

SCREENING OF INDIGENOUS KNOWLEDGE OF HERBAL REMEDIES FOR SKIN DISEASES AMONG LOCAL COMMUNITIES OF NORTH WEST PUNJAB, PAKISTAN

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Abstract

The aim of this study was to conduct an ethnobotanical survey of North Western Punjab to identify medicinal plants traditionally used to treat skin infections and to determine their antimicrobial potential against skin-infecting pathogens. Methanolic extracts of selected plants were screened against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* using the well diffusion method. Some plants are traditionally used in combination with other plants and chemicals like vinegar and olive oil. Therefore, antimicrobial screening was also done for these combinations in different proportions. Results showed that out of 12 studied plants, six showed inhibitory effect against *Staphylococcus aureus* and *Candida albicans*. *Azadirachta indica* and *Mentha arvensis* showed high antibacterial activity against *Staphylococcus aureus* with similar minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 2.5 and 5mg/ml, respectively. *Azadirachta indica*, *Cassia angustifolia*, *Phoenix dactylifera* and *Lawsonia inermis* were found to be effective against the fungus *Candida albicans*, with MIC values of 0.625, 1.25, 0.625, 0.625 mg/ml and MBC values of 1.25, 2.5, 1.25 and 1.25 mg/ml, respectively. None of the plants showed antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The results of the combination experiment demonstrated that antimicrobial activity exhibited by combinations of plant extracts and chemicals was imparted by chemicals like vinegar.

Introduction

Plant-based medicines have a respectable position today, especially in developing countries where modern health services are not sufficient. Indigenous remedies are gaining popularity in both rural and urban areas because they are effective, safe and inexpensive. Information collected from ethnic groups or indigenous traditional medicine has played an important role in the discovery of new products from plants as chemotherapeutic agents (Katewa *et al.*, 2004). Through ethnobotanical surveys, indigenous knowledge from local people and practitioners is collected and documented in order to identify plants that can be a source of drugs against infectious diseases (Sarwat *et al.*, 2012).

People inhabiting the tribal localities and villages have used indigenous plants as medicines for generations because this knowledge is based on experience. The tribes and villages also have no health facilities as they are far away from cities. Most inhabitants are poor or middle class and they cannot afford expensive synthetic drugs (Shinwari & Khan, 1998).

About 80% of the world's population depends on traditional systems of health care (Ahmad, 2005). In Pakistan, about 84% people depend upon traditional medicine for almost all their medicinal needs (Hocking, 1958). All traditional medicine systems have their roots in home remedies and this information is transmitted from one generation to another with the passage of time (Shinwari, 1996).

Pakistan has an area of about 80,943 km² and it lies between 60° 55' to 75° 30' E longitude and 23° 45' to 36° 50' N latitude. As Pakistan has an altitude ranging from 0 to 861 lm, it has a variety of climatic zones and is unique in biodiversity. In Pakistan there are about 6,000 species of higher plants. It has been reported that 600 to 700 species are used for medicinal purposes (Shinwari, 2010). It has

also been anticipated that 70% of the total species are uni-regional and about 30% are bi-or pluri-regional. The country is divided into four phytogeographical regions: (i) Irano-Turanian (45% of species); (ii) Sino-Himalayan (10%); (iii) Saharo-Sindian (9.5%); (iv) Indian element (6%). Even though the Saharo-Sindian Region has the biggest area, the diversity of species restricted to this area is the lowest for any phytogeographical region (Ali & Qaiser, 1986). Pakistan is quite rich in medicinal herbs that are spread over a large area due to its salubrious climate. Pakistan has a rich flora, but a large portion of this indigenous plant knowledge still remains unexplored. The present investigation contributes to further comprehensive biological studies on these medicinal plants along with their biological standardization.

The area of study for the ethnobotanical survey is the North-Western part of the Punjab province. It represents the plains of the western part of the salt-ranges near the Sakesar hill (Iftikhar, 1964). Its boundaries are within Bhakkar, Khushab, D.I. Khan and Bannu districts. It is part of Sargodha Division and has a population of more than one million. It has been estimated that 79.22% of people live in the rural areas while 20.78% of people live in the urban areas (Anonymous, 1998). Literacy rate of study area is 25%.

