

Full Length Research Paper

Screening for antimicrobial activity of thirty-three medicinal plants used in the traditional system of medicine in Pakistan

Farnaz Malik¹, Shahzad Hussain¹, Tahira Mirza¹, Abdul Hameed², Safia Ahmad², Humayun Riaz³, Pervaiz Akhtar Shah⁴ and Khan Usmanghani^{5*}

¹Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad-45500, Pakistan.

²Department of Microbiology, Quaid-i-Azam University, Islamabad-45500. Pakistan.

³University of Sargodha, Sargodha, Pakistan.

⁴School of Pharmacy, University of Punjab, Lahore, Pakistan.

⁵Hamdard University, Karachi.

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Ninety-nine aqueous, ethanolic and n-hexane extracts of thirty three medicinal plants from twenty six families used in the unani system of medicines in Pakistan were tested against ten commonly prevalent gram negative and gram positive bacteria's particularly the enteric pathogens and yeast using agar diffusion disc method for their antimicrobial activity. Aqueous, ethanolic and n-hexane extracts of fifteen medicinal plants have no activity while other 18 plants have moderate to good antimicrobial activity. The aqueous and alcoholic extract of *Adhatoda vasica* Nees, *Allium sativum* Linn., *Embelia ribes* Burm. f. *Mallotus philippensis* Muell., *Picrorrhiza kurrooa* Royle ex Benth., *Ricinus communis* Linn., *Elaeagnus hortensis* M. Bieb., *Swertia chirata* Buch. Ham. and exhibited good activity against all or most of the microorganisms. The alcoholic extracts of *Solanum nigrum* Linn, *Centella asiatica* Linn, *Solanum xanthocarpum* Schrad and Wendl., *Oxtostagia limbata* Benth., *Cassia angustifolia* Vahl, *Polygonum viviparum* Linn. have moderate activity against some of the microbial strains. Antibacterial activity of some of the plant extracts can be compared with some antibiotics. This shows that Pakistan has a rich flora, having good potential of antimicrobial agents which can be used as a substitute of antibiotics and need for investigation of unexplored flora and screening for new antimicrobial agents.

Key words: Antimicrobial properties, crude extracts, Pakistani medicinal plants.

INTRODUCTION

Plants have been used throughout the world by human beings as drugs and remedies for various diseases since time immemorial. In the sub-continent administration of decoction of plants is still in practice (Nadharm, 1954; Chopra, 1958; Said, 1969). Traditionally, this treasure of knowledge has been passed on orally from generation to generation without any written document (Perumal and Ignacimuthu, 1998, 2000). Reduced susceptibility to antibacterial drugs is continuously increasing which is attributed to indiscriminate use of broad-spectrum antibacterials and immunosuppressive agents (Dean, 1996). This situation provided the impetus to the search

for new antimicrobial substances from various sources like medicinal plants (Cordell, 2000) and screening of these plants may result in the discovery of new effective compounds and can raise the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (Tomoko et al., 2002). There is a need to search for new infection-fighting strategies to control microbial infections. Several studies have been carried out on various medicinal plants screening for their antimicrobial activity (Firas et al., 2008; Yoshikawa et al., 2008; Morales et al., 2008; Parekh and Chanda, 2007; Pesewu et al., 2008). Past two decades, antibacterial properties of various plants and plant parts like root, stem, leaves, seeds and flowers have been well documented for some of the medicinal plants (Nandagopal et al., 2007; Parekh and Chanda,

*Corresponding author. E-mail: ugk_2005@yahoo.com.

2007; Akinpelum and Onakoya 2006). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005).

In our study, we choose thirty three promising medicinal plants used traditionally in Unani and folks medicines in Pakistan and evaluated them for potential antimicrobial activity in order to confirm their use and to detect the new sources of antimicrobial agents.

