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PHYTOCHEMICAL SCREENING AND FREE REDICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *RHODODENDRON ARBOREUM* (FLOWERS)

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ABSTRACT

Cancer prevention agents expel free radicals from the body which can run wild and really harm cells, causing genuine sickness. Numerous wellbeing experts use them for medicines of stroke and neurodegenerative illnesses, for example, Alzheimer's and Parkinson's. They have additionally been useful in treating mind damage and may slow and even anticipate advancement of malignancies. 1,1-diphenyl-2-picryl hydrazyl (DPPH) was utilized to decide the free radical rummaging movement. IC_{50} value of standard ascorbic acid corrosive for DPPH was 4.46µg/ml and the IC_{50} of the methanol concentration of *Rhododendron arboreum* was 12.06µg/ml that was huge at all contrast and ascorbic acid corrosive. In the present examination, methanol concentration of *Rhododendron arboreum* was screened to assess its free radical rummaging impact.

KEYWORDS: Antioxidants, Free radicals, 1,1-diphenyl-2-picryl hydrazyl (DPPH), Ascorbic acid, *Rhododendron arboretum.*

INTRODUCTION

Oxidative stress is mainly caused by an imbalance between the activity of endogenous pro-oxidation enzymes (such as NADPH oxidase, xanthine oxidase or the mitochondrial dismutase, glutathione peroxidase, heme oxygenase, thio redoxin peroxidase/ peroxiredoin, catalase and paraoxonase). Endogenous reactive intermediates including photoexcited states of tissue chromophores, reactive oxygen species (ROS), reactive carbony species (RCS), transition metal ions and Schiff bases have been implicated in the initiation and progression of diverse human pathologies including tumorgenesis, atheroscelorosis, diabetes and neurodegenerative, disease. Oxidative stress is also implicated in the cognitive deterioration associated with normal aging as well as neurodegenerative disorders such as Alzheimer's and Parkinson's disease.[1] Endothelial cells control vascular homeostasis by generating paracrine factors that regulate vascular tone, inhibit platelet function, prevent adhesion of leukocytes and limit proliferation of vascular smooth muscle. The dominant factor responsible for many of those effects is endothelium derived nitric oxide. Endothelial dysfunction characterized by enhanced inactivation or reduced synthesis of NO, alone or in combination, is seen in conjunction with risk factors for cardiovascular diseases. Endothelial dysfunction can promote vasospasm, thrombosis, vascular inflammation and proliferation of the intima.[1-2] Vascular oxidative stress and increased production of reactive oxygen species contributes to mechanisms of vascular dysfunction and has been implicated to play an important role in a number of cardiovascular pathologies, including hypertension, atherosclerosis, myocardial infarction, ischemia/reperfusion injury, and restenosis after angioplasty or venous bypass grafting.[3] Autoimmune diseases such as type 1 diabetes mellitus (DM1) are believed to result from the failure of immunological tolerance to protein self antigens. It has been proposed that alterations in self antigens could initiate the process of autoimmunity.[4] If the mitochondria are dysfunctional or cells are under stress, such as during high metabolic demand, viral infections, or exposure to certain cytokines/toxins, cells may produce a sufficient amount of radical oxygen or radical nitrogen species (ROS/RNS) to overwhelm the antioxidant systems that normally neutralize these free radicals. A number of oxidative protein modifications have been described in autoimmune diseases.[5] Oxidative modifications produced high molecular weight complexes of glutamic acid decarboxylase (GAD) and sera from type 1 diabetic patients bound these complexes much more strongly than the monomer GAD autoantigen.[6] It has been shown that several of the autoantigens targeted in diffuse scleroderma are uniquely susceptible to cleavage by ROS.[7] Oxidation of beta-2-glycoprotein, a target of antiphospholipid antibodies with hydrogen peroxide rendered this protein able to activate immature monocyte-derived dendritic cells.[8] It has been shown that the insulinproducing beta cells in the islet of Langerhans are particularly vulnerable to damage by free radicals.[9] Involvement of cytochrome P450 (CYP)