Average maximum temperature per annum of this area is 47°C and minimum temperature is 19°C. Mean annual rainfall is 3.3 mm and the maximum rainfall of about 6.6 cm occurs in the month of July. The three types of soil in this district are sandy, clay and loamy. Wheat, barley, oat, mustard, Eruca, fennel, peanut, are important crops of the area (Anonymous, 2000; Iftikhar, 1964). Due to cutting of forests for fuel and timber purposes, the area covered by forest is low. The study area is primarily semi-arid, with a very small area irrigated by canals of the river Indus (Iftikhar, 1964).

Skin diseases can be caused by a number of microbes and the skin is a haven for many microbes. Medicinal plants have been used in traditional treatments of skin infections worldwide. The present study focused on the antimicrobial activity of some medicinal plants against bacterial and fungal infections of the skin. Common skin infections include impetigo, boils, carbuncles, cellulitis, and complications from burns (Gelfand, 1984). Common pathogens causing infection include *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Baggett & Hennessy, 2004; Toshkova & Annemuller, 2001; Wysocki, 2002). Candidal infections (a fungal infection) commonly occur in warm moist body areas. Usually skin effectively blocks yeasts, but any breakdown or cuts in the skin may allow this organism to penetrate.

Medicinal plants are rich sources of antimicrobial agents. Prior to the development of Western medicine, traditional medicinal plants were used as remedies to cure various diseases including infectious diseases. Currently, most of the drugs that were isolated from natural resources, including medicinal plants are used for treatment of various bacterial and other infections (Sarwat *et al.*, 2012). Plants are used medicinally and are widely used as ethnomedicine around the world in different countries as they are sources of many potent and powerful

drugs (Srivastava *et al.*, 1996). A number of plants have been known for their biological (Grover *et al.*, 2002; Gajera *et al.*, 2005) and antimicrobial properties.

In an attempt to expand the spectrum of antimicrobial agents from natural resources, 10 different plants, *Lawsonia inermis*, *Albizia lebbek*, *Saussurea lappa*, *Nigella sativa*, *Azadirachta indica*, *Cassia angustifolia*, *Dalbergia sissoo*, *Punica granatum*, *Phoenix dactylifera*, *Curcuma longa*, *Mentha arvensis* and *Trigonella foenum-graecum*, were selected based on their traditional uses in the study area to assess their antibacterial potential. Some plants are used alone and some plants are used in combination with other plants or with chemicals.

Materials and Methods

Ethnobotanical survey: The Northwest part of Punjab was explored during the ethnobotanical survey. Information was collected through investigations and by interviewing the local people during various field trips. According to ethnobotanical investigations, different parts of plants are used for the treatment of various skin infections. Plants with their local name, botanical name, family, part of plant used and ethnobotanical uses are reported in Table 1.

Table 1. Ethnobotanical importance of plants from North West Punjab.

No.	Common name	Botanical name	Family	Part of plant used	Ethnobotanical use
1.	Siris	<i>Albizia lebbek</i>	Mimosaceae	Bark	The bark of <i>Albizia lebbek</i> and root of <i>Curcuma longa</i>
2.	Haldi	<i>Curcuma longa</i>	Zingiberaceae	Root	(Haldi) are ground with water and used to cure acne
3.	Kust	<i>Saussurea lappa</i>	Compositae	Root	The root powder of <i>Saussurea lappa</i> and vinegar are mixed and used for boils treatment
4.	Kalonji	<i>Nigella sativa</i>	Ranunculaceae	Seed	The paste prepared by mixing ground <i>Nigella sativa</i> seeds in water is used for the treatment of boils
5.	Neem	<i>Azadirachta indica</i>	Meliaceae	Leaves	<i>Azadirachta indica</i> leaves are used for acne treatment.
6.	Mehndi	<i>Lawsonia inermis</i>	Lythraceae	Leaves	The leaves of <i>Lawsonia inermis</i> are mixed with olive oil and are used for furuncle treatment The leaves of <i>Cassia angustifolia</i> and <i>Lawsonia inermis</i> are boiled in vinegar and are used for the treatment of furuncles
7.	Seena	<i>Cassia angustifolia</i>	Caesalpiniaceae	Leaves	Paste of the dried leaves of <i>Cassia angustifolia</i> in vinegar is used for acne, eczema and pimples. <i>Saussurea lappa</i> and <i>Caessia angustifolia</i> are boiled in vinegar and used for treatment of fungal infections
8.	Khajur	<i>Phoenix dactylifera</i>	Palmae	Leaves	<i>Phoenix dactylifera</i> leaves are used for healing of wounds
9.	Anar	<i>Punica granatum</i>	Lythraceae	Seed	The seeds of <i>Punica granatum</i> are ground and are applied on wounds for their treatment.
10.	Shesham	<i>Dalbergia sissoo</i>	Papilionaceae	Leaves	Leaves of <i>Dalbergia sissoo</i> are used for treatment of boils
11.	Podina	<i>Mentha arvensis</i>	Labiatae	Leaves	The juice of <i>Mentha arvensis</i> leaves is used for treatment of boils
12.	Mathery	<i>Trigonella foenum-graecum</i>	Papilionaceae	Seed	<i>Nigella sativa</i> is boiled along with <i>Trigonella foenum-graecum</i> in vinegar and this mixture is used to cure fungal infections

