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STUDY OF THE ANTIOXIDANT POTENTIAL OF SOLVENT EXTRACTS OF RHIZOMES OF *BERGENIA STRACHEYI*

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ABSTRACT

Medicinal plants are gaining global attention owing to the fact that the herbal drugs are cost effective, easily available and with negligible side effects. The beneficial effects of the medicinal plants in health care can be well judged from the WHO estimate that around 80% of the world population uses them in some form or the other. It is important to note that homeopathy and modern medicine have their roots in medicinal plants. The compounds derived from medicinal plants form the ingredients of analgesics, antibiotics, heart drugs, laxatives, anti-cancer agents, ulcer treatments, contraceptives, diuretics etc. Compounds from plants are referred as plant secondary metabolites, phytochemicals, anti-nutritional factors, plant xenobiotics etc. In the present investigation, *in vitro* antioxidant activity of solvent extracts of *Bergenia stracheyi* was determined by total phenolic content, DPPH radical scavenging method, superoxide anion radical scavenging assay and total antioxidant activity. The results confirmed that, polar extracts possessed higher antioxidant activity followed by non polar extracts. It was found that, TPC in ethyl acetate, aqueous and methanol were found to be 246, 235 and 232µg/g gallic acid equivalents followed by petroleum ether and hexane extracts viz. 187 and 134µg/ml. IC_{50} values of ethyl acetate, aqueous and methanol extracts were found to be 22.5, 25.56 and 35.23µg/ml followed by 52.57 and 85.56µg/ml of petroleum ether and hexane extracts respectively. With reference to superoxide anion radical scavenging method polar extracts showed 78-82% inhibition of superoxide followed by non polar extracts having 54-63% inhibition. Total antioxidant activity also followed the same order. Ascorbic acid was used as the standard antioxidant having IC_{50} value 55.45µg/ml in DPPH radical scavenging method and 87.80% inhibition of superoxide. Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the solvent extracts. The ethyl acetate extracts possessed tannin, steroids, saponin and glycosides, alkaloids and flavanoids while aqueous and methanol extracts showed the absence of saponin and glycosides. Petroleum ether extracts possessed only steroids and saponin while hexane extracts showed the presence of saponin only.

KEYWORDS: Antioxidant activity, solvent extracts, *Bergenia stracheyi*, phytochemical screening.

INTRODUCTION

Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of some known drugs. Components with medicinal properties from plants play an important role in conventional Western medicine. In 1984, at least 25% of the Western medicine issued in the US and Canada were derived from or modeled after plant natural products and 119 secondary metabolites were used globally as drugs.[1] It has been estimated that 14-28% of higher plant species are used medically. Only 15% of all angiosperms have been investigated chemically and 74% of pharmaceutically active plant derived components were discovered after following up on ethno medical use of the plant.[2] The traditions of collecting, processing and applying plant and plant-based medications have been handed down from generation to generation. In many African countries, traditional medicines, with medicinal plants as their most important components, are sold in marketplaces or prescribed by traditional healers (without accurate dose value) in their homes. Because of this strong dependence on plants as medicines, it is important to study their safety and efficacy. The value of ethno-medicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine because the search for new potential medicinal plants is frequently based on an ethno-medicinal basis.[3-5] The plants and plant molecules are the rich source of antioxidants. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability.

Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. The present study deals with the determination of antioxidant potential of solvent extracts of the rhizomes of the plant, *Bergenia stracheyi* (Pashan-Bhed) by conventional *in vitro* methods.

MATERIALS AND METHODS

Collection and identification of plant material

The selected plants were collected from Garhwal region of Uttarakhand, India. The plants were further identified from Botanical Survey of India, Dehradun.

Preparation of different solvent extracts

Plant parts was separated, washed with distilled water, dried under shade and pulverized. The method[6] was adopted for preparation of plant extracts with little modifications. Briefly 20g portions of the powdered plant material was soaked separately in different solvents viz. hexane, petroleum ether, methanol, ethyl acetate and distilled water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30^0C.