enzymes in the pathogenesis of autoimmune hepatitis type 2, occurring via molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytotoxic T cell recognition, is well appreciated.[10] In addition, two different cytochrome P450 enzymes are believed to be the adrenal antigens in autoimmune polyendocrine syndrome type I and Addison's disease.[11] Free radicals are highly reactive molecules or chemical species capable of independent existence. Generation of highly reactive oxygen species (ROS) is an integral feature normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation and fertilization. The production however, multiplies several folds during pathological conditions. The release of oxygen free radicals has also been reported during the recovery phases from many pathological noxious stimuli to the cerebral tissues.[12] The emerging challenge in understanding the pathogenesis of Parkinson's disease includes abnormalities in cellular protein transport, interaction between proteins and protein aggregation (1). Recent advances in both molecular genetics and neurochemistry have shown involvement of excitotoxicity and oxidative stress in cell death.[13] Parkinson's disease is pathologically characterized by loss of catecholaminergic neurons in the brainstem. Numbers of biochemical processes are involved in pathogenesis and progression of neurological disorders. The concept of oxidative stress and antioxidants may be directly or indirectly involved in the pathogenesis of Parkinson's disease.[14-16] *Rhododendron arboreum*, the tree rhododendron,[18] otherwise called Burans or Laligurans or essentially Gurans in Nepal, is an evergreen bush or little tree with a gaudy showcase of brilliant red blooms. It is found in Bhutan, China, India, Myanmar, Nepal, Sri Lanka, Pakistan and Thailand. Rhododendron arboreum is the national bloom of Nepal; in India it is the state tree of Uttarakhand and state blossom of Himachal Pradesh and Nagaland. *Rhododendron arboreum's* nectar is brewed to make wine and is effective in diarrhoea and dysentery. Its Corolla is administered in case of fishbone stuck in the gullet. Snuff made from the bark of the tree is excellent cold reliever. Young leaves can be processed into paste and applied on the forehead to alleviate headaches.

MATERIALS AND METHOD

Plant material

The plant *Rhododendron arboreum* was gathered from Nepal.

Preparation of the crude extract

Cold extraction (Methanol extraction)

The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 180 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained.

Phytochemical Screening[18]

Phytochemical studied of methanol extract of plant material extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book.

Screening for the antioxidant activity

Antioxidant activity of the extract was determined on the basis of their scavenging potential of the stable DPPH free radical in quantitative assay.

Antioxidant tests[19-21]

Stock solution of the plant extract was prepared in methanol (10mg/ml) from which a serial dilution was carried out. At first 6 volumetric flasks are taken to make 6 different types of concentration 1, 5, 10, 50, 100 and 500 µg/ml. Test tubes and volumetric flasks are rapped with foil paper. In 6 volumetric flasks serial dilution of extract is done and marked them respectively.

1ml of sample from each concentration and 3ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. Then solution is kept in dark place for 45 minutes with raping each test tube with foil paper. In another test tube 3ml 0.004% DPPH & 1ml methanol is taken to prepare blank solution. Then absorbance is taken by UV Spectroscopy. The percent of inhibition is calculated by using following formula__

$$\%\text{inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

RESULT AND DISCUSSION

Phytochemical Screening

Results of the phytochemical screening of the Methanol Extract of *Rhododendron arboretum.*

Table 1: Results of Phytochemical Screening.

Chemical Groups	Methanolic Extract of *Rhododendron arboreum*
Saponin	+
Glycoside	+
Flavonoids	+
Tannin	-
Alkaloids	+

Note: (+) = Indicates the presence and (−) = Indicates the absence of the tested group.

Result of Anti-oxidants test

DPPH scavenging assay

Table 2: % inhibition of ascorbic acid and *Rhododendron arboreum*.

