



**PHYTOCHEMICAL SCREENING AND EVALUATION OF FREE RADICAL
SCAVENGING ACTIVITY OF EXTRACT PREPARED FROM LEAVES OF PLANT
LEUCAS LANATA**

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ABSTRACT

The present study deals with the phytochemical screening and free radical scavenging activity of leaf extract of ethno medicinally important plant *Lucas lanata*. The phytochemicals from the leaf extract of *Lucas lanata* were extracted qualitatively in methanol and confirmed by performing various chemical tests. The leaves extract showed presence of various biologically important phytochemicals like cardiac glycosides, flavonoids, alkaloids, saponins, invert sugars, and phenolic compounds. The free radical scavenging activity of leaves extract was performed by using DPPH method with solvents of different polarity viz. petroleum ether, methyl acetate and methanol. The IC₅₀ (50 % inhibition of DPPH) value of free radical scavenging activity of leaf extract was calculated from the graph for each solvent. The IC₅₀ value of DPPH free radical scavenging activity in petroleum ether, methyl acetate and methanolic extract of leaves was found 0.505 ± 0.003 mg/ml, 0.523 ± 0.004 mg/ml and 0.943 ± 0.006 mg/ml respectively. The present study reveals that *Lucas lanata* is medicinally important plant and it can be well exploited for making a diverse range of pharmaceutical preparations.

KEYWORDS: *Lucas lanata*, cardiac glycosides, flavonoids, saponins, DPPH.

INTRODUCTION

Plants have always been recognized as the rich source of food and medicines. Plants contains primary and secondary metabolites.^[1-3] The metabolites found in plants 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. 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 *Lucas lanata*, belongs to the family Lamiaceae. It is an herbaceous plant having a life span of two to three 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 1500m from sea level. The plant *Lucas lanata* 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, alkaloids, reducing sugars and phenolic compounds.

In present study the free radical scavenging activity of antioxidants was determined by DPPH method using ascorbic acid as standard.^[4,20-21] The free radical scavenging activity was evaluated in leaves extract with different solvents viz. petroleum ether, methyl acetate and methanol having different polarity. The IC₅₀ value of DPPH free radical scavenging activity was also calculated from graph in different solvents Viz. petroleum ether, methyl acetate and methanolic extract of leaves.

MATERIAL AND METHODS

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

Collection of Plant

The plant *Lucas lanata* was collected from Chamoli district of Uttarakhand state in India at an elevation of 1500m 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 grams of powder was mixed with 500 ml of methanol and extracted with the help of soxhlet apparatus at 60-85°C for 24 hours. The suspension was then filtered and the methanolic extract is 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. H₂SO₄ 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.^[5-7]

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 minutes, generation of foam layer confirmed the presence of saponins in plant extract.

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.^[8-12]

Test for invert sugars

5 mg of dried plant extract was mixed with 5 ml of distilled water followed by the addition of 2 ml of Fehling Solution and heating of solution. Development of red colour confirmed the presence of invert sugars.

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 *Neguemfo et al.* using ascorbic acid as standard. DPPH method has been quite frequently used in determining free radical scavenging activity.^[13-18]

Preparation of leaf extract

Fine powder of 350 grams of shade dried leaves of *Lucus lanata* was extracted separately by Soxhlet apparatus at 60-85°C for 24 hours with different solvents viz. Methanol, Methyl Acetate and Petroleum ether. The extracts were dried on reduced pressure by rotavapor at 60-85°C.

Preparation of DPPH

0.1mM 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.*^[19]). The solution was prepared in dark and was protected from light.

Preparation of Samples

Sample of 100, 200, 400, 800 and 1000 µg/ml concentrations of dried leaf extract of *Lucus lanata* were prepared separately in different solvents viz. Methanol, Methyl Acetate and Petroleum ether.

Preparation of Standard

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

Measurement of absorbance

One 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.

$$\% \text{ Activity} = \frac{A-B}{A} \times 100$$

A= Absorbance of Control at 0 min

B= Absorbance of test after 30 min

RESULTS AND DISCUSSION

The phytochemicals of *Lucus lanata* were extracted from the methanolic extract of leaves. 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 viz. cardiac glycosides, flavonoids, saponins, sugars, and phenolic compounds.

