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Phytochemical Screening and Free-Radical Scavenging Activity of *Bergenia stracheyi*

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In an effort to reduce the undesirable consequences of synthetic food conservatives in human health and food industries, scientists have recently changed their interest to search new conservatives. Antioxidants are important inhibitors of lipid per-oxidation not only as a defense mechanism of living cells against oxidative damage but also for food preservation. The extracts prepared from *Bergenia stracheyi* herb were screened for flavonoids, tannins, alkaloids, saponins, terpenoids and glycosides. The free-radical scavenging activities of prepared extracts of *Bergenia stracheyi* were carried out using DPPH (1,1–Diphenyl-2-Picryl-Hydrazyl). The results of phytochemical screening of extracts confirmed the presence of amino acids, proteins, carbohydrates, glycosides, phenolics, steroids, and terpenoids in *Bergenia stracheyi.* The results showed that all extracts of *Bergenia stracheyi* had radical scavenging activity. The free-radical scavenging activities of *Bergenia stracheyi* extracts may be due to its polyphenol content. Further research should be carried out to isolates compounds with radical scavenging capacity for industrial applications.

Keyword: Antioxidants, Free Radical, Scavenging Activity, and Bergenia Stracheyi

1. Introduction

Bergenia stracheyi is a perennial herb belongs to genus Bergenia (family- Saxifragaceae). In indigenous and folklore system of medicine, the species of genus *Bergenia* are used for the treatment of various diseases. The many species of Bergenia have been studied for various biological studies[1]. Reactive oxygen species (ROS), such as peroxyl radicals, superoxide radicals, and hydroxyl (OH) radicals, are natural byproducts of the normal metabolism of oxygen in living organisms[2-3]. However, extravagant concentration of ROS may be a primary condition of bio-molecular oxidation and may end in significant damage to cell structure, contributing too many diseases, such as diabetes, stroke, cancer and degenerative processes associated with ageing[4-5].

It has been realized in the biomedical field that a balance exists between antioxidants and pro-oxidants in biological systems and disturbance of that balance in favor of the first can result in "oxidative stress"[6]. Many factors were identified which disturb this balance leading to formation of free radical thought to be associated with pathological and physiological phenomena, such as declining immunity, aging, and many diseases including cardiovascular diseases (CVD), carcinogenesis, metabolic and inflammatory alterations, osteoporosis and age related macular degeneration (AMD). Moreover, environmental factors such as exposure to explosion-generated shock waves[7] and inhalation of oxidant air

pollutants were proposed to be associated with free radicals formation, and antioxidant depletion in living organisms[8]. Antioxidants are important inhibitors of lipid per-oxidation, not only, as a defense mechanism of living cells against oxidative damage but also for food protection[9]. Halliwell and Gutteridge explained antioxidants as a substance that inhibits or suspends the oxidation of the compounds, when present in low concentration in relation to the oxidants. The significance of antioxidants in the safeguarding of health, and the protection from cancer and coronary heart diseases has been established[10]. Antioxidants have been reported to prevent or delay oxidation processes in food products and human body[11]. Antioxidants also prevent the destruction of cells[12-13].

Aromatic plants are rich in phenolic compounds, usually known to as polyphenols, which are prevalence components of herbs and plants. Polyphenols are antioxidants with redox properties, which allow them to act as singlet oxygen quenchers, hydrogen donators, and reducing agents. Some antioxidants showed metal chelation and antimicrobial activities[14-16]. Plant polyphenols with antioxidant capacity could reduce oxidative damage as well as scavenge reactive chemical species. Some plant polyphenols are important components of both human and animal diets and they are safe to be consumed[17]. Antioxidants, whether from pharmacological supplementation or diet, gained important popularity among scientists and lay public in recent years. Antioxidants were claimed to protect or treat numerous ailments. Food antioxidants such as carotenoids, peptides, flavonoids, α-tocopherol, ascorbic acid, amino acids, proteins, and other phenolic compounds might also play a important role as dietary and physiological antioxidants[18].

In an effort to reduce the undesirable consequences of synthetic food conservatives in human health, and food industries, scientists have recently changed their interest to search new conservatives. Aromatic plants are well known for their antioxidant and antimicrobial potential that inhibit food alteration and degeneration[19]. Natural antioxidants are also shown to exhibit a wide range of biological activities including anti-inflammatory, antibacterial, antiallergic, antiviral, vasodilatory and antithrombotic activities[20]. In our previous study, it was observed that no previous work has been done on free radical scavenging activity of *Bergenia stracheyi*[1]. Therefore, this present work was undertaken to determine the radical scavenging potential of *Bergenia stracheyi*.

