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QUALITATIVE AND QUANTITATIVE ANALYSIS OF SECONDARY PHYTOCHEMICAL IN GYMNEMA SYLVESTRE

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ABSTRACT

Present investigation deals with the qualitative analysis and quantitative estimation of secondary phytochemicals in leaves of important medicinal climber plant *Gymnema sylvestre*. Qualitative analysis was carried out to identify the different classes of secondary metabolites in various chemical extracts such as acetone, water, methanol and chloroform. Phytochemical analysis of the extracts proved the presence of secondary phytochemicals such as Alkaloids, Flavanoids, Phenols, Saponins, Tannins, steroids and cardiac glycosides. Qualitatively estimation showed that total alkaloids and total saponins was more 36 mg/g and 23 mg/g in leaf as compare to stem barks10 mg/g and 14 mg/g however total flavonoid was estimated more in stem 49 mg/g than leaf 34 mg/g.

KEYWORDS: *Gymnema Sylvestre*, Qualitative and Quantitative Secondary Phytochemicals.

Herbal medicine denotes therapeutic uses of plants. Plants are one of the major sources of drugs due to its secondary derivative bioactive chemical constituents which play vital role to cure many ailments. Herbal medicines in long course for permanent health care benefits are considered to be safe. World Health Organization reported in that, approximately 80% world population use plant parts for their primary health care. *Gymnema sylvestre* a woody climber plant belongs to the family *Asclepiadaceae* commonly known as Gurmar in hindi which mean sugar killer. The plant is distributed in tropical forests of central and southern India and plant also grows in parts of African continent. *G. sylvestre* is valued for its medicinal importance. Many authors have reported large number of ethanomedicinal properties of *G. sylvestre,* such as the leaves is particularly used as antiviral, anti allergic, diuretic, hypoglycemic, hypolipidemic, for the treatment of obesity and dental cares. It is also used as antibiotic, in stomach pains, as a blood purifier and in rheumatism. *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. Present investigation deals with the qualitative and quantitative estimation of major class of secondary phytoconstituents present in leaves and stem bark of *G. sylvestre* though biochemical method.

MATERIALS AND METHOD

Collection and Processing

Fresh leaves of *Gymnema sylvestre* was collected from forest nursery of Godhi village near Mandir Hassaud Raipur Chhattisgarh, India during the summer season in the month of March- April 2016. This leaf sample was washed thoroughly with tap water, shade dried and grinded finely in to the powder form, which was then used for various form of extract preparation. The plant was identified with help of flora of H.H. Hains. .

Preparation of Extract

20g per 200ml of each solvent such as Acetone, methanol, water and chloroform extracts of leaf of *Gymnema sylvestre* were prepared separately with the help of soxhlet apparatus. After extraction the extracts were filtered through Whatman No. 41 filter paper. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard preliminary phytochemical methods given by (Trease & Evans, 2009; Harbone, 1998; Kokate *et al.,* N. Raaman ,2006)

Preparation of Reagent

All the reagent and chemicals including Dragendorff's, Wagner's, Mayer's, Hager's and keddr's were prepared according to available literature(Flinn scientific 2011)

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Qualitative Secondary Phytochemical Analysis

Test for Alkaloids

i). Wagner's test: Extract was treated with few drops of Wagner's reagent. The reddish brown precipitate indicated the presence of Alkaloids.

ii). Mayer's test: Extract was treated with few drops of Mayer's reagent. The white or pale precipitate indicated the presence of Alkaloids.

iii). Hager's test: Extract was treated with hager's reagent, appearance of yellow colour precipitate indicated the presence of Alkaloids.

Test for Flavonoids

i). Shinoda test: Crude extracts was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicated the presence of flavonoids.

ii). Sodium hydroxide test: Few quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid which indicated the presence of flavonoids.

iii). Zn test: 2 ml extract was treated with Zn dust and conc. HCl development of red colour indicated presence of Flavonoid

Test for Phenols

i). Ferric chloride test: Extract was treated with 2ml of water and 10% aqueous ferric chloride solution. Blue or green colour precipitate indicated presence of the phenol.

ii). Lead acetate test: Ethanolic extract was added to about 1% solution of gelatin containing 10% NaCl. Formation of white precipitate indicated presence of the phenol.

Test for Saponins

i). Foam test: 5ml of filtrate was diluted with 20ml of water and vigorously shaken. The test tube was observed for the presence of stable foam upon standing.

Test for Steroids

Liebermann Burchardest test: Concentrated extracts was added with 2ml of acetic anhydride and 1 ml of concentrated sulphuric acid development of colour bluish to green indicated the presence of steroids.

