Original Article

Evaluation of Antioxidant Potential of *Barleria prionitis* Leaf and Stem

Piush Sharma*[1], Ganesh N. Sharma[2], B. Shrivastava[2] and Hemant R. Jadhav[3]

[1]Maharishi Arvind College of Pharmacy, Ambabari, Jaipur (Raj), India-302023
[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Raj), India-302025
[3]Department of Pharmacy, Birla Institute of Technology & Science, Pilani, Rajasthan, India

Address for Correspondence

Maharishi Arvind College of Pharmacy, Ambabari, Jaipur (Raj), India-302023.
E-mail: joshipiush@gmail.com

ABSTRACT

The aim of the present work was to investigate the antioxidant potential of different extracts of *Barleria prionitis* leaf and stem. The successive extraction of individual plant part was carried out using solvents of different polarity viz. n-hexane, ethyl acetate, methanol and water. The preliminary Phytochemical screening of all the extracts was done. The present total phenolic contents were estimated by Folin- Ciocalteu reagent method and expressed as µg/mg of gallic acid equivalent. The antioxidant potential and reducing power of all the prepared extracts were measured against DPPH as compared to standard ascorbic acid, and BHA respectively. The result data indicated that the phenolic contents were higher in methanolic extracts of leaf (103.51±0.38 mg/g) followed by ethyl acetate (52.91±0.28 mg/g), aqueous extract (42.02±0.36 mg/g) and n-Hexane (12.48±0.27 mg/g). The similar pattern in stem part was also observed, i.e. methanolic extracts (94.37±0.18 mg/g), ethyl acetate (44.31±0.45 mg/g), water (32.82±0.31 mg/g) and n-Hexane (8.33±0.21 mg/g). The antioxidant capacity of methanolic extract of both the part, i.e. leaf and stem were founded highest as IC50 values were 63.41±0.32, 81.69±0.40 respectively. The reducing power was also highest in the methanol extract of both parts. The result data conclude that the higher antioxidant as well as reducing power may be due to present phenolic contents.

Keywords: *Barleria prionitis*, Antioxidant, Reducing power, DPPH, IC50.

INTRODUCTION

Free-radicals are species that are capable of independent existence which contain one or more unpaired electrons. These include superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), thiyl ($RS^{\bullet}$) or trichloromethyl ($CCl_3^{\bullet}$,) in which the unpaired electron is delocalized between both atoms. These free radicals are fundamental to any biochemical process and

also represent an essential part of aerobic life and metabolism. These are continuously produced in body's normal utilization of oxygen, such as respiration and some cell-mediated immune functions, and may also be generated through environmental pollutants, automobile exhaust fumes, pesticides, cigarette smoke, radiation, air pollutants etc. [1], which become part and parcel of our daily inhaling/ingesting life, and appears no escape from them[2].

Antioxidants are fighters against harmful free radicals, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The toxic activity of free radicals and peroxides can be counteracted to some extent by the cellular antioxidant defense system of the body. Antioxidants are involved in the prevention of cellular damages, and the pathway is common for ageing, cancer and a variety of diseases[3]. A wide range of antioxidants, both natural and synthetic are utilized in the treatment of diseases, where the role of oxygen free radicals has been implicated[4,5]. Primary antioxidant enzymes like superoxide dismutase (SOD), catalases, and glutathione peroxidase (GPX) are involved in direct elimination of active oxygen species, while secondary antioxidant enzymes like glutathione-S-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (GGPD) helps in detoxification of reactive oxygen species by decreasing peroxide levels (GST) or by maintaining a steady supply of metabolic intermediates like NADPH (GGPD) and glutathione (GR) for the primary antioxidant enzymes[6,7]. Non-enzymatic antioxidants such as Vit.-E, β-carotene, Vit.-C, cysteine, and ceruloplasmin are also present[8]. Plant derived constituents also contain various micro constituents such as organosulphur compounds, phenolics, flavonoids etc. Antioxidant property of these phytochemical is well established[9-11]. Natural antioxidants are well known to exhibit a wide range of biological effects including anti-inflammatory, antibacterial, antiviral, antithrombic, antiallergic, and vasodilatory activities[12].

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[13,14]. Beside this the *Barleria prionitis* have also been found effective against many ailments and have been reported with anti-fertility[15], anti-inflammatory[16,17] Hepatoprotective[18,19], anti-microbial[20], anthelmintic[21], antidiabetic[22], antidiarrhoeal[23], and many more potentials. The main objective of this study was to compare phenolic content, free radical scavenging activity and reducing power assay of the leaves and stem of *Barleria prionitis* extracted successively from n-Hexane, Ethyl Acetate, Methanol and Water.

