

Study Of Antioxidant Potential Of Medicinal Plants From High Altitude Areas Of Northwest Himalayan Garhwal Region, Uttarakhand State, India

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Abstract

Medicinal plants are the storage houses of natural molecules and metabolites which are nutraceutical and antioxidant agents. These molecules have the ability to fight against free radicals which gets generated within the body via different metabolic processes leading to ageing, cardiovascular diseases and other serious threats. Large-scale research projects are conducted to identify and study various medicinal plants from various geographical areas, particularly in the high altitudes and in the Himalayan region of Garhwal. The present studies were performed to investigate the antioxidant properties of polar and non polar solvent extracts viz. Methanolic, Aqueous and Petroleum ether of medicinal plants viz. *Andrographis paniculata*, *Artemis annua*, *Xanthium strumarium*, *Rheum australes* and *Valeriana jatamansi*. Results of antioxidant activity follows the pattern as that of- Methanolic extract > Aqueous extract > petroleum ether extract. The phytochemical screening of methanolic extracts was performed to qualitatively determine the active molecules present. Further studies are however needed to isolate the possible active molecules responsible for antioxidant activities.

Keywords: Medicinal plants, polar and non polar solvent extracts, antioxidant activity.

Introduction

Plants have been used as medicine in India, China, and Egypt since ancient times, and their importance dates back to the Neolithic era. Plant-derived medicines have been used to treat and prevent disease for over 5,000 years. The use of medicinal plants has been widespread around the world, but their wide use has been restricted to China, India, Japan, Pakistan, Sri Lanka, Thailand and a few African countries. India is known as “the Botanical Garden of the World” because it is the biggest producer of medicinal herbs in the world. India is one of the 12 biodiversity centers in the world, and it is home to over 45,000 plant species. India is unique in the world in that it has 16 agro-climatic zones, 10 vegetation zones, 25 biotic provinces, and 426 biomes, of which about 15,000-200,000 are of good medicinal value. India's Uttarakhand state is renowned for its wide variety of medicinally significant plants and related traditional knowledge. The rural populations in Uttarakhand's Chamoli district have unique lifestyles within their own social and cultural contexts. Regardless matter how complicated or simple they are, many tribes have extensive customs around medicinal plants. Due to the underdeveloped western medical system in this area, people are forced to rely on their traditional medical practices for both the diagnosis and treatment of physiological diseases as well as for the maintenance of health.

¹⁻⁴ Natural antioxidants are mostly sourced from plants and are well-known for their ability to reduce damage caused by reactive species such as nitrogen, oxygen, or even chlorine. Due to their numerous health advantages, antioxidants are regarded as significant nutraceutical.⁵⁻¹² The present investigation is about the screening of solvent extracts of plants of high altitude of North West Himalayan Garhwal region for phytochemical screening and antioxidant potential.

Materials and Methods

Sample collection

The plant parts of *Andrographis paniculata*, *Artemis annua*, *Xanthium strumarium*, *Rheum australes* and *Valeriana jatamansi* were collected from the meadows of Chamoli district of Uttarakhand at an altitude of 2000-2800 meters. The plant material was identified from the Botanical Survey of India, Dehradun, Uttarakhand, India.

Extraction procedure

The extraction procedure was adopted with some modifications¹³. The tubers were washed with running water followed with distilled water in order to remove dust and other contaminants. These plant parts were shade dried at an ambient temperature and coarsely powdered with the help of an electric blender, passed via sieve no. 40 and stored in a closed container for further use. Different organic solvents (petroleum ether, methanol and water) were used for the extraction of polar and non-polar organic compound (s). The extraction was carried out by using 100 g of powdered material of plant parts. The powdered material was extracted with different solvents using soxhlet apparatus for a period of 72 hours at an ambient temperature on the basis of increasing polarity. The extracts were concentrated and dried using vacuum rotator evaporator in order to obtain crude extracts.

Phytochemical screening

The phytochemical screening of solvent extracts viz. petroleum ether, methanol and distilled water was performed for identification of the chemical constituents such as alkaloids, glycosides, carbohydrates, proteins, phenolics, flavonoids, saponins, amino acids, steroids and triterpenoids was performed according to the standard and conventional methods¹⁴⁻¹⁶.

