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Research Article

Phytochemical Studies on Selected *Gymnema* Species from Kerala

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ABSTRACT

Phytochemical studies were carried out in four *Gymnema* species such as *Gymnema sylvestr*e, *Gymnema hirsutum*, *Gymnema elegans* and *Gymnema khandalense* collected from Kerala. Preliminary chemical tests were conducted using various qualitative analyses. Phenolics, flavonoids and alkaloids were found as major class of compounds in all the species. Total phenolics content (TPC) was determined by Folin Ciocalteu Spectrophotometric method and was expressed as mg Equivalent of Gallic acid. Total flavonoid content (TFC) was also calculated by aluminium chloride colorimetric method and was expressed as mg equivalent of Quercetin. Among the different parts, roots showed maximum phenolics for all the four species. The highest TPC were found in the root of *G. khandalense* (3.175 mg E Ga) and the least TPC was observed in leaves of *G. elegans* (0.84 mg E Ga). Flavonoid to phenolic ratio (F/P) was also calculated for the evaluation of specificity of flavonoids farther than phenolics.

Key words: Phytochemical, *Gymnema* species etc.

INTRODUCTION

Gymnema sylvestre (Retz.) R.Br. is known as Chakkarakolli in Malayalam for its distinctive property of temporarily destroying the taste of sweetness. It is a large woody, branched climber. Its distribution is Indo-malesian and grows in varying soil conditions. It is well recognized in traditional medicine as a remedy for diabetes mellitus and as stomachic and diuretic. The entire plant is used as medicine. Under the name 'meshasringi', it is there in Ayurvedic classical texts since ancient times. It was Sushruta who described the antidiabetic property of the drug. Due to the high medicinal value of the plant, the plant is used extensively. *Gymnema khandalense* is very rare and endemic to the Western Ghats of Maharashtra and Kerala. *Gymnema elegans*, endemic to Peninsular India, is rare. *Gymema hirsutum* is reported only from India. *Gymnema sylvestre* is widely used against diabetes. (Chattopadhay, 1999; Otaet et al. 1998; Shimizu et al., 1997).

Phenolic compounds are plant substances which possess in common an aromatic ring bearing one or more hydroxyl groups. There are about eight thousand naturally occurring plant phenolics and about half of this number are Flavonoids (Jeffrey *et al* 1993). Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anti carcinogenic as well as ability to modify the gene expression.Phenolics are the largest group of phytochemicals that account for most of the anti oxidant activity in plants or plant products.(Okpuzor *et al*, 2009).

MATERIALS AND METHODS

Collection: Botanical surveys were conducted in different forest areas of Western Ghats of Kerala and collected four species of the Genus *Gymnema* such as *Gymnema sylvestre* (Retz.) R.Br. ex Schult. *Gymnema* hirsutum Wight & Arn., *Gymnema elegans* Wight & Arn., and *Gymnema khandalense* Sant. Except *Gymnema sylvestre* all are endemic. The voucher specimens were deposited in CMPR herbarium, Arya Vaidya Sala, Kottakkal.

Extraction: Five grams of each of the dried leaves, stem and root of Gymnema *sylvestre, Gymnema hirsutum, Gymnema elegans* and *Gymnema khandalense* were pulverized into coarse powder and subjected to extraction with methanol using soxhlet apparatus. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure. The dried residues were then dissolved in 100 ml of methanol.

Preliminary Phytochemical Screening: The methanolic extract of different parts was screened for the detection of major class of compounds by carrying out specific qualitative tests

Total Phenolic assay: The total phenolics content was determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). An aliquot (1 ml) of extracts or standard solution of Gallic acid (20, 40, 40, 60, 80 and 100µg/ml) was added to 25 ml of volumetric flask, containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE)

Total Flavonoid Assay: Total flavonoid content was measured by the aluminium chloride colorimetric assay (J Zhishen *et al*). An aliquot (1ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of

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Table 1: Preliminary phytochemical screening of *G. sylvestre*

Sl No.	Tested for	Leaf	Stem	Root
1.	Carbohydrates			
	Molish	+++	++	+++
	Fehling's test	+++	++	+++
2.	Phenols			
	Phoshomolybdic acid test	+	+	++
3.	Flavonoids			
	Shinoda test	++	+	+
	Lead acetate test	++	+	+
4.	Tannins			
	Braemer's test	+	+	++
5.	Alkaloids			
	Dragendorff's test	++	++	+++
	Hager's test	++	++	+++
6.	Glycosides			
	Legal's test	+++	+++	+++
7.	Saponins			
	Foam test	+	+	+++
8.	Anthraquinones			
	Borntrager's test	++	++	+++
9.	Aminoacids			
	Ninhydrin test	+++	++	+
10.	Fixed Oils	+	+	+