MATERIAL AND METHODS

Reagents and chemicals

All the chemicals used in the study were of analytical grade. The reagents used in the preliminary phytochemical study were freshly prepared. Folin Ciocalteu reagent (Qualigens), Gallic acid (Fluka), DPPH (Sigma), Ascorbic acid, Iron chloride, Butylated hydroxy anisole (BHA), Trichloroacetic acid (Merck), Potassium ferricyanide (Thomas Baker) were incorporated into the study.

Collection and authentification of plant parts

The leaves and stem of *Barleria prionitis* were collected from *tehsil* Aamer, Jaipur (Raj.) India, in the month of September to November and was authenticated by Joint Director, Botanical survey of India, Jodhpur. Specimen samples

are stored at the herbarium of Maharishi Arvind college of Pharmacy, Ambabari, Jaipur.

Preparation of plant part extracts

The leaf and stem part was cleaned and shade dried. The samples were broken into small pieces with cutter mill, powdered and passed through sieve no. 44. The leaf and stem samples separately; were extracted successively using a soxhlet apparatus with n-hexane, ethyl acetate and methanol. Finally remaining marc was extracted with water. The collected extracts were vacuum dried and were labeled as indicated in table 1.

Preliminary phytochemical screening of extracts

The collected extracts were subjected to preliminary Phytochemical screening for qualitative determination of phytoconsti-tuents[24,25].

Estimation of total phenolic content

The total polyphenolic content of all extracts was measured by Folin- Ciocalteu reagent method. The absorbance was measured at 760 nm using a UV spectrophotometer (Jasco V530 – UV/VIS/NIR) and they were expressed as µg/mg of gallic acid equivalent[26].

Estimation of free radical scavenging activity by DPPH method

Scavenging free radical potential of collecting extracts was evaluated against a methanolic solution of 1, 1- diphenyl- 2-picryl hydrazyl (DPPH) as method describes by Cengiz 2008[27] and Bhatnagar *et al*, 2014[28]. The 200µM methanolic solution of DPPH was prepared. 1ml of different concentration (10µg to 4 mg/ml) of extract solution and standard Ascorbic acid solution (10- 60 µg/ml) were taken in different values. To this 1 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 min. A blank was also prepared in the similar way and the absorbance was measured at 517nm. Scavenging activity was expressed as the percentage inhibition calculated using the formula.

% Anti-radical activity = Control Abs - Sample Abs X 100 / Control Abs.

Reducing power assay

The reducing capability was measured by the transformation of Fe^{3+}- Fe^{2+} in the presence of different extracts. Different concentrations of extracts (250-2500 µg) in 1ml of water were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50^0C for 20 min. 2.5 ml (10%) of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. 2.5 ml of the upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% Fecl3 solution and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased, reducing power[29, 30]. BHA solution (1000 µg/ml) prepared in phosphate buffer was used as standard.

Statistical analysis

All the extracts of leaf and stem part were analyzed in triplicate to determine antioxidant and reducing power against standard compound(s). The resulting data are presented as Mean ± S.D.

RESULT AND DISCUSSION

% Yield of extracts

The yield of *Barleria prionitis* extract was found to be increased with the increase in polarity of solvent used for the extraction It was found to be minimized with n -hexane (Leaf 0.79%, Stem 0.88%), and was maximum for aqueous extract (Leaf 8.40%,

Stem 6.73%). The resulting data are given in table 2.

Preliminary phytochemical screening results

The Phytochemical screening result data are shown in table 3, which implies that the alkaloids, carbohydrates, glycosides, steroids, flavanoids and phenolic compounds are present in different extracts of leaf and stem, Both. The saponins were found only in leaf extract. Beside this amino acids and proteins were not found present in extracts of both the part.

Total polyphenolic content determination

Barleria prionitis methanolic extract has a maximum value (103.51mg/g from the leaves, 94.37mg/g from stem) while its hexane extract shows 12.48mg/g of leaves, 8.33mg/g for stem. The present phenolic contents were found as given in table 4.

Antioxidant assay of extracts

Results for DPPH free radical scavenging activity

Scavenging activity of various extracts and ascorbic acid was studied against DPPH radicals. All the samples were analyzed in triplicate.