MATERIALS AND METHODS

Plant material and extract preparation

All plants included *Swertia chirata* C.B. Clarke (Gentianaceae), *Solanum jacquini* Willd. (Solanaceae), *Peganum harmala* L. (Zygophyllaceae), *Cannabis sativa* L. (Cannabaceae), *Adhatoda vasica* Nees (Acanthaceae), *Dodonaea viscosa* Jacq. (Sapindaceae), *Datura alba* Nees. (Solanaceae), *Elaeagnus hortensis* M. Bieb. (Elaeagnaceae), *Ephedra vulgaris* Rich. (Ephedraceae), *Brassica nigra* (L.) W. D. J. Koch. (Brassicaceae), *Dalbergia sissoo* Roxb. (Leguminosae), *Xanthium strumarium* L. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), *Ficus glomerata* Roxb. (Moraceae), *Withania somnifera* (L.) Dunal. (Solanaceae), *Ziziphus mauritiana* Lam. (Rhamnaceae), *Mallotus philippensis* (Lam.) Müll. Arg. (Euphorbiaceae), *Picrorrhiza kurroa* Royle ex Benth. (Serophulariaceae), *Polygonum viviparum* Linn. (Polygonaceae), *Carica papaya* Linn. (Caricaceae), *Cassia angustifolia* Vahl. (Caesalpinaceae), *Allium sativum* Linn. (Liliaceae), *Cassia fistula* Linn. (Caesalpinaceae), *Grewia tenax* (Tiliaceae), *Oxtostagia limbata* (Benth.) Boiss. (Oxtostagia) Labiatae, *Ziziphus jujuba* Mill. (Rhamnaceae), *Solanum xanthocarpum* Schrad. Wendl. (Solanaceae), *Embelia ribes* Burm. F. (Myrsinaceae), *Centella asiatica* (L.) Urb. (Umbelliferae), *Cichorium intybus* Linn. (Compositae), *Solanum nigrum* Linn. (Solanaceae), *Saussurea lappa* (Compositae), *Colocythis vulgaris* (Cucurbitaceae) were collected from various parts of Pakistan that is Islamabad, Punjab, Sargodha Division, Kalar Kahar, and Hazara Division and dried (GACP and FCP guidelines, 2004). All species were identified in the Herbarium of National Agricultural Council, Islamabad, Pakistan. The plant extracts were prepared using the modified method (Alade and Irabi, 1993). Three 100 g portions of the each powdered plant were separated for (a) aqueous extraction in Soxhlet apparatus; (b) ethanolic extraction by maceration of the powdered material in ethanol from 7 to 10 days; (c) n-hexane extraction with maceration. The extracts are then passed through Whatman filter 1. The filtrates obtained were evaporated under vacuum at 40°C using rotary vacuum evaporator to obtain the dry extracts. The last traces of water and/or solvent were evaporated using water bath.

The stock solutions of the dried materials of aqueous, ethanolic and n-hexane extracts were prepared in their respective solvents, in an appropriate concentration of 100 mg/ml. The WHO (2004) reference standards of antibiotics that is, tetracycline, streptomycin and chloramphenicol were used for comparison and a positive control 100 mcg/ml dilution were prepared in their respective solvents/buffers.

Microorganisms

The following strains of bacteria were used: *Bacillus subtilis*, ATCC#6633, *Escherichia coli*, ATCC, #10536, *Klebsiella pneumoniae*, ATCC #10031, *Micrococcus pyogenes*, ATCC #6538,

Salmonella typhi, ATCC# 19430, *Shigella dysenteriae*, ATCC# 11835, *Staphylococcus aureus*, ATCC #6538, *Staphylococcus epidermidis*, ATCC # 12228, *Saccharomyces cerevisiae*, ATCC #9763, *Vibrio cholerae*, ATCC # 25870. For the sub culturing of the microorganisms nutrient agar and Sabouraud dextrose agar were used; and was autoclaved at 121°C for 15 min. The final pH was 7.0 ± 0.2. All the culture media were prepared and treated according to the manufacturer guidelines (DIFCO). The inoculums were prepared in N/saline or broth.

Antibacterial activity

The agar plate diffusion method for aqueous, ethanolic, n-hexane extracts and solvent extract (Baur et al., 1966; Perez et al., 1990) were employed for the determination of antibacterial activity, in which the wells are used as a reservoir of the sample dilutions and the standard dilutions. The reservoir containing the sample dilutions were brought into contact with the inoculated medium and after incubation for 18 to 24 h, the diameter of the clear zones of inhibition around the reservoirs was measured with vernier caliper (Rios et al., 1988). However, in case of no activity of the sample, no zone of inhibition will develop. The concentration of the extracts employed was 100 mg/ml. The dilution medium for the positive controls was sterile distilled water. The test was carried out by triplicate.

