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STUDY OF PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *BARLERIA PRIONITIS*

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ABSTRACT

The objectives of this study are to screen the phytochemicals, estimate the content of phenolic and flavonoid compounds and determine the antioxidant capacity of the *Barleria prionitis* flower. *Barleria prionitis* is a famous perennial plant commonly known as porcupine flower or Vajradanti. It is a shrub with yellow flowers and two flat seeds shielded with matted hairs, inhabit most parts of India. Various parts of the plant such as leaves, roots, aerial parts, flowers, and stems are used in the traditional system of medicine. Conventionally, various infusions are prepared using the plant parts and utilized for the treatment of different kinds of diseases. The hydro alcoholic extract of flower of *Barleria prionitis* was studied for antioxidant activity on different *in vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay. Phytochemical analysis revealed the presence of Alkaloid, Glycosides, phenols and flavonoids.. Ascorbic acid used as standards was also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. *Barleria prionitis* flower extract showed IC_{50} value 51.32µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=18.01µg/ml). The present study describes the phytochemical profile and antioxidant activity of *Barleria prionitis* which will further used for medicinal applications.

Keywords: *Barleria prionitis*, phytochemical, Antioxidant activity.

INTRODUCTION

Throughout the human body, free radicals or extremely reactive oxygen species are produced by exogenous chemicals or endogenous metabolic processes. They can oxidize bio-molecules from nucleic acids, enzymes, lipids and DNA and may induce multiple degenerative diseases such as neurological conditions, obesity, emphysema, cirrhosis, atherosclerosis, arthritis, etc. (Halliwell and Gutteridge, 1984; Maxwell, 1995). Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders (Rice-Evans et al., 1996). With the aid of enzymes such as super-oxide dismutase, catalase, and

antioxidant compounds viz, almost all organisms are protected to some extent by free radical disruption. Ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione (Prior and Cao 1992), indicated that antioxidant supplements or dietary antioxidants protect against the harmful effects of free radicals. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuro pharmacology (Dhawan, 1995). Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of *Barleria prionitis* which are traditionally well known for their various activities. *Barleria prionitis* L. (Acanthaceae) is one of the important annual shrub, which is native to tropical areas of East Africa and Asia (India and Sri Lanka), and in South Africa also. The plant has been found abundantly in term of present phytoconstituents and secondary metabolites (Sunil et al., 2010). Beside this the *Barleria prionitis* have also been found effective against many ailments and have been reported with anti-fertility (Pramod et al., 2005), anti-inflammatory (Singh et al., 2003)**.** Hence Present investigation deals with Phytochemical screening and antioxidant potential of hydroalcoholic extract of *Barleria prionitis.*

MATERIAL AND METHOD

Plant material

The flower of *Barleria prionitis*were collected from Vindhya herbal Bhopal, (M.P.). Plant material (flowers) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals

and solvent used in this study were of analytical grade.

Defatting of plant material

Powdered bark of *Barleria prionitis* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

50gm of dried plant material were exhaustively extracted with Hydroalcoholic solvent (ethanol: water: 80:20) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening of the extract

The extract of *Barleria prionitis*was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids (Roopashree et al., 2008).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometer with slightly modification of method (Olufunmiso and Afolayan, 2011). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. 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. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Results and discussions

The percentage yields of Pet ether and hydroalcoholic extract obtained from *Barleria prionitis* are depicted in the Table 2. Preliminary phytochemical studies of the extract were done according to the published standard methods. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging

activity the inhibitory concentration 50% (IC_{50}) value of *Barleria prionitis* hydroalcoholic bark extract was found to be 60.22µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed Table 3. Extracts was capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur containing molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper (I), cobalt (II) and nickel (II) also take part in the process. Thus, the removing is very important for antioxidant defense in cell or food systems.

Table 1 % Yield of barks of *Barleria prionitis*

S. No.	Solvents	% Yield
1	Pet ether	0.85
2.	Hydroalcoholic	6.47

Table 2 Phytochemical screening of extract of *Barleria prionitis*

S. No.	Constituents	Hydroalcoholic extract of Flower of *Barleria prionitis*
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 +Ve +Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve -Ve
4.	Saponins A) Froth Test:	 -Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

Table 3 % Inhibition of ascorbic acid and *Barleria prionitis* hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	42.56	32.56
2	20	53.36	41.45
3	40	60.85	49.98
4	60	72.32	55.65
5	80	79.98	58.89
6	100	86.65	62.23
IC 50		18.01	51.32

Conclusion

The numerous antioxidant extract behavior shown in this analysis clearly shows the possible application benefit of the two plants. However, until its potential use as an antioxidant element, either in animal feeds or in human health products, the in vivo protection of plant needs to be extensively examined in experimental rat model. The above results showed that *Barleria prionitis* hydroalcoholic flower extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

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