The DPPH assay of BPLH extract found to produce no response (% inhibition) even at concentration 4000µg/ml. The BPLEA, BPLM and BPLW extract at the level of 100µg/ml, 80µg/ml and 120µg/ml showed > 50% inhibition respectively, along with a concentration dependent response. The methanolic extract of *Barleria prionitis* leaves exhibited a maximum DPPH scavenging activity. IC50 value was 63.41±0.32 µg/ml followed by the ethyl acetate and water whose scavenging activities (IC50) were 81.05±0.33 and 119.50±0.43 µg/ml, respectively. The IC50 value of standard Ascorbic acid was also 36.14±0.26.

BPSH extract also produces similar results upto concentration 4000µg/ml as of BPLH extract i.e. no response was generated. The BPSEA, BPSM, BPSW extract, at the concentration level of 120µg/ml, 100µg/ml and 150µg/ml was found to exhibit > 50% inhibition, which was a concentration dependant response. The IC50 value of methanolic extract of stem part was, 81.69±0.40, followed by ethyl acetate and water, whose IC50 values were 123.62±0.66, and 153.31±0.35 µg/ml respectively.

Results for Reducing Power Activity

Result of reducing power assays for antioxidant activity have been recorded for *Barleria prionitis* leaves extracts and compared with standard drug Butylated Hydroxy Anisole (BHA). BPLEA, BPLM and BPLW generated responses were quite good, and were maximum with methanolic extract. Although BPLH also produced somewhat reducing effect, yet it was not upto the mark. The similar finding was also observed with different extracts of *Barleria prionitis* stem. The resulting data were found as in table 5 and 6.

CONCLUSION

A free radical based mechanism for various human diseases are more prone for their acceptance, as they may act on various target levels. Many sources of antioxidants of plant origin have been studied in recent years, as antioxidant based therapy may play major role in coming years in the management of these disorders.

The *Barleria prionitis* most often found on roadsides, and due to the presence of spines it is ignored by cattles and considered as a weed. In the present work we have been trying to establish the antioxidant measures of the said plant, which can be helpful in establishing its therapeutic values. The leaf and stem part of the plant was extracted successively using solvents of different polarity. The extracts were subjected to phytochemical screening to confirm presence of phytoconstituents and secondary

metabolites. The results data regarding the presence of total phenolic contents imply that the methanolic extract of both the parts was most abundant, as compared to other extracts. From this we can conclude that the phenolic compounds can be better isolated with solvents of higher polarity range. The antioxidant potential as well as reducing power of the extracts was determined and was found better in methanolic extract. At the same time the results also demonstrate that the IC50 values of different extracts may be co-related to present phenolic content as it was in proportion to that of concentration of the extract and present phenolic moieties. The results confirm the superiority of the different leaf extracts than the stem part extracts. In this study, it seemed that, the higher total phenolic content of plant extracts resulted in higher antioxidant activity as similarly reported by earlier[31-33].

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Table 1. Labels of successive extracts of leaf and stem of *Barleria prionitis*

Plant part	n-Hexane	Ethyl acetate	Methanol	Water
Leaf	BPLH	BPLEA	BPLM	BPLW
Stem	BPSH	BPSEA	BPSM	BPSW

Table 2. % yield of extracts by successive extraction

Plant part	Extract (% w/w)			
	n-Hexane	Ethyl acetate	Methanol	Water
Leaf	0.79	0.97	2.55	8.40
Stem	0.88	0.84	3.08	6.73

Table 3. Preliminary phytochemical screening of *Barleria prionitis*

Sr. No.	Test	*Barleria prionitis*							
		Leaf				Stem			
		n-Hexane	Ethyl acetate	Methanol	Water	n-Hexane	Ethyl acetate	Methanol	Water
Alkaloids	Dragendroff's test	+	+	+	-	-	+	+	-
	Mayer's test	+	+	+	-	-	+	+	-
	Hager's test	+	+	+	-	-	+	+	-
	Wagner's test	+	+	+	-	-	+	+	-
Carbohydrates	Molish test	-	-	+	+	+	+	+	+
	Fehling's test	-	-	+	+	+	+	+	+
	Barfoed's test	-	-	+	+	+	+	+	+
Glycosides	Kellar-Killani test	-	+	+	-	+	+	+	-
	Borntrager's test	+	+	+	+	-	-	-	-
	Legal's test	+	+	+	+	+	+	+	+
Flavonoids	Shinoda test	-	+	+	+	-	+	+	-
Saponin	Foam test	-	-	+	+	-	-	-	-
	Haemolytic test	-	-	+	+	-	-	-	-
Sterols/Steroids	Salkowaski reaction	+	+	+	-	-	-	+	-
Tannins and Phenolic compounds	5% Ferric chloride solution	-	-	+	+	+	+	+	+
	Lead acetate solution	-	-	+	+	+	+	+	+
	Dil. Potassium permanganate solution	-	-	+	+	+	+	+	+
	Bromine water	-	-	+	+	+	+	+	+
Amino acids	Ninhydrin test	-	-	-	-	-	-	-	-
Proteins	Biuret test	-	-	-	-	-	-	-	-