Test for Terpenoids

Salkowski test: 2 ml of each extracts was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of red colour indicated the presence of steroids

Test for Tannins

i). Ferric chloride test: The ethanolic extract is treated with 2 ml of $FeCl_3$ solution. The blue black precipitation is observed.

ii). Alkaline reagent test: Extract was treated with 10% NaOH solution formation of intense yellow colour indicated the presence of tannins.

iii). Gelation test: Extract was treated with aqueous solution of gelatin and added sodium chloride, white buff colour precipitate indicated the presence of tannins.

Test for Cardiac glycosides

i). Keller-Killani test: Extracts was treated with 1ml of $FeCl_3$ reagent (mixture of 5% of $FeCl_3$ and 99 volume of glacial acetic acid). To this solution few drop of cons. H_2So_4 was added appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.

ii). Legal test: Extracts was treated with few ml of pyridine add 2 drop of nitroprusside and 1 drop of 20% sodium hydroxide solution deep red colour indicated the presence of cardiac glycosides.

Quantitative Secondary Phytochemical Estimation

Total Alkaloid Estimation by using Harborn (1973) Method

5g of each plant parts sample was weighed separately into a 250 ml capacity beaker and added 200ml of 10% solvent of acetic acid in ethanol then covered the beaker to check evaporations of solvent and allowed to stand for 4 hour. This was filtered and extracts was concentrated on water bath to ¼ of

original volume then cons. ammonium hydroxide was added drop wise into concentrated extracts until the precipitation was completed. The solution was allowed to settle the precipitate and filtered. Filtered precipitate washed with dil. ammonium hydroxide and then again filtered. This precipitate residue is alkaloid which was dried and weighed.

Weight of total alkaloids: $\frac{W2-W1}{W3}$ g, % Yields of Alkaloid $\frac{W2-W1}{W3}\times 100$

Where, W1 = weight of crucible, W2 = weight of crucible with alkaloids, W3 = initial weight of plant sample taken for estimation.

Total Flavonoids Estimation by Using Boham and Kocipai (1994) Method

10g of leaf powder sample was weighed into a 250ml capacity beaker and added 100ml of 80% aqueous methanol for extraction at room temperature. This was filtered through Whatman No. 42(125mm) filter paper and extracts was collected into another 250ml capacity beaker. Extraction procedure repeated in same used sample separately and extract was recollected. Collected extract then transferred into crucible and evaporated till dryness on water bath and weighed.

Weight of total flavonoids: $\frac{W2-W1}{W3}$ g, % Yields of Flavonoids $\frac{W2-W1}{W3}\times 100$

Where, W1 = weight of crucible, W2 = weight of crucible with flavonoids, W3 = initial weight of plant sample taken for estimation.

Total Saponin Estimation By Using Nahapetian and Bassiri (1975) Method

Suspension was prepared of 10g of leaf powder sample in 100ml of 20% ethanol. This sample suspension was heated over water bath for 4 hour at 55ºC with continuous stirring. This sample was filtered and extract was collected in 200ml capacity of beaker. Obtained residue re- extracted with 100ml of 20% ethanol. Combine extracts heated over water bath at about 90 till volume was reduced to 40ml. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 30 ml of n-butanol was added. The combine n- butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven and weighted.

Weight of total saponins: $\frac{W2-W1}{W3}$ g, % Yields of Saponins $\frac{W2-W1}{W3}\times 100$

Where, W1 = weight of crucible, W2 = weight of crucible with saponins, W3 = initial weight of plant sample taken for estimation.

RESULTS AND DISCUSION

Secondary metabolites were qualitatively analyzed using leaf extracts in different solvents such as chloroform, methanol, acetone and aqueous. Out of these four extracts methanolic extract showed maximum number of phytoconstituents such as alkaloids, flavonoids, saponins, phenol, tannins, cardiac glycosides followed by aqueous extracts which showed alkaloids, flavonoids, saponins, phenol and tannin. Very few numbers of phytochemicals were found to be present in chloroform extract such as steroids, tannin and phenol results are presented in table 1. It was observed that alkaloids were present in all extracts except chloroform extracts. Aqueous extracts was positive for all tests of alkaloids. Flavonoids were positively analyzed for all extracts except chloroform extracts but all extracts were positively detected for ferric chloride test of phenol. Methanol and water extracts revealed the presence of saponin in G*ymnema salvestre* Qualitatively estimated total alkaloids and total saponins was more 36 mg/g and 23 mg/g in leaf as compare to stem barks10 mg/g and 14 mg/g however total flavonoid was estimated more in stem 49 mg/g and then leaf 34 mg/g.

