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COMPARATIVE ESTIMATION OF PHYTOCHEMICALS IN *LAVATERA CASHMERIANA*: AN IMPORTANT MEDICINAL PLANT OF KASHMIR HIMALAYA

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ABSTRACT

Lavatera cashmeriana Camb. is an endemic and essential medicinal plant species of Kashmir Himalaya. Owing to the medicinal properties it is widely used locally and is sold as crude drug in Kashmir markets. However, despite its conventional usage there is limited information pertaining to phytochemical accumulation in different parts of *L. cashmeriana.* So, the present investigation was carried out to study the variation of phytochemical contents in different parts of the species. Soxhlet extraction was carried out and the extract obtained was screened for different phytochemicals. Standard methods were followed for screening and quantification of the various secondary metabolites. Phytochemical analysis showed the presence of important classes of the secondary metabolites viz. alkaloids, terpenoids, phenols, flavonoids, saponins etc. in different extracts, which are helpful in curing various diseases. The results of the quantitative estimation revealed that the phytochemical constituents varied significantly among different parts of the plant. The results also depicted that most of the phytochemicals screened were abundant in the roots of the plant and least in the leaves.

Keywords: *Lavatera cashmeriana,* Phytochemical, Quantitative estimation, Secondary metabolites, Screening

INTRODUCTION

The plant kingdom harbors a colossal pool of biologically active ingredients. These active constituents such as alkaloids, steroids, flavonoids, terpenoids, tannins, and many others are present in smaller quantities in plants with different chemical structures and disease preventive properties. Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. World health organization (WHO) pointed out that more than 80% of world's population depends on plants to meet their primary health care needs **[1]**. Phytoconstituents are naturally occurring bioactive compounds that are responsible for colour, odor and therapeutic potential of plants. Plants produce these chemical compounds as arms for defense against biotic and abiotic stresses. Phytochemical screening is an essential tool in identifying chemical compounds of medicinal and industrial importance and to accomplish this plant parts (root, stem, leaf, flower, etc.) and types of extraction procedure followed, play fundamental roles **[2, 3]**.

Lavatera cashmeriana (Kashmir mallow) a member of family Malvaceae, is a perennial herb which dwells in forest clearings, shrubberies, wet meadows, and sunny open rocky slopes. It is a medicinal plant widely used in traditional folk medicine such as, abdominal disorders and renal colic **[4]**, flowers for common cold and mumps **[5]** and seeds as antiseptic etc. **[6]**. Four protease inhibitors viz LC-pi I, II, III and IV were isolated from seeds of *L. cashmeriana* which inhibited trypsin, chymotrypsin and elastase *in vitro* **[7]**. Antibacterial activity is also reported **[8]**. The decoction of flowers of *L. cashmeriana* mixed with leaves of *Salix alba* is given to cure the skin irritation in pregnant women **[9]**.

Inspite of its tremendous medicinal importance a lesser amount of information is available on the comparative estimations of phytochemicals of *L. cashmeriana.* Therefore, the present work is an attempt to provide comprehensive report on the phytochemicals present in the different parts of the plant. In this connection, various parts (leaves, stem, root, flowers and seeds) of the plant were examined following standard methods.

MATERIAL AND METHODS

Plant collection

Giving due cognizance to the threat status, healthy and disease free mature flowering plants of *Lavatera cashmeriana* were collected quiet judiciously from Aharbal area of Jammu and Kashmir. The specimens were identified and

deposited in Kashmir University Herbarium (KASH) under voucher number 2038-KASH.

Preparation of plant extract

The identified plant materials were dried under shade at room temperature for 15-20 days. After shade drying, the plant materials were cut into small pieces and powdered using grinder and passed through sieve. The powdered plant materials were sequentially extracted with hydroalcohol (70% ethanol) by using Soxhlet apparatus for 2-3 days. The extracts were filtered through Whatmann filter paper No.1. The pellet was discarded and the supernatant was collected and concentrated under thermostat water bath at required temperature. Finally the extracts were dried, labeled and stored at 4˚C in storage vials for further experimental uses.