Conc. (µg/ml)		Absorbance (nm)		% of Inhibition	
	Blank	Ascorbic Acid	*Rhododendron arboreum*	Ascorbic Acid	*Rhododendron arboreum*
1	0.712	0.612	0.680	14.50	4.50
5		0.438	0.587	38.50	17.50
10		0.163	0.211	77.10	70.36
50		0.075	0.118	89.46	83.42
100		0.073	0.105	89.74	85.25
500		0.062	0.011	91.29	98.45

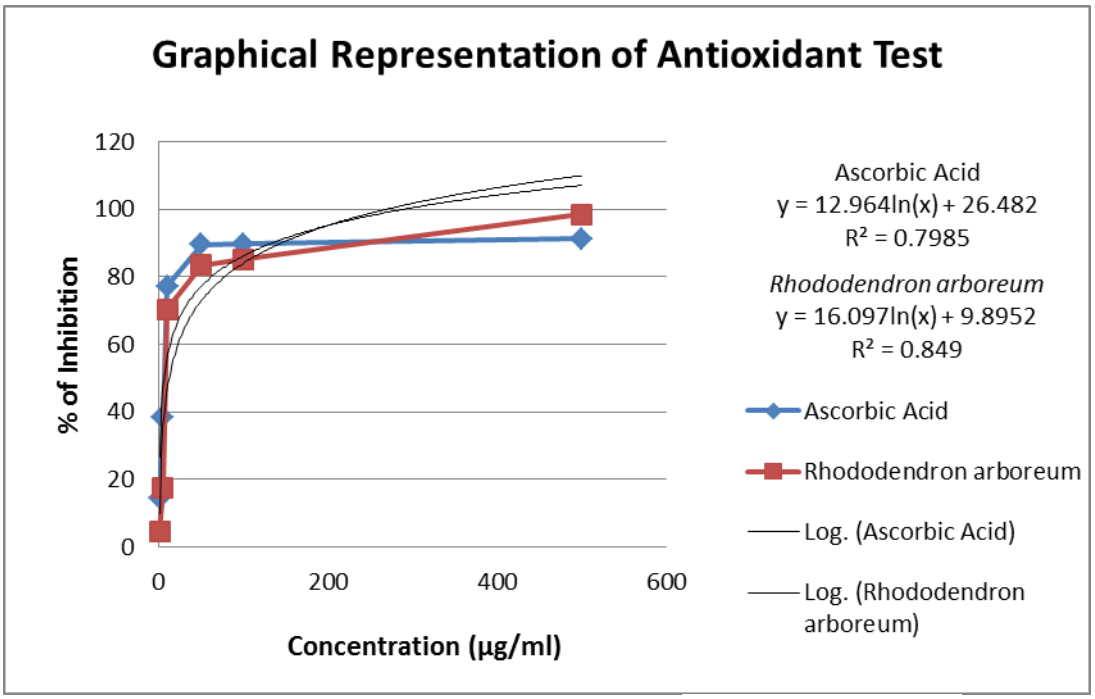

Fig. 1: Anti-oxidant activity of ascorbic acid and *Rhododendron arboreum*.

Table 3: IC_{50} values of the extracts of Ascorbic Acid and *Rhododendron arboreum*.

Test Samples	Regression line	R^2 Value	IC_{50} (µg/ml)
Ascorbic Acid	y = 12.964ln(x) + 26.482	R² = 0.798	6.14
Rhododendron arboreum	y = 16.097ln(x) + 9.8952	R² = 0.849	12.06

DISCUSSION

The cancer prevention agent movement of the methanol separate Rhododendron arboreum was assessed utilizing DPPH free radical rummaging action strategy. DPPH stable free extreme technique is a touchy method to decide the cell reinforcement action of plant extracts.[22-23] Ascorbic acid going about as a chain breaking cancer prevention agent debilitates with the development of free radicals during the time spent arrangement of intracellular substances all through the body, including collagen, bone lattice and tooth dentine.[24-25] The phenols contain hydroxyls that are liable for the radical rummaging impact principally because of redox properties.[26] The methanol concentrate of *Rhododendron arboreum* leaf has noteworthy enemy of oxidant movement. The IC50 of the *Rhododendron*

arboreum is 12.06µg/ml, while IC50 of Ascorbic Acid is 6.14 µg/ml.

CONCLUSION

The present examination revealed that concentrates of the Rhododendron arboreum can be used as a wellspring of cancer prevention agent. At long last we can say that further assessment is required to do in-vivo cancer prevention agent action and find the causative metabolites of Rhododendron arboreum and possible part.

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