

Efficacy of Ethanol, Methanol and Aqueous Extracts of *Irish kashmiriana* and *Lavatera cachemiriana* for Potential Antimicrobial Activity

Mohd. Zakir¹, Narendra Kumar², Akash³

¹Himalayan University, Itanagar, Arunachal, Pradesh, India

²Alpine Group of Colleges, Uttarakhand, India

³H.R. Degree College, Uttarakhand, India

Abstract— Plants have been used as source of medicine and remedies since many years. Plants are recently being used in various pharmacologically activities due to their efficacy to treat the diseases. More than 25000 products from plants have been obtained from medicinal plants to treat various diseases. So a study has been planned to elucidate the antimicrobial efficacy and phytochemical screening of the two medicinal plants from Kashmir valley viz. *Irish kashmiriana* and *Lavatera cachemiriana*. These two medicinal plants were obtained from Kashmir valley and tested against *Escherichia coli*, *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. It was observed from the present study that ethanol, methanol and aqueous extract of *Irish kashmiriana* has shown the better results in terms of antibacterial activity against *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* except in case of methanol whereas in case of *Lavatera cachemiriana*, aqueous extract has shown no activity against any of the four tested pathogens. The preliminary screening of whole plants of *Lavatera cachemiriana* different root extracts of *Lavatera cachemiriana* and *Irish kashmiriana* reveals the presence of various phytochemical like tannins, saponins, flavonoids, Terpenoid, Phenol and Carbohydrate which can be used in further novel drug discovery.

Keywords— *Irish kashmiriana*, *Lavatera cachemiriana* medicinal plants, antimicrobial activity.

I. INTRODUCTION

The use of medicinal plants has been recorded for many centuries in various medical systems such as Ayurveda, Siddha and Unani. India is a great repository of various medicinal plants since the plants has been used in different diseases from millions of years. More than 2,500 traditional plants used as a healing agent in India which are used for various human ailments (1). Plants were used as ethnomedicine from generation after generation due to the continuous use and by practices in oral form without any written documents (2a,3). So ethnomedicinal studies focussed on the significant used of plant species as source of medicine and illuminate the indigenous knowledge of plants which further helps in the discovery of novel drug (2b, 8b). The documentation traditional knowledge on plants species has made significant contribution (4).

Irish kashmiriana belongs to the family Iridaceae and in Kashmir, India, it is locally known as Mazarmund. It is an important medicinal plant of the family Iridaceae since it has been used as source of medicine to treat various disease like malaria, flu, common cold, tooth problems as well as in viral and bacterial infections. *Irish kashmiriana* has been used extensively due to the presence of various biological agents in its root, stem and in leaves. It has many biological compounds like tannins, saponins, isoflavonoids and flavonoids. The pharmacological study revealed that it has great quantity of secondary isoflavonoids, metabolites triterpenoids, quinines, stilbene glycoside and stilbene glycoside (5). The other species of *Irish* is *Irish ensata* which were also used traditionally as a source of medicine. About 1500 species of the family Iridaceae which has been used as a medicine in

different ailments since millions of year ago. The other species of the present study is *Lavatera cachemiriana* which belongs to the family Malvaceae. This species is endemic to the Kashmir Himalaya (6). *Lavatera cachemiriana* is also used in traditional folk medicine since the existence of civilization. In Kashmir valley, *Lavatera cachemiriana* is locally known as *Lavatera cachemiriana* (7). It is recently recorded in western Himalaya along with Uttar Pradesh and in other state (8a,9). *Lavatera cachemiriana* is a perennial flowering herb which generally grow in humus rich soil (7,8). Further it root, is recently has been observed in different pharmacologically activity by the workers (8,9) flowers in common cold and mumps and seed as antiseptic by different workers (21). So the present study has made an attempt to study the antimicrobial efficacy of the two essential medicinal plants from Kashmir valley due to their great biological compounds for screening.

II. MATERIALS AND METHODS

Plants like *Irish kashmiriana*, *Lavatera cachemiriana* plant was collected locally from Srinagar and identified from Botanical Survey of India. The herbarium was deposited in department of life Science, Himalayan University, Itanagar, India

Extraction of Plant Material

Plant was dried (about 50 g) and converted into dried form. The crude extraction was done with ethanol, methanol, and aqueous solvent in Soxhlet assembly to get the extract which dried and kept for further analysis.

