Investigation of Biochemical Analysis And Antibacterial Properties of Lavatera Cachemiriana

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Abstract- *The current investigation was carried out to analyze preliminary composition of phytocompounds and antimicrobial activities in the roots and leaves of L.cashmeriana using solvents of different polarities. Antimicrobial properties were carried out by agar well diffusion method. Phytochemical analysis indicated presence of coumarins, sterioids, phenolics etc. among different extracts. It was found that extracts displayed significant variation in antimicrobial activities. The methanolic extract of L.cashmeriana leaf showed more sensitivity (69%) against K. pneumoniae followed by (64%) against C.perfringens. Therefore, it is concluded here that the biochemical evaluation of current study has validated antimicrobial activities of L.cashmeriana which could act as a safe & natural source of antimicrobial agents against various pathogenic microorganisms.*

Keywords- L.cachemiriana, phytochemical analysis, antibacterial, antimicrobial, biochemical evaluation.

I. INTRODUCTION

In Himalayan regions especially in Jammu & Kashmir, there is a prevalence of different traditional plants which are being used for medicinal use & evidences suggest that such plants are having antimicrobial activities. In the present work, parts of two different medicinal plants each belonging to different families were evaluated for their phytochemical activities and antimicrobial properties. These well known medicinal plants are Saffron (*Crocus sativus* L.) and *Lavatera cachemiriana* (*Cashmeriana*) cambess. Owing to the increasing demand of food as medicine and issue of multiple drug resistance, the plant derived drug market is picking up immensely. Thus, there was a systematic scientific program needed to compare & assess the phytochemical as well as antimicrobial activity of these plants against selected human pathogens, also to assess their antimicrobial potential in comparison to well known antibiotics already available in the market. The study would led scientific fraternity to be acquainted with the new information about the comparative knowledge of phytochemical analysis along with antimicrobial activities of the plant materials taken in this study, so that this would help in future to develop global enterprise involving the sale, production and use of plant extracts against commonly encountering human microbes.

Classification:

Kingdom : Plantae
Family : *Malvaceae*
Subfamily : Malvoideae
Genus : *Lavatera*
Species : *Lavatera cashmeriana*

Lavatera cashmeriana Cambess is a semi-evergreen, woody- based perennial herb with stem upto 2 m tall belonging to Malvaceae family & commonly known as tree mallow. It grows in habitat with humus rich soils (Polunin and Stainton, 1984) in meadows and forest clearings at 1800 - 3600 metres hight (Kaul, 1997). It is in flowering stage from July to August, and the seeds ripen from Aug to September. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade and requires dry or moist soil. The plant can tolerate maritime exposure. The Leaves are 3-5 lobed, lower leaves rounded heart-shaped with 5 shallow and toothed lobes. Flowers bright-pink with dark petals on terminal spikes. The plant is grown in the gardens due to its presence of beautiful pink coloured flowers. The seeds germinate at room temperature, exposure to light may be helpful & seed ripens late July. This is an easily grown plant, succeeding in any ordinary garden soil in sun or partial shade, prefers a light well-drained moderately fertile soil in full sun. A soil that is too rich encourages foliar growth at the expense of flowering. This species is considered by some botanists to be no more than a minor variant of *L. thuringiaca* (Huxley , 1992). The parts of plant (Root, Leave & Seed) have been used traditionally for various medicinal purposes like throat

problems, as a mild laxative (purgative), demulcent, pectoral etc & roots of this plant are sold as a crude drug in the market. The young leaves are edible as raw or cooked. A mild flavour, but they are tough and not very worthwhile. When cooked they have a somewhat slimy consistency. Also, A strong fibre is obtained from the stems, it is used for making string, bags, paper etc. The germplasm of this plant is maintained by The Germplasm Resources Information Network's (GRIN) under the National Plant Germplasm System (NPGS) supported by united states Department of Agriculture (USDA) agriculture research services (Ars-grin). It has been found recently that the plant possesses anticancer/ antibacterial properties as well as contains protease inhibitors. This plant has been declared as Endangered by International Union for Conservation of Nature (IUCN) (Molur and Walker, 1998 and Pereira *et al.*, 2008). Therefore, it is important to assess the biochemical evaluation and bioactivities of this plant species, so as to validate its ethno medicinal use which could pave the way to use this plant species sustainably.

