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Phytochemical Screening and Antioxidant Potential of solvent extracts of *Rheum webbianum*

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ABSTRACT
Plants diversity represents a significant untapped source of structurally novel compounds that could serve as the lead for the production of novel drugs. The phytochemicals available in medicinal plants are the source of different pharmaceuticals. These medicinal molecules are the significant agents to eliminate the free radicals getting generated in the body due to metabolic processes, thus empowers the ethnomedicinal diversity of antioxidants. The present study showed the antioxidant activity of solvent extracts of *Rheum webbianum*. The results of the study showed that, determined the presence of alkaloids, flavonoids, steroids, saponin and reducing sugars. The results of the study also showed antioxidant potential of the polar extracts which were found significant in comparison to non polar extracts.

Keywords: Antioxidant potential, solvent extracts, Rheum webbianum, ethnomedicinal diversity.

INTRODUCTION
Medicinal plants possessed high potential to showcase its significance as a research ground for novel plants-based molecule to the medical and clinical world. So far, not much phytomedicine (herbal medicine) based research has been conducted in the region. Most of the flora of Garhwal is still untouched and enormous biodiversity has to be explored. Plants also served for the synthesis of some known drugs as a source of new pharmaceutical products and are inexpensive starting materials [1-3]. Within traditional Western medicine components with medicinal properties from plants play a significant role. In the present study, the phytochemical screening and antioxidant potential of solvent extracts of *Rheum webbianum* was evaluated.

MATERIALS AND METHODS
Preparation of Plant extracts
Plant parts were separated, washed with distilled water, dried under shade and pulverized. The method [4] was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of the powdered plant material were soaked separately in different solvents i.e. petroleum ether, hexane, methanol and distilled water based on increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatman filter paper No. 1 (Whatman, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C .

Phytochemical screening of the extract
The portion of the dry crude extracts of the plants utilized for the study were subjected to phytochemical screening using the method adopted. Phytochemical screening will be performed to test for alkaloids, saponin, tannins, flavonoids, steroids, sugars and cardiac glycosides [5-9].

Test for alkaloids
The 0.5 g 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/phenolic compounds
About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few milli liters of 6% Ferric chloride was added to the filtrate. Deep green color appeared confirmed the presence of Tannins.

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Test for Flavonoids
About 0.2 gm 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 Indicator of the flavonoids.

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 a evidence for the presence of saponin.

Test for Steroids, Terpenoids and Triterpenoids
Salkowaski method was adopted for the detection of steroids. About 0.5 g of extract was dissolved in 3 ml 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.

Test for Cardiac glycoside
About 0.5 g of the extract was dissolved in 2ml 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 2 ml 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.

Test for fats
a) Oil Stain test: Petroleum ether extracts were pressed between two filter papers. Oil stains on the papers indicated the presence of fixed oils.
b) Saponification test: To the petroleum ether extracts, few drops of 0.5 N alcoholic potassium hydroxide and a drop of phenolphthalein were added and heated on a water bath for 1-2 hours. Formation of soap and/or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for Resins
1 ml of solvent extracts were treated with few drops of acetic anhydride solution followed by one ml of concentrated sulphuric acid. Resins give colouration ranging from orange to yellow.

Determination of In vitro Antioxidant activity
DPPH free radical scavenging activity
Different solutions of the extracts for the DPPH test [10] were prepared by re-dissolving 0.2 g of sample in 10 ml of the specific solvent. The working solution of DPPH solution was prepared after mixing 0.025 g of DPPH in 1000 ml of methanol. From the above working solution of DPPH, 2 ml of the DPPH solution was mixed with 40 µl of each of the sample solution and transferred to a cuvette. The reaction solution was monitored at 515 nm, 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 min -AbsT=30 min)/ AbsT=0 min ×100
Where, AbsT=0 min was recorded as absorbance of DPPH at zero time and AbsT=30 minutes was recorded as the absorbance of DPPH after 30 minutes of incubation.
Ascorbic acid (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of active constituent solution to the ascorbic acid equivalent. IC50, concentration of the sample required to scavenge 50% of DPPH free radicals was also determined.

Superoxide Anion Radical Scavenging Activity
Superoxide anion radical scavenging Activity was measured with some modifications [11] The active constituent was mixed with 3 ml of reaction buffer solution (pH 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µmolar NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using a spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control.

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The superoxide anion radical scavenging activity (%) will be calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

Where, Ao = absorbance of positive control
As = absorbance of sample

Scavenging of Hydrogen peroxide (H_2O_2)

Percent scavenging of H_2O_2 was determined [12]. A solution of H_2O_2 40 mM was prepared in phosphate buffer (pH, 7.4). H_2O_2 concentration was determined spectro-photometrically from absorbance at 230 nm by using UV-VIS spectrophotometer. Active constituent was added to H_2O_2 solution. The absorbance of H_2O_2 at 230 nm was observed after 10 minutes against a blank solution containing phosphate buffer without H_2O_2. Ascorbic acid was used as a positive control. The % scavenging H_2O_2 was determined as:

$$\frac{Ao - As}{Ao} \times 100$$

Where, A0 = the absorbance of positive control
As = the absorbance of sample

RESULTS AND CONCLUSION

The study was performed in order to determine the antimicrobial activities, metabolites (active principle)/chemical composition and genes responsible for the expression of desired metabolites of *Rheum webbianum*. The phytochemical screening of the polar extracts showed the presence of alkaloids, flavonoids, steroids, saponin and reducing sugars **(Table 1).** The antioxidant activity of solvent extracts were determined via different invitro methods which revealed that the polar extracts showed significant activity in comparison to non-polar extracts **(Table 2).** The results determined the presence of alkaloids, flavonoids, steroids, saponin and reducing sugars. The results of the study also showed antioxidant potential of the polar extracts which were found significant in comparison to non polar extracts.

Table 1: Phytochemical screening of polar extracts of whole plant of *Rheum webbianum*

Solvent extracts	**Phytochemical constituents**						
	Alkaloids	**Flavonoids**	**Tannins**	**Steroids**	**Saponin**	**Glycosides**	**Reducing sugars**
Hydro-alcoholic extract	+	-	-	+	+	-	+
Ethanolic extract	+	-	-	+	+	-	+
Methanolic extract	+	-	-	+	+	-	+

+, present; -, absent

Table 2: Antioxidant activity assays of solvent extracts of *Rheum webbianum*

Extracts and Standard (1 mg/ml)	**Assays of determination of antioxidant activity**		
	DPPH free radical scavenging activity (IC50)	**Superoxide anion radical scavenging activity (Percent inhibition)**	**Scavenging of hydrogen peroxide (Percent inhibition)**
Ethanolic extract	22.45±1.57	23.04±2.08	35.23±2.21
Methanolic extract	20.12±1.67	45.34±1.92	40.12±1.79
Hydro-alcoholic extract	7.56±0.018	72.12±0.015	82.12±0.012
Hexane extract	15.02±0.035	73.45±0.026	72.56±0.045
Chloroformic extract	18.23±0.042	0.83±0.056	63.34±0.035
Standard (Ascorbic acid)	15.67±0.025	1.56±0.03	85.23±0.034

*±SD; Level of significance, $p<0.05$

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