2. Materials and Methods

2.1 Plant Collection And Extraction

The plants were collected from the Alpine slope of Kumaun Himalaya of Uttarakhand State, India. The plant was identified as *Bergenia stracheyi* by the Department of Botany, D.A.V. (P.G.) College, Dehradun, Uttarakhand. In the laboratory the plant tissues were dried under controlled conditions before extraction. The dried roots, rhizomes, and leaves of *B. stracheyi* were subjected to reduction to coarse powder using hammer grinding mill. The coarse powder of *Bergenia stracheyi* plants was extracted with organic solvents of different polarity (i.e. petroleum ether, chloroform, ethyl acetate and ethanol) in a Soxhlet apparatus. The crude extracts were evaporated to dryness in rotator evaporator under low temperature and reduced pressure.

2.2 Phytochemical Screening

The prepared plant extracts were screened for flavonoids, tannins, alkaloids, saponins, terpenoids and glycosides. Preliminary phytochemical analysis was carried out according to the methods of Sofowora and Harborne[21-22]. The following qualitative chemical tests, for identifying various phyto-constituents present, were carried out on various extracts of *Bergenia stracheyi*.

2.3 Free Radical Scavenging Activity

Free radical scavenging **activity** of various extracts of *Bergenia stracheyi* was carried out using DPPH (1-1 –Diphenyl-2-Picryl-Hydrazyl). DPPH is a molecule containing a stable free radical.

2.4 Reaction with 1-1 –Diphenyl-2-Picryl-Hydrazyl (DPPH)

In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical to free DPPH radical decays and the change in absorbency at 517nm is followed either spectrophotometrically. This simple test can provide information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. In cases where the structure of the electron donor is not known (eg- a plant extract), this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials.

2.5 Free Radical Scavenging Capacity on DPPH Radical (Mechanism)

Free radical scavenging potential of the extracts was tested against a methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1-1-diphenyl -2-picryl hydrazine .The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant.

2.6 Procedure

For the present study the samples were prepared in different concentrations i.e. 10-70µg/ml in AR grade methanol. The samples of above concentrations were mixed with 2ml of 90µM of DPPH prepared in AR grade methanol and make up the final volume up to 4ml with AR grade methanol .The absorbance of the resulting solutions and the blank (with same chemicals except sample) were recorded after 1 hour at room temperature, against BHT .The disappearance of DPPH was read spectrophotometrically at 517nm using a UV-Visible Spectrophotometer. Radical Scavenging Capacity (RSC) in percent was calculated by following equation.

$$RSC = 100\ X\ \frac{A_{blank} - A\ sample}{A_{blank}}$$

Where, RSC = Radical Scavenging Capacity

A_{blank} = Absorbance of blank.

A_{sample} = Absorbance of sample.

From the obtained RSC values the IC_{50} were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization. The all extracts of *Bergenia stracheyi* were studied for free radical scavenging activity.

3. Results and discussion

3.1 Phytochemical screening of various extracts of *Bergenia stracheyi*

The results of preliminary phytochemical screening are given in table no. 1.1. The ethanol extract shows the positive results for the presence of amino acids, carbohydrates, glycosides, phenolic, and steroids. The water and ethyl acetate extracts reveal the presence of amino acids, carbohydrates, glycosides, and phenolic compounds. Chloroform extract shows the positive results for the presence of steroids. The ether extract shows positive results for the presence of terpenoids in *Bergenia stracheyi.* The results show the presence of amino acids, proteins, carbohydrates, glycosides, phenolics, steroids, and terpenoids in *Bergenia stracheyi.*

3.2 Free Radical Scavenging Capacity

The results of free radical scavenging capacity of *Bergenia stracheyi* are shown in table no.1.2. The free radical scavenging capacity was observed at 5 µg, 10 µg, 15 µg, 20 µg, 25 µg, 30 µg, and 35 µg of ethanol, chloroform, ethyl acetate, and ether extracts. The results showed that all extracts of test plant had radical scavenging activity. The ethanolic extract had maximum radical scavenging capacity at 13.86 percent at lowest concentration 5 µg. No plant extracts had 50 percent radical scavenging capacity (IC_{50}) at 5µg, and 10µg concentrations. The free radical scavenging capacity of at 20 µg concentration ethyl acetate and ether extract was observed

