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ANTIMICROBIAL ACTIVITY OF SOME ETHNOMEDICINAL PLANTS USED IN KASHMIR, INDIA

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ABSTRACT: Antimicrobial activity of 11 ethnomedicinal plant extracts was evaluated against five bacterial strains (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris*) and one fungal strain (*Candida albicans*). Plants were collected from different regions of Kashmir Valley and the ethnomedicinal data were gathered from traditional healers and elders of the study area. Cold percolation method was used to obtain hexane and methanol extracts and the antimicrobial activity was found using paper disc diffusion method. The results indicated that all plants, exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5 and 5 mg/disc. Among the plants tested, *Nepeta cataria, Allium consangium, Artemisia biensis* and *Ocimum sanctum* were most active. The highest antifungal activity was exhibited by hexane extract of *Nepeta catariaa*gainst *Candida albicans*. Compared to hexane extract, methanol extract showed significant activity against tested organisms. This study also showed that *Nepeta cataria, Allium consangium, Artemisia biensis* and *Ocimum sanctum* could be potential sources of new antimicrobial agents. Among the tested microbial strains, bacteria were found to be more susceptible to many plant extracts than fungi.

INTRODUCTION: Plant based therapies have long history for their use in various ailments. Being comparatively harmless, the natural products have attracted the attention of modern researchers in the treatment of various challenging diseases. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Generations of use of these plants has resulted in gathering of huge knowledge bank regarding benefits and harms of these plants.

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The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Village communities of Kashmir, depend on plant resources mainly for herbal medicines and other daily uses. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections. Eleven plant species used in folk medicine were chosen to determine their antimicrobial activity **Table 1**. In general, these plants are used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory

problems and nervous disorders. Properties of the collected plants are also provided in same table. Evidently, there are not many scientific studies that confirm the antimicrobial properties for most of the plants collected for this study. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants.

There are a few reports on the use of plants in traditional healing by either tribal people or indigenous communities of Kashmir [1, 2, 3]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. This paper reports the results of a survey that was done based on folk uses by traditional practitioners in Kashmir Valley along with bioassay test for antimicrobial activity.

METHODOLOGY:

Ethnobotanical Survey: Plants were selected for this study based on their medicinal use. Fresh plant parts were collected from, Kashmir Valley, located between 33°20' and 34°54'N latitudes and 73°55' and 75°35'E longitudes, India in April-August 2017 [4]. The ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through questionnaire, interviews and discussions among the tribal practitioners in their local language. Duplicates of properly identified and authenticated voucher specimens were deposited in the herbarium of Department of Botany, Islamia College of Science and Commerce, Kashmir, India. Flora of British India was mainly used for identification purposes, and the description drawn by studying the specimens in the laboratory and by adding field notes [5]. Duplicate voucher specimens of *Allium consangium* [GAG 217], *Artemisia biensis* [GAG 218], *Urtica parviflora* [GAG 220], *Geum elatum* [GAG 221], *Ocimum sanctum* [GAG 224], *Ranunculus muricatus* [GAG 230], *Rumex dentatus* [GAG 231], *Polygonum hydropiper* [GAG 234], *Sisymbrium irio* [GAG 235], *Iris germanica* [GAG 237], *Nepeta cataria* [GAG 239] have been deposited in the Herbarium of Department of Botany, Islamia College of Science and Commerce, Srinagar.

Preparation of Hexane and Methanol Extract: Cold percolation method was used to prepare plant extracts. The plant materials were dried under shade and ground into fine powder using electric blender. 50 g of dried powder was soaked in 300 ml hexane for 48 h with intermittent shaking. The plant extracts were filtered through Whatman no. 1 filter paper into pill vials. The filtrates were dried until a constant dry weight of each extract was obtained. The residues were stored at 4 °C for further use. The remaining plant residue was dried and soaked in 300 ml of methanol as above and the extract was collected as described earlier.

Antimicrobial Screening: The hexane and methanol extracts of 11 plants were screened against a total of 5 bacterial strains and one fungal strain. The test organisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and one fungal strain *Candida albicans* obtained from the stock cultures of Department of Pharmaceutical Sciences, University of Kashmir, India.

Preparation of Inoculums: Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 h at 37 °C and 25 °C respectively. The cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria and $2.0 \cdot 10^5$ spore/ml for fungal strains.