II. MATERIALS AND METHODS

2.1. Plant material: Collection, authentication and processing

Roots and leaves from *Lavatera cashmeriana*/*cachemiriana* were collected from Pampore town of Kashmir Himalaya, India. *Lavatera cashmeriana* roots & leaves were collected in the month of May- June 2010 from a local field of Pampore Kashmir, India. Both leaves as well as roots were shade dried & ground into powder by electric blender. The ground powders were passed through a mesh sieve & kept separately in light protected bottles at 5^0C till further down streaming.

2.2. PREPARATION OF CRUDE EXTRACTS

The powdered form of roots and leaves of *L.cashmeriana* were successively extracted using different solvents (petroleum ether-PE, chloroform - CH, methanol - MeOH and ethanol- EOH) using Soxhlet apparatus for 5h in each solvent and maceration as cold extraction using shaker incubator at room temperature for 3 days. The extracts obtained were subjected to dryness using rotary evaporator and stored in dark at 5^0c till further analysis.

2.3. PRELIMINARY PHYTOCHEMICAL ANALYSIS

The extracts were subjected to phytochemical analysis using standard methods(Harborne, 1998; Evans, 1996; Cannell, 1998; Tiwari *et al*., 2011; Raaman,2006; Sayeed, 2007).

2.4. ULTRAVIOLET-VISIBLE (UV/Vis) ABSORPTION SPECTRUM

UV-Vis absorption spectrum was carried out for all the samples using UV-Vis Single Beam Spectrophotometer 6.89 so as to get an idea about the presence of major phytochemicals present in the samples as well as to get a confirmation regarding the presence of phytoconstituents which showed positive during preliminary phytochemical analysis. UV-Vis spectrophotometry is related to the spectroscopy of photons in the UV-visible region and colour of the chemicals involved directly affects the absorption in the visible ranges (Harborne,1998).

2.5. ANTIMICROBIAL ACTIVITIES

The microorganism were collected and inoculated in an exceedingly nutrient broth at 37^0C for twenty-four hour in pteri plates. 25ml of Muller Hinton agar (Himedia) was mixed with H_2O and sterilized in autoclave at 15lbs pressure for 15 min. Wells of equal size (8 millimeter diameter) were cut with correct gaps within the medium using sterile borer and therefore the plates with wells were used for the antimicrobial studies.

The antibacterial activity of plant extracts were determined by well diffusion method (Perez *et al*., 1990) and minimum inhibition concentration (MIC) was performed by well diffusion method (Okeke *et al*., 2001). Serial dilutions of root and leaf extracts of *L.cashmeriana* were subjected to MIC in the concentration range of 160 µg/disc to 1000 µg/disc and from 83 µg/disc to 1400 µg/disc respectively. Commercial antibiotics like Ampicillin (250µg/disc), Gentamycin (500 µg/disc) metronidazole (800µg/disc) were used as standards for positive antibacterial control. Each experiment was carried out in triplicates, and the diameter of the zone of inhibition surrounding each well was recorded at the respective concentrations. The inhibition zones were recorded after 24 hours of incubation at 37°C.

III. RESULTS AND DISCUSSION

Percentage yield of crude extract has demonstrated highest % yield of 2% (Methanol root extract) and 9.50% (Methanol leaf extract) (Table-1). The phytochemical analysis of root and leaf extracts of *L.cashmeriana* has displayed diverse class of phytocompounds *Viz.* steroids/triterpenes, saponins, glycosides, alkaloids, flavonoids , gums and mucilages etc (Table-2). Each compound absorbs light at a particular wavelength. UV-visible spectroscopy can be used to determine many physicochemical characteristics of compounds and thus can provide information as to the identity