RESULTS

Aqueous, ethanolic and n-hexane extract of thirty three medicinal plants from twenty six families used in the unani system of medicines were screened for their antimicrobial activity against ten commonly prevalent gram negative and gram positive bacteria's particularly the enteric pathogens and yeast that is *E. coli*, *S. typhi*, *S. dysenteriae*, *V. Cholerae*, *K. pneumoniae*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *M. pyogenes*, *S. cerevisiae*. Table 1 shows the common uses of thirty three medicinal plants in the unani system of medicines in Pakistan. Aqueous, ethanolic and n-hexane extracts of fifteen medicinal plants have no activity while other 18 plants have moderate to good antimicrobial activity against some of the medicinal plants. The aqueous and alcoholic extract of *E. ribes* Burm. f., *P. kurroa* Royle ex Benth., *A. sativum* Linn., *phillippensis* Muell., *R. communis* Linn., *E. hortensis* M. Bieb., *S. chirata* Buch. Ham. and *A. vasica* Nees. have good activity against most of the microorganisms. The alcoholic extracts of *S. nigrum* Linn., *C. asiatica* Linn., *S. xanthocarpum* Schrad and Wendl., *O. limbata* Benth., *C. angustifolia* Vahl., *P. viviparum* Linn has moderate activity against some of the microbial strains (Table 2). The hexane extracts of all the plants showed no activity. Comparison of antibacterial activity of plant extracts with antibiotics is given in.

DISCUSSION AND CONCLUSIONS

In our study, 99 aqueous, alcoholic or n-hexane extracts of 33 commonly used medicinal plants from 26 different families were tested for the presence of antimicrobial

Table 1. Common uses of medicinal plants used in “traditional system” of medicines in Pakistan.