Determination of antioxidant activity

Estimation of Total phenolic content (TPC) of extracts

The total phenolic content of each extract of the plant material was determined by the method.[7] The phenolic content was expressed as mg/g gallic acid equivalents. In brief 100µl aliquots of the sample were added to 2ml of 0.2% (w/v) Na_2CO_3 solution. After 2 minutes of the incubation. 100µl of 500ml/1 Follin-Ciocalteu reagent was added and the mixture was allowed to stand for 30 minutes at 25^0C. The absorbance was measured at 750nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The total phenolic content (TPC) was determined using the standard gallic acid calibration curve.

Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The extract solution for the DPPH test[8] was prepared by re-dissolving 0.2g of each of the dried crude extract in 10ml of the specific solvent in which the extract was prepared. The concentration of DPPH solution was 0.025g in 1000ml of methanol. Two ml of the DPPH solution was mixed with 20µl of the plant extract solution and transferred to a cuvette. The reaction solution was monitored at 515nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible Systronics spectrophotometer.

The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

Inhibition%= (Abst=0 minutes- Abst=30 min)/ Abst=0 minutes ×100

Where, Abst=0 minutes was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5mM) was dissolved in methanol and used as a standard to convert the inhibition capability of plant extract solution to the ascorbic acid equivalent. IC_{50} is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Determination of Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity was measured with some modifications.[9] The various extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3µM riboflavin, 0.02 M methionine and 5.1uM NBT separately. The reaction solution was illuminated by exposure to 30 W fluorescent lamps for 20 minutes and the absorbance was measured at 360 nm using a spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control. The superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{A_0 - A_S}{A_0} X\ 100$$

Where, A_0 = absorbance of positive control; A_S = absorbance of sample.

Determination of Total antioxidant Activity

Total antioxidant activities of extracts and ascorbic acid were determined. An aliquot (0.1M) of the extracts were combined with 1ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated at 95^oC for 90 minutes. After that the sample were cooled at 25^oC, the absorbance was measured at 695 nm against blank. The blank contained 1ml of reagent solution without sample. The total antioxidant activity was expressed as an absorbance value at 695nm. Higher absorbance value indicates the maximum antioxidant activity.

Phytochemical screening of the extract

The portion of the dry extract obtained was subjected to the phytochemical screening using the method adopted.[10,11] Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides.[12,13]

Test for alkaloids

The 0.5g of the plant extract was dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent. Turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for Tannins
About 0.5g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% $FeCl_3$ was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins.

Test for Flavanoids
About 0.2gm of the extract was dissolved in methanol and heated for some time. A chip of magnesium metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was an indicator of the flavanoids.

Test for Saponin
About 0.5 g of the plant extract was stirred with water in the test tube. Frothing persists on warming was taken as an evidence for the presence of saponin.

Test for Steroids
Salkowaski method was adopted for the detection of steroids. About 0.5g of extract was dissolved in 3ml of chloroform and filtered. To the filtrate, conc. H_2SO_4 was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring.[14]

Test for Cardiac glycoside
About 0.5g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% $FeCl_3$. This was under laid with conc. H_2SO_4. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing Sugars
1ml each of Fehling's solutions, I and II was added to 2ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

RESULTS
The present study suggests that solvent extracts of rhizomes of the plant, *Bergenia stracheyi* had potent antioxidant potential as determined by conventional methods studied. The results suggest that polar extracts had significant antioxidant potential in comparison to non polar extracts of rhizomes of the plant. It was also suggested that, ethyl acetate is the significant and most appropriate solvent for extraction of antioxidant molecules as the antioxidant activity determined by different assays was found to be maximum in ethyl acetate extract in comparison to aqueous and methanol extracts. Also it was suggested that, hexane extracts are not significant to extract antioxidant molecules in comparison to petroleum ether extract as lowest antioxidant activity was found in the hexane extract. The antioxidant activity of the extracts thus follows the order ethyl acetate>aqueous>methanol>petroleum ether>hexane. The results showed that, TPC in ethyl acetate, aqueous and methanol were found to be 246, 235 and 232µg/g gallic acid equivalents followed by petroleum ether and hexane extracts viz. 187 and 134µg/ml **(Table 1; Figure 1).** IC_{50} values of ethyl acetate, aqueous and methanol extracts were found to be 22.5, 25.56 and 35.23µg/ml followed by 52.57 and 85.56µg/ml of petroleum ether and hexane extracts respectively as determined by DPPH free radical scavenging activity. The results suggested that lower the IC50 values, dominant is the antioxidant activity **(Table 2; Figure 2).** With reference to superoxide anion radical scavenging method, polar extracts showed 78-82 % inhibition of superoxide followed by non polar extracts having 54-63 % inhibition **(Table 3; Figure 3).** Total antioxidant activity also followed the same order **(Table 4; Figure 4).** Ascorbic acid was used as the standard antioxidant having IC_{50} value 55.45µg/ml in DPPH radical scavenging method and 87.80 % inhibition of superoxide. The results of phytochemical screening showed the presence of tannin, steroids, saponin and glycosides, alkaloids and flavanoids in ethyl acetate extract while aqueous and methanol extracts showed the absence of saponin and glycosides. Steroids and saponin were found present in petroleum ether extract while hexane extracts showed only the presence of saponin **(Table 5).**