of a particular compound. UV-VIS absorption spectrum was determined for all the four samples so as to provide a relative evidence for the presence of particular molecules/compounds in different solvent extracts. The compounds which were found positive during preliminary phytochemical analysis, many of them showed maximum absorption at various wavelengths. Different absorption patterns were found. For sterols which absorbs the UV light at the wavelength of 219 to 315 nm (Badgujar and Jain, 2009), 791nm for alkaloids (Sivakumar *et al*., 2011), polyphenolics absorb at 320nm to 330nm and coumarins at 507nm to 585nm (Harborne, 1998).
The current study showed varying degrees of antimicrobial activity against human pathogenic bacteria like *E. coli, B. cereus, S. aureus, P.aeruginosa, K. pneumoniae and C. perfringens* (Table-3). Petroleum ether, chloroform, methanol and ethanol extracts of *L.cashmeriana* roots showed antimicrobial activity against 4, 5, 2 and 3 microorganisms respectively out of 6. While as petroleum ether, chloroform, methanol and ethanol extracts of *L.cashmeriana* leaves showed antimicrobial activity against 1, 2, 4 and 3 microorganisms respectively out of 6. More number of extracts showed activity against gram negative microbes as compared to gram positive microbes. No inhibition zones were observed with aqueous extracts of all the samples against the selected microorganisms. The highest zone of inhibition (30mm) was shown by the chloroform and methanolic extracts of *L.cashmeriana* against *Klebsiella pneumonia* and *Clostridium perfringens.* Also, greater inhibition zones were shown by ethanolic extracts of affron stamen (25mm) and methanolic extracts of *L.cashmerina* (20mm) against *Bacillus cereus* .

Also, root & leaf extracts of *L.cashmeriana* showed the zone of inhibitions between 5 mm to 30 mm at concentration range between 2.5 mg/ml to 6 mg/ml. The variation in the effectiveness of the extracts against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. The extracts which showed antimicrobial activities were subjected to minimum inhibitory concentration (MIC) at a concentration between 183 µg/disc to 1400 µg/disc, these where also compared with respective standard antibiotics prevalent in the market i.e. AMP (250µg ampicillin/disc), GEN (500 Gentamycin µg/disc) and Metronidazole (800 µg/disc). (Table-4) he petroleum ether, methanol and ethanol extracts of stamen

Greater inhibition zones (Fig.1)were shown by the petroleum ether (10.2mm), chloroform (9 mm and 10 mm) and ethanolic (9mm) root extracts of *L.cashmeriana* at lower concentrations 175 µg/disc, 190 µg/disc and 175 µg/disc respectively against *E.coli, Bacillus Cereus and Klebsiella pneumoniae* when compared to inhibition zone shown by respective antibiotics. The outcome of the current research work will provide a better understanding of antimicrobial activities of *L.cashmeriana* and thus will pave the way for the further investigation of safe, potent & natural source of antimicrobial compounds against diverse therapeutically challenging microbial pathogens.

IV.CONCLUSION

The present study investigation revealed that samples under this study contain diverse pool of potential phytoconstituents.The zone of inhibition varied between 5 mm to 30 mm at concentration range between 0.52mg/ml to 6 mg/ml. It is also concluded that roots and leaves of *L.cashmeriana* if exploited properly, could play a role as antimicrobial source for pharmaceutical, food and cosmaceutical industries.

V. ACKNOWLEDGMENTS

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VI. CONFLICT OF INTEREST

The authors declare no conflict of interest.

Figures and Tables

Table 1. Percentage yield extract values (%w/w) of *Lavatera cachemiriana* (Root and leaf).

Solvent	Root	Leaf
Petroleum ether	1.05%	5.50%
Chloroform	1.11%	6.00%
Methanol	2%	9.50%
Ethanol	1.40%	7.25%
Water	1.10%	4.50%

Table 2. Preliminary phytochemical analysis of different extracts of roots and leaves of *L.cashmeriana*

S.NO	Phytoconstituents	Root Extracts*					Leaf Extracts*				
		PE	CH	ME	EH	AQ	PE	CH	ME	EH	AQ
1	Carbohydrates	-	-	+	+	+	-	-	+	-	+
2	Proteins	-	-	-	-	-	-	-	-		-
3	Fats	+	-	-	-	-	+	-	-	-	-
4	Starch	-	-	-	-	+	-	-	-	-	-
5	Steroids and Trierpenes	+	-	-	-	-	+	-	-	-	-
6	Glycosides	-	-	-	-	-	-	-	-	-	-
7	Saponins	-	-	-	-	-	-	-	-	-	-
8	Alkaloids	-	-	-	-	-	-	-	-	-	-
9	Tannins/ Phenolic compounds	-	-	-	-	+	-	+	-	-	+
10	Flavonoids	-	-	-	-	-	-	-	-	-	-
11	Gums and mucilages	-	-	+	+	+	-	+	+	+	+
12	Coumarins	+	-	+	+	+	+	+	+	-	-

