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IN VITRO ANTIOXIDANT ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ETHANOLIC LEAF EXTRACT OF *GYMNEMA SYLVESTRE* R.BR.

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ABSTRACT

The present study was carried out to evaluate the *in vitro* antioxidant activity and preliminary phytochemical analysis of ethanolic leaf extract of *Gymnema sylvestre* R.Br. The *in vitro* antioxidant activity was evaluated by DPPH radical scavenging activity method. The *Gymnema sylvestre* R.Br. ethanolic extract showed antioxidant activity by inhibiting DPPH. Significant antioxidant activity of ethanolic leaf extract of *Gymnema Sylvestre* R.Br. was found to which might be due to the presence of acidic compound, tannins, saponins, phenols, flavonoids and alkaloids found in the preliminary phytochemical analysis.

Keywords: *Gymnema sylvestre*, antioxidant, phytochemical analysis, DPPH.

INTRODUCTION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [1].

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances [2]. The search for antioxidants from natural sources has received much attention and efforts have been put into identify compounds that can act as suitable antioxidants to replace synthetic ones. Medicinal plants are a source for a wide variety of natural antioxidant [3]. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [4].

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second [5]. Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [6].

Gymnema sylvestre R. Br. commonly known as gudmar belongs to the family Asclepiadaceae. It is a woody, climbing herb grown in India, China, Indonesia, Japan, Malaysia, Srilanka, Vietnam and South Africa [7]. *Gymnema sylvestre* called as Gurmar in Hindi and Periploca of the Woods in English is a highly effective anti-diabetic medicinal herb. Leaves contain lupeol, β-amyrin, stigmasterol, pentriacontane, hentricontane, α and β chlorophyll, resin, tartaric acid, gymnemic acid (anti sweet compounds) the mixture of triterpene saponins, anthraquinone derivatives, alkaloids, betain, choline and trimethylamine [8]. Antisweet constituent of the leaves has been found to be a mixture of triterpene saponins. The sugar residues are glucuronic acid and galacturonic acid while ferulic and angelic acids have been attached as the carboxylic acid. Chewing of leaves reduces sensitivity to sweet substances [9].

The active principles which have been identified

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as glycosides (7 gymnemic acids) suggest that the topical and selective anaesthetic effect of the plant might result from the competition of the receptor sites between glycosides and the sweet substances [10]. In the present investigation an attempt has been made to evaluate in vitro antioxidant activity and prelimnary phytochemical analysis of *Gymnema sylvestre* R.Br.

MATERIALS AND METHODS

Plant materials: Fresh leaves of Gymnema *sylvestre* R.Br. (with Field No - 4789/24.4.94) were collected from the botanical garden of Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India (Figure-1).

Preparation of Plant extracts: The leaves were dried under shade condition and powdered mechanically. 100 g of dry powdered leave samples of *G.sylvestre* was extracted for 8 hours with ethanol in soxhlet apparatus. The collected solutions were filtered through Whatman No-1 filter paper. The extract was evaporated to dryness under reduced pressure at 90^0c by Rotary evaporator and stored at -18^0c in a freeze until used for further analysis.

Preparation of Gymnema Stock Solution. Alcoholic extracts of gymnema prepared at the concentration of 1,000 µg/ml in ethanol. From the stock solution different concentration viz. 20, 40, 60, 80, 100 and 120 µg/ml were prepared in ethanol and used for antioxidant studies.

Preparation of Standard Stock Solution of Ascorbic Acid. Ascorbic acid used as standard for the study and its stock solution was prepared in the concentration of 1,000 µg/ml in ethanol. It was prepared freshly and used immediately for the study. From the stock solution different concentration viz. 20, 40, 60, 80,100 and 120 µg/ml were prepared in ethanol and used for antioxidant studies.

Qualitative Phytochemical analysis: Freshly prepared extracts were subjected to preliminary phytochemical analysis to find the presence of the followings phytoconstituents; alkaloids, cardiac glycosides, Anthraquinoes, Tannins, Phenols, Terpenoids, Steroids, Saponins and Flavonoids. Qualitative tests were carried out using standard procedures to identify the constituents [11,12]**.**

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 [13]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent [14]. 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 2 ml 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 characteristic 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: Bomtregers test was used for the detection of anthraquinones. 5 gm of plant extract was shaked with 10 ml 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 anthroquinones.

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml 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 H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for saponin: About 2 g 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 [15]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was observed indicating 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 3 min. 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.