Sample collection: Fresh leaves, bark and roots of 12 different plants, *Lawsonia inermis*, *Albizia lebbek*, *Saussurea lappa*, *Nigella sativa*, *Azadirachta indica*, *Cassia angustifolia*, *Dalbergia sissoo*, *Punica granatum*, *Phoenix dactylifera*, *Curcuma longa*, *Mentha arvensis* and *Trigonella foenum-graecum*, were

collected from different areas of North West Punjab as well as from Hakeem (traditional healers). The leave, bark and roots were washed with tap water and then with sterile distilled water. Leaf, bark and root material was then air-dried under shade.

Antimicrobial activity

Grinding of samples: Dried samples of *Lawsonia inermis*, *Albizia lebbek*, *Saussurea lappa*, *Nigella sativa*, *Azadirachta indica*, *Cassia angustifolia*, *Dalbergia sissoo*, *Punica granatum*, *Phoenix dactylifera*, *Curcuma longa*, *Mentha arvensis* and *Trigonella foenum-graecum* were ground into powder using an electric blender.

Extraction of samples: About 10g of dried plant powder was extracted in 100ml methanol and kept on a rotary shaker for 24h. The extract was filtered by using filter paper and then dried under reduced pressure by using a rotary evaporator. The dried samples were dissolved in DMSO for preservation. Then samples were then filter sterilized using Millipore Syringe filters of 0.2 μ m pore size. The extract was stored at 4°C in airtight bottles.

Combination of samples: All the plants were used alone and some plants were used in combination with other plants and chemicals for antimicrobial screening. Combinations of plant extracts and chemicals were prepared by mixing the plant extract and chemical in different proportions like plants 25%, 50% and 75% and chemicals 75%, 50% and 25%, respectively.

The plants used in combination with other plants as well as with chemicals were combined in different ratio such as 25% of 1st plant, 50% 2nd plant and 25% chemical, 25% of 1st plant, 25% 2nd plant and 50% chemical, 50% of 1st plant, 25% 2nd plant and 25% chemical.

Plant used in combination with two other plants and chemicals were also combined in different proportions for checking the antifungal activity of medicinal plants in combination.

Microorganisms tested: The bacterial strains used to assess the antibacterial properties of crude methanol extract of all plants and combination of these plants with other plants as well as with chemical, as they are ethnobotanically used. For antibacterial assay microbial strains were obtained from PIMS (Pakistan Instituted of Medical Sciences) Islamabad. One Gram positive bacteria *Staphylococcus aureus* and two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were studied for antibacterial activity. For antifungal activity *Candida albicans* strain was also collected from PIMS. The organisms were maintained on nutrient agar slope at 4°C and sub-cultured before use.