TABLE 1. Description of the plants

S. No	Botanical name	Common name	Family	Part used
1	<i>Irish kashmiriana</i>	Mazarmund	Iridaceae	Whole plant
2	<i>Lavatera cachemiriana</i>	sazakul	Malvaceae	Whole plant



Fig. 1. *Irish kashmiriana* and *Lavatera cachemiriana*

Tested Microorganism

In present study, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were used. 200mg/ml concentration of each plant extract was used in testing on mullarhinton agar plate.

Antimicrobial Activity

The antibacterial activity test was carried out using the disk diffusion method (10).

Phytochemical Analysis

Plant extract was tested for Phytochemical analysis in which major phytoconstituents were recorded by using standard method (11,12). All the plant extracts were screened for the presence or absence of biologically active compounds such as Tanin, Saponin, Steroid Flavonoid, Terpenoid, Napthoquinone, Phenol, Carbohydrate, Carbohydrate and starch.

III. RESULT AND DISCUSSION

Antimicrobial Efficacy

The use of folk medicine use is widespread across the world as a remedial measure or a source of alternate medicine in various diseases or ailments. It is well known that knowledge of folk medicinal plants play very essential role in modern medicinal system. Various novel compounds have been isolated from plants due to their anti-inflammatory, antibacterial, antifungal and anticancer activities (2a,13). The result indicated that ethanol and methanol extract of both plants showed significant antimicrobial activity as compared to the aqueous extract against the pathogens thus inhibited

high inhibitory zone of *Escherichia coli*, *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. In present study, the antimicrobial activities was tested in 200 mg/ml concentrations of different crude extract of *Irish kashmiriana* and *Lavatera cachemiriana* against different bacteria like *Escherichia coli*, *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Inhibitory effects of all the zone was observed in mm with Gentamycin as positive control for bacteria. It was observed for earlier studies that family Malvaceae has great potential of different compounds (14), and every species of the family has different composition of the biological compounds due to different phases of accumulation, biosynthesis, and developmental stages (15). *Lavatera cachemiriana* of Malvaceae and *Irish kashmiriana* of Iridaceae is most important plant in plant kingdom which have huge antimicrobial potential and traditionally in curing various diseases.

In present study, it was observed that ethanol, methanol and aqueous extract of *Irish kashmiriana* has shown the better results in terms of antibacterial activity against *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* except in case of methanol whereas in case of *Lavatera cachemiriana*, aqueous extract has shown no activity against any of the four tested pathogens. There was no concentration activity dependent of all extract in the present study as we have tested all the plant extract of 200 mg/ml. It was observed from different studies that plant extract also showed different activities in various concentrations. At lower concentration, lesser antimicrobial compound resulting into the low activity. Although in present study, we have tested all the plant extract of 200 mg/ml against the selected pathogens in which ethanol extract of *Irish kashmiriana* and methanol extract of *Lavatera cachemiriana* has shown great antibacterial activity.

In present study, it was observed that ethanol extract of *Irish kashmiriana* has showed very effective against *Pseudomonas aeruginosa* (32 ± 0.30 mm) than other *Escherichia coli* (28 ± 0.34 mm), *Bacillus amyloliquefaciens* (28 ± 0.32 mm) and *Staphylococcus aureus* (25 ± 0.55 mm), whereas in methanol extract no activity has been recorded for *Escherichia coli*, *Pseudomonas aeruginosa* but moderate inhibitory zone was observed for *Bacillus amyloliquefaciens* (13 ± 0.35 mm) and *Staphylococcus aureus* (23 ± 0.32). On the other hand, aqueous extract of *Irish kashmiriana* has showed promising antibacterial effects against *Escherichia coli* (25 ± 0.34 mm), *Bacillus amyloliquefaciens* (25 ± 0.34 mm), *Pseudomonas aeruginosa* (30 ± 0.32 mm), *Staphylococcus aureus* (28 ± 0.55 mm).