Determination of antioxidant activity

DPPH free radical scavenging activity

The antioxidant activity of crude extracts of different solvents viz. petroleum ether, methanol, and aqueous was determined using DPPH assay¹⁷. Decline in absorbance at 517 nm reflected the decrement of DPPH radical. Ascorbic acid was considered as reference. Scavenging capacity was estimated as –

$$\text{DPPH Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

Ascorbic acid (0.5 mM) was used as the positive control. The antioxidant activity was determined on the basis of IC₅₀ (inhibitory concentration). IC 50 is the amount of concentrated solution of sample needed to inhibit 50% of DPPH free radicals.

Superoxide anion radical scavenging Activity

Superoxide anion radical scavenging activity was measured with some modifications¹⁸. 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 nitro blue tetrazolium (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.

$$\frac{A_o - A_s}{A_o} \times 100$$

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

Where, A_o = absorbance of positive control; A_s = absorbance of sample

Scavenging of Hydrogen peroxide (H₂O₂)

Percent scavenging of H₂O₂ was determined¹⁹. A solution of H₂O₂ 40 mM was prepared in phosphate buffer (pH, 7.4). H₂O₂ concentration was determined spectrophotometrically from absorbance at 230 nm by using UV-VIS spectrophotometer. Active constituent was added to H₂O₂ solution. The absorbance of H₂O₂ at 230 nm was observed

after 10 minutes against a blank solution containing phosphate buffer without H₂O₂. Ascorbic acid was used as a positive control. The % scavenging H₂O₂ was determined as:

$$\frac{A_0 - A_s}{A_0} \times 100$$

Where, A₀ = the absorbance of positive control

A_s = the absorbance of sample

Results and Discussion

Phytochemical screening

The methanolic extracts of all the plants were used for phytochemical screening. The results showed the presence of all phytochemicals viz. carbohydrates, alkaloids, glycosides, flavonoids, phenols, saponins and tannins in the methanolic extracts of the plants. The results are shown in **Tables 1-5**.

Antioxidant activity

The results of the antioxidant activity suggest that, polar extracts of plants had significant antioxidant potential in comparison to non-polar extracts. The result of antioxidant activity follows the order as-

Methanolic extract > water (aqueous) extract > petroleum ether extract. The results are shown in **Tables 6-10**

The results of the study suggest the importance of plant extracts of the plants as per phytochemical screening and antioxidant activity. The results of the study are in correlation with the previous findings²⁰⁻²².

Table 1: Phytochemical screening of methanolic extract of *Andrographis paniculata*

S.No.	Test	<i>Andrographis paniculata</i>
1.	Carbohydrates Molisch's test	+
	Fehling's test	
	Benedict's test	+
2.	Alkaloids Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
3.	Glycosides Killani's test	+
4.	Phenols Folin – Cioclteau's test	-
5.	Flavonoids H ₂ SO ₄ /Mg test	+
6.	Saponin Foam's test	+

7.	Tannins Gelatin's test	+
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*+, present; -, absent

Table 2: Phytochemical screening of methanolic extract of *Artemis annua*

S.No.	Test	<i>Artemis annua</i>
1.	Carbohydrates Molisch's test	+
	Fehling's test	
	Benedict's test	+
2.	Alkaloids Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
3.	Glycosides Killani's test	+
4.	Phenols Folin – Cioclteau's test	-
5.	Flavonoids H ₂ SO ₄ /Mg test	+
6.	Saponin Foam's test	+
7.	Tannins Gelatin's test	+

*+, present; -, absent

Table 3: Phytochemical screening of methanolic extract of *Xanthium strumarium*

S.No.	Test	<i>Xanthium strumarium</i>
1.	Carbohydrates Molisch's test	-
	Fehling's test	
	Benedict's test	+

2.	Alkaloids Mayer's test	+
	Wagner's test	-
	Dragendroff's test	+
3.	Glycosides Killani's test	+
4.	Phenols Folin – Cioclteau's test	+
5.	Flavonoids H ₂ SO ₄ /Mg test	+
6.	Saponin Foam's test	+
7.	Tannins Gelatin's test	+

*+, present; -, absent

Table 4: Phytochemical screening of methanolic extract of *Rheum australes*

S.No.	Test	<i>Rheum australes</i>
1.	Carbohydrates Molisch's test	+
	Fehling's test	+
	Benedict's test	+
2.	Alkaloids Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
3.	Glycosides Killani's test	+
4.	Phenols Folin – Cioclteau's test	+
5.	Flavonoids H ₂ SO ₄ /Mg test	+

