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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF EXTRACT PREPARED FROM LEAVES OF *DIGITALIS PURPUREA*

YOGESH CHANDRA NAINWAL*

Department of Chemistry, Dr. Shivanand Nautiyal Government Post Graduate College, Karnprayag, Uttarakhand, India.
Email: yogeshcnainwal@gmail.com

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ABSTRACT

Objective: The present study deals with the phytochemical screening and free radical scavenging activity of leaf extract of *Digitalis purpurea*.

Methods: The phytochemicals from the leaf extract of *D. purpurea* were extracted qualitatively in methanol and confirmed by performing various chemical tests. The free radical scavenging activity of leaves extract was performed using DPPH method with solvents of different polarity, namely, petroleum ether, ethyl acetate, and methanol. The IC_{50} (50 % inhibition of DPPH) value of free radical scavenging activity of leaf extract was calculated from the graph for each solvent.

Results: The leaves extract showed presence of various biologically important phytochemicals such as cardiac glycosides, flavonoids, saponins, anthraquinone, carbohydrates, steroids, phenolic compounds, and aminoacids. The IC_{50} value of DPPH free radical scavenging activity in petroleum ether, ethyl acetate, and methanolic extract of leaves was found 0.982±0.009 mg/mL, 0.538±0.006 mg/mL and 0.480±0.01 mg/mL respectively.

Conclusion: The present study reveals that *D. purpurea* is medicinally important plant and it can be well exploited for making a diverse range of pharmaceutical drugs. The present investigations also give an insight to scientists and researchers to come up with innovative ideas for effective eradication of different diseases.

Keywords: *Digitalis purpurea*, Cardiac glycosides, Flavonoids, Saponins, DPPH.

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INTRODUCTION

Plants are the rich source of primary and secondary metabolites [1]. The primary metabolites help in growth while the secondary metabolites show remarkable physiological effects on animals. The antioxidants found in plant show good efficacy of removing or neutralizing the activity of reactive oxygen species also called free radicals (viz. Hydroxy radicals [HO.], Hydroperoxy radicals [HOO.], Lipid radicals, Protein radicals, etc.). The free radicals are formed in body as a result of metabolic activities. Several studies in past have shown that antioxidants prevent harmful oxidative process by transferring electron to free radicals thereby suppressing the activity of free radicals and thus, help in maintaining Human health.

The plant *Digitalis purpurea*, commonly called Fox glove, belongs to the family *Plantaginaceae*. It is an herbaceous plant having a life span of 2–3 years. The plant is grown in different parts of Europe and Asia. For conducting the present study, the plant was collected from chamoli district of Uttarakhand state in India at an elevation of 1800 m from sea level. The leaves are spirally arranged on the plant, the flowers are tubular in shape and purple or violet in color. The flowers appear in long hanging racemes. The plant *D. purpurea* is a rich source of various primary and secondary metabolites. The major secondary metabolites extracted from the methanolic extract of leaves of plant involve cardiac glycosides, flavonoids, saponins, anthraquinone, steroids, and phenolic compounds.

In the present study, the free radical scavenging activity of antioxidants was determined by DPPH method using ascorbic acid as standard [2]. The free radical scavenging activity was evaluated in leaves extract with different solvents, namely, petroleum ether, ethyl acetate, and methanol having different polarity. The IC_{50} value of DPPH free radical scavenging activity was also calculated from graph in different solvents, namely, petroleum ether, ethyl acetate, and methanolic extract of leaves.

METHODS

All chemicals of analytical grade were used in the entire experiment.

Collection of plant

The plant *D. purpurea* was collected from Chamoli district of Uttarakhand state in India at an elevation of 1500 m from sea level.

Sample preparation

The plant was washed with water to remove any undesirable material and dried in dark for 20 days at room temperature. Fine powder of the dried leaves was prepared with the help of grinder. 100 g of powder was mixed with 500 mL of methanol and extracted with the help of soxhlet apparatus at 60–85°C for 24 h. The suspension was then filtered and the methanolic extract was dried on rotavapor drier at 60–85°C. The dried leaf extract was dissolved in suitable solvent for further phytochemical analysis.

Phytochemical screening of plant extract

Test for cardiac glycosides

Keller Killiani test

5 mg of dried plant extract was mixed with 5 mL of water followed by addition of 2 mL of glacial acetic acid having one drop of ferric chloride solution. Now, 1 mL of Conc. H_2SO_4 was added from the side wall of test tube. Generation of brown or purple ring confirmed the presence of cardiac glycosides. The cardiac glycosides show remarkable therapeutic action in various diseases [3-5].

