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Research Article

Screening of phytoactives and antioxidant potentiality in *Gymnema sylvestre*

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ABSTRACT

Gymnema sylvestre is wellknown as “gurmar” for its discrete function as sugar destroyer. It is an excellent herb in the Ayurvedic system of medicine. The herb exhibits a wide range of healing effects as an effectual natural remedy for diabetes, arthritis, asthma, anemia, cardiopathy, constipation diuretic, hypercholesterolemia, indigestion, microbial infections, osteoporosis etc. Realising its importance, The current study was carried out to reveal the distinct phytoactive potentiality in *Gymnema sylvestre*. It suggested that the antioxidant activities of *Gymnema sylvestre* can be used as a natural antioxidant and might be effective to diminish oxidative stress associated with different pathophysiological conditions.

Keywords: *Gymnema sylvestre*, Phytochemicals, antioxidant activity.

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INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover et al., 2002). A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of ondiann traditional health care systems (Scartezini and Sproni., 2000). In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004).

Gymnema sylvestre is a perennial woody vine that grows in tropical areas of India, Africa, and Australia and has been used for medicinal purposes in Ayurvedic medicine. Common names include gymnema (Duke, 2002), Australian cow plant, and Periploca of the woods, and the Hindi term gurmar which means “sugar destroyer” (Quattrocchi U, 1999). The leaves and extracts contain gymnemic acids, the major bioactive constituents that interact with taste receptors on

the tongue to temporarily suppress the taste of sweetness. (Ulbricht C et al., 2011).

The sequencing projects of whole genome and functional exposition of pathway genes have made important contributions in deciphering the biological role and properties of biomolecules. With the useful categorization of genes, their significance in the plant and efficient role in the bioactivity of phytoactives are being recognized. Information about such genes which code for economically feasible bioactive molecules holds vast prediction in crop engineering. The expansion of genetic transformation systems will provide a platform in the proliferation and continuation of such pharmacologically vital plant having applications in drug discovery and development (Pragya et al., 2014).

MATERIALS AND METHODS

Plant Material

The leaves of *Gymnema sylvestre* were collected from Dhanvanthri Vana, Department of Indian System of Medicine and Homeopathy, Karnataka Forest Department. The material was thoroughly washed with tap water to remove soil particles and then shade dried on filter paper. The leaves were chopped into small pieces and were ground to fine powder (100g) and stored in polythene containers at room temperature until required for further use.

Qualitative Phytochemical analysis

Freshly prepared extracts were subjected to preliminary phytochemical analysis to find the presence of the followings phytoconstituents; alkaloids, glycosides, Anthraquinones, Tannins, Phenols, Terpenoids, Steroids, Saponins and Flavonoids. Qualitative tests were carried out using standard procedures to identify the constituents (Lachman et al., 1989, Mojab et al., 2003, Mondal et al. 2006, Nahak et al., 2011).

Test for Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Nahak et al., 2011). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Mondal et al., 2006). The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Anthraquinones: Bomtreger's test was used for the detection of anthraquinones. 5 gm of plant extract was shaken with 10ml of benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet color in the lower phase indicated the presence of free hydroxyl anthraquinones.

Test for tannins: About 0.5g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Phenols: The solvent plant extract was treated with few drops of neutral ferric chloride solution 5% intense color developed which indicated the presence of phenols.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2 ml H₂SO₄. The

colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for saponin: About 2g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample (Lachman et al., 1989, Nahak et al., 2011). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicated the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

RESULTS AND DISCUSSION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Siddique et al., 1997). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases (Sofowora et al., 1993). The result revealed that the ethyl acetate fraction of *Gymnema sylvestre* exhibited the highest DPPH radical scavenging activity with 83.41±0.13% at 120 µg/ml concentration (which is nearly close to the value of Ascorbic acid i.e. 89.65±0.26%) followed by 66.28±0.11%, 52.22±0.17%, 40.33±0.16%, 31.17±0.18% and 19.10±0.13% at the concentrations of 100 µg/ml, 80µg/ml, 60 µg/ml, 40 µg/ml and 20 µg/ml respectively. Similarly in case of methanolic extract the highest inhibition activity i.e 81.17 ± 0.26 % was found at 120 µg/ml followed by 65.15±0.14%, 45.35±0.11%, 39.07±0.12%, 26.26±0.17% and 14.02±0.23% at different range of concentration (100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml and 20 µg/ml) respectively. The order of percentage of scavenging activity in case of aqueous leaf extract of *Gymnema sylvestre* were as follows: 72.11±0.18%, 60.31±0.19%, 45.21±0.36%, 33.37±0.31%, 24.23±0.11% and 13.10±0.14. The antioxidant capacity is also expressed as 50% inhibitory concentration (IC₅₀). A lower IC₅₀ value means a higher antioxidant capacity of the sample. Significantly lowest IC₅₀ value 76.59 µg/ml was observed in ethanolic extracts of *Gymnema sylvestre* which is close to 76.49 µg/ml obtained in the standard ascorbic acid.

Table 1: DPPH scavenging activity of *Gymnema sylvestre* R.Br. leaf extracted in different solvents

Concentration of extracts (µg/ml)	Antioxidant activity (%)			
	Ascorbic Acid	Methanol	Ethyl acetate	Water
20	24.25±0.11	14.02±0.23	19.10±0.13	13.10±0.14
40	34.19±0.13	26.26±0.17	31.17±0.18	24.23±0.11
60	41.37±0.15	39.07±0.12	40.33±0.16	33.37±0.31
80	50.29±0.35	45.35±0.11	52.22±0.17	45.21±0.36
100	65.11±0.17	65.15±0.14	66.28±0.11	60.31±0.19
120	86.65±0.26	81.17±0.26	83.41±0.13	72.11±0.18

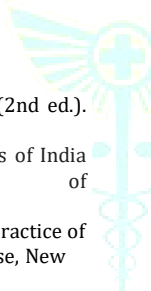
Values represent means ± SD, n=3

CONCLUSION

Gymnema sylvestre is a significant medicinal plant of the family *Asclepiadaceae*. In the existing study Methanol, ethanol and ethyl acetate extracts of shade dried leaves were prepared using standard procedures and used for phytochemical and antioxidant studies. Qualitative analysis of alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates and glycosides was done. Antioxidant activity of leaf extracts was done by DPPH method. The present results revealed that the ethyl acetate leaf extract of *Gymnema sylvestre* R.Br. 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 leaf extract of *Gymnema sylvestre* R.Br. can be used as an accessible source of natural antioxidant agent. Thus the findings of present investigation support the traditional ethanomedicinal claims of *Gymnema sylvestre* for the treatment of diverse infections. All the *Gymnema sylvestre* extracts showed the presence of alkaloids, flavonoids and saponins. This study also leads to the further research in the way of isolation and identification of the active compound from the *Gymnema sylvestre*. The antioxidant activities of *Gymnema sylvestre* can be used as a natural antioxidant and might be effective to diminish oxidative stress associated with different pathophysiological conditions. Moreover, such screening of plants can provide a source of new bioactive compounds with functional properties beneficial to restore health.

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