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Estimation Of Antioxidant Activity Of Two Locally Available Medicinal Plants Namely *Berginia Ciliata* And *Urtica Dioica* From Darjeeling Hills

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Abstract: In the present study two local medicinal plants namely *Berginia ciliata* and *Urtica dioica* from Darjeeling have been screened for its antioxidant properties. The antioxidant activities were measured *in vitro* by 1, 1-diphenyl-2- Picryl-hydrazyl (DPPH) assay and Phosphomolybdate method using taking ascorbic acid and α-tocopherol as the standard respectively. It could be traced out from that ethanolic extract of *Urtica dioica* exhibited a highest radical scavenging activity at concentration 200µg/ml as 38.7% and methanolic extract of *Urtica dioica*, a relatively high inhibition percentage of 56.9%. A high value of 56.9% activity was observed at concentration 200µg/ml by methanolic extract of *Berginia ciliata*. The total antioxidant value of ethanolic and methanolic extract of *Urtica dioica* are 2.7 and 2.45 µg α-tocopherol equivalent/mg respectively and that of *Berginia ciliata* is high as 5.43 and 7.03 µg α-tocopherol equivalent /mg respectively. From the results obtained, it could be concluded that *U. dioica* and *B. ciliata* from Darjeeling Hills bear significant antioxidant property which could probably contribute to be used as antioxidants.

Keywords- antioxidant, *Berginia ciliata, Urtica dioica,* Darjeeling

1. INTRODUCTION

Indian medicinal plants represent a rich source of antioxidants. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found to have antimicrobial properties *in vitro* (Dahanukar *et. al.,* 2000). In addition to this, work on antioxidant activities of medicinal plants has been carried out by various scientists (Jadhav and Bhutani, 2002; Tajner *et. al*., 2020). Hence, this present study deals with the estimation of radical scavenging activity and total antioxidant activity of two local medicinal plants namely *Berginia ciliata* and *Urtica dioica* from Darjeeling Hills.

2. MATERIALS AND METHODS

2.1 Area of Study

Darjeeling District (27˚ 13'' N to 26˚ 27'' N and 88˚ 53" E to 87˚ 59" E), is the northernmost District of West Bengal. The area under Darjeeling District is flanked by North Dinajpur District from South, Bangladesh from South-East, Bihar from South-West, Nepal from West, Sikkim from North and Bhutan from North-East.

2.2 Collection of medicinal plant samples:

The medicinal plants under study were collected from profusely grown places of Darjeeling and surrounding areas.

2.3 Extraction of samples

Each medicinal plant sample was washed to remove debris, dried and ground to powder and was stored in sterile glass bottle in the refrigerator. The 10g portions of sieved powder were added to 100 ml of solvents (ethanol and methanol), sonicated for 30 min and left overnight at room temperature. The crude extract was prepared by decanting, followed by filtration through muslin cloth and further filtered with Whatman No. 1 filter paper to obtain a clear filtrate. Fifty ml of the filtrate was evaporated to obtain 10 ml of concentrated extract and sterilized by membrane filtration using 450 nm bacteriological filters. (50 ml) filtrates were concentrated to paste in reduced pressure at 40°C using a rotary evaporator and were used for the determination antioxidant assays.

Table1. List of medicinal plant samples

Sl No.	Name of lichens and medicinal plants	Extraction solvent	Extract code
1.	*Berginia ciliata*	ethanol	BERE
		methanol	BERM
2.	*Urtica dioica*	methanol	URRE
		methanol	URRM

2.4 Statistical analysis

Statistical analysis was calculated using Excel software (Microsoft 2007) and SPSS version 21.0 for Windows **2007**. All the results are shown as mean ± standard deviation (SD) of three parallel measurements.

Fig 1 and Fig 2 *Berginia ciliata* and *Urtica dioica*

2.5 Estimation of DPPH radical scavenging activity of medicinal plants

The free radical scavenging activity of the extracts was measured *in vitro* by 1, 1-diphenyl-2- Picryl-hydrazyl (DPPH) assay (Nagarajan *et. al.*, 2008). Solution having strength of 0.3 mM DPPH in ethanol and methanol was prepared and 1 ml of this solution was added to 3 ml of the extract residue dissolved in ethanol/ methanol at different concentrations (25-200 µg/ml). This mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm. Ascorbic acid was taken as reference. The ability to scavenge DPPH radical was calculated by the following equation (Adedapo *et. al.*, 2009):

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(Abs_{control} - Abs_{sample})]}{(Abs_{control})]} \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + solvent; Abs_{sample} is the absorbance of DPPH radical + sample extract /standard.

For the study of combined activities respective solvents extracts from different samples were mixed in equal proportion and used.

2.6 Determination of total antioxidant capacity

The total antioxidant capacity of the extracts was determined with phosphomolybdate method using α-tocopherol as the standard (Nagarajan *et. al.*, 2008). An aliquot of 0.5 ml of the extracts solution was combined with 5 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. The extracts concentration (25-200 µg/ml) was prepared by dissolving the extract residue in respective solvents (ethanol and methanol). After the samples had cooled to room temperature, the absorbance was measured at 695 nm against the blank using an UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. All tests were performed in triplicate, the total antioxidant capacity was expressed as µg equivalents of α-tocopherol by using the standard α-tocopherol graph (Y=0.141x-0.039; R^2 = 0.941).

