In- Vitro antioxidant activity of *Primula denticulata* aerial parts extracts

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Abstract- In this study, we identify the anti-oxidant impact of ethanolic and aqueous extract of aerial parts of *Primula denticulata* and to evaluate the anti-oxidant effect of ethanolic and aqueous extract and compare to it with ascorbic acid and BHT by DPPH Radical Scavenging Assay methods because there is no scientific data available on the anti-oxidant activity of plant. The current investigation also showed that *Primula denticulata* aerial portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may have enormous potential for use as a treatment for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence.

Key words: Oxidative Stress, Anti-oxidants, *Primula Denticulata*, DPPH, Ascorbic acid, BHT.

Introduction

The generation of reactive oxygen species (ROS) and the intracellular ability to eliminate ROS are out of balance, which leads to oxidative stress. It causes excessive damage to all biomolecules, including lipids, proteins, DNA, and RNA[1]. This damage can set off the onset of numerous illnesses, including cancer, oxygen toxicity, ageing, atherosclerosis, lipofuscinosis, and liver injury[2,3]. Anti-oxidants are substances that prevent oxidation or oxidative damage caused by free radicals. As a Results, they can potentially neutralize reactive oxygen species or free radicals. The presence of these phytochemicals in plant products has also led to recent investigations revealing them as possible antioxidants against a range of diseases caused by free radicals[4]. The antioxidant activity of plants is caused by these phytochemicals, which interact with other organisms in the environment to prevent the growth of bacteria or fungi. Because these compounds inhibit infections and have little toxicity to host cells, they are thought to provide the foundation for the development of new antimicrobial medications[5].

Primula denticulata, a member of the Primulaceae family, is a rumbustious, easily growing species that is native to the

Himalayan meadows and lightwoods. Another name for it is drumstick primula. The ideal conditions for *Primula denticulata* growth are partially shaded areas with summertime soil that doesn't dry up. Primrose species are utilized as hepatoprotective, antitumor, diuretic, anti-inflammatory, and bactericidal medicines due to its antioxidant activity[6]. Denticin, denticulatin, and flavonoids are among the triterpene glycosides found in Primula denticulata[7]. The study aimed to explore the anti-oxidant impact of ethanolic and aqueous extract and to evaluate the anti-oxidant effect of ethanolic and aqueous extract by DPPH Radical Scavenging Assay methods because there is no scientific data available on the anti-oxidant activity of the plant.

Material and Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and Butylated Hydroxy Toluene (BHT) were purchased from Shaila enterprise. Analytical grade reagents were utilized in all other cases.

Plant material collection and authentication

Healthy and disease free plants of *Primula denticulata* were collected in August month from the Mussoorie region. The healthy plant species were randomly collected by hand- picking and later identified number is 114999.

Preparation of plant extract

The shade-dried plant/selected parts of plant material was powdered and extracted by (percolation method) soaking in solvent, it also known as cold extraction or in Soxhlet extractor with successive solvents from non-polar to polar. The extract was concentrated to dry under reduced pressure and controlled temperature (40-50ºC). On concentration, yielded respective solvent extracts. The extracts were preserved in a refrigerator and used for phytochemical test to know the class of chemicals present and used for biological screening (Anti-diabetic, wound healing and antioxidant activity). Extract found to be active were further used to isolate the active constituents present in it.

Successive solvent extraction

About 500g of the air-dried powder of the plant's material was extracted successively with petroleum ether (65-85°C), benzene, chloroform, acetone and ethanol (70% v/v) in a Soxhlet apparatus. Each time before extracting with next solvent, marc was air dried below 50°C. Finally, marc was macerated with water (with small quantity of chloroform) for 24 hrs. to obtain an aqueous extract. The aqueous extract was filtered, the solvent was reduced by evaporation and the extracts were weight correctly. Finally, the extractive value (%) was calculated of air dried drug by suitable method.

In-vitro Antioxidant activity

The antioxidant activity of plant extracts was determined by 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) free radical scavenging assay**.** All the assays were carried out in triplicate and average values were considered.

DPPH Radical Scavenging Assay

The free radical scavenging capacity of the ethanolic and aqueous extracts of Tricosanthes tricuspidata roots, primula denticulata arial parts, determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% ethanol. Ethanolic and

aqueous extracts of given plants were mixed with 95% ethanol and water respectively to prepare the stock solution (10 mg/100ml). From this stock solution 1ml., 2ml. & 3ml., of solution were taken in three-three test tubes respectively & by serial dilution with the same solvent, the final volume of each test tube was made up to 10 ml. whose concentration was then 10 µg/ml., 20 µg/ml., 30 µg/ml. respectively for all extracts. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes and after 10 min, the absorbance was taken at 517 nm. using a spectrophotometer (Double beam UV–visible spectrophotometer). Ascorbic acid and Butylated Hydroxy Toluene (BHT) were used as reference standards and dissolved in distilled water to make the stock solution with the same concentration. A control sample was prepared containing the same volume without any extract and reference standards. % scavenging of the DPPH free radical was measured using the following equation.