Antioxidant assay

DPPH Radical Scavenging Activity*:* Plant extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 20, 40, 60, 80, 100 and 120 µg/ml was added to 3 ml of a 0.004% ethanol solution of DPPH. An equal amount of ethanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance of the samples was measured at 517 nm. Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100.$$

The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations [16].

RESULTS AND DISCUSSION

Qualitative Estimation of Phytochemicals

Phytochemical analysis is very useful in the evaluation of some active biological components of medicinal plants. The phytochemical screening carried on the leaves extract of *Gymnema sylvestre* revealed the presence of some active ingredients such as alkaloids, cardiac glycosides, tannins, saponins, anthroquinones, phenols and flavonoids. (Table-1). This analysis determines the biologically active compounds that contribute to the flavour, colour and other characteristics of leaves. The phytochemical screening on qualitative level showed that the leaves of the plant *Gymnema sylvestre* were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity.

For the qualitative analysis results, below is the discussion. The research work that was carried out on the medicinal plant *Gymnema sylvestre* showed the presence of various types of phytochemicals constituents which were shown in Table-1. It shows that tannins, saponins, phenols, flavonoids, alkaloid, anthroquinone and Cardiac glycoside were present where as steroid and terpenoids were found to be absent in *Gymnema sylvestre*. The presence of various active ingredients (secondary plant metabolites) [17] as revealed by the phytochemical screening (Table-1) supports the resourcefulness of the plant extracts [18].

Antioxidant activity

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage [19] Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases [20].

Table-2 shows the results of the free radical (DPPH) scavenging activity in (%) inhibition. The result revealed that the ethanol fraction of *Gymnema sylvestre* exhibited the highest DPPH radical scavenging activity with 87.74% at 120 µg/ml concentration (which is nearly close to the value of Ascorbic acid i.e. 98.25%) followed by 69.41%, 52.25%, 43.37%, 34.26% and 19.10% at the concentrations of 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml and 20µg/ml respectively (Table-2 and Figure-2). Ascorbic acid was taken as a standard for studying the antioxidant activity. It was observed that the antioxidant values were increased with increase in concentration of crude extracts which may be indicated that antioxidant values may be dependent on the presence of different phytochemicals such as alkaloids, flavonoids, saponins, tannins etc. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant [21]. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as alkaloids, flavonoids, phenols and tannins [22,23].

Table 1. Qualitative Phytochemical analysis of ethanolic leaf extract of *Gymnema sylvestre*

Phytochemicals	***Gymnema sylvestre* Ethanolic leaf extract**
Alkaloids	+
Cardiac glycoside	+
Anthraquinone	+
Tannins	+
Phenols	+
Terpenoids	--
Steroids	--
Saponins	+
Flavonoids	+
(+) denotes present and (–) denotes absent	

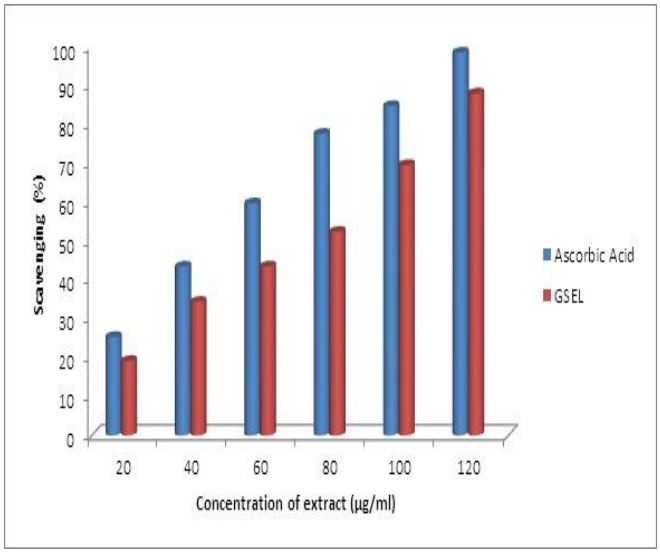
Table 2. DPPH scavenging activity in *Gymnema sylvestre* R.Br. ethanolic leaf extracts

Concentration of extracts (µg/ml)	**Antioxidant activity (%)**	
	Ascorbic Acid	***Gymnema sylvestre* leaf extract**
20	25.25	19.10

40	43.32	34.26
60	59.53	43.37
80	77.40	52.25
100	84.53	69.41
120	98.25	87.74

Figure 1. *Gymnema sylvestre* Plant

Figure 2. DPPH Radical Scavenging activity of *Gymnema sylvestre* ethanolic leaf extract
GSEL denotes – *Gymnema sylvestre* ethanolic leaf extract



CONCLUSION

The present results revealed that the ethanolic 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.

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