Test for alkaloids

Development of yellow color on mixing 5 mL of plant extract with 5 mL of Meyer's reagent (Potassium mercuric iodide) shows presence of alkaloids.

Test for saponins

Foam test

2 mL of plant extract was mixed with 5 mL of distilled water and shaking was carried out for 15 min, generation of foam layer confirmed the presence of saponins in plant extract.

Test for steroids

Salkowaski test

2 mL of plant extract was mixed with 5 mL of $CHCl_3$ and Conc. H_2SO_4 was added from the side wall of test tube. The upper chloroform layer showed red color while lower H_2SO_4 layer showed greenish-yellow glow and thus, confirmed the presence of steroids.

Test for anthraquinone

Borntrager test

To 2 mL of leaf extract, 2 mL of 10% NH_3 solution was mixed and shaken vigorously for 1 min; generation of red color indicated the presence of anthraquinone.

Test for flavonoids

Alkaline reagent test

The detection of flavonoids was carried out by adding 10% aqueous sodium hydroxide solution to 4 mL of methanolic leaf extract of plant, development of yellow color which turned colorless on addition of hydrochloric acid confirmed the presence of flavonoids.

Shinoda test

To 4 mL of methanolic plant extract added few pieces of Mg ribbon and 1 mL of HCl. Generation of red color confirmed the presence of flavonoids. The flavonoids act as growth regulators and help in combating oxidative stress [6-10].

Test for carbohydrates

5 mg of dried plant extract was mixed with 5 mL of distilled water followed by the addition of 2 mL of Molisch's reagent (α –naphthol dissolved in ethanol) and few drops of Conc. H_2SO_4. Development of purple ring confirmed the presence of carbohydrates.

Test for aminoacids

Generation of violet color on heating for few minutes after adding ninhydrin reagent with 2 mL of aqueous plant extract indicates the presence of amino acids.

Test for phenolic compounds

50 mg of dried plant extract was dissolved in 5 mL of distilled water and few drops of 5% neutral ferric chloride were added to it. Development of green color shows presence of phenolic compounds.

Free radical scavenging activity

The free radical scavenging activity of leaf extract in different solvents was carried out by DPPH method as stated by using ascorbic acid as standard. DPPH method has been quite frequently used in determining free radical scavenging activity [11-16].

Preparation of leaf extract

Fine powder of 300 g of shade dried leaves of *D. purpurea* was extracted separately by Soxhlet apparatus at 60–85°C for 24 hours with different solvents, namely methanol, ethyl acetate and petroleum ether. The extracts were dried on reduced pressure by rotavapor at 60–85°C.

Preparation of DPPH

0.1 mM DPPH solution was prepared in methanol as it is insoluble in water (in case of aqueous mixture the water content must not exceed 60% as described by Stasco *et al.*[17]). The solution was prepared in dark and was protected from light.

Preparation of Samples

Sample of 100, 200, 400, 600, 800, and 1000 µg/mL concentrations of dried leaf extract of *D. Purpurea* was prepared separately in different solvents, namely methanol, ethyl acetate and petroleum ether.

Preparation of Standard

Absorbance of ascorbic acid of (100, 200, 400, 600, 800, and 1000 µg/mL) was also measured during the experiment.

Measurement of absorbance

1 mL of each concentration was added to 3 mL of solvent-DPPH solution followed by vigorous shaking. The mixture was now kept in dark for 30 min at room temperature. The absorbance was measured for three replicates of same concentration by double beam UV-VIS spectrophotometer at 517 nm.

Calculation

The % free radical scavenging activity of each concentration of sample and standard was calculated from following formula.

$$\%\,Activity = \frac{A \quad B}{\quad} \times 100$$

A= Absorbance of Control at 0 min
B=Absorbance of test after 30 min

RESULTS AND DISCUSSION

The phytochemicals of *D. purpurea* were extracted with different solvents viz. methanol, ethyl acetate and petroleum ether. The extraction was carried out on Soxhlet apparatus at 60–85°C for 24 hours. The extract was dried under reduced pressure on rotavapor at 60–85°C. The extract showed the presence of various primary and secondary metabolites, namely cardiac glycosides, flavonoids, saponins, anthraquinone, carbohydrates, steroids, phenolic compounds, and amino acids.

The cardiac glycosides were qualitatively analyzed by *Keller Killiani test* showing brown ring. Development of yellow color confirmed the presence of alkaloids using Meyer's reagent (Potassium mercuric iodide). The anthaquinones in the extract were evaluated by Borntrager test showing appearance of red color. Development of yellow color which turned colorless on addition of hydrochloric acid confirmed the presence of flavonoids and the Shinoda test was also performed to confirm flavonoids. Development of purple ring confirmed the presence of carbohydrates on addition of *Molisch's reagent* (α – naphthol dissolved in ethanol).