TABLE 2. Antimicrobial activity of *Irish kashmiriana* and *Lavatera cachemiriana* (diameter in mm)

Tested microorganism	<i>Irish kashmiriana</i>			<i>Lavatera cachemiriana</i>		
	Ethanol 200 mg/ml	Methanol 200 mg/ml	Aqueous 200 mg/ml	Ethanol 200 mg/ml	methanol 200 mg/ml	Aqueous 200 mg/ml
<i>Escherichia coli</i>	28 ± 0.34	-	25 ± 0.34	-	32 ± 0.32	-
<i>Bacillus amyloliquefaciens</i>	28 ± 0.34	13 ± 0.35	25 ± 0.34	32 ± 0.32	23 ± 0.37	-
<i>Pseudomonas aeruginosa</i>	32 ± 0.30	-	30 ± 0.32	-	-	-
<i>Staphylococcus aureus</i>	25 ± 0.55	23 ± 0.32	28 ± 0.55	13 ± 0.55	28 ± 0.55	-

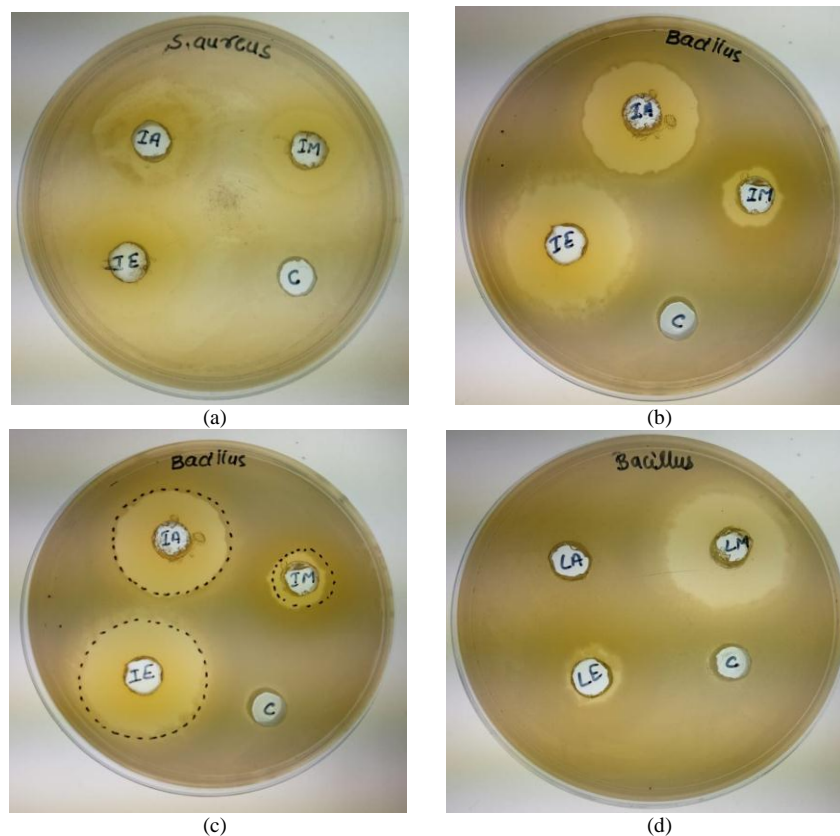


Figure 2: Antimicrobial potential through disc diffusion assay showing the zone induced by Gentamicin (control) (a). *S. aureus* (b). *B. amyloliquefaciens* *S.aureus* (c) (d) *B. amyloliquefaciens* (d) *B. amyloliquefaciens* (Note: a-c= *Lavatera cachemiriana* d= *Lavatera cachemiriana*)

In case of *Lavatera cachemiriana*, ethanol extract has showed no activity against *Escherichia coli* and *Pseudomonas aeruginosa* whereas the extract has been observed for high inhibitory zone against *Bacillus amyloliquefaciens* (32 ± 0.32 mm), and *Pseudomonas aeruginosa* (13 ± 0.55 mm), whereas methanol extract has been observed for high antibacterial efficacy against *Escherichia coli* with a diameter zone of 32 ± 0.32 mm, followed by *Staphylococcus aureus* (28 ± 0.55 mm) and *Bacillus amyloliquefaciens* (23 ± 0.37 mm). Aqueous extract of *Lavatera cachemiriana* has showed no activity against of all the four tested bacteria.