Table 4. Total polyphenolic content in plant extracts

Plant part	Total phenolic content mg/g equivalent to gallic acid			
	n-Hexane	Ethyl acetate	Methanol	Water
Leaf	12.48±0.27	52.91±0.28	103.51±0.38	42.02±0.36
Stem	8.33±0.21	44.31±0.45	94.37±0.18	32.82±0.31

Table 5. Reducing power of *Barleria prionitis* leaves extracts

S. No.	Conc (µg/ml)	BHA	BPLH	BPLEA	BPLM	BPLW
1	31.2	0.306±0.001	0.007±0.002	0.078±0.002	0.083±0.001	0.070±0.001
2	62.5	0.551±0.002	0.015±0.002	0.124±0.002	0.131±0.002	0.112±0.002
3	125	0.985±0.001	0.027±0.001	0.237±0.002	0.278±0.002	0.190±0.001
4	250	1.631±0.001	0.036±0.001	0.501±0.002	0.606±0.001	0.422±0.002
5	500	2.603±0.002	0.032±0.002	0.760±0.002	0.893±0.002	0.705±0.003

n=3, Data are given as Mean± S.D.

Table 6. Reducing power of *Barleria prionitis* stem extracts

S. No.	Conc (µg/ml)	BHA	BPSH	BPSEA	BPSM	BPSW
1	31.2	0.306±0.001	0.007±0.001	0.067±0.001	0.074±0.001	0.060±0.001
2	62.5	0.551±0.002	0.011±0.002	0.093±0.002	0.119±0.001	0.102±0.002
3	125	0.985±0.001	0.018±0.001	0.186±0.002	0.267±0.002	0.209±0.002
4	250	1.631±0.001	0.021±0.001	0.424±0.002	0.596±0.001	0.441±0.002
5	500	2.603±0.002	0.021±0.002	0.654±0.002	0.882±0.002	0.659±0.003

n=3, Data are given as Mean ± S. D.