Antibacterial activity: Antibacterial activity of the plant extracts was tested on the selected organism of Gram-positive and Gram-negative bacteria by Agar well diffusion method (Shinwari *et al.*, 2009). Mueller Hinton medium was prepared at a concentration of 2.0g/l agar. Twenty ml of Mueller Hinton agar medium was poured into each 20 x 90 mm petri plate and allowed to solidify. The plates were kept in an incubator for 24 hr and checked for contamination. Nutrient broth cultures of the test bacteria, prepared 24 hr previously, were swabbed evenly on solidified sterile Mueller Hinton agar using a sterile cotton swab. Wells were made in the agar by using

a sterile cork borer. Plant extract (50 μ l) was loaded in each well. A control antibiotic disc of 30 μ g chloramphenicol was also used to compare the activity of plant extract with antibiotics. The plates were incubated at 37°C in duplicate in the incubator for 24 h. Zone of inhibition for each extract was then measured and the results were recorded.

Minimum inhibitory concentration (MIC): The MIC of the crude extracts was determined using the method described by Akinpelu & Kolawale (2004). Each of the extracts was reconstituted into nutrient broth (5 mg/ml) in test tubes and the 5 mg/ml was taken as the initial concentration. Four more tubes of 2 ml nutrient broth were set up and 2ml of 2.5mg/ml of the extract were taken and used for two-fold dilution of the four tubes of nutrient broth, forming concentrations of 2.5, 1.25, 0.625 and 0.3125mg/ml.

Normal saline was used for the turbid suspensions of the microbes. This bacterial suspension was then compared to the 0.5 McFarland standards. If the bacterial suspension was not of the same density as the McFarland 0.5 standard, the turbidity was decreased by adding sterile saline or by adding more bacterial suspension. When the suspension matched the standard, it was assumed to be 1.5×10^8 cfu/ml. An aliquot (0.1 ml) of the cell suspension was introduced into each of the tubes with the varied extract concentrations. All the tubes were incubated for 24 h at 37°C. The tube with the lowest concentration of extract but with no growth (turbidity) of the microbes was taken to be the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration (MBC): The minimum bactericidal concentration (MBC) of the plant extract was determined using the Spencer and Spencer method (2004). A drop of inocula from the tubes of the MIC that showed no growth of the microbes was dropped on nutrient agar plates. The plates were incubated for about 24h at 37°C. The lowest concentration of the extract that showed no colony growth on the agar plates was taken as the MBC.

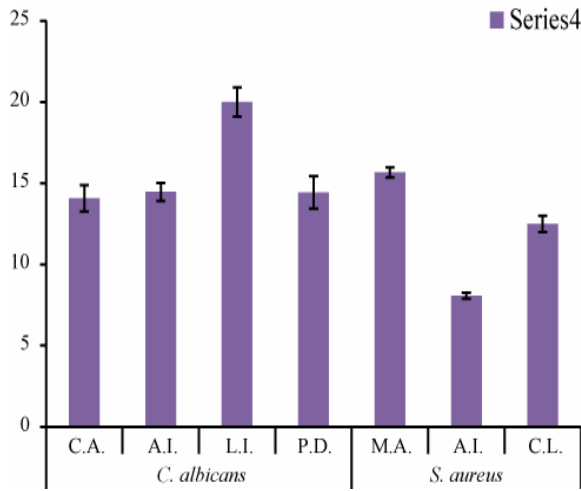
Results

The ethnobotanical survey of traditionally used medicinal plants to treat skin infections conducted in North West Punjab collected useful information. Ethnobotanical data, plant parts used along with their common name, their botanical name and family are given in Table 1. Twelve plants were selected for the study based on their importance to the indigenous community where the survey was conducted.

Among the 4 microorganisms used in the present study, *C. albicans* was found to be sensitive of all microorganisms used in the study. Antimicrobial screening of individual plants against *C. albicans* revealed that the microbe was sensitive to only 4 plants. It was most sensitive to *Lawsonia inermis* (20 \pm 1) while sensitivities to *Cassia angustifolia*, *Azadirachta indica* and *Phoenix dactylifera* were not found to be significantly different from each other and produced zones of inhibition of 14.06 \pm 0.90,