Qualitative Estimation of Phytochemical Constituents

In present study, the results of phytochemical screening showed the presence of various compounds which can be used in further anti-oxidant, anti-microbial and anti-hyperglycemic activities. These compounds have great potential of biological activities like anti-proliferative, anti-cancer, anti-inflammatory, anti-cancer (16,17). Studies also has been conducted by the different workers for biological effects of phytochemical in terms of antibacterial agent (18), antilipoxygenase (19), urinary disorders (20), antiseptic (21), anticancerous properties (22) might be due to the presence of various phytochemicals and essential compounds in *Lavatera* sps.

The preliminary screening of whole plants of *Lavatera cachemiriana* different root extracts of *Lavatera cachemiriana* and *Irish kashmiriana* reveals the presence of various phytochemical like tannins, saponins, flavonoids, Terpenoid, Phenol and Carbohydrate. It was observed from the studies that the amount of phytochemical in a particular medicinal plants is depend upon the extraction method, polarity of compounds and in the tested samples as well as the polarity of the solvent (23). The highest number of compounds released in methanol for *Lavatera cachemiriana* and other compound didn't show any major compounds. In case of *Irish kashmiriana*, methanol and ethanol extract of the plant has showed moderate rate of phytochemicals. *Lavatera cachemiriana* and *Irish kashmiriana* have great medicinal property due to the presence of high biological compounds. Phytochemical screening of ethanol extract of *Lavatera cachemiriana* showed the presence of Saponin, Tanin, Terpenoid, Phenol and Carbohydrate. Ethanol extract showed Saponin, Tanin, Terpenoid, Phenol and Carbohydrate whereas aqueous extract showed only Terpenoid, Tanin, Phenol and Carbohydrate. On the other hand, *Lavatera cachemiriana* showed the presence of Terpenoid, Tanin, Phenol and Carbohydrate in its methanol and ethanol extract whereas in aqueous extract only Terpenoid, Phenol and Carbohydrate has been recorded.

TABLE 3. Qualitative analysis of secondary metabolites in *Irish kashmiriana* and *Lavatera cachemiriana*

S. No	Test	<i>Irish kashmiriana</i>			<i>Lavatera cachemiriana</i>		
		Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous
1	Saponin	+	+	-	-	-	-
2	Tanin	+	+	+	+	+	-
3	Steroid	-	-	-	-	-	-
4	Flavonoid	-	-	-	-	-	-
5	Terpenoid	+	+	+	+	+	+
6	Napthoquinone	-	-	-	-	-	-
7	Insulin	-	-	-	-	-	-
8	Phenol	+	+	+	+	+	+
9	Carbohydrate	+	+	+	+	+	+
10	Phlobatannin	-	-	-	-	-	-
11	Starch	-	-	-	-	-	-

IV. CONCLUSION

Ethanol, methanol and aqueous extract of *Irish kashmiriana* and *Lavatera cachemiriana* has been observed maximum inhibitory activity against all the tested for pathogens like *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* except in case of methanol extract whereas in case of *Lavatera cachemiriana*, aqueous extract has shown no activity against any of the four tested pathogens. Phytochemical screening revealed that both plants showed presence of tannins, saponins, flavonoids, Terpenoid, Phenol and Carbohydrate. Although antibacterial activities in some extracts were lower inhibitory zone than standard reference compounds, but further study need to clarify in different concentration of plant extract. Further, phytochemical studies need to be analyzing for future drug discovery.