Qualitative screening

Qualitative phytochemical screening of hydroalcoholic (70% ethanol) extracts of *L. cashmeriana* for major bioactive constituents like alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, carbohydrates, glycosides, proteins and aminoacids was undertaken using following standard methods.

i) Tests for alkaloids

- **Wagner's reagent test:** A fraction of extract was treated with 3-5 drops of Wagners reagent and observed for formation of reddish brown precipitate or colouration **[10]**.
- **Dragendroff's test:** To the plant extract Dragendorff's reagent was added, reddish brown precipitate indicated presence of alkaloids **[11]**.
- **Hager's test:** To the crude plant extract Hager's reagent was added, yellow precipitate indicated presence of alkaloids **[12]**.

ii) Test for flavonoids

- **Alkaline reagent test:** 2ml of extract was treated with few drops of 20% sodium hydroxide solution. The formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid indicated the presence of flavonoids **[13]**.
- **Shinoda test:** Pieces of zinc turnings and concentrated HCl were mixed with crude plant extract after few minutes pink colored scarlet appeared that indicated the presence of flavonoids **[14]**.

iii) Tests for phenolics

- **Ferric chloride test:** A fraction of extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour **[15]**.

- **Lead acetate test:** A fraction of extract was treated with 1-3 drops of lead acetate solution and observed for formation of white precipitate **[12]**.

iv) Test for tannins

- **Ferric chloride test:** 0.5ml of extract was taken and 1ml of distilled water was added and it was treated with few drops of ferric chloride and observed for blue or green black coloration **[16]**.

v) Test for saponins

- **Foam test:** To 2ml of extract was added 6ml of water in a test tube. The mixture was vigorously shaken and observed for the formation of persistent foam that confirms the presence of saponins **[17]**.

vi) Test for terpenoids

- **Salkowski's test:** 1ml of chloroform was added to 2ml of extract followed by few drops of conc. sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids **[18]**.

vii) Tests for Glycosides

- **Keller-Kiliani test:** A solution of glacial acetic acid (4.0ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10ml aqueous plant extract and 1ml H_2SO_4 concentrated. A brown ring formed between the layers indicated the presence of cardiac glycosides **[12]**.
- **Borntrager's test:** The methanolic extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform was added. After thorough shaking the organic solvent layers was separated and ammonia solution was added. The change of ammonia layer to pink or red color indicated the presence of glycosides **[11]**.
- **Legal's test:** To the plant extracts, pyridine and sodium nitroprusside were added and development of pink or red color indicated the presence of cardiac glycosides **[19]**.

viii) Tests for Carbohydrates

- **Molisch test:** 2 ml of Molisch's solution was shaken with the plant extracts then 2 ml of H_2SO_4 concentrated was added and poured carefully along the side of the test tube. A violet ring appeared at the inter phase of the test tube which indicated the presence of carbohydrates **[14]**.
- **Benedict's test:** 2 ml of Benedict's reagent was boiled with crude plant extracts, a reddish brown color indicated the presence of the carbohydrates **[14]**.

ix) Test for Proteins and Amino acids

- **Ninhydrin test:** 2 ml of 0.2% Ninhydrin solution was boiled with the plant crude extracts, appeared violet color indicated the presence of proteins **[12]**.
- **Xanthoproteic test:** To the (5ml) of extract, 1ml of concentrated nitric acid was added and boiled, yellow precipitate is formed. After cooling it, 40% sodium hydroxide solution was added, orange colour is formed which indicated the presence of amino acids **[20]**.