55.23 and 58.72 percent respectively. All *Bergenia stracheyi* extracts inhibit 50 percent RSC at 30µg concentration. At the concentration 35µg the ether extract had maximum RSC value (i.e., 86.94 percent), while ethyl acetate had 77.27 percent. The results indicated that the plant of *Bergenia stracheyi* has good radical scavenging capacity. The other plants show antioxidant activities mainly due to the presence of phenyl propanoid derivatives, like polyphenols, besides other secondary metabolites widely distributed in plant kingdom. The free radical scavenging activities of *Bergenia stracheyi* may be due to its polyphenol content.

Table: 1.1 Results of phytochemical screening of various extracts of *Bergenia stracheyi*

Nature	Water	Ethanol	Chloroform	Ethyl acetate	Petroleum Ether
Alkaloids	Negative	Negative	Negative	Negative	Negative
Amino acids	Positive	Positive	Negative	Positive	Negative
Carbohydrates	Positive	Positive	Negative	Positive	Negative
Glycosides	Positive	Positive	Negative	Positive	Negative
Phenolics	Positive	Positive	Negative	Positive	Negative
Steroids	Negative	Positive	Positive	Positive	Positive
Terpenoids	Negative	Negative	Negative	Negative	Positive

Table- 1.2: Free Radical Scavenging Activity of *Bergenia stracheyi* extracts on DPPH radical

Concentration	Radical Scavenging Concentration Values (%)				
	B.H.T.	Ethanol	Chloroform	Ethyl acetate	Ether
5 µg	26.5	13.86	7.25	12.8	13.53
10 µg	42.65	21.67	15.45	24.56	27.25
15 µg	58.56	30.42	25.48	38.36	43.43
20 µg	67.34	39.57	36.32	55.23	58.72
25 µg	79.25	47.48	45.43	67.54	69.56
30 µg	88.12	58.74	56.27	75.62	78.35
35 µg	97.94	67.23	66.18	84.3	86.94
Mean values	65.76571	39.85286	36.05428571	51.20142857	53.96857143

Key: DPPH = 1,1-Diphenyl Picryl Hydrazyl, and B.H.T = Butylated Hydroxy Toluene.

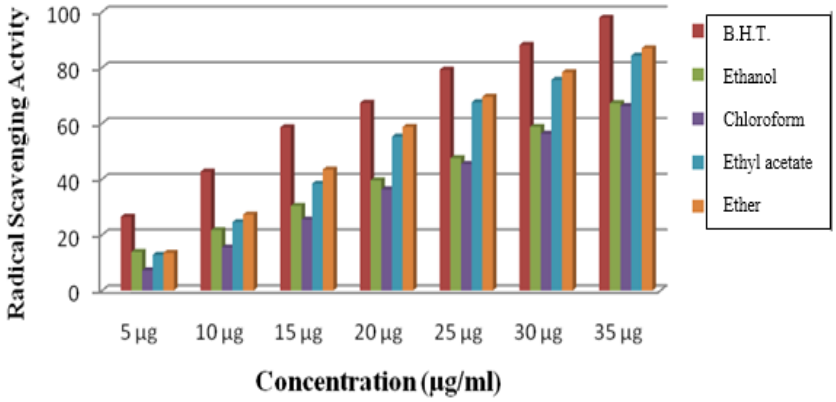

Fig 1.1: Free radical scavenging activity of Bergenia stracheyi extracts on DPPH radical

4. Conclusion

The results of preliminary phytochemical screening of different extracts of *Bergenia stracheyi* reveals the presence of amino acids, proteins, carbohydrates, glycosides, phenolics, steroids, and terpenoids. The free radicals scavenging activity results show that *Bergenia stracheyi* has compounds of radical scavenging potential. The extracting solvents significantly affected the free-radical scavenging property of *Bergenia stracheyi*. Optimal antioxidants can only be obtained by extracting *Bergenia stracheyi* with medium polar solvents like ethyl acetate or acetone. The results of this study indicate that selective extraction from plant material, by an appropriate solvent, is important for obtaining fractions with high antioxidant capacity. The information from this study may direct the pharmaceutical industry to concentrate on radical scavenging capacity of *Bergenia stracheyi*. In future further research should be carried out to isolates the compounds with their free radical scavenging potentials of *Bergenia stracheyi*.

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