6.	Saponin Foam's test	+
7.	Tannins Gelatin's test	+

*+, present; -, absent

Table 5: Phytochemical screening of methanolic extract of *Valeriana jatamansi*

S.No.	Test	<i>Valeriana jatamansi</i>
1.	Carbohydrates Molisch's test	+
	Fehling's test	
	Benedict's test	+
2.	Alkaloids Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
3.	Glycosides Killani's test	+
4.	Phenols Folin – Cioclteau's test	+
5.	Flavonoids H ₂ SO ₄ /Mg test	+
6.	Saponin Foam's test	+
7.	Tannins Gelatin's test	+

*+, present; -, absent

Table 6: Antioxidant activity of methanolic extract of *Andrographis paniculata*

Extracts and Standard (1 mg/ml)	Results for determination of antioxidant activity
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	DPPH free radical scavenging activity (IC50)	Superoxide anion radical scavenging activity (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)
Methanolic extract	0.04±0.056	68.12±0.042	60.07±0.045
Aqueous extract	0.05±0.062	56.08±0.056	52.56±0.058
Petroleum ether extract	0.08±0.087	40.02±0.075	45.05±0.078
Standard (Ascorbic acid)	0.02±0.034	86.56±0.038	85.23±0.03

*Level of significance, $p < 0.05$

Table 7: Antioxidant activity of methanolic extract of *Artemis annua*

Extracts and Standard (1 mg/ml)	Results for determination of antioxidant activity		
	DPPH free radical scavenging activity (IC50)	Superoxide anion radical scavenging activity (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)
Methanolic extract	0.067±0.056	70.20±0.045	64.27±0.058
Aqueous extract	0.082±0.065	56.12±0.058	54.02±0.052
Petroleum ether extract	0.090±0.085	34.04±0.072	38.23±0.075
Standard (Ascorbic acid)	0.02±0.034	86.56±0.038	85.23±0.03

*Level of significance, $p < 0.05$

Table 8: Antioxidant activity of methanolic extract of *Xanthium strumarium*

Extracts and Standard (1 mg/ml)	Results for determination of antioxidant activity		
	DPPH free radical scavenging activity (IC50)	Superoxide anion radical scavenging activity (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)
Methanolic extract	0.068±0.056	80.12±0.045	68.26±0.058
Aqueous extract	0.072±0.065	52.08±0.058	50.45±0.052
Petroleum ether extract	0.075±0.085	45.12±0.072	25.12±0.075
Standard (Ascorbic acid)	0.02±0.034	86.56±0.038	85.23±0.03

*Level of significance, $p < 0.05$

Table 9: Antioxidant activity of methanolic extract of *Rheum australes*

Extracts and Standard (1 mg/ml)	Results for determination of antioxidant activity		
	DPPH free radical scavenging activity (IC50)	Superoxide anion radical scavenging activity (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)

Methanolic extract	0.052±0.048	65.07±0.052	78.12±0.045
Aqueous extract	0.065±0.052	48.02±0.058	65.02±0.056
Petroleum ether extract	0.082±0.075	40.01±0.078	45.05±0.082
Standard (Ascorbic acid)	0.02±0.034	86.56±0.038	85.23±0.03

*Level of significance, $p < 0.05$

Table 10: Antioxidant activity of methanolic extract of *Valeriana jatamansi*

Extracts and Standard (1 mg/ml)	Results for determination of antioxidant activity			
	DPPH free radical scavenging (IC50)	Superoxide anion radical scavenging activity (Percent inhibition)	Scavenging of hydrogen peroxide (Percent inhibition)	
Methanolic extract	0.067±0.056	64.27±0.058	0.067±0.056	
Aqueous extract	0.082±0.065	54.02±0.052	0.082±0.065	
Petroleum ether extract	0.090±0.085	38.23±0.075	0.090±0.085	
Standard (Ascorbic acid)	0.02±0.034	86.56±0.038	85.23±0.03	

*Level of significance, $p < 0.05$

Conclusion

The results of the present study suggest that, the medicinal plants and their parts used in the study viz. *Andrographis paniculata*, *Artemis annua*, *Xanthium strumarium*, *Rheum australes* and *Valeriana jatamansi* are the source of effective phytochemical and nutraceutical agents. The methanolic extracts were found to extract almost all important molecules from the respective plant parts. The results of the study can be utilized to formulate novel antioxidants or poly herbal nutraceutical preparations that can be utilized to scavenge free radicals getting generated in the body. It is also thus advised to utilize the studied plants or their parts in daily diets or as a medicine to minimize the risks of free radicals generation within the body.

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