Quantitative estimation

i) Alkaloid determination

2.5g of the powder was extracted using 100ml of 20% acetic acid in ethanol. The solution was covered for almost 4hrs. Filtrate was concentrated to 25ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed **[21, 22]**.

ii) Saponin determination

10g of sample was mixed with 100ml of 20% aqueous ethanol. The mixture was kept for 4hrs on water bath shaker at 55°C. Filtrate was again extracted in same manner. The combined extracts were concentrated to 40ml over water bath at 90°C. Concentrate obtained was transferred into a separating funnel and 10ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated. To the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Latter it was dried in oven at 60ºC to a constant weight **[21, 23]**.

iii) Phenolics determination

100mg of the extract of the sample was weighed accurately and dissolved in 100ml of triple distilled water (TDW). 1ml of this solution was transferred to a test tube, then 0.5ml 2N of the Folin-Ciocalteu reagent and 1.5ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2hrs after which the absorbance was taken at 765nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid **[24]**.

iv) Flavonoid determination

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method **[25]**. The sample contained 1ml of methanol solution of the extract in the concentration of 1mg/ml and 1ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and a dilution series of rutin of concentration 0.01, 0.02, 0.03, 0.04 and 0.05mg/ml was prepared and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in the extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

v) Terpenoid determination

10g plant powder were taken separately and soaked in alcohol for 24hrs. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids **[26]**.

vi) Tannin determination

The Folin-Ciocalteu protocol was followed for the determination of total tannin content. The plant extracts were prepared in the 1mg/ml concentrations. A dilution of the sample extracts were taken in test tubes followed by the addition of 7.5ml of distilled water, 0.5ml of Folin-Ciocalteu reagent and 1ml of 35% Na_2CO_3 solution. Final volume (10ml) was made with distilled water. The reaction mixtures were vigorously shaken and incubated for 30min at room temperature. The same protocol was followed for standard solutions of gallic acid. Absorbances were measured against the blank at 725nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract **[27, 28]**.

RESULTS

Qualitative screening of *L. cashmeriana*

The phytochemical screening of different parts (leaf, stem, root, flower and seed) of *L. cashmeriana* was carried out using hydroalcoholic solvent (70% ethanol). During the present study it has been observed that various preliminary tests carried showed positive results. Alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, carbohydrates, proteins and amino acids are present in most of the plant parts screened. Glycosides (anthraquinone and cardiac glycosides) are absent in all the plant parts of

L. cashmeriana. The screening of the various plant parts is shown in the **Table 1**.

Quantitative estimation of phytochemicals in *L. cashmeriana*

In the present study mature plants of *L. cashmeriana* were subjected to quantitative estimation for the presence of various phytochemicals by using different spectrophotometric techniques. The proportion of the phytochemicals viz, phenolics, flavonoids, tannins, terpenoids, saponins and alkaloids undergo distinct periodical changes and vary significantly in different parts of the plant.

The results obtained depict that the terpenoids are most abundant in root followed by seed, stem, flower and least in leaves. Alkaloid content is highest in the root and least content of alkaloids was found to be in the leaves. Regarding the saponin content it is observed that among the different plant parts studied it is highest in the root and least in the leaves **(Table 2 and Figure 1)**.

The results obtained also reveal that the total phenolics, flavonoid and tannins are most abundant in root and least abundant in the leaves **(Table 2 and Figure 2)**.

Table 1: Phytochemical screening of different parts of *Lavatera cashmeriana*

S. No.	Phytochemicals	Test	Leaf	Stem	Root	Flower	Seed
1	Terpenoids	Salkowskis test	+	+	+	+	+
2	Alkaloids	Dragendrofs test	+	+	+	+	+
		Wagners test	+	+	+	+	+
		Hagers test	+	+	+	+	+
3	Phenolics	Lead acetate test	-	+	+	+	+
		Ferric chloride test	-	+		+	+
4	Flavonoids	Shinoda test	+	+	+	+	+
		Alkaline reagent test	+	+	+	+	+
5	Tannins	Ferric chloride test	+	+	-	+	+
6	Saponins	Foam test	+	+	+	+	+
7	Glycosides	Borntragers test	-	-	-	-	-
		Keller- Killani test	-	-	-	-	-
8	Cardiac glycosides	Legals test	-	-	-	-	-
9	Carbohydrates	Molisch test	+	+	+	+	+
		Benedicts test	-	+	+	+	+
10	Proteins and amino acids	Ninhydrin test	+	+	+	+	+
		Xanthoproteic test	+	-	+	+	+