* + Positive, - Negative, PE - Petroleum ether, CH – Chloroform, ME - Methanol, EH – Ethanol, AQ – Aqueous

Table 3. Antimicrobial activities of different extracts of Roots and Leaves of *L.cashmeriana.*

S.No	Microorganisms	Zone of Inhibition (mm)									
		Root Extracts					Leaf Extracts				
		PE	CH	ME	EH	AQ	PE	CH	ME	EH	AQ
		5.25*	5.75*	6*	5*	5.5*	5.5*	6*	5.5*	2.5*	2.75*
1	*E.coli*	10.3^{+}	10.5	20	5	NI	NI	NI	25.5	NI	NI
2	*Bacillus cereus*	10.15	10.2	NI	10.7	NI	NI	5	20	5	NI
3	*Staphylococcus aureus*	NI^{-}	10	NI	NI	NI	NI	NI	NI	NI	NI
4	*Pseudomonas aeruginosa*	5	20	NI	NI	NI	NI	NI	NI	5	NI
5	*Klebsiella pneumoniae*	10	30	5	NI	NI	10	NI	30	10.2	NI
6	*Clostridium perfringens*	NI	NI	NI	16	NI	NI	10.3	30	NI	NI

*mg/ml concentration of extracts PE - Petroleum ether, CH – Chloroform, ME - Methanol, EH –Ethanol, AQ – Aqueous. NI^{-} No inhibition, $^{+}$ Inhibition zone.

Table 4. Minimum inhibitory concentration (MIC) of different extracts of *L.cashmeriana* roots and leaves.

S. No.	Microorganisms	Minimum inhibitory concentrations (µg/disc)								
		Root Extracts				Leaf Extracts				
		PE	CH	ME	EH	PE	CH	ME	EH	Positive control *
1	*E.coli*	525 (10.8)	760 (8)	800[+] (8)[$]	160 (9)	ND	ND	900 (8)	ND	250 13(AMP)
2	*Bacill*	175 (10.2)	190 (9)	ND	640 (7)	ND	1400 (8)	1100 (7.5)	83 (10.2)	500 20 (GEN)
3	*Staphylococcus aureus*	ND	950 (7)	ND	ND	ND	ND	ND	ND	500 25(GEN)
4	*Pseudomonas aeruginosa*	875 (8)	950 (8)	ND	ND	ND	ND	ND	250 (9)	500 25(GEN)
5	*Klebsiella pneumoniae*	350 (7)	190 (10)	1000 (7)	ND	916 (9)	ND	183 (10.5)	916 (9)	250 10 (AMP)
6	*Clostridium perfringens*	ND	ND	ND	ND	ND	600 (8)	183 (14)	ND	800 10.4(MET)

[+]Concentration (µg/disc), [$]-Inhibition zone in mm; ND**-Not done; *AMP (250µg Ampicillin/disc), GEN (500 Gentamycin µg/disc) and Metronidazole (800 µg/disc).

