difference in different ecological conditions. The results of quantitative estimation of proteins per gram of dry weight in the leaf and stem of *Gymnema sylvestre* respectively are as follows - Amboli (0.30% & 2.01%,), Dapoli (0.19% & 1.55%), botanical garden (0.25% & 2.00%), Khambataki Ghat (0.58% & 1.02%) and Mulshi (0.28% & 2.33%). The result indicates that the values are less in leaves while more in stem of all the plants. It is also indicated that it is more in quantity in Mulshi followed by Amboli, botanical garden of Department of Botany, University of Pune, Dapoli and at last the Khambataki Ghat locality **(Table 2)**. The results of alkaloids also show difference in the leaf and stem of *Gymnema sylvestre*. The corresponding values are as followes - Amboli (1.22% & 0.74%), Dapoli (1.85% & 0.93%), botanical garden (1.80% & 1.55%), Khambataki Ghat (0.67% & 0.84%) and Mulshi (1.95% &1.12%). The quantity of Alkaloids is less in stems while more in leaves of all the plants. It is also indicated that it is more in quantity in botanical garden of followed by Mulshi, Dapoli, Amboli and Khambataki Ghat **(Table 3)**.

In addition, quantitative estimations of gymnemic acid in both the leaf and stem of *Gymnema sylvestre* is found out and their corresponding values are as follows - Amboli (1.35% & 0.56%,), Dapoli (1.64% & 0.68%), botanical garden (2.03 % & 0.91%), Khambataki Ghat (1.67% & 0.75%) and Mulshi (2.08% & 1.74%). Value of gymnemic acid is present less stem while more in leaves of all the plant collected from different localities. It is also indicated that it is more in quantity in Mulshi followed by botanical garden, Khambataki Ghat, Dapoli and at last Amboli **(Table 4)**.

Table 1. *Phytochemical tests of Gymnema sylvestre*

Sr. No.	Tests	Reagents used	Results of stem	Results of leaf
A)	**Water Extractives**			
1.	Starch	I2-KI	-ve	+ve
2.	Tannins	Acidic $FeCl_3$	+ve	-ve
3.	Saponins	H_2SO_4 + Acetic unhydride	+ve	+ve
4.	Proteins	Million's test	+ve	+ve
5.	Anthraquinones	Benzene + 10%NH_4OH	+ve	+ve
6.	Reducing sugars	Benedict's	-ve	+ve
B)	**Alcoholic Extractives**			
1.	Alkaloids	Mayre's	+ve	+ve
		Wagner's	+ve	+ve
		Dragnendorff's	+ve	+ve
2.	Flavonoides	HCl + Mg turnings	+ve	+ve
3.	Glycosides	Benzene+hot ethanol	-ve	+ve

Table 2. *Estimation of proteins in Gymnema sylvestre collected from different localities*

	Plant Part Used		
Name of the Locality	Leaf % / g / dry weight	Stem % / g / dry weight	Mean % / g / dry weight
Amboli	O.30	2.01	2.31
Dapoli	0.19	1.55	1.74
Department Garden	O.25	2.00	2.25
Khambataki Ghat	O.58	1.O2	1.60
Mulshi	0.28	2.33	2.61

The results are mean of three different readings.

Table 3. *Estimation of alkaloids in Gymnema sylvestre collected from different localities*

	Plant Part Used		
Name of the Locality	**Leaf** **% / g / dry weight**	Stem % / g / dry weight	**Mean** % / g / dry weight
Amboli	1.22	O.74	1.96
Dapoli	1.85	0.93	2.78
Department Garden	1.80	1.55	3.35
Khambataki Ghat	0.67	0.84	1.15
Mulshi	1.95	1.12	3.07

The results are mean of three different readings.

Table 4. *Estimation of Gymnemic acids in Gymnema sylvestre collected from different localities*

	Plant Part Used		
Name of the Locality	**Leaf** **% / g / dry weight**	Stem % / g / dry weight	**Mean** % / g / dry weight
Amboli	1.35	0.56	0.96
Dapoli	1.64	0.68	1.16
Department Garden	2.03	0.91	1.47
Khambataki Ghat	1.67	0.75	1.21
Mulshi	2.08	1.74	1.91

The results are mean of three different readings.

Results indicated that plants growing under various ecological conditions showed histological similarities, but size and thickness of leaves shows difference in plants growing at different ecological conditions. The results of quantitative microscopy of the leaf of plants collected from various localities indicated that the values of stomatal number and stomatal index, Vien - islets and Veinlet - termination number are nearly same in all plants. But values of palisade ratio showed a little difference in all plants collected from different localities **(Table 5)**. The results of root zone soil analysis indicated that **(Table 6)** the soil found in Amboli, Dapoli, botanical garden, Khambataki Ghat and Mulshi contains PH is suitable for the growth of crops. (13). E.C content in the Amboli, Dapoli, botanical garden, Kambataki Ghat and Mulshi soils is less than 1 and good for the growth of crops (13, 14). Results indicated Organic carbon in the Amboli, Dapoli and Mulshi soils is more than sufficient, in botanical garden soil is more and in Kambataki Ghat soil is moderate (14). Results showed Nitrite content in Amboli soil is moderate, in Kambataki Ghat, Dapoli and botanical garden soil is more, in Mulshi soils is more than sufficient (15). Phosphorous in Amboli, botanical garden and Mulshi soils is medium while in Dapoli and Kambataki Ghat soil is less (16). Potassium in Amboli soil is good, Khambataki Ghat is medium and in botanical garden, Dapoli and Mulshi soils is more than sufficient (13). Sodium in Amboli, Dapoli and Mulshi soils is very less, in botanical garden and Khambataki Ghat soil is very good (13). Calcium carbonate in Amboli soils is very less, in Dapoli and Mulshi soils is more, in botanical garden and Khambataki Ghat soil is less (14, 17).