REFERENCES

- Revathi P, Parimelazhagan T. Traditional knowledge on medicinal plants used by the Irula Tribe of Hasanur Hills, Erode District, Tamil Nadu, India. *Ethnobot Leaflets*, 2010;(14):136-60.
- Akash, Navneet, Bhandari BS. editors. *Ethnomedicinal plant use and practice in traditional medicine*. IGI Global, USA, 2020; 1-300. doi: <https://doi.org/10.4018/978-1-7998-1320-0>.
- Akash, Navneet, Bhandari BS. editors. *Ethnobotany the traditional medical science for alleviating the human ailments and suffering*. 2020, DOI: 10.4018/978-1-7998-1320-0.ch003. 38-57
- Nasrullah AB, Nisar M, Suliman and Ali Z. Ethnobotanical wealth of Jandool Valley, Dir Lower, Khyber Pakhtunkhwa (Kpk), Pakistan. *Int. J. phytomedicine*. 2012;4:351-4.
- Mahmood AAM, Malik RN, Shinwari ZK. Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan. *J. Ethnopharm*. 2013;148:714-23.
- Koka JA, Wani AH, Bhat MY, Wani TA, Parveen S. Antimycotic activity of ethanolic and aqueous leaf extracts of *Ajuga bracteosa* Wall. ex Benth. (Lamiaceae) and *Iris kashmiriana* Baker (Asparagales: Iridaceae) against some vegetable rot fungi. *Brazilian Journal of Biological Sciences*, 2018, v. 5, No. 9, p. 75-84.
- Molur, S. and Walker, S. *Biodiversity Conservation Prioritization Project (BCPP) India*, Endangered Species Project. Zoo Outreach Organisation, 1998, 51.
- Vidyarthi S., O.P. *Forest Flora Of Kashmir*. Working Plan Circle, Jammu and Kashmir Forest Department, 2010: 91-92.
- Sharma R. *Medicinal plants of India: an encyclopaedia*. Daya Books; 2003.
- Sharma R. *Medicinal plants of India: an encyclopaedia*. Daya Books; 2003.
- Kaul, M.K. *Medicinal Plants of Kashmir and Ladhak*. Indus Publishing Co., New Delhi, 1997, 144.
- Kaul MK. *Medicinal plants of Kashmir and Ladakh, temperate and cold-arid Himalaya*. New Delhi: Indus Publishing Co; 1997. 173.
- Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standard single disc diffusion method. *Am J Clin Pathol* 1966;36: 493e6.
- NCCLS-National Committee for Clinical Laboratory Standards Approved standard M7-A2. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Villanova, Pa; 1990.
- Yan X, Murphy BT, Hammond GB, Vinson JA, Neto CC. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *Journal of agricultural and food chemistry*. 2002 Oct 9;50(21):5844-9.
- Razia M, Rajalakshmi BS, Lavanya K, Karthiga V, Bernala W, Deboral P. GC-MS, FTIR and in vitro antibacterial activity of *Abutilon indicum*. *International Journal of Biological and Pharmaceutical Research*. 2013;4(4):256-60.
- Dulf FV, Pamfil D, Baciu AD and Pintea A. Fatty acid composition of lipids in pot marigold (*Calendula officinalis* L.) seed genotypes *Chem Cent J*; 2013, 201-36
- Atanassova M, Bagdassarian V. Rutin content in plant products. *Journal of the University of Chemical Technology and Metallurgy*. 2009;44(2):201-3.
- Porwal, V., Singh, P., Gurjar, D. (2012). A comprehensive study on different methods of extraction from guajava leaves for curing various health problem. *Int J Eng Res*. 6, 490- 496.
- Rakashanda S, Mubashir S, Qurishi Y, Hamid A, Masood A, Amin S. Trypsin inhibitors from *Lavatera cashmeriana* Camb. seeds: Isolation, characterization and in-vitro cytotoxicity activity. *International Journal of Pharmaceutical Science Invention*. 2013;2(5):55-65.
- Khattak S, Saeed-Ur-Rehman, Ullah Shah H, Khan T, Ahmad M. In vitro enzyme inhibition activities of crude ethanolic extracts derived from medicinal plants of Pakistan. *Natural product research*. 2005 Sep 1;19(6):567-71.
- Ballabh B, Chaurasia OP, Ahmed Z, Singh SB. Traditional medicinal plants of cold desert Ladakh—used against kidney and urinary disorders. *Journal of ethnopharmacology*. 2008 Jul 23;118(2):331-9.
- Dar GH, Bhagat RC, Khan MA. *Biodiversity of the Kashmir Himalaya*. Valley Book House; 2002.
- Dar M, Yousuf M, Qurishi M, Ansari A, Ahmad S, Shawl A, Saxena A, Qazi G, inventors; Council of Scientific, Industrial Research (CSIR), assignee. Novel anticancer diterpene compounds, process and uses thereof. United States patent application US 10/401,404. 2004 Sep 30.
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. Phytochemical screening and extraction. *A Rev. IPS*. 2011., 1, 99-100.