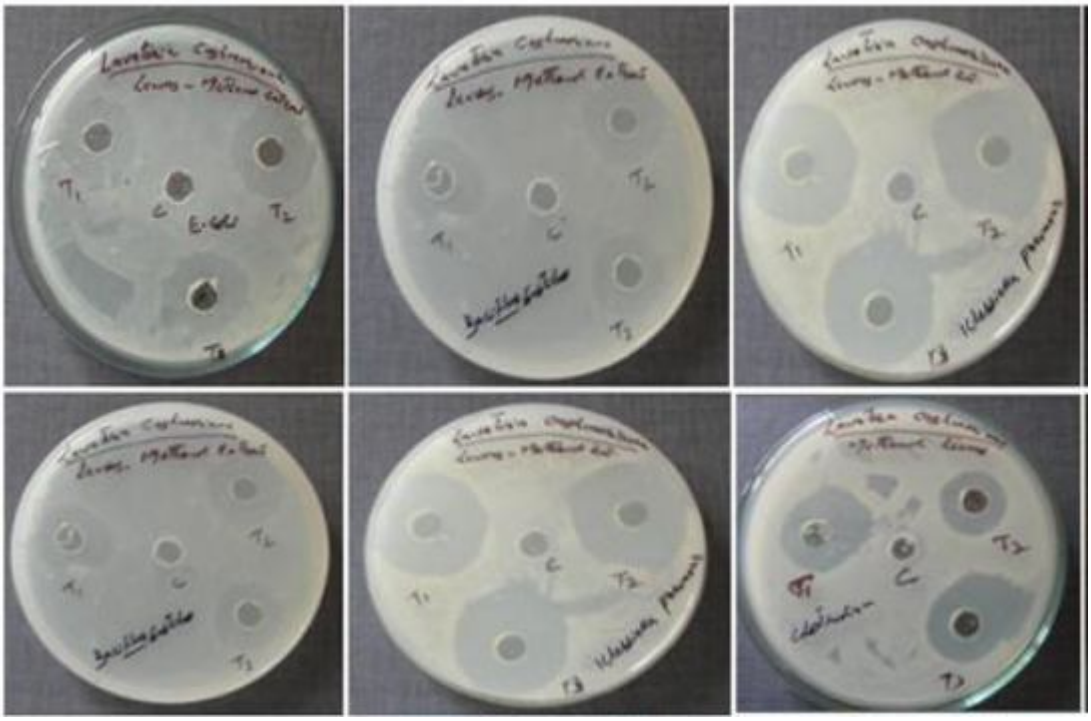

Fig 1. Representative plates of antimicrobial activities of *L.cashmeriana* sample extracts against various bacterial strains.

REFERENCES

[1] Badgujar, V.B. and Jain, P.S., 2009. A phytochemical analysis of the medicinal plant: Helicteresisora. *Int J PharmTech Res*, *1*(4), pp.1376-1377.

[2] Cannell, R.J. ed., 1998. *Natural products isolation* (Vol. 4). Springer Science & Business Media.Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4),

[3] Evans W.C., 1996. *Trease and Evans Pharmacognosy*. (14th Edition, Ed. Bailiere Tindall).W.B.Sauders Company Ltd: London. pp.224–575.

[4] Harborne, J.B., 1998. *Phytochemical methods*, Chapman and Hall Ltd., LondonHuxley, ed., 1992. *The New Royal Horticultural Society Dictionary of Gardening*, MacMillan Press Ltd., London, 3, pp. 25-26.

[5] Kaul, M.K., 1997. *Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya*. Indus publishing.

[6] Molur, S. and Walker, S. 1998. Biodiversity Conservation Prioritisation Project (BCPP) India,

[7] Endangered Species Project. *Zoo Outreach Organisation*. 51.

[8] Okeke, M.I., Iroegbu, C.U., Eze, E.N., Okoli, A.S. and Esimone, C.O., 2001. Evaluation of extracts

[9] Pereira, J.A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P.B., Ferreira, I.C., Ferreres, F., Bento,

[10] Perez, C., Pauli, M., Bazerque, P., 1990. An antibiotic assay by the agar well diffusion method. *Acta Biol Med Exp. 15*, pp. 113-115.

[11] Polunin, O. and Stainton, A., 1984. *Flowers of the Himalaya*. Oxford University Press, Delhi, India.

[12] Raaman, N., 2006. *Phytochemical techniques*. New India Publishing.

[13] Sayeed A., 2007. *Pharmacognosy- Introduction of Plant Constituents and their Tests*. Department

[14] Sivakumar, P., Senthilkumar, K.L. and Varma, J.P., 2011. Phytochemical studies on Polygonumglabrum (willd.). *International Journal of Pharma and Bio Sciences*, *2*(2), pp.169-175.

[15] Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H., 2011. Phytochemical screening andextraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), pp.98-106.