+ denotes presence; - denotes absence

Table 2: Comparative estimation of different phytochemicals in various parts of *Lavatera cashmeriana*

Phytochemicals	Plant parts					ANOVA	
	Leaves	Stem	Root	Flower	Seed	F	P
Alkaloids (mg/g)	67.43±1.1*	75.86±1.43	156.70±1.73	71.5±0.88	96.50±1. 08	2487.8	.000
Terpenoids (mg/g)	10.96±1.2	29.53±1.5	69.73± 1.3	20.53±0.92	29.93±1.4	851.14	.000
Saponins (mg/g)	6.43±1.12	14.90±1.21	30.03± 1.56	12.40±1.3	14.43±1.06	142.52	.000
Phenolics (mg of *1GAE/g)	13.96±1.4	23.9±1.6	32.5± 2.4	14.1±1.6	26.7± 1.4	63.27	.000
Flavonoids (mg of *2RU/g)	30.01±2.2	35.03±1.3	37.83±1.9	31.54±2.2	36.14±1.7	8.51	.000
Tannins (mg of GAE/g)	14.5±0.83	20.6±1.8	25.6±0.81	18.5±0. 9	22.7±1.6	32.57	.000

***Mean (triplicates) ±SD; *1GAE (gallic acidequivalent); *2RU (rutin equivalent)**

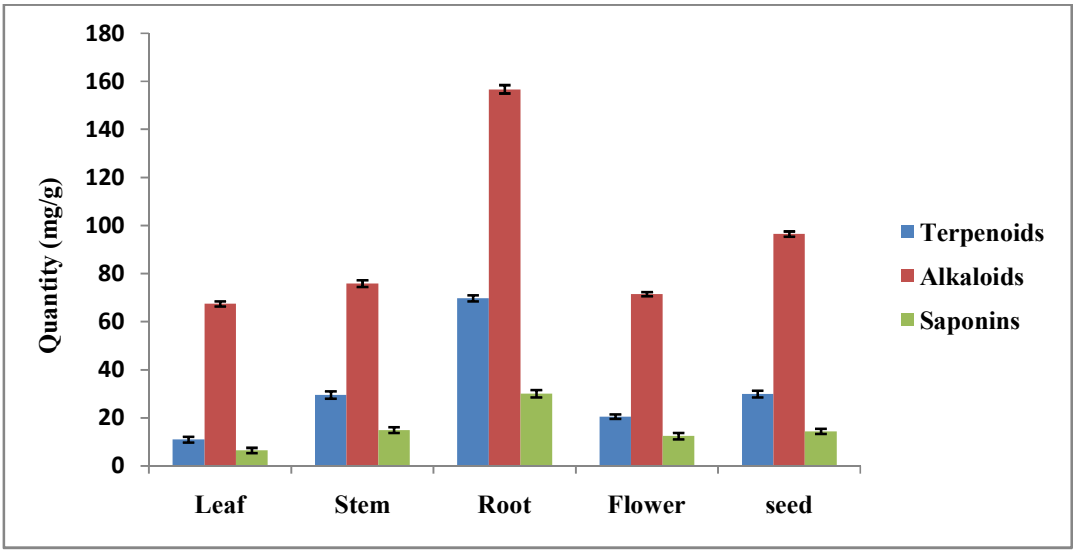

Figure 1: Comparative quantitative estimation of terpenoids, alkaloids and saponins in different parts of *Lavatera cashmeriana*