Medicinal value of plant is due to presence of secondary phytoconstituents that have a definite physiological function in the human body. Different phytochemicals have been found to possess a wide range of medicinal properties. Gymnemagenol a type of saponins which is found in Gymnema was found to show a high degree of inhibition to theproliferation of *HeLa* cancer cell line medication in cancer therapy (V. Khanna and K. Kannabiran, 2009):. Group of

active compound of plant known as gymnemic acid which play vital role to control blood sugar by regeneration of the *beta* cells in insulin-dependent diabetes mellitus and may also in obesity, obesity is usually caused by an abnormality of feeding regulatory mechanism (Mall *et al* 2009). Flavonoids and phenolic acids were isolated from the aerial parts which exhibit interesting antiviral and antimicrobial properties both *in vitro* and *in vivo (*M. Senthilkumar 2015). Previous author reported that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as alkaloids, flavonoids, phenols and tannins(Rice-Evans *et a*l1997).

Table 1: Extractive values of different chemical extracts of leaf of *Gymnema sylvestre.*

S. No.	Solvent system	% Yield of extracts
1.	Water	4.20±0.115
2.	Acetone	2.53±0.223
3.	Methanol	5.93±0.088
4.	Chloroform	2.11±0.116

Mean ± SE of three replicates

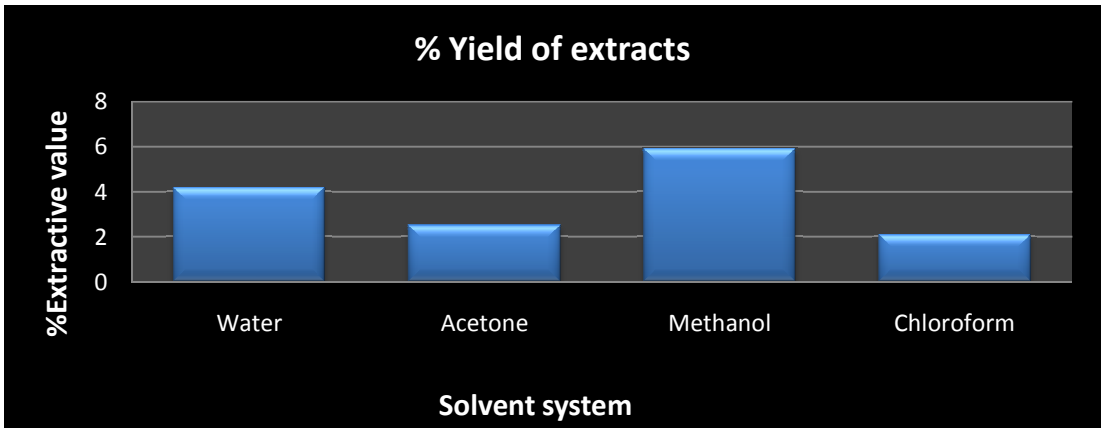


Table 2: Qualitative phytochemical analysis in the different chemical extracts of leaves of *Gymnema sylvestre.*

S. No.	Phytoconstituents	Test	Various chemicals and aqua. Extracts of leaves of Gymnema			
			Aqua. Extracts	Acetone extracts	methanol extracts	Chloroform extracts
1.	Alkaloid	i). Wagner's	+	-	+	-
		ii). Mayer's	+	-	-	-
		iii). Hager's	+	+	+	-
2.	Flavonoid	i). Shinoda	-	-	+	-
		ii). NaOH	+	+	+	-
		iii). Zn	-	-	-	-
3.	Phenols	i). Ferric chloride	+	+	+	+
4.	Saponins	ii). Foam	+	-	+	-
5.	Steroids	i).Liebarmann's Burchardest	-	-	-	+
6.	Terpenoids	i). Salkowski	-	-	-	-
7.	Tannins	i).Alkaline reagent	+	-	+	-
		ii). Gelation	+	-	+	-
		iii). Ferric chloride	+	+	+	+
8.	Cardiac Glycosides	i). Keller killani	-	-	+	-
		ii). Legal	-	-	-	-