Table 1: TPC (µg/g gallic acid equivalents) of solvent extracts of *Bergenia strachyei.*

Rhizomes extracts	TPC (µg/g gallic acid equivalent)
Ethyl acetate extract (EA)	246
Aqueous extract (AQ)	235
Methanol extract (MET)	232
Petroleum ether extract (PET)	187
Hexane extract (HEX)	134

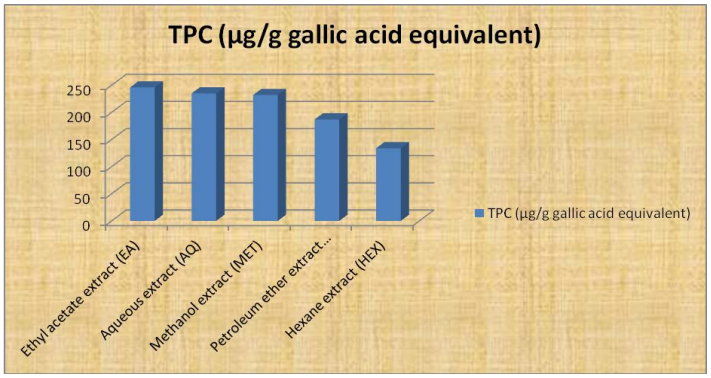

Figure 1: Graphical representation of TPC (µg/g gallic acid equivalents) of solvent extracts of *Bergenia strachyei.*

Table 2: IC50 values of solvent extracts of *Bergenia strachyei.*

Rhizomes extracts	IC50 (µg/ml)
Ethyl acetate extract (EA)	22.5
Aqueous extract (AQ)	25.56
Methanol extract (MET)	35.23
Petroleum ether extract (PET)	52.57
Hexane extract (HEX)	85.56

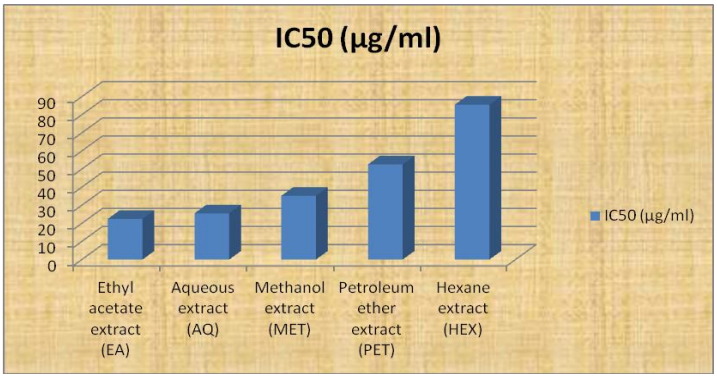

Figure 2: Graphical representation of IC50 values of solvent extracts of *Bergenia strachyei.*

Table 3: Percent inhibition of superoxide anion radical scavenging activity of solvent extracts of *Bergenia strachyei.*

Rhizomes extracts	Percent inhibition of superoxide anion radical scavenging activity
Ethyl acetate extract (EA)	82.0
Aqueous extract (AQ)	76.56
Methanol extract (MET)	72.0
Petroleum ether extract (PET)	63.0
Hexane extract (HEX)	54.0