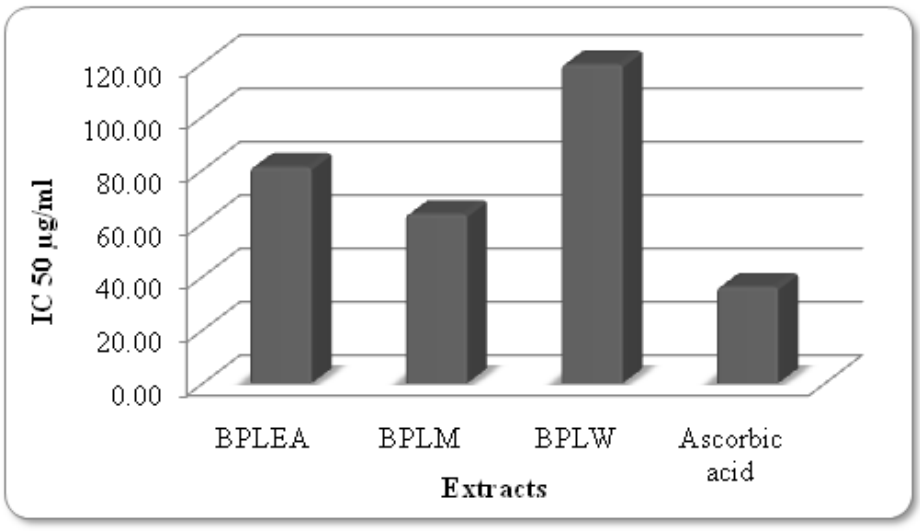

Figure 1. IC 50 of *Barleria prionitis* leaves extracts and ascorbic acid

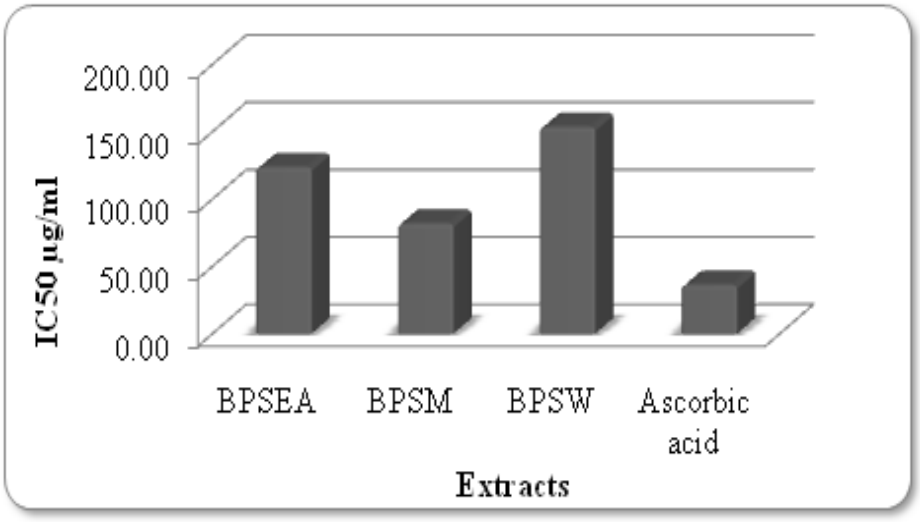

Figure 2. IC 50 of *Barleria prionitis* stem extracts and ascorbic acid

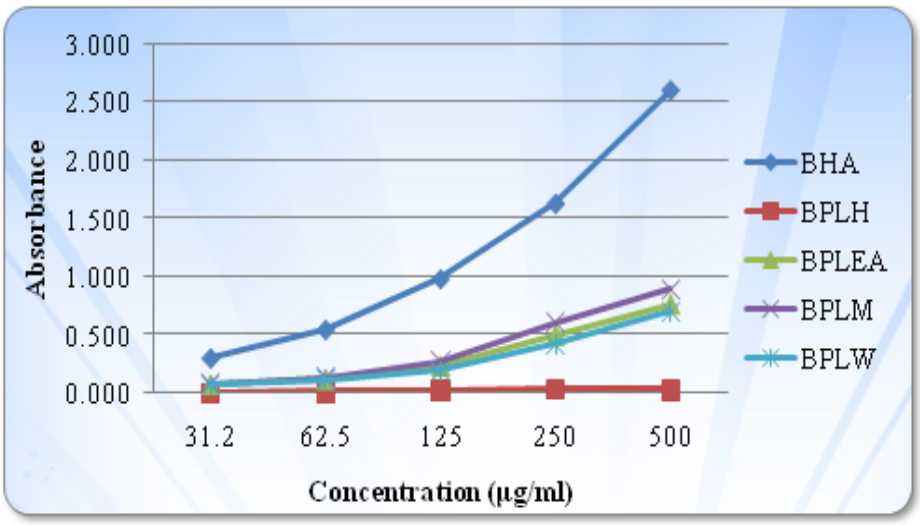

Figure 3. Reductive potential of different concentrations of *Barleria prionitis* leaves extracts

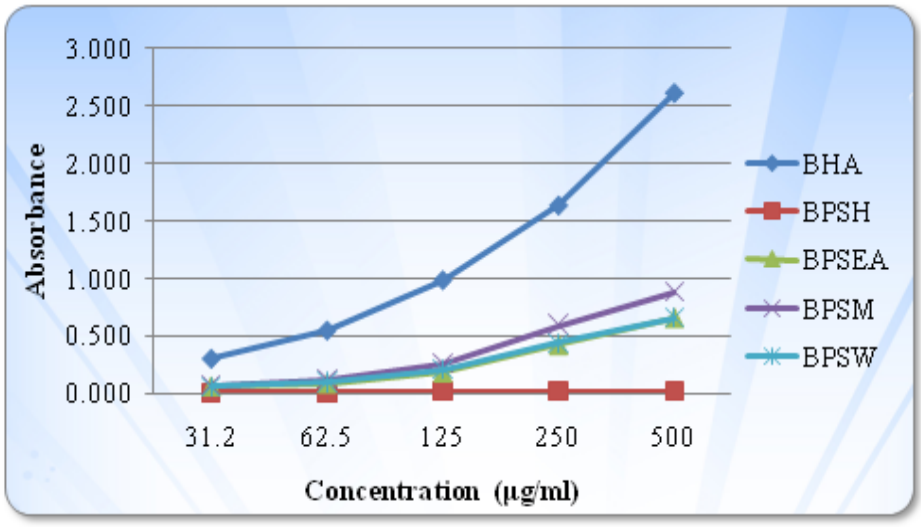

Figure 4. Reductive potential of different concentrations of *Barleria prionitis* stem extracts