Antimicrobial Susceptibility Test: The disc diffusion method was used to screen the antimicrobial activity [6]. *In-vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min.

The different concentrations of extracts (1.25, 2.5 and 5 mg/disc) were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The same procedure was followed for the fungus also. These studies were performed in triplicate.

RESULTS AND DISCUSSIONS: Table 1 provides the botanical name, family, local name, plant parts used together with their traditional therapeutic uses and properties for the 11 ethnomedicinal plants collected from Kashmir Valley. **Table 2** provides results wherein it is found that all the plants tested for antimicrobial activity showed positive activity by inhibiting one or more micro-organisms.

Methanol extracts exhibited higher degree of antimicrobial activity than hexane extracts. However *Ocimum sanctum* and *Nepeta cataria* showed promising activity for its hexane extracts. *Allium consangium* showed highest activity against all microbes except *Proteus vulgaris*.

Highest zones of inhibition were formed by *Nepeta cataria* against *Staphylococcus aureus* and *Escherichia coli*, and by *Ocimum sanctum* against *Bacillus subtilis* and *Escherichia coli*.

TABLE 1: USES AND PROPERTIES OF ETHNOMEDICINAL PLANTS COLLECTED FOR ANTIMICROBIAL SCREENING

Botanical name and Family [voucher specimen]	Local name	Mode of preparation, parts used and Ethnobotanical use	Properties
Allium consangium Kunth. Alliaceae [GAG 217]	Rohan Haak	Decoction of leaves prepared in water is taken internally to treat dysentery. Root and leaf paste is prepared in water and applied externally to treat skin diseases	Stomachic and attenuate
Artemisia biensis Willd. Asteraceae [GAG 218]	Magrass	Leaf powder is mixed with coconut oil and applied on hair for better growth	Insecticide
Urtica parviflora Roxb. Urticaceae [GAG 220]	Soi	Root powder is mixed with lime juice and applied topically on the affected places to treat skin diseases	Antiparasitic, astringent, purgative
Geum elatum Wall ex Hook.f. Rosaceae [GAG 221]	Goji-mool	Fruit powder is mixed with honey and the seeds of *Prunus amygdalus* and rhizome of *Crocus sativus* and taken orally to stimulate body stamina	Astringent
Ocimum sanctum Linn. Lamiaceae [GAG 224]	Tulsi	Powder of whole plant is mixed with the leaves of *Nepeta floccosa* and *Platinus orientalis* (bark)are applied externally to treat rheumatism	Cardiac tonic, febrifuge, astringent and alternative
Ranunculus muricatus Linn. Ranunculaceae [GAG 230]	Kawkhor	Decoction of stem bark prepared in water and is taken internally to treat fever and cough	Febrifuge
Rumex dentatus Linn. Polygonaceae [GAG 231]	Obuj	Root powder and dried fruits of *Juglans regia* are mixed with water and taken internally to get relief from indigestion	Laxative, tonic
Polygonum hydropiper Linn. Polygonaceae [GAG 234]	Machren	Decoction of leaves prepared in water is taken orally by pregnant women during delivery to reduce delivery pain	Tonic
Sisymbrium irio Linn. Brassicaceae [GAG 235]	Danda haakh	Fruit powder prepared in water is made into paste and taken internally with the fruits of *Berberis lycium*, leaves of *Rumex hastata*, rhizome of *Crocus sativus* to strengthen the body	Diuretic, stomachic, tonic and astringent
Iris germanica Linn. Iridaceae [GAG 237]	Sosan	Leaf and stem powder is mixed with the flowers of *Peganum harmala* and fruits of *Daucus carota* and taken internally toinduce abortion and applied externally to treat skin diseases	Anthelmintic and narcotic
Nepeta cataria Benth. Lamiaceae [GAG 239]	Gandh Soi	Leaf paste prepared in water is taken internally to treat stomachache. Powder of bark from stem is used as tooth powder and also used to treat toothache	Aromatic-tonic, stimulant, antiperiodic, pungent, stomachic