(+) indicate present and (-) indicate absent

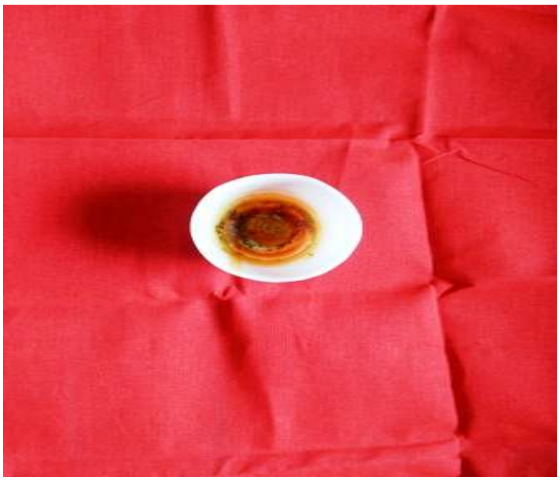


Figure B: Quantitative total saponins in leaf

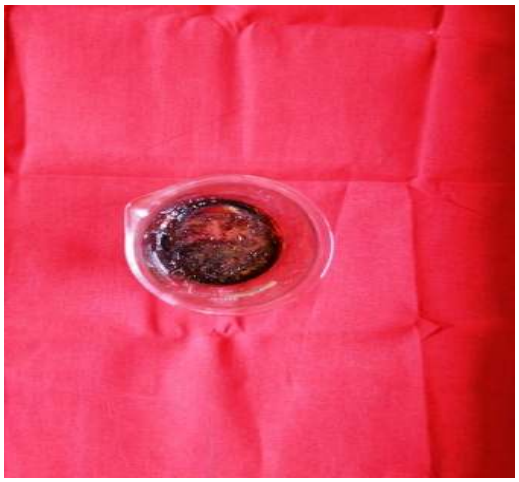


Figure C: Quantitative total flavonoids in leaf

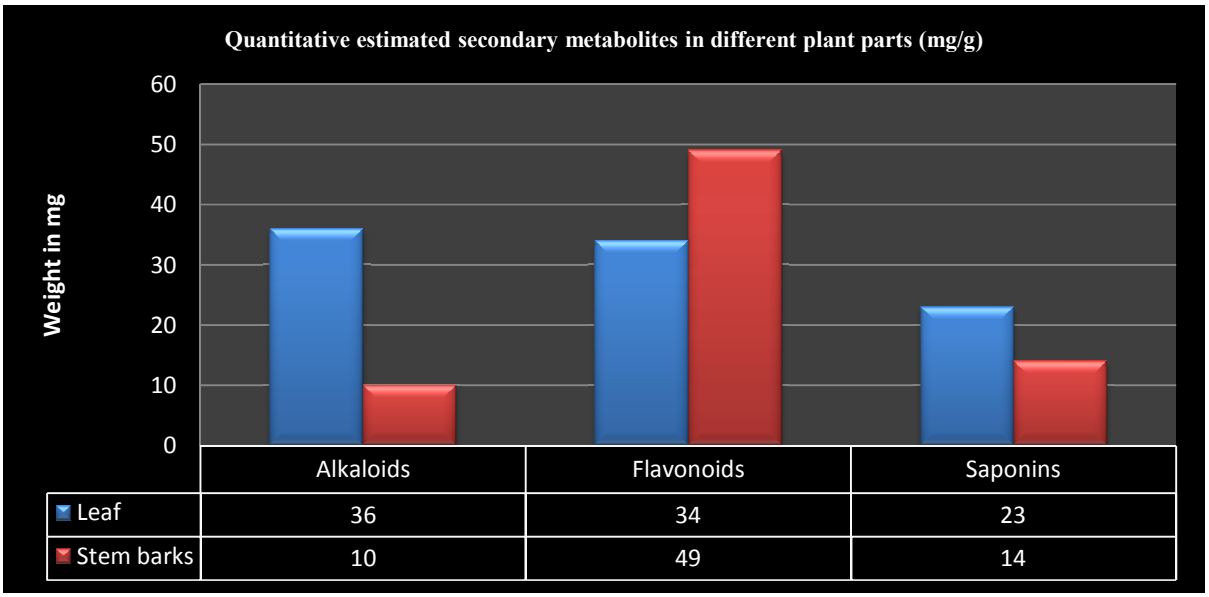
Figure D: Qualitative phytochemical screening in leaf

Table 3: Quantitative phytochemical estimation of leaves of *Gymnema sylvestre*.

S. No.	Secondary metabolites	Total estimated secondary metabolites in gm	Estimated S.M. in per gram of dried powder form of leaf	% Yields of secondary metabolites
1.	Alkaloids	0.18	0.036	3.6%
2.	Flavonoids	0.34	0.034	3.4%
3.	Saponins	0.23	0.023	2.3%

Table 4: Quantitative phytochemical estimation of Stem bark of *Gymnema sylvestre*.

S. No.	Secondary metabolites	Total estimated secondary metabolites in gm as per method	Estimated S.M. in per gram of dried powder form of stem bark	% Yields of secondary metabolites
1.	Alkaloids	0.05	0.010	1.0%
2.	Flavonoids	0.49	0.049	4.9%
3.	Saponins	0.14	0.014	1.4%



CONCLUSION

According to result of preliminary qualitative phytochemical screening it is concluded that the plant contained much number of secondary bioactive compound such as alkaloids, flavonoids, Phenols, tannins, steroids, cardiac glycosides. These secondary metabolites were detected to be present in the leaves which can be used to cure various ailments (above mention) traditionally as well as used to prepare drug by pharmaceutical industries.

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