The cardiac glycosides were qualitatively analysed by *Keller Killiani test* showing brown ring. Development of

yellow color confirmed the presence of alkaloids using Meyer's reagent (Potassium mercuric iodide).

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 red colour confirmed the presence of invert sugars on addition of *Fehling reagent* and heating of solution. Presence of saponins was evaluated by *Foam test*. Development of green color on addition of few drops of neutral ferric chloride confirmed the presence of phenolic compounds in the extract [Table 1].

Table 1: Qualitative Phytochemical Analysis.

Active Ingredient	Observation	Inference
cardiac glycosides	Keller-Killiani test	Positive
Alkaloids	Meyer's test	Positive
Saponins	Foam test	Positive
Flavonoids	1)Shinoda test 2)Alkaline reagent test	Positive
Invert Sugar	Fehling test	Positive
Phenolic Compounds	Ferric Chloride test	Positive

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 53.51 ± 0.29% activity [Table 2] in 1000 µg/ml sample concentration.

Table 2: Free radical scavenging activity of Pet. Ether extract of *Lucus lanata*.

S.No.	Conc. µg/ml	% Activity ± SD (n=3)
1	100	12.28 ± 0.29
2	200	16.08 ± 0.29
3	400	22.12 ± 0.43
4	800	41.81 ± 0.29
5	1000	53.51 ± 0.29

The methyl acetate extract of plant leaves showed 81.66 ± 0.44% activity [Table 3] in 1000 µg/ml sample concentration.

Table 3: Free radical scavenging activity of Methyl Acetate extract of *Lucus lanata*.

S. No.	Conc. µg/ml	% Activity ± SD (n=3)
2	100	22.22 ± 0.29
3	200	32.00 ± 0.43
4	400	38.46 ± 0.28
5	800	76.54 ± 0.43
6	1000	81.66 ± 0.44

The methanolic extract of plant leaves showed 83.24 ± 0.42% activity [Table 4] in 1000 µg/ml sample concentration.

Table 4: Free radical scavenging activity of Methanol extract of *Lucus lanata*.

S. No.	Conc. µg/ml	% Activity ± SD (n=3)
2	100	25.14 ± 0.27
3	200	32.87 ± 0.27
4	400	39.68 ± 0.42
5	800	78.08 ± 0.24
6	1000	83.24 ± 0.42

The free radical scavenging activity of test samples was compared with standard ascorbic acid [Fig. 1]. The IC₅₀ value (Showing 50% inhibition of DPPH) was calculated from the graph for each leaf extract. The IC₅₀ value for petroleum ether extract of leaves was found 0.982±0.009 mg/ml. The IC₅₀ value for methyl acetate and methanolic extract of leaves was calculated as 0.538±0.006 mg/ml and 0.480± 0.01mg/ml [Fig. 1] respectively.

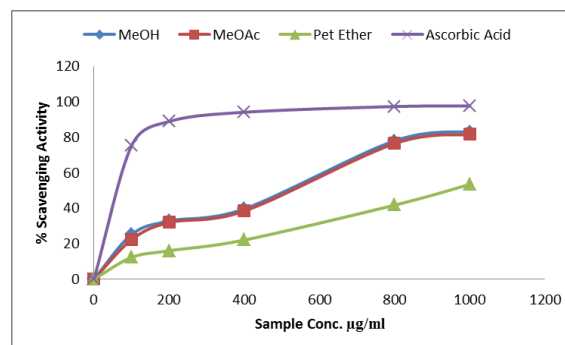


Fig. 1: Comparison of DPPH free radical scavenging activity in different solvents along with ascorbic acid.

CONCLUSION

The phytochemical screening of plant *Lucus lanata* 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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CONFLICTS OF INTERESTS

No conflict of interest was reported by the author in this experimental work.

AUTHORS CONTRIBUTION

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

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