Table 5. *Comparisonbetween quantitative microscopy of different leaf in Gymnema sylvestre collected from different localities*

Name of the Locality	Amboli	Dapoli	Botanical Garden	Khambataki Ghat	Mulshi
1. Palisade Ratio	14.98 per cell	14.45 per cell	15.60 per cell	15.94 per cell	15.51 per cell
2. Stomatal Index	12.04 per mm² area	12.04 per mm² area	12.04 per mm² area	12.04 per mm² area	12.04 per mm² area
3. Stomatal Number	10 per mm² area	10 per mm² area	10 per mm² area	10 per mm² area	10 per mm² area
4. Vein- Islets Number	20 per unit area	20 per unit area	20 per unit area	20 per unit area	20 per unit area
5. Vein Termination Number	7 per unit area	7 per unit area	7 per unit area	7 per unit area	7 per unit area

The values are mean of three different observations.

Table 6. *Root zone soil analysis*

Name of the Locality	Amboli	Dapoli	Department Garden	Khambataki Ghat	Mulshi
PH	6.07	6.39	7.72	6.55	6.15
E. C.	0.48	0.09	0.19	37	0.05
Organic Carbon%	1.23	1.44	0.94	0.31	2.14
Nitrogen kg/ha	338	691	451	689	1027
Phosphorous kg/ha	48	30	36	17	44
Potassium kg/ha	323	420	600	234	650
Sodium kg/ha	0.9	0.63	1.99	3.0	0.63
Calcium Carbonate kg/ha	0.4	10.2	8.00	8.09	11.12

The results are mean of three different readings.

DISCUTION

To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (1, 2). Every detail of the composition and development of vegetation is dependent on the environmental factors. A reciprocal relationship is built up between plants and their environment, and this can lead to changes in the conditions of the habitat and therefore, to a change in the conditions experienced by the plants. Results indicated that Plants growing under various ecological conditions showed histological similarities, but size and thickness of leaves, quantity of gymnemic acids, alkaloids and proteins content were different. The results of root zone soil analysis of plant *G. sylvestre* growing under various ecological conditions indicated that, the quantity of available k was more in soils at Mulshi and botanical garden than other localities while values for gymnemic acids were also higher in plants growing at Mulshi and botanical garden of Department of Botany. Thus, there was a positive correlation between available k and gymnemic acids content of *G. sylvestre*. The results of this investigation will be helpful for the correct botanical identification of plants and also different sources of medicine and Pharmaceutical industries.

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REFERENCES

1. Karthikeyan, A, Shanthi, V, Nagasathaya A., Pre-liminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica L. *Int Journal Green Pharm.* 3: 78-80, 2009.
2. Lozoya M. and Lozoya X., Pharmacological properties in vitro of various extracts of Mimosa pudica Linn. *Tepescohuite Arch Invest Mex*. 87-93, 1989.
3. Anonymous, Pharmacopoeia of India, 1nd ed. Government of India Ministry of Health Manager Publications, Delhi, pp 370 – 864, 1955.
4. Yoshikawa, K., Amimoto K, Arihara S, et al. Structure studies of new antisweet constituents from Gymnema sylvestre. *Tetrahedron Letters*, 30 (9) :1103 -1106. 1989.
5. Singh, N., Lakshminarasimhan, P., Karthikeyan, S. and Prasanna, P., Flora of Maharashtra State, Dicotyledones. The Director, Botanical Survey of India. 334 – 342 : 362-364, 2001.
6. Jagtap, A. and Singh, N., Fasicles of Flora of India. The Director, Botanical of India. 24 ; 1-6, 82-91, 62-71, 1999.

7. Johnsen, D., Plant Microtechnique. Mc Graw Hill Book Co. Inc. New York. 154, 1940.
8. Trease, G. and Evans W., Phamacognosy, Baillene Tindall, London. 735-738, 1982.
9. Wallis, T., A textbook of pharmacognosy. 3rd Edn. J and A Churchill Ltd, London. 1976.
10. Harborne, J., Phytochemical Methods. Chapman and Hill Tokyo Japan. 1973.
11. Manikam A. and Sadashivam, S., Biochemical Methods in Agriculture Scince. Wiley Eastern Indian Ltd., India. 1991.
12. Passera C., Pedrotti, A. and Ferrari, G., Chromatography. 14 : 289, 1964.
13. Jackson, M., Soil Chemical Analysis, Prentice Hall of India Pvt. Ltc., New Delhi, 134 – 182, 1973.
14. Black, C. Methods in Soil Analysis Part - I & II, Amer. Soc. of. Agron. Inc. Pub. Madison, Wisconsin, USA, 3, 1965.
15. Subbiah, B. and Asija, G., A rapid procedure for the estimation of available nitrogen in soil, *Curr. Sci.* 25 (8): 259 – 260, 1956.
16. Bray, R. and Kurtz, L., Determining to total organic and available forms of phosphates in soil. *Soil Science Society of America Journal*, 39 – 45, 1945.
17. Piper C., Soil and Plant Analysis. Inter Science Publishers, INC, New York ,368, 1950.
18. Cragg, G., Newman, D. and K. Snader, Natural products in drug discovery and development. *Journal. Nat. Prod*. 52-60, 1997.