% DPPH radical-scavenging = (Absorbance of control - Absorbance of test Sample) X 100 (Absorbance Of control)

Results

Table-1 Preliminary phytochemical investigation of *Primula denticulata*

S.No.	Compound	Test	DP		
			CE	EE	WE
01	Alkaloid	Hager's	-	+	+
		Dragendroff's	-	-	+
		Wagners test	-	+	+
02	Flavonoid	Fecl3	-	-	+
		Zn-HCL	-	+	-
		Alkaline	-	-	-
03	Triterpenoids	Salwkowski	-	-	-
		Libbermann- Burchards	-	-	-
04	Carbohydrate	Molish's test	-	-	+
		Barfoed test	-	+	+
		Anthrone test	-	-	+
05	Protein	Millon's test	-	-	-
06	Saponis	Froth test	-	+	+

Phytochemical screening

Preliminary phytochemical screening of *Primula denticulata* aerial extract showed that the plant is rich in various active ingredients (secondary plant metabolites). The Results of the phytochemical screening revealed strong to moderate presence of alkaloids flavonoids saponins and carbohydrates (Table-1).

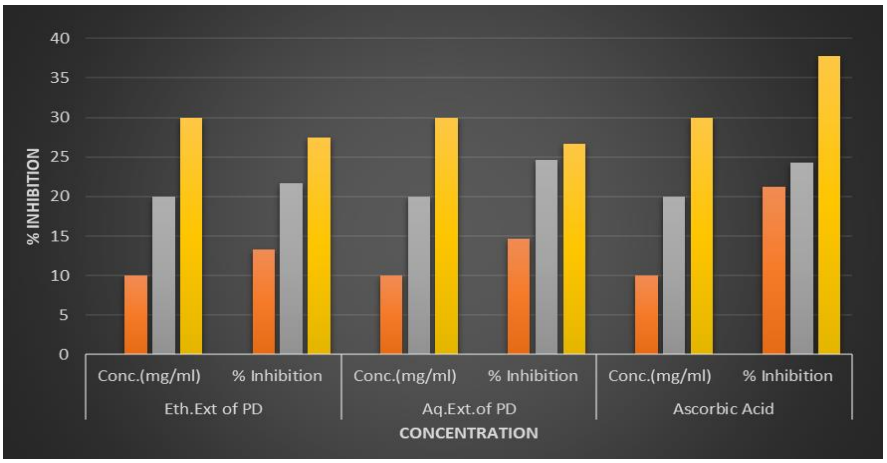
In-Vitro Antioxidant Activity

DPPH radical scavenging activity

DPPH radical scavenging potential of *Primula denticulata* extract at different concentrations investigated in the present study was determined together with standard antioxidant (ascorbic acid Butylated Hydroxy Toluene (BHT) at the same concentrations. *Primula denticulata* extracts (ethanolic and aqueous extracts) showed significant scavenging effect on DPPH free radical in concentration dependent manner. When compared with standard antioxidants used in the experiment, the extract showed relatively lower DPPH free radical scavenging potential.

Table-2 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

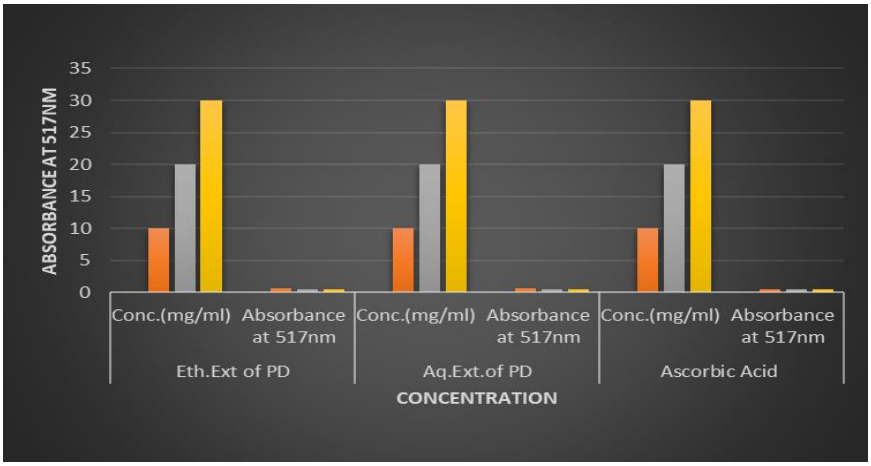
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		Ascorbic Acid	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
01	10	13.34	10	14.69	10	21.28
02	20	21.73	20	24.58	20	24.28
03	30	27.43	30	26.68	30	37.78