Plant and family	Voucher number	Local name	Part used	Common uses
<i>Swertia chirata</i> (Gentianeaceae)	118-P	Chireta shirin	P	Bitter tonic, antiperiodic, anthelmintics.
<i>Solanum Jacquini</i> (Solanaceae)	119-F	Kateli	F	Carminative properties, asthma, cough, fever, pain in chest.
<i>Peganum harmala</i> (Zygophyllaceae)	120-L	Hermal	L	Cough, jaundice, neuralgia, diuretic, laxative, antiseptic.
<i>Cannabis sativa</i> (Cannabinaceae)	121-L	Bhang	L	Stimulant, aphrodisiac and sedative.
<i>Adhatoda vasica</i> (Acanthaceae)	122-L	Arusa	L	Antispasmodic, cough, cold, bronchitis, asthma. Anthelmintics, vermifuge, diarrhoea and dysentery.
<i>Dodonaea viscosa</i> (Sapindaceae)	123-L	Sanata	L	Alterative, laxative, tonic-also used as poultices in gout, rheumatism and snake bite.
<i>Datura alba</i> (Solanaceae)	124-L	Dhatoora	L	Whole plant narcotic, anodyne, antispasmodic, causes dilation of when locally.
<i>Eleagnus hortensis</i> (Eleagaceae)	125-L	Sirijib	L	Locally used for rheumatism and digestion.
<i>Ephedra vulgaris</i> (Genetaceae)	126-Sm	But Shur	Sm	Diuretic, Stomachic and tonic, dilates pupil of eye.
<i>Brassica nigra</i> (Cruciferae)	127-L	Kalo Rai	L	Emetic, digestion, plasters used in sciatica, urticaria and gout.
<i>Dalbergia sissoo</i> (Leguminosae)	128-L	Shisham	L	Astringent, bleeding, piles and varicose veins.
<i>Xanthium strumarium</i> (Compositae)	129-L	Kullan Chotta gokkro	L	Whole plant is diaphoretic, diuretic, used in urinary and renal complains, malarial fever.
<i>Ricinus communis</i> (Euphorbiaceae)	130-L	Arand	L	Oil is purgative, root used in various diseases, rheumatism, lumbago and sciatica.
<i>Ficus glomerata</i> (Urticaceae)	131-L	Gular	L	Astringent, carminative, stomachic and vermicide.
<i>Withania somnifera</i> (Solanaceae)	132-L	Asgand nigori	L	Tonic, astringent, nervine sedative, cough, asthma and uterine diseases, Antiseptic.
<i>Ziziphus napica</i> (Rhamnaceae)	133-R	Kakoli	R	Aphrodisiac, diseases of bile, cures burning of skin and fever.
<i>Mallotus philippensis</i> (Euphorbiaceae)	134-F	Kambli	F	Cathartic, anthelmintics, aphrodisiac and purgative.
<i>Picrorrhiza kurroa</i> (Serophulariaceae)	135-Ri	Kali Kutki	Ri	Used for treatment of epilepsy, paralysis, skin diseases, antiperiodic.
<i>Polygonum viviparum</i> (Polygonaceae)	136-R	Maslum	R	Root is astringent, used in diarrhoea, dysentery, fever, sore throated haemoptysis.
<i>Carica papaya</i> (Caricaceae)	137-Fp	Pajata	Fp	Dyspepsia, intestinal irritation, anthelmintics for round worms.
<i>Cassia angustigolia</i> (Casesal piniaceae)	138-L	Hindi Sana	L	Laxative and purgative. Cathartic properties, increases peristaltic movements of the colon.
<i>Allium sativum</i> (Liliaceae)	139-B	Lason	B	Hot, stimulant, carminative ant- rheumatic, cough and fever.
<i>Cassia fistula</i> (Caesalpiniaceae)	140-L	Sonhali	L	Purgative, liver diseases, laxative, ulcers
<i>Grewia tenax</i> (Tiliaceae)	141-L and Sm	Gwarji	L and Sm	Useful in treatment of diarrhoea and dysentery. A decoction of the wood ginstolcure, cough and pain.
<i>Oxtostagia limbata</i> (Ostostagia) Labiatae	142-L	Bhui	L	Leaves are applied to gums in ophthalmia
<i>Ziziphus jujuba</i> (Rhamnaceae)	143-L	Baer	L	Stomachic, fruit purifies blood and assists in digestion.
<i>Solanum xanthocarpum</i> (Solanaceae)	144-L and St	Kateli	L and St	Respiratory disorder, stomach disorder, throat sore, paste of roots applied on gums, in snake bite and scorpion bite.
<i>Embelia ribes</i> (Myrsinaceae)	145-F	Babrug	Fruit	Antiseptic, intestinal worms, biter piles, locally tooth ache, head ache, skin diseases and lung inflammation.
<i>Centella asiatica</i> (Umbelliferae)	146-L	Barhami	L	Skin diseases, syphilitic, rheumatism, dysentery and fevers.
<i>Cichorium intybus</i> (Compositae)	147-Sm and L	Kasani	Sm and L	Acne, diarrhoea, fever and vomiting, diuretic.
<i>Solanum nigrum</i> (Solanaceae)	148-F	Mukod	F	Skin disorders, wound, fevers, eye diseases.
<i>Saussurea lappa</i> (Compositae)	149-R	Kuth	R	Anthelmintics, skin diseases, leprosy ulcers.
<i>Colocyntis vulgaris</i> (Cucurbitaceae)	150-R	Indra –yan	R	Ascites, jaundice, urinary diseases and rheumatism.

L (leaves), F (fruit), Ap (aerial parts), B (bark), Rf (rind of fruit), Ri (rhizome), S (seeds), Sm (stem), R (root) and St (shoots).

Table 2. Contd.