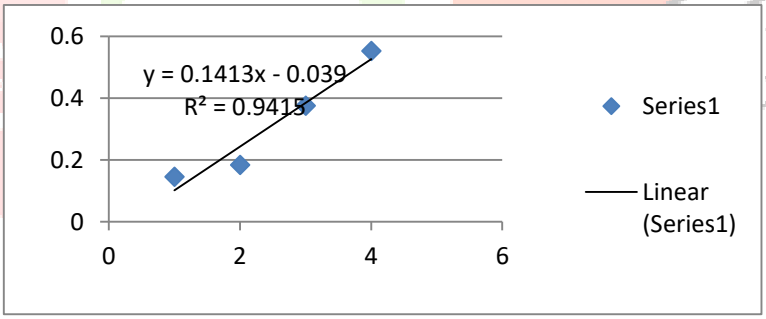

Fig 3.Standard α-tocopherol graph

3 RESULTS AND DISCUSSION

3.1 DPPH radical scavenging activity of medicinal plants

Table 2. DPPH radical scavenging activity of medicinal plants with ascorbic acid as standard.

DPPH Radical Scavenging activity (%)					
Concentration (µg/ml)	Ascorbic acid (Control)	URRE	URRM	BERE	BERM
25	18.87±0.06	11.2±0.02	21.6±0.35	17.6±0.17	15.2±0.51
50	28.37±0.06	22.09±0.16	38.6±0.55	21.8±0.41	28.0±0.01
100	38.78±0.06	30.9±0.2	53.3±0.34	37.9±0.20	49.9±0.48
200	70.58±0.06	38.7±0.11	56.9±0.91	49.4±0.26	56.9±0.50

Two medicinal plants were screened for its radical scavenging activity. The ethanolic and methanolic extracts overall possessed a moderate radical scavenging activity. But it could be traced out from (Table.2), that URRE exhibited a highest radical scavenging activity at concentration 200µg/ml as 38.7% and URRM a relatively high inhibition percentage of 56.9% (Table .2).

The ethanolic and methanolic extract of *B. ciliata* also possessed an appreciable radical scavenging activity. The percentage of activity increased with increase in concentration of extract. A high value of 56.9% activity was observed at concentration 200µg/ml by BERM

3.2 Discussion

Urtica dioica which is a member of Urticaceae class, its Latin name is Nettle it has been used traditionally in treatment of many diseases. There are many reports which show this plant is very effective in the treatment of blood pressure, diabetes and prostate hyperplasia, rheumatoid arthritis and allergic rhinitis (Fathi *et. al*., 2005). DPPH which can be used as indicators for radical scavenging abilities of biological samples are widely used (Wang and Zhang, 2003).

The methanolic extract of *U. dioica* had greater DPPH radical scavenging activity than of the ethanolic extract and standard ascorbic acid at concentrations 25, 50 and 100 µg/ml respectively. Similarly in a screening conducted by Gulcin *et. al*., (2004), the scavenging effect of water extract of *U. dioica* was 32% at a concentration of 60 µg/ml and that of standards quercetin and BHA on the DPPH radical were 93% and37% respectively.

B. ciliata is considered to be one important medicinal plant. Its rhizome extracts is proved to have anti-bacterial and anti-tussive properties. It is reported to be helpful in dissolving kidney stones. *B. ciliata* is used in traditional ayurvedic medicine for the treatment of several diseases in Nepal, India, Pakistan, Bhutan and some other countries. Methanolic extract to be more active radical scavenger than aqueous extract. Similar findings are also reported by Rajkumar *et. al*.,(2010). The obtained results signify that the radical scavenging ability of medicinal plants vary significantly with ascorbic acid.

3.3 Total antioxidant activity of medicinal plants under study

. Table 3. Total antioxidant activity of medicinal plants extracts in at conc. 200 µg/ ml with α-tocopherol as standard

Medicinal plants	µg α-- tocopherol equivalent/ mg
α-tocopherol	4.19±.005
URRE	2.7±0.012
URRM	2.45±0.005
BERE	5.43±0.005
BERM	7.03±0.007

The total antioxidant value of ethanolic and methanolic extract of *Urtica dioica* are 2.7 and 2.45 µg α-tocopherol equivalent/mg respectively (Table.3) On the other hand the antioxidative value of ethanolic and methanolic extract of *Berginia ciliata* is high as 5.43 and 7.03 µg α-tocopherol equivalent /mg respectively (Table.3).

3.4 Discussion

In a study conducted by Gulcin *et. al*., (2003) water extract of nettle *Urtica dioica* (WEN), was subjected to for antioxidant, antimicrobial, antiulcer and analgesic properties including antimicrobial activity against nine microorganisms and antioxidant activity. The total antioxidant activity of WEN increased concentration dependently. WEN (50, 100 and 250 µg) showed higher antioxidant activities than that of α-tocopherol 100 µg. But the antioxidant activity of ethanolic and methanolic extract of *U. dioica* was less than that of the standard α-tocopherol.

In our findings the antioxidant value of ethanolic and methanolic extract of *Berginia ciliata* was higher than the standard α-tocopherol at concentration 200µg/ml. The obtained results show that there is significant difference between the total antioxidant activity of two medicinal plants extracts and the standard compound (α-tocopherol), however ethanolic extract of *B. ciliata* had no significant difference with α-tocopherol.

4. CONCLUSION

Antioxidants derived from medicinal plants have been increasingly investigated for their various health benefits. This present study provides the useful information about proximate composition, antioxidant properties of two local medicinal plants, which are used for the therapeutic purposes in hilly areas. The findings of this study support the fact that some medicinal plants commonly consumed in India are promising sources of potential antioxidants.

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