Graphical representation showing the % inhibition of DPPH radicals by extracts of *P. denticulata*

Table-3 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

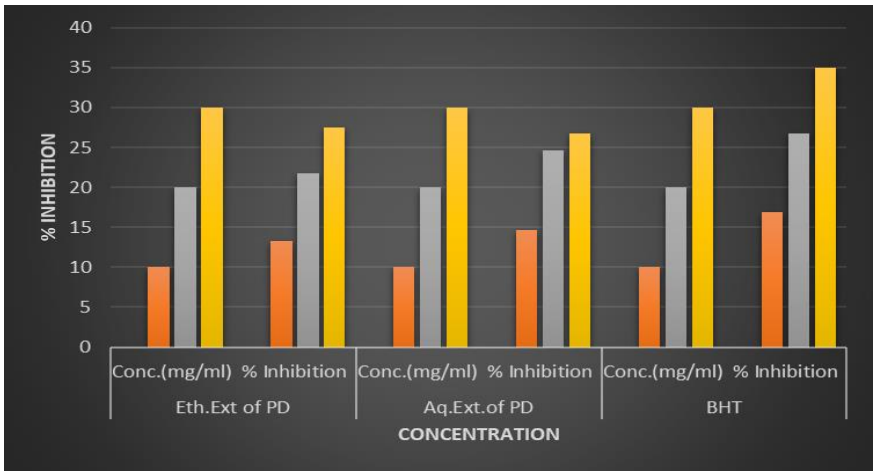
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		Ascorbic Acid	
	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm
01	10	0.578	10	0.569	10	0.525
02	20	0.522	20	0.503	20	0.505
03	30	0.484	30	0.489	30	0.415



Graphical representation showing the absorbance of DPPH radicals by extracts of *P. denticulata*

Table-4 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula denticulata* by DPPH method.

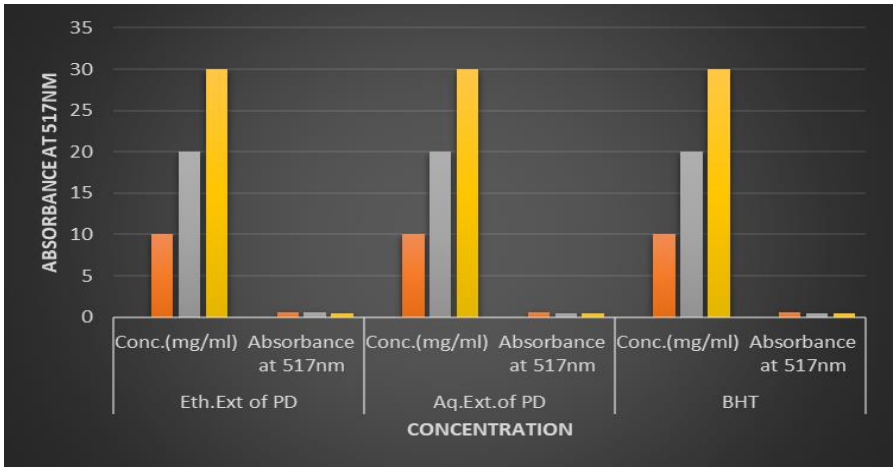
S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		BHT	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
01	10	13.34	10	14.69	10	16.94
02	20	21.73	20	24.58	20	26.68
03	30	27.43	30	26.68	30	34.93



Graphical representation showing the % inhibition of DPPH radicals by extracts of *P. denticulata*

Table-5 Antioxidant activity of aqueous and ethanolic extract of aerial parts of *Primula Denticulata* by DPPH method.

S.NO.	Eth. Ext. of PD		Aq. Ext. of PD		BHT	
	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm	Conc. (µg/mL)	Absorbance at 517nm
01	10	0.578	10	0.569	10	0.554
02	20	0.522	20	0.503	20	0.489
03	30	0.484	30	0.489	30	0.434



Graphical representation showing the absorbance of DPPH radicals by extracts of *P. denticulate*

Discussion

At normal temperature, DPPH is a purple stable free radical with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles the nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired[8]. Aerial extracts from Primula denticulata demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a Results, in vulnerable biological and food systems, *Primula denticulata* leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins, polyunsaturated fatty acids (PUFA), and carbohydrates. The highly reactive species known as hydroxyl radical (HO•) is produced in biological systems and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removing hydrogen atoms from membrane lipids' polyunsaturated fatty acids, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell [9]. The aerial parts extract of *Primula denticulata* demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (Ascorbic acid and BHT) at the same dosages[10].

Conclusion

The current investigation also showed that *Primula denticulata* aerial portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may have enormous potential for use as a treatment for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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