			0	0	0	0	0	0	0	0	0	0	0
<i>Centella asiatica</i> (L.) Urb. (Umbelliferae)	Barhami	146-L	+	+	0	0	+	0	+	0	+	0	0
			0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
<i>Cichorium intybus</i> Linn. (Compositae)	Kasani	147-Sm and L	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
<i>Solanum nigrum</i> Linn. (Solanaceae)	Mukod	148-F	+	+	+	+	++	0	+	0	+	+	+
			0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
<i>Saussurea lappa</i> (Compositae)	Kuth	149-R	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
<i>Colocyntis vulgaris</i> (Cucurbitaceae)	Indra –yan	150-R	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0

(A) *Staphylococcus aureus*, (B) *Salmonella typhi*, (C) *Shigella dysenteriae*, (D) *Vibrio cholerae* (E), *Escherichia coli*, (F) *Klebsiella pneumonia*, (G) *Staphylococcus epidermidis*, (H) *Micrococcus pyogenes*, (I) *Bacillus subtilis*, (J) *Sacetromyees cerevisiae*. Key: - denotes zone of inhibition. '+' 2 to 16 mm; '++' 17 to 20 mm; '+++' 21 to 24 mm; '++++' 25 to 30 mm.

activity against 10 g positive and gram negative bacteria's especially enteric pathogens and yeast which was the distinctive feature of this study. The use of these plants in Pakistan for treating with the most frequent medicinal uses being astringent, anthelmintics, antispasmodic, laxative, stomachic, diuretic etc has been already reported (Said, 1969). In other studies conducted in Turkey, Cambodia, Yemen, Peru, Brazil, Mexico and Cameron, screening of antibacterial activity were conducted. In these studies, either the number of extracts were fewer than our study or the microorganisms were less in number against whom the antibacterial activity was performed (Turker and Usta, 2008; Chea et al., 2007; Kong

et al., 2007; Al-Fatimi et al., 2007; Duarte et al., 2007; Kloucek et al., 2007; Molina-Salinas et al., 2007; Gangoué-Piéboji et al., 2006). In another citation the antibacterial activity of aqueous, ethanol and acetone extracts of *Corriander sativum*, *Abutilon indicum*, *Boerhavia diffusa*, *Andrographis paniculata*, *Plantago ovata*, *Bacopa monnieri*, *Bauhinia variegata*, *Flacouratia ramontchi*, *Euphorbia ligularia*, *Zinziber officinale*, *Terminalia chebula*, *Azadirachta indica*, *Ocimum sanctum* and *Cinnamomum cassia* was determined against *E. coli* and the acetone and ethanol extracts exhibited significant activity (Anjana et al., 2009). The antimicrobial activity of crude extracts of medicinal plants used in traditional Indian

medicine was tested against five important pyogenic bacteria. They are *S. aureus*, *E. coli*. The most effective antimicrobial plant was identified as *Glycyrriza glabra* followed by *Dathura metal*, *Coccinia grandis* (Bagyalakshmi et al., 2009). In present study, the antibacterial activity of some medicinal plants against *S. aureus*, *S. typhi*, *S. dysenteriae*, *V. cholerae*, *E. coli*, *K. pneumoniae*, *S. epidermidis*, *M. pyogenes*, *B. subtilis* and *S. cerevisia* has been studied. Total of thirty three plants were studied. Seventeen plants (*S. chirata* C.B.Clarke, *A. vasica* Nees, *D. viscosa* Jacq., *E. hortensis* M.Bieb., *X. strumarium* L., *Ricinus communis* L., *M. philippensis* (Lam.), *P. kurrooa* Royle ex Benth, *P. viviparum* Linn., *Carica*

papaya Linn., *Cassia angustigolia* Vahl., *A. sativum* Linn., *Oxtostagia limbata* (Benth.), *S. xanthocarpum* Schrad. Wendl., *E. ribes* Burm. F., *Centella asiatica* (L.) Urb., *S. nigrum* Linn.) have displayed antibacterial activity against *S. aureus*.

Seventeen plants (*Swertia chirata* C.B.Clarke, *A. vasica* Nees., *D. viscosa* Jacq., *E. hortensis* M.Bieb., *X. strumarium* L., *Ricinus communis* L., *M. philippensis* (Lam.) Müll. Arg., *P. kurrooa* Royle ex Benth., *P. viviparum* Linn., *C. papaya* Linn., *C. angustigolia* Vahl., *A. sativum* Linn., *O. limbata* (Benth.), *S. xanthocarpum* Schrad. Wendl., *E. ribes* Burm. F., *C. asiatica* (L.) Urb., *S. nigrum* Linn.) have antibacterial against *S. typhi*. Fourteen plants (*S. chirata* C. B. Clarke, *A. vasica* Nees, *E. hortensis* Bieb., *R. communis* L., *M. philippensis* (Lam.) Müll.Arg. *P. kurrooa* Royle ex Benth., *P. viviparum* Linn., *C. papaya* Linn. *C. angustigolia* Vahl. *A. sativum* Linn. *O. limbata* (Benth.) Boiss., *S. xanthocarpum* Schrad. Wendl. *E. ribes* Burm. F. *S. nigrum* Linn.) showed anti-bacterial activity against *S. dysenteriae*. Thirteen plants (*S. chirata* C.B.Clarke, *A. vasica* Nees. *E. hortensis* Bieb., *R. communis* L., *M. philippensis* (Lam.) Müll.Arg., *P. kurrooa* Royle ex Benth., *P. viviparum* Linn., *C. angustigolia* Vahl., *A. sativum* Linn., *O. limbata* (Benth.) Boiss., *S. xanthocarpum* Schrad. Wendl., *E. ribes* Burm. F., *S. nigrum* Linn.) have antibacterial activity against *V. cholerae*. Thirteen plants (*S. chirata* C.B.Clarke., *A. vasica* Nees., *D. alba* Nees., *R. communis* L., *M. philippensis* (Lam.) Müll.Arg., *P. kurrooa* Royle ex Benth., *P. viviparum* Linn., *C. papaya* Linn., *A. sativum* Linn., *O. limbata* (Benth.) Boiss., *E. ribes* Burm. F., *C. asiatica* (L.) Urb., *S. nigrum* Linn.) have antibacterial activity against *E. coli*. Three plants (*E. hortensis* M.Bieb., *A. sativum* Linn., and *E. ribes* Burm. F.), exhibited antibacterial activity against *K. pneumoniae*.

Four plants (*A. sativum* Linn., *E. ribes* Burm. F., *C. asiatica* (L.), *S. nigrum* Linn.), have antibacterial activity against *S. epidermidis*. Three plants (*S. chirata* C.B.Clarke, *A. sativum* Linn., *E. ribes* Burm. F. have antibacterial activity against *Micrococcus pyogens*. Fifteen plants (*S. chirata* C.B.Clarke, *A. vasica* Nees., *E. hortensis* M.Bieb., *R. communis* L., *M. philippensis* (Lam.), *P. kurrooa* Royle ex Benth., *P. viviparum* Linn. *C. papaya* Linn., *C. angustigolia* Vahl., *A. sativum* Linn., *O. limbata* (Benth.) Boiss., *S. xanthocarpum* Schrad. Wendl., *E. ribes* Burm. F., *C. asiatica* (L.), *S. nigrum* Linn.) have antibacterial activity against *B. subtilis*.

Three plants (*S. chirata* C.B.Clarke, *E. ribes* Burm. F., *S. nigrum* Linn.) gave antibacterial activity against *S. cerevisiae*. All these results clearly corroborate with the findings on an antibacterial activity as cited in the text, with the plants presented herewith. It can be concluded from our study that most of the plants had potential of antibacterial activity and is comparable to antibiotics like tetracycline, streptomycin and chloramphenicol. They can offer an alternative method to treat drug resistant enteropathogens. Medicinal plants have a great importance as 80% of the population in developing countries, which is

the 65% of the total population of the world rely on "traditional" herbal medicines for their basic health care needs (Fabricant and Farnsworth, 2001). As Pakistan is rich in medicinal and aromatic plants, and have good potential of antimicrobial agents, these can be used as a substitute of antibiotics and as natural health care products in "traditional systems" of medicine prevalent in Pakistan.

Medicinal plants are growing abundantly in the wild that is Hazara, Malakand, Kurrum Agency, Murree Hills, Azad Kashmir, Northern Areas, Sindh and Baluchistan, or are cultivated on farmlands in the Punjab, Sindh, NWFP, Baluchistan and Azad Kashmir. There is also need for the investigation of unexplored flora and screening for new antimicrobial agents and determination of their phytochemical studies to determine the types of compounds responsible for the activity. It will help in alleviating the health problems of the peoples especially living in far flung areas and did have the access to modern health care system.

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