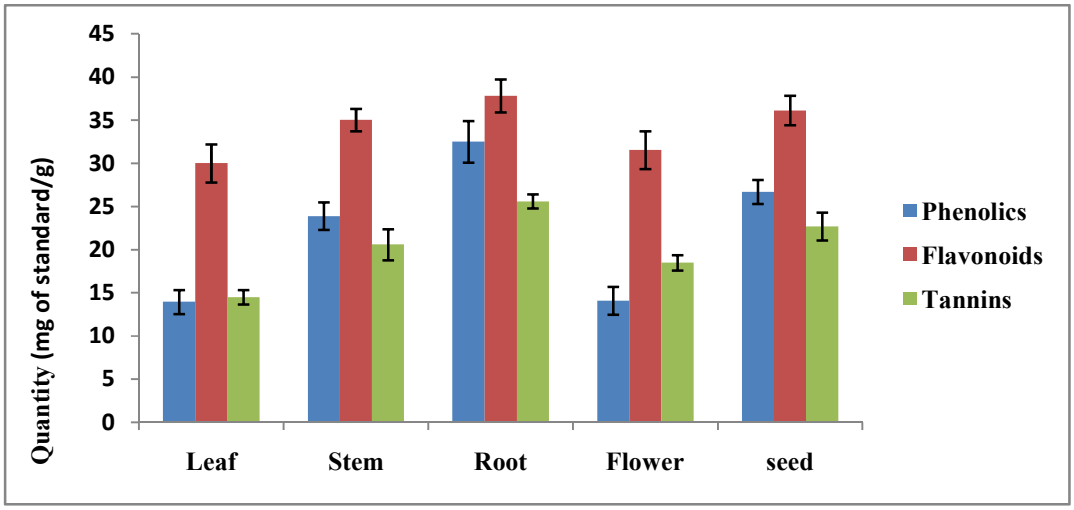

Figure 2: Comparative quantitative estimation of phenolics, flavonoids and tannins in different parts of *L. cashmeriana*

DISCUSSION

Present investigation revealed the presence of the phytochemical constituents like phenolics, flavonoids, alkaloids, tannins, terpenoids, saponins etc. and their quantity varied significantly ($p \leq 0.05$) in different parts of *L. cashmeriana.* The similar phytochemicals have been reported earlier in several species of genus *Lavatera* **[29, 30]**. In *L. cashmeriana* the results obtained revealed that the total phenolics, flavonoids and tannins are most abundant in root and least abundant in the leaves. Moreover, it was observed that the terpenoids, alkaloids and saponins were most abundant in root followed by seed, stem, flower and least in

leaves. Similar studies were carried out by other workers in the family Malvaceae which revealed presence of many bioactive compounds in different parts of the studied plants. For instance, Shehata and Galal **[31]** carried out their work on *Malva parviflora* and revealed significant difference in the active compounds between the different organs (leaves, stems and roots) of the same plant. Mishra and Thorat **[32]** studied *Abutilon indicum* and revealed significant variation in different phytochemicals from different parts (root, stem, leaves and whole plant separately) of the plant.

CONCLUSION

The results have shown the presence of various versatile bioactive constituents such as phenols, alkaloids, tannins, terpenoids, flavonoids, saponins etc. in all the plant parts. Also, crude methods and spectrophotometric analysis disclose variations in relative content of phytochemicals from different parts of the plant. These phytochemicals serve as a chief source for pharmaceutical products, so the plant species can hold an enormous potential to serve as therapy for various chronic diseases. The qualitative and quantitative estimations reported in this study signify that scientific explorations are helpful in the confirmation of traditional medicinal practices for the improvement of new remedial agents from medicinal herbs. Thus, the present investigation has provided the chemical basis for the ample use of these plants as healing agent for treating diverse ailments. However, there is additional need to carry out advanced studies in order to explicate the structure of these chemical compounds.

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