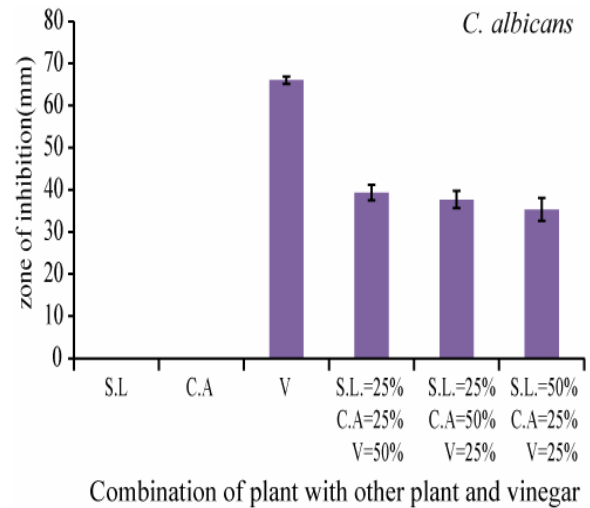
14.46±0.61, and 14.43±1.10 mm, respectively (Fig. 1). The organism was found to be resistant to the rest of the plants. The ethnobotanical survey indicated that some plants were applied in combination with other plants and chemicals. The results of the combination of *Cassia angustifolia*, *Saussurea lappa* and vinegar in different proportions showed that the zone of inhibition decreased as the concentration of vinegar decreased in the combination from 50 to 25%. When vinegar was 100% the zone of inhibition was (66±1), in combination with *Cassia angustifolia* (25%), *Saussurea lappa* (50%) and vinegar (25%), the inhibition zone was 39.3±2.08, when *Cassia angustifolia* (25%), *Saussurea lappa* (25%) and vinegar(50%), the

inhibitory zone was 37.6±6.8, while the combination of *Cassia angustifolia* (50%), *Saussurea lappa* (25%) and vinegar (25%) resulted in an inhibition zone of 35.3±3.05 (Fig. 2). The results of antimicrobial activity of a combination of *Nigella sativa*, *Trigonella foenum-graecum*, *Lepidium sativum* and vinegar in different ratios showed a similar trend as the other vinegar combination, that is, a decrease in inhibition zone with the decrease in the amount of vinegar in the mixture (Fig. 3). A combination of *Lawsonia inermis* with olive oil showed that the zone of inhibition formed by the combination (20±1) was not different than the one formed by the plant extract alone (Fig. 4).



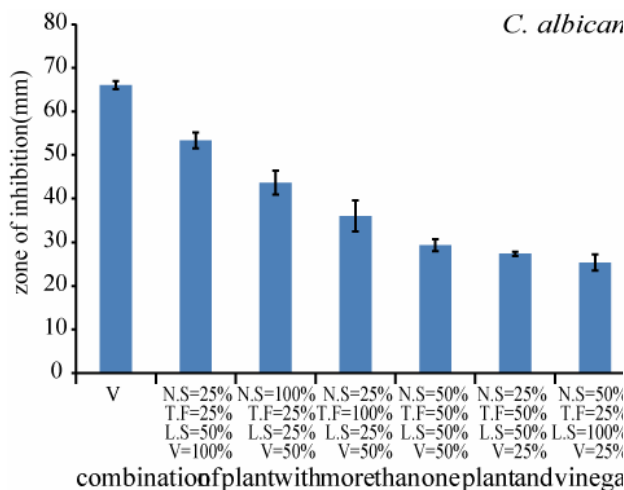
Azadirachta indica (A.I.), *Lawsonia inermis* (L.I.), *Cassia angustifolia* (C.A.), *Mentha arvensis* (M.A.), *Phoenix dactylifera* (P.D.), *Curcuma longa* (C.L.).

Fig. 1. Zones of inhibition of the extracts against the selected pathogen in (mm) at a concentration of 5mg/ml.