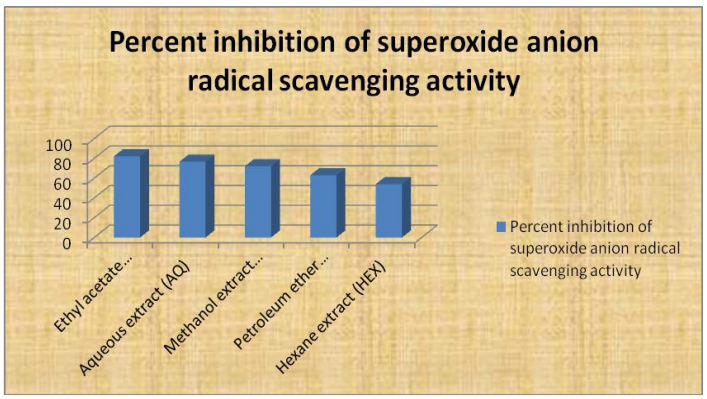

Figure 3: Graphical representation of percent inhibition of superoxide anion radical scavenging activity of solvent extracts of *Bergenia strachyei.*

Table 4: Total antioxidant activity of solvent extracts of *Bergenia strachyei.*

Rhizomes extracts	Total antioxidant activity (Absorbance values @ 695 nm)
Ethyl acetate extract (EA)	0.085
Aqueous extract (AQ)	0.075
Methanol extract (MET)	0.056
Petroleum ether extract (PET)	0.034
Hexane extract (HEX)	0.010

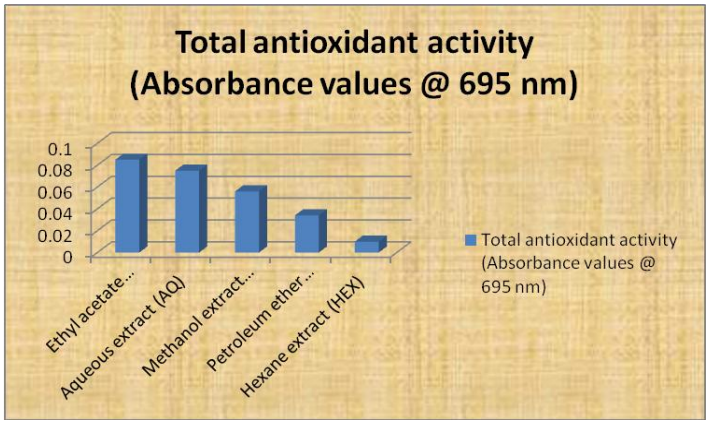

Figure 4: Graphical representation of total antioxidant activity of solvent extracts of *Bergenia strachyei.*

Table 5: Phytochemical screening of the solvent extracts of *Bergenia strachyei.*

	Phyto-constituents						
Solvent extracts	**Alkaloids**	**Tannins**	**Reducing Sugar**	**Flavanoids**	**Saponin**	**Steroids**	**Cardiac Glycosides**
Ethyl acetate extract (EA)	+	+	+	+	+	+	+
Aqueous extract (AQ)	+	+	+	+	-	+	–
Methanol extract (MET)	+	+	+	+	-	+	–
Petroleum ether extract (PET)	–	–	–	–	+	+	–
Hexane extract (HEX)	–	–	–	–	+	–	–

+, present; -, absent.

DISCUSSION AND CONCLUSION

The present study suggests, the effectiveness of the solvent extracts of rhizome of *Bergenia Strachyei* as per the antioxidant potential. The results provide an approach for the isolation and characterization of novel molecules that can be utilized in formulation of antioxidant agents or as an antioxidant molecule as such. Previous studies reported the antibacterial, antioxidant, anti-inflammatory, anti-diabetics and antiviral activities of *Bergenia ciliate*.[15-17] Potential drugs that used against tumors for chemoprevention or chemotherapy are derived from Bergenia ciliate rhizome, methanolic and aqueous extract.[18] *B. ciliate* have potential to work against neoplastic activities due to which act as defensive medicine.[19] Studies also reported that the rhizome of *B. ciliate* work against urolithiasis infections, inflammations, oxidative processes and bacterial infections.[20] Previous studies reported different pharmacological properties of different medicinal plants from Garhwal region of North west Himalayan region.[21-25]

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