Table 2: Preliminary phytochemical screening of *G. hirsutum*

Sl No.	Tested for	Leaf	Stem	Root
1.	Carbohydrates			
	Molish	+++	++	+++
	Fehling's test	+++	++	+++
2.	Phenols			
	Phoshomolybdic acid test	++	++	+++
3.	Flavonoids			
	Shinoda test	+++	+	+
	Lead acetate test	+++	+	+
4.	Tannins			
	Braemer's test	++	++	+++
5.	Alkaloids			
	Dragendorff's test	++	++	+++
	Hager's test	++	++	+++
6.	Glycosides			
	Legal's test	+++	+++	+++
7.	Saponins			
	Foam test	+	+	+++
8.	Anthraquinones			
	Borntrager's test	++	++	+++
9.	Aminoacids			
	Ninhydrin test	+++	++	+
10.	Fixed Oils	+	+	+

distilled water. To the flask was added 0.30 ml 5% $NaNO_2$ and after five minutes 0.3 ml 10 % $AlCl_3$. After five minutes, 2 ml IM NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed (Table 1 to 4) the chemical pattern of different parts of selected species. All the species contain phenolics, flavonoids, alkaloids and glycosides as major group of phytochemicals. Saponins and anthraquinones were also detected.

The results of total phenolic and total flavonoid content and the ratio of total flavonoids/ total phenolics in the studied plant extracts are presented in table 5. The plants selected for the present studies possess significant

Table 3: Preliminary phytochemical screening of *G. elegans*

Sl No.	Tested for	Leaf	Stem	Root
1.	Carbohydrates			
	Molish	+++	++	+++
	Fehling's test	+++	++	+++
2.	Phenols			
	Phoshomolybdic acid test	++	++	+++
3.	Flavonoids			
	Shinoda test	+++	+	+
	Lead acetate test	+++	+	+
4.	Tannins			
	Braemer's test	++	++	+++
5.	Alkaloids			
	Dragendorff's test	++	++	+++
	Hager's test	++	++	+++
6.	Glycosides			
	Legal's test	+++	+++	+++
7.	Saponins			
	Foam test	+	+	+++
8.	Anthraquinones			
	Borntrager's test	++	++	+++
9.	Aminoacids			
	Ninhydrin test	+++	++	+
10.	Fixed Oils	+	+	+

Table 4: Preliminary phytochemical screening of *G. khandalense*

Sl No.	Tested for	Leaf	Stem	Root
1.	Carbohydrates			
	Molish	+++	++	+++
	Fehling's test	+++	++	+++
2.	Phenols			
	Phoshomolybdic acid test	++	++	+++
3.	Flavonoids			
	Shinoda test	+++	+	+
	Lead acetate test	+++	+	+
4.	Tannins			
	Braemer's test	++	++	+++
5.	Alkaloids			
	Dragendorff's test	++	++	+++
	Hager's test	++	++	+++
6.	Glycosides			
	Legal's test	+++	+++	+++
7.	Saponins			
	Foam test	+	+	+++
8.	Anthraquinones			
	Borntrager's test	++	++	+++
9.	Aminoacids			
	Ninhydrin test	+++	++	+
10.	Fixed Oils	+	+	+

quantity of phenolics and flavonoids.

The highest phenolics were observed in the root of *G. khandalense* (3.175 mg E Ga) whereas least was noticed in leaves of *G. elegans* (0.84 mg E Ga). The maximum flavonoid was also observed in *G. khandalense* (2.01 EQ). The least flavonoid content was found in the root of *G. elegans* (0.71 QE). Comparing the different parts, root showed higher phenolics for three species except for *G.hirsutum.* In *G.hirsutum* leaves showed maximum phenolics (3.425 mg E Ga). In *G. sylvestre* TPC varied as 1.6, 1.65 and 2.31 for leaf, stem and root respectively and TFC varied as 0.91, 1.11 and 1.42. The highest F/P ratio was observed in leaves of *G. khandalense* (0.90). The Flavonoids / Phenolics (F/P) ratio indicates the specificity of flavonoids among the phenolic compounds (Sulaiman and Balachnadran, 2012). The TPC of *G.hirsutum* was found as 3.425, 1.615 and 1.075 for leaf, stem and root respectively. Leaves of *G.elegans* showed minimum

Table 5: Total Phenolic and Total Flavonoid Content

Plant	Investigated part	Total phenolics Mg GAE/ g	Total flavonoids Mg QE/ g	Flavonoids/ phenolics (F/P ratio)
G. sylvestre	Leaves	1.6	0.91	0.57
	Stem	1.625	1.11	0.68
	Root	2.31	1.42	0.61
G.hirsutum	Leaves	3.425	0.91	0.26
	Stem	1.615	1.43	0.88
	Root	1.075	0.91	0.85
G. elegans	Leaves	0.84	0.71	0.84
	Stem	2.84	0.84	0.29
	Root	2.625	0.71	0.27
	Leaves	1.825	1.66	0.90
G. khandalense	Stem	3.16	1.725	0.54
	Root	3.175	2.01	0.63

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

phenolic and flavonoid contents but its stem and root showed considerable phenolics.

The current study established the phenolic composition of selected *Gymnima* species collected from Kerala. Phenolics are largest group of phytochemicals and account for most of the anti oxidant activity in plants or plant products. Phenolic compounds are the major contributors towards the free radical scavenging activities of plant extracts. The present investigation showed that the selected four species are rich source of naturally occurring antioxidant phenolic compounds.

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