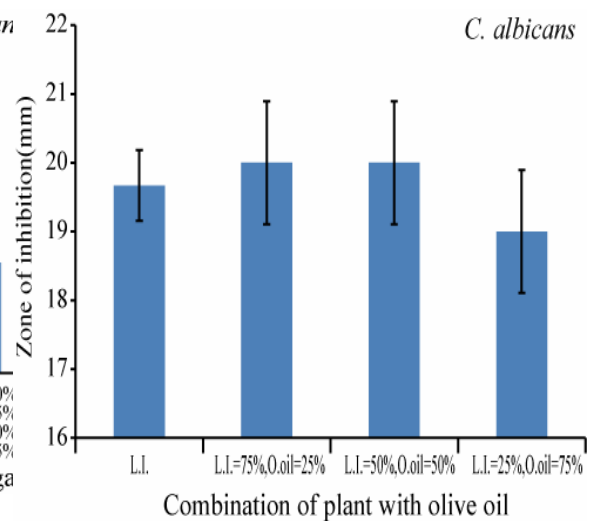
Saussurea lappa (S.L.), *Cassia angustifolia* (C.A.), Vinegar (V)

Fig. 2. Zones of inhibition of plant extracts with vinegar against selected fungus (mm)



Nigella sativa (N.S.), *Trigonella foenum-graecum* (T.F.), *Lepidium sativum* (L.S.), Vinegar (V).

Fig. 3. Zones of inhibition of the plant extract with more than one plant and vinegar against selected fungus (mm).



Lawsonia inermis (L.I.), Olive oil (O.oil).

Fig. 4. Zones of inhibition of the extracts with olive oil against fungus (mm)

Plant extracts exhibiting antimicrobial potential against *C. albicans* were analyzed to determine their MIC and MBC. *Azadirachta indica* and *Phoenix dactylifera* presented similar MIC value of 0.625mg/ml and MBC values of 1.25mg/ml. MIC of *Cassia angustifolia* was found to be 1.25mg/ml and MBC was 2.5mg/ml. The lowest MIC and MBC values were obtained from *Lawsonia inermis*, 0.3125 and 0.625mg/ml, respectively (Table 2).

Among the bacteria used in the present study, *S. aureus* was found to be resistant to most plant extracts. The antimicrobial screening assay revealed that the microbe was sensitive to only two plants. It was most sensitive to *Mentha arvensis* (15.66±0.351), followed by *Azadirachta indica* (7.96±0.35) (Fig. 1).

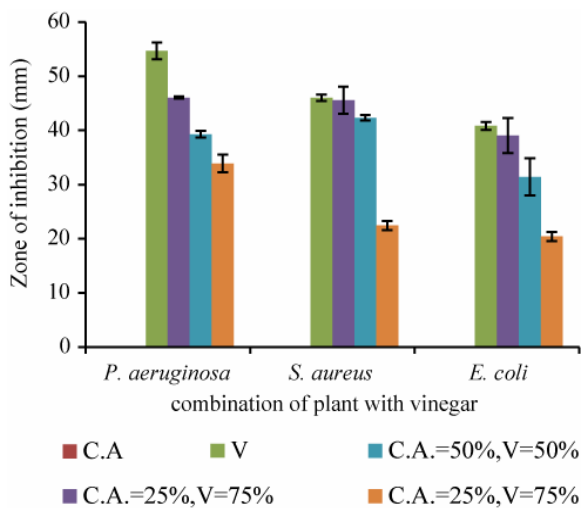
Following information obtained from the ethnobotanical survey, different recipes used for the treatment of bacterial infections were also tested. The ethnobotanical survey showed that some plants were applied in combination with other plants and chemicals. *Pseudomonas aeruginosa* was found to be most sensitive to a combination, followed by *S. aureus* and *E. coli*. Fig. 4

shows results of antimicrobial screening of *Cassia angustifolia* with vinegar in different proportions. The zone of inhibition formed against *P. aeruginosa* decreased with the decrease in concentration of vinegar from 100 to 25%. The zone of inhibition when vinegar = (100%) was 54.67±1.52, in a combination of *Cassia angustifolia* (50%) and vinegar (50%), the inhibitory zone was 46.06±0.20, for the combination of *Cassia angustifolia* (75%) and vinegar (25%) the zone of inhibition was 39.3±0.60SD, and sensitivity of the combination of *Cassia angustifolia* (25%) and vinegar (75%) was 33.86±1.62 mm. *Staphylococcus aureus* and *E. coli* were also found to be sensitive against a combination of *Cassia angustifolia* with vinegar. The *S. aureus* and *E. coli* zones of inhibition decreased with the decrease in vinegar from 100 to 25% and zones were 46±0.57 and 45.5±2.5 at 100%, 42.3±0.50 and 22.4±0.85 at 75%, 40.8±0.73 and 39.03±3.25 at 50%, and 31.4±3.42 and 20.4±0.85 at 25%, respectively. (Fig. 6) shows the combination of *Saussurea lappa* with vinegar the results were the same as those shown by Fig. 5.