TABLE 2: ANTIMICROBIAL ACTIVITY OF THE HEXANE AND METHANOL EXTRACTS OF COLLECTED ETHNOMEDICINAL PLANTS

Plant Name	Solvent	Conc. (mg/disc)	Zone of Inhibition (mm)					
			Bs	Sa	Ec	Pa	Pv	Ca
Allium consangium	H	1.25			-		-	-
		2.5			-		-	-
		5			-		-	-
	M	1.25	12	10	9	12	-	11
		2.5	13	13	12	13	-	13
		5	16	15	14	15	-	15
Artemisia biensis	H	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.25	7	9	8	6	-	10
		2.5	10	11	12	9	-	12
		5	12	13	15	13	-	15
Urtica parviflora	H	1.5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.25	5	5	11	-	-	-
		2.5	7	8	13	-	-	-
		5	9	10	16	-	-	-
Geum elatum	H	1.5	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.5	-	-	-	-	-	-
		2.5	-	5	3	-	-	-
		5	5	7	5	-	-	-
Ocimum sanctum	H	1.25	15	9	15	-	-	-
		2.5	17	11	19	-	-	-
		5	20	13	24	-	-	-
	M	1.25	-	-	-	-	-	17
		2.5	10	11	8	-	-	23
		5	13	14	10	-	-	30
Ranunculus muricatus	H	1.25	-	-	-	-	-	-
		2.5	-	-	-	9	-	-
		5	-	-	-	12	-	-
	M	1.25	11	-	14	-	-	
		2.5	14	-	18	-	-	5
		5	16	-	22	-	-	9
Polygonum hydropipper	H	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.25	9	-	-	-	-	-
		2.5	12	10	-	10	-	-
		5	14	13	-	13	-	-
Rumex dentatus	H	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.25	11	12	-	-	-	8
		2.5	14	15	8	-	-	13
		5	18	19	9	-	-	18
Iris germanica	H	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	M	1.25	-	11	-	-	-	-
		2.5	10	15	-	-	-	-
		5	13	18	-	-	-	-
Sisymbrium irio	H	1.25	8	-	-	8	-	-
		2.5	10	-	-	11	-	-
		5	12	-	-	14	-	-
	M	1.25	-	-	-	-	-	-
		2.5	8	8	-	8	-	-
		5	10	10	-	10	-	
Nepeta cataria	H	1.25	-	17	15	-	-	20
		2.5	8	23	20	-	-	25
		5	10	30	25	-	-	30
	M	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-

Bs- *Bacillus subtilis*, Sa- *Staphylococcus aureus*, Ec- *Escherichia coli*, Pa- *Pseudomonas aeruginosa*, Pv- *Proteus vulgaris*, Ca- *Candida albicans*

Lowest antimicrobial activity was showed by *Geum elatum* extract which showed only slight activity against *Staphylococcus aureus* and *Escherichia coli*.

Urtica parviflora showed activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* but no activity against *Pseudomonas aeruginosa, Proteus vulgaris* and *Candida albicans.*

Bacillus subtilis was found to be the most susceptible which was inhibited either by methanol or hexane extracts. Some organisms exhibited only slight susceptibility.

Escherichia coli was not effected by *Polygonum hydropipper* and *Sisymbrium irio* extracts but was significantly influenced by *Ocimum sanctum, Ranunculus muricatus* and *Nepeta cataria*

Staphylococcus aureus showed highest susceptibility to *Nepeta cataria* besides being affected by other plants as well but showed no sensitivity to *Ranunculus muricatus.*

Candida albicans showed high susceptibility to *Nepeta cataria* besides being significantly affected by *Rumex dentatus, Ocimum sanctum, Artemisia biensis, Allium consangium* but exhibited no sensitivity to *Sisymbrium, Iris, Geum* and *Urtica* extracts.

In general, among the tested microbial strains, bacteria were found to be more susceptible to many plant extracts than fungi.

CONCLUSION: The processing of the plants performed in this study was not comparable to the traditional approach where the Kashmir is use water for extracts whereas we have used hexane and methanol for extraction. As methanol extracts were more effective than hexane extract, it is likely that water extracts as were will be effective as well and possibly more so.

Among the medicinal plants tested in this work, *Nepeta cataria, Allium consangium, Artemisia biensis* and *Ocimum sanctum* showed the most promising antimicrobial properties indicating the potential for discovery of antibacterial principles. Further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these species. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results.

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CONFLICT OF INTEREST: The author declares no conflict of interest.

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