The steroids in leaf extract were detected by performing Salkowaski test. The presence of saponins is evaluated by Foam test. The appearance of blue color by Ninhydrin reagent confirmed the presence of amino acids. Development of green color on addition of few drops of neutral ferric chloride confirmed the presence of phenolic compounds in the extract (Table 1).

The free radicals promote harmful oxidative processes in the body; hence, the free radicals have to be captured and eliminated from the body. The free radical scavenging activity of the extract of leaves was studied with the help of UV-VIS spectrophotometer at 517 nm in different solvents having different polarity. It was observed that the free radical scavenging activity increases with the increase of polarity of solvent. The decrease in absorbance of solvent- DPPH solution with the increase of sample concentration also reveals the presence of antioxidants in the extract. The highest free radical scavenging activity was observed in 1000 µg/mL sample concentration.

The petroleum ether extract of plant leaves showed 52.51±0.30% activity (Table 2) in 1000 µg/mL sample concentration.

Table 1: Qualitative phytochemical analysis

Active ingredient	Observation	Inference
Cardiac glycosides	Keller-Killiani test	Positive
Alkaloids	Meyer's test	Positive
Saponins	Foam test	Positive
Steroids	Salkowaski test	Positive
Anthraquinone	Borntrager test	Positive
Flavonoids	Shinoda test Alkaline reagent test	Positive
Carbohydrates	Molisch test	Positive
Amino acids	Ninhydrin test	Positive
Phenolic Compound	Ferric Chloride test	Positive

Table 2: Absorbance of pet. ether extract of *Digitalis purpurea*

S. No.	Conc. µg/mL	% Activity±SD (n=3)
1	100	12.09±0.30
2	200	15.34±0.29
3	400	21.73±0.45
4	600	28.02±0.29
5	800	41.49±0.46
6	1000	52.51±0.30

Table 3: Absorbance of ethyl acetate extract of *Digitalis purpurea*

S. No.	Conc. µg/ml	% Activity±SD (n=3)
2	100	22.19±0.45
3	200	30.60±0.44
4	400	36.27±0.45
5	600	57.18±0.29
6	800	74.39±0.44
7	1000	79.08±0.44

Table 4: Absorbance of methanol extract of *Digitalis purpurea*

S. No.	Conc. µg/mL	% Activity±SD (n=3)
2	100	25.22±0.41
3	200	32.94±1.02
4	400	40.68±0.60
5	600	60.48±0.73
6	800	80.96±0.60
7	1000	88.31±0.44

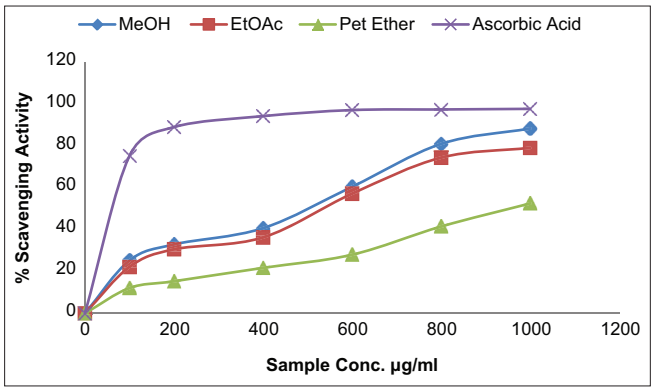

Fig. 1: Comparison of DPPH free radical scavenging activity in different solvents along with ascorbic acid

The ethyl acetate extract of plant leaves showed 79.08±0.44% activity (Table 3).

The methanolic extract of plant leaves showed 88.31±0.44% activity (Table 4) in 1000 µg/mL sample concentration.

The free radical scavenging activity of test samples was compared with standard ascorbic acid (Fig. 1).

The IC_{50} value (Showing 50 % inhibition of DPPH) was calculated from the graph for each leaf extract. The IC_{50} value for petroleum ether extract of leaves was found 0.982±0.009 mg/mL. The IC_{50} value for ethyl acetate and methanolic extract of leaves was calculated as 0.538±0.006 mg/mL and 0.480±0.01 mg/mL (Fig. 1), respectively.

CONCLUSION

The phytochemical screening of plant *D. purpurea* reveals that it contains potentially rich bioactive secondary metabolites. The presence of antioxidants as free radical scavengers and cardiac glycosides provide valuable information regarding the further exploitation of plant in formulations of various medicines of future.

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AUTHORS CONTRIBUTION

The entire experimental work was carried out by the single author.

CONFLICTS OF INTERESTS

No conflicts of interest were reported by the author in this experimental work.

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