Table 2. Minimum inhibitory concentration (MIC) of the extracts against the microbes (in mg/ml).

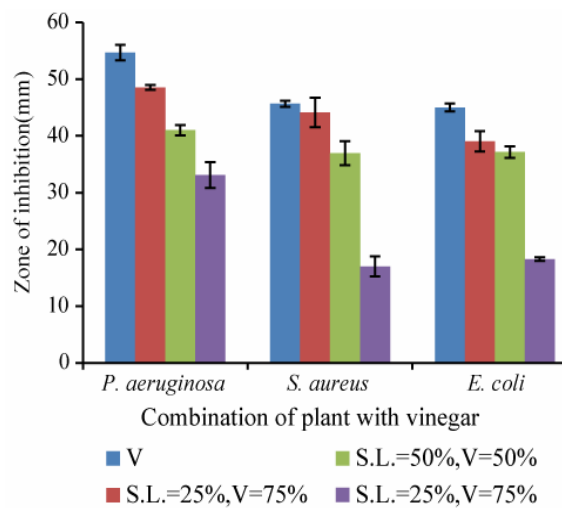
Species	Microorganism tested									
	<i>S. aureus</i>					<i>Candida albicans</i>				
	5	2.5	1.25	0.625	0.3125 mg/ml	5	2.5	1.25	0.625	0.3125 mg/ml
<i>Azadirachta indica</i>	y	x	+	++	+++	-	-	y	x	+
<i>Cassia angustifolia</i>						-	y	x	+	++
<i>Mentha arvensis</i>	y	x	+	++	+++					
<i>Phoenix dactylifera</i>						-	-	y	x	+
<i>Lawsonia inermis</i>						-	y	x	+	+

KEY: - = No growth (turbidity), ++ = Moderate growth, x = MIC, y = MBC +++ = High growth, + = Light growth



Cassia angustifolia (C.A.), Vinegar (V)

Fig. 5. Zones of inhibition of plant extracts with vinegar against the selected bacteria (mm).

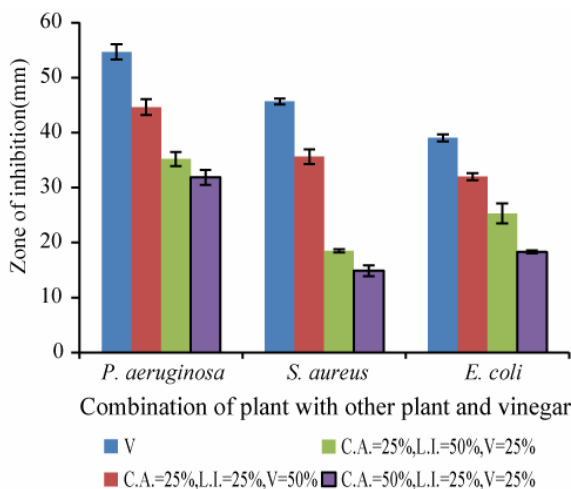


Saussurea lappa (S.L.), Vinegar (V)

Fig. 6. Zones of inhibition of plant extract with vinegar against the selected bacteria in (mm)

Pseudomonas aeruginosa was also found to be sensitive for a combination of *Cassia angustifolia*, *Lawsonia inermis* and vinegar with a zone of inhibition of 54.67 ± 1.36 , 44.6 ± 1.41 , 35.2 ± 1.30 or 31.86 ± 1.34 when the concentration of vinegar decreased from 50 to 25%. *Staphylococcus aureus* and *E. coli* were sensitive to vinegar, with a zone of inhibition of 45.67 ± 0.51 and 41 ± 0.73 , respectively. The zones of inhibition formed against *S. aureus* and *E. coli* for a combination of *Cassia angustifolia* (25%), *Lawsonia inermis* (