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RESEARCH ARTICLE

STUDY OF PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF GYMNEMA SYLVESTRE

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ABSTRACT:

Gymnema sylvestre (Asclepiadaceae), popularly known as “gurmar” for its distinct property as sugar destroyer, is a reputed herb in the Ayurvedic system of medicine. The present study was carried out to evaluate the *in vitro* antioxidant activity and preliminary phytochemical analysis of ethanolic leaves extracts of *Gymnema sylvestre*. The qualitative analysis indicated the presence of alkaloids, phenolic compounds, flavonoids, saponins, in the ethanolic extracts.

Keywords: Phytochemical, Antioxidant activity, *Gymnema sylvestre*.

INTRODUCTION:

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects. The antioxidants present in dietary mushrooms are of

great interest as possible protective agents to help the human body reduces oxidative damage without any Interference[1].

Gymea Sylvester is an herb native to the tropical forests of southern and central India and Sri Lanka.[2] Common names include Gymea, Cow plant, Australian Cow plant, and Periploca of the woods. The name itself signifies as it kills sugar. Hence, popularly Gudmar is known as 'the insulin plant'. The plants are available in nurseries and the tablets of this are prepared by pharmaceutical companies. It rises up as a woody climber in tropical forests of central and southern India. So it was easy for them to use it as natural treatment for diabetes for more than two millennium Chemical constituents are gymnemic acid, innositol, hentriacontane, pentatriacontane. According to the horticultural department at Purdue University, it has been used in India for the treatment of diabetes for 2000 years. These drug constituents are useful for the control and treatment of diabetes.[3]

Material and Method: -

Plant Material

Plant material (leaves) of *Gymnema sylvestre* was collected from ruler area of Bhopal (M.P), India in the months of January 2015.

Extraction Procedure [4]

Following procedure was adopted for the preparation of ethanolic extracts from the shade dried and powdered herbs:

Defatting of Plant Material

Powdered plant material of *Gymnema sylvestre* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction by hot continuous percolation process

100 g. of *Gymnema sylvestre* dried plant material were exhaustively extracted with ethanol solvent. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts

Qualitative phytochemical tests [5]

A. Test for Carbohydrates

Following tests were carried out for carbohydrates.

a) **Molisch's test:** In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

b) **Benedict's test:** In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

c) **Barfoed's test:** The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

d) **Anthrone test:** To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

B. Test for Alkaloids

a) **Dragendorff's Test**: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

b) **Wagner's test**: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

c) **Mayer's Test**: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

d) **Hager's Test**: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

C. Test for Steroids and Sterols

Liebermann's Burchard reaction: The test extract solution was dissolved in 2 ml of chloroform in a dry test tube. Now 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.

D. Test for Glycosides

a) **Legal's test**: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

E. Test of Saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

F. Test for Flavanoids

a) **Shinoda test**: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

G. Test for Tannins

a) To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

b) To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

c) To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

H. Test for Triterpenoids

a) In the test tube, 2 or 3 granules of tin was added, and dissolved in 2 ml of thionyl chloride solution and test solution was added. Pink colour was produced which indicates the presence of triterpenoids.

I. Test for Protein and Amino acid

a) Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

b) Ninhydrin's test.

c) Xanthoprotein test

d) Millon's test.

e) Lead Acetate test.

J. Test of Resins

Dissolved the extract in the acetone and pore the solution in the distilled water. Turbidity indicated the presence of resin.

K. Test of Fats or Fixed oils

a) Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

b) Using sodium hydrogen sulphate:

c) Saponification.

Estimation of total Phenolic and flavanoid Content

Total Phenolic content estimation

Principal: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.[6]

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 125µg/ml was prepared in methanol.

Preparation of Extract: 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoid content estimation

Principal: Determination of total flavonoids content was based on aluminium chloride method.[6]

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 125µg/ml were prepared in methanol.

Preparation of extract: 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

***In-Vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH):[6]**

It is a **s**table free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

Procedure:

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of control. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes.

Protocol for DPPH free radical scavenging activity:

a) Preparation of stock solution of test sample: 100 mg of the extract was dissolved in 100 ml of methanol to get 1000 µg/ml solution.

b) Dilution of test solution: 10, 20, 40, 60, 80 and 100µg/ml solution of the test samples were prepared from stock solution.

c) Preparation of DPPH solution: 15 mg of DPPH was dissolved in 10 ml of methanol. The final solution was covered with aluminum foil to protect from light.

Estimation of DPPH radical scavenging activity:

- 75 µl of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading.
- 75 µl of DPPH and 50 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken.
- Absorbance at zero time was taken for each concentration.
- Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Calculation of % Reduction = $\frac{\text{Control Absorbance - Test absorbance}}{\text{Control Absorbance}} \times 100$

RESULTS AND DISCUSSION

Result of Percentage Yield of Different Extract

Yield of Extraction: The crude extracts so obtained after the soxhlet extraction process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from samples using ethanol solvent is depicted in the table. 1.

Table No. 1 Result of Percentage Yield of Different Extract

S. No.	Solvents	Percentage Yield (%)
1.	Pet. Ether	1.2%
2.	Ethanol	4.1%

Results of phytochemical Testing

A small portion of the dried extracts were subjected to the phytochemical test using methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. The outcomes of the results are discussed separately in the table 2.

Table No. 2 Result of Phytochemical Screening of Extracts

S. No.	Constituents	Ethanolic extract
1.	Alkaloids	+
2.	Glycosides	-
3.	Flavonoids	+
4.	Diterpenes	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	-
8.	Proteins	-
9.	Saponins	+

From the results obtained it is clear that the *Gymnema sylvestre* plant shows the presence of alkaloids, glycosides, saponins, tannins, flavonoids, amino acid terpenoids, were found present in leaves parts when extracted with different solvents usingsoxhlet extraction procedure. The phytochemical analysis of *Gymnema sylvestre* plant indicates the presence of phenols and flavonoids present in sufficiently enough quantity according to preliminary phytochemical analysis. Phenolic and Flavonoids are the phytochemicals that are present in ethanol.

Results of Total Phenolic and flavonoid content

Total Phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) and total tannin content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.008x + 0.009$, $R^2 = 0.999$,** where × is the absorbance and y is the Gallic acid equivalent (GAE).

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.008 X+0.007, R^2=0.999**, where X is the absorbance and Y is the quercetin equivalent (QE). The results are given in Table 3.

The total phonolic content in ethanolic extract was 107.47mg of galic acid equivalent/g of dry extract and total flavonoid content was 160.74 mg of quercetin equivalent/g of dry extract. The results are given in Table 3.

Table No. 3 Results of Total Phenolic and flavonoid content

S. No.	Extracts	Total Phenol (mg/gm)	Total Flavanoid (mg/gm)
1.	Ethanolic	107.43	160.74

The results revealed that phenolic and flavanoid content was observed in ethanolic extract *Gymnema sylvestre* 107.43 mg/gm and 160.74 mg/gm respectively.

Result *in vitro* free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl - DPPH)

Table No. 4 Result of *in vitro* free radical scavenging activity

S. No		Ascorbic acid	Plant Extract
	Conc.	% Inhibition	
1	10	25.48	33.29
2	20	51.52	38.55
3	40	68.10	42.76
4	60	84.45	45.31
5	80	87.78	48.10
6	100	88.89	55.17
IC_{50} (µg/ml)		31.56	189.58

Anti Oxidant activity of Plant extract (DPPH Method) percentage Inhibition Vs Concentration

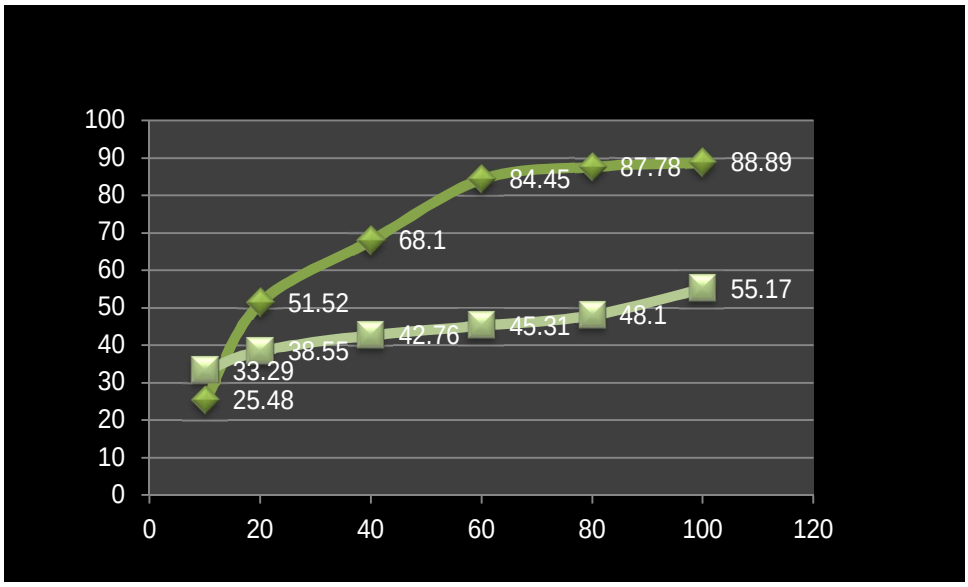

Figure 1: Graph of *in vitro* free radical scavenging activity

DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation. IC50 for standard ascorbic acid was found to be 31.56 µg/ml and for ethanolic extract of *Gymnema sylvestre* was found to be 189.58 µg/ml. Thus the anti-oxidant activity of sample was less than the standard.

Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known. Therefore there observation can be used in pharmaceutical to explore new drugs. Thus the present aim is to assess the antioxidant activity of *Gymnema sylvestre* by DPPH method and also compared the % antioxidant activity with standard ascorbic acid.

Conclusion

The present results revealed that the ethanolic leaves extract of *Gymnema Sylvestre* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the leaves of *Gymnema sylvestre* is very much rich in different types of phytochemical constituents especially

alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. So it can be concluded that ethanolic leaves extract of *Gymnema Sylvestre* can be used as an accessible source of natural antioxidant agent.

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