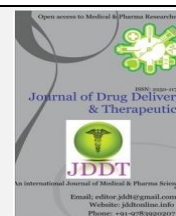


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Research Article

Phytochemical Analysis of *Gymnema* Complex from Maharashtra

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ABSTRACT

Plants are the major source of drugs comprising to different groups such as anti-diabetic, anti-cancer, antispasmodics, anti-microbial, etc. A number of plants are claimed to possess anti-diabetic properties in the traditional systems and extensively used by tribal people as well as local inhabitants. Out of these 'Gud-mar'/'Gulmar' is one of the groups. Ethnobotanical data revealed that, *Gymnema sylvestre* (Retz.) R.Br. ex Sm., *Gymnema cuspidatum* (Thunb.) Kuntze, *Gymnema latifolium* Wall. ex Wight, *Gymnema inodorum* (Lour.) Decne., *Gymnema montanum* Hook. f. and *Ichnocarpus frutescens* (L.) W.T. Aiton are called Gulmar and used as anti-diabetic plants in Maharashtra. Therefore, phytochemical screening was carried out using standard experimental procedures in dry leaf powder of *Gymnema sylvestre*, *G. cuspidatum*, *G. latifolium* and *Ichnocarpus frutescens*. This study scientifically validates the use of these plants in traditional medicines and the phytochemical profile is helpful for standardization of indigenous drug.

Keywords: Phytochemicals, *Gymnema* Complex, Maharashtra.

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INTRODUCTION

The genus *Gymnema* R. Br. is one of the important medicinal plants used in therapeutic applications and commonly referred as sugar killer due to anti-diabetic potential and commonly known as *Gulmar*/*Gudmar*/*Bedkichapala*/*Aphumari*. The genus comprises 49 species distributed widely in Asia, Africa and Australia. In India, it is represented by 14 species and two varieties⁸. Among these six are distributed in Maharashtra viz., *Gymnema cuspidatum* (Thunb.) Kuntze, *G. latifolium* Wall. ex Wight (Syn. *Gymnema khandalense* Santapau), *G. montanum* Hook. f., *G. sylvestre* (Retz.) R. Br. ex Sm., *G. inodora* (Lour.) Decne. (Syn. *Gymnema tingens* Roxb. ex Spreng), and *G. elegans* (Wight & Arn.) Decne. All these species are used as alternative drugs against *G. sylvestre* in traditional medicine or ethno-medicine for the treatment of diabetes^{1, 10-13}. Beside this, *Ichnocarpus frutescens* (L.) W.T. Aiton is also used on diabetes and called as *Aphumari*. Out of these species, *G. cuspidatum*, *G. latifolium*, *G. sylvestre* and *Ichnocarpus frutescens* are commonly available in local market and collected as 'Gulmar' by local peoples. Therefore, these species are considered as '*Gymnema* complex'. These traditional medicines are receiving a renewed interest in recent years due to adverse effects of synthetic medicines. Of these taxa only *G. sylvestre* has been widely studied and reported to have potent primary metabolites like proteins, amino-acids and secondary metabolites consisting of

alkaloids, flavonoids and phenolic compounds⁴. It also reported to have biochemically active antioxidant, anti-mutagenic and anti-carcinogenic properties⁹⁻¹⁰. Rest of the crude drug plants are less studied and there was a need of evaluation of these valuable resources. Therefore, a study was undertaken to evaluate the above mentioned taxa for phytochemical analysis and the results are presented.

MATERIALS & METHODS

The materials of *Gymnema* complex drugs were collected from different localities of Maharashtra during the years 2016 to 2018. The identification and authentication of these species was done with the help of available literature and herbarium^{2-3, 6-10, 15}. The herbarium specimens are deposited in Herbarium of Department of Botany, Balasaheb Jadhav Arts, Commerce and Science College, Ale.

Leaf materials after collection were cleaned and dried in shade condition for two weeks. The materials were grinded to coarse powder by using household electric blender and passes through sieve no.25. For extraction of components 10 gm of each powder were subjected for extraction by a hot percolation method with water, ethanol and methanol in Soxhlet apparatus for 72 hrs. Each solvent extraction step was carried out for 24 hrs. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure and stored at 4°C for further study^{5, 18}.

Preliminary phytochemical screenings were done using extracts. The leaf extracts in different solvents i.e., water, ethanol and methanol were used for detection of different compounds by using qualitative tests.

Total Phenolic assay: Folin-Ciocalteu assay¹⁶.

1. An aliquot (1ml) of extracts or standard solution of Gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to 25ml of volumetric flask, containing 9ml of distilled water.
2. Reagent blank using distilled water was prepared.
3. Add 1 ml of Folin-Ciocalteu phenol reagent and mix sample well.
4. After 5 minutes 10 ml of 7% Na₂CO₃ solution was added to the mixture. Make the final volume 25 ml by using distilled water.
5. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV Visible spectrophotometer.
6. Total phenolic content was expressed as mg Gallic acid Equivalents (GAE).

Estimation of total Flavonoid by Aluminium Chloride Colorimetric Method¹⁹.

An aliquot (1ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water.

1. Add 0.30 ml 5% NaNO₂ and after 5 minutes 10% AlCl₃ and mix well.
2. After 5 minutes add 2 ml of 1 N NaOH and make the final volume 10 ml by using distilled water.
3. The solution was mixed and absorbance was measured against blank at 510 nm.
4. The total flavonoid content was expressed as mg quercetin equivalents (QE).

RESULT AND DISCUSSION

Present study revealed that, the presence of bioactive compounds viz., alkaloids, phenolics, flavonoids and glycosides are the major groups in all the species. Saponins and anthraquinones also present. The phytochemical characters of all species are summarized in table-1.

Table 1: Phytochemical Screening

Sr. No.	Phyto-Compounds tested for	<i>Gymnema sylvestre</i>			<i>Gymnema latifolium</i>			<i>Gymnema cuspidatum</i>			<i>Ichnocarpus frutescens</i>		
		M	E	W	M	E	W	M	E	W	M	E	W
1	Carbohydrates												
	Molisch	+	+	+	+	+	+	+	+	+	+	+	+
	Fehling's	+	+	+	+	+	+	+	+	+	-	-	-
2	Phenols												
	Phosphomolybdic acid test	+	+	+	+	+	+	+	+	+	-	-	-
3	Flavonoids												
	Shinoda test	+	+	+	+	+	+	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+	+	+	+	+	+	+
4	Tannins												
	Braemer's test	-	-	+	+	+	+	-	-	+	-	-	-
5	Alkaloids												
	Dragendroff's test	+	+	-	+	+	+	+	+	+	+	+	-
	Mayer Test	+	+	-	+	+	+	+	+	+	+	+	-
	Hanger's test	+	+	-	+	+	+	+	+	+	+	+	-
6	Glycosides												
	Legal's test	+	+	+	+	+	+	+	+	+	-	-	-
	Keller killans Test	+	+	+	+	+	+	+	+	+	-	-	-
7	Saponins												
	Foam test	+	+	+	+	+	+	+	+	+	-	-	-
8	Anthraquinones												
	Borntrager's test	+	+	+	+	+	+	+	+	+	-	-	-
9	Amino-acids												
	Ninhydrine test	+	+	+	+	+	+	+	+	+	+	+	+
10	Fixed oils	+	+	+	+	+	+	+	+	+	+	+	+
11	Test for protein	+	+	+	+	+	+	+	+	+	+	+	+
12	Test for steroids and terpenoids	+	+	+	+	+	+	+	+	+	-	-	-

M- Methanol extract, E- Ethanol extract, W- water extract.

Table 2: Total Phenolics and total Flavonoid content in Leaf

Parameters	<i>Gymnema sylvestre</i>	<i>Gymnema latifolium</i>	<i>Gymnema cuspidatum</i>	<i>Ichnocarpus frutescens</i>
Total Phenolics mg GAE/mg	1.8	1.625	3.84	3.89
Total Flavonoids mg QE/mg	0.94	1.66	2.82	2.46
Flavonoids/Phenolics (F/P ratio)	0.52	1.02	0.73	0.63

The absorbance against the reagent black was determined at 550nm and 510nm with an UV-Visible spectrometer for phenolics and flavonoids respectively. The phenolics content was expressed as mg of Gallic acid Equivalents (GAE) and total flavonoid content was expressed as mg quercetin equivalent (QE)

In alcoholic extracts of all the species, alkaloids are prominently present than that in the water extract. Water extract of *Gymnema latifolium* showed positive test for alkaloids. In ethanol and methanol extract phenols showed positive test except *Ichnocarpus frutescens*. Tannins are mainly observed in water extracts. Anthraquinones mainly present in *Gymnema cuspidatum*. Methanol extracts show maximum positive results for alkaloids, phenols, flavonoids and saponins; whereas, glycosides are found in water extracts. The highest phenolics were observed in *I. frutescens* (3.89 mg Ga E) and *Gymnema cuspidatum* (3.84 mg Ga E) whereas least was recorded in *G. latifolium* (1.625 mg Ga E). The maximum flavonoids also observed in *G. cuspidatum* (2.82 EQ) and least flavonoids content found in *G. sylvestre*. The highest F/P ratio recorded in *G. latifolium*. It indicates specific flavonoids among the phenolic compounds¹⁸. This study scientifically validates the use of plants in traditional medicine and phytochemical data will be helpful for standardization of Indigenous drug.

CONCLUSION

From the present investigation, it is conformed that alkaloids, glycosides and flavonoids present in alcoholic extract and tannins, saponins and anthroquinones present in water extract of leaf. Maximum number of biochemical compound occurs in *G. cuspidatum* and *G. latifolium* than *G. sylvestre*. As compare these species *I. frutescens* showed less biochemical compounds. Both the genera viz. *Gymnema* and *Ichnocarpus* showed different compounds. These compounds are used as identification markers for crude drug. This study also revealed that *G. latifolium* and *G. cuspidatum* have more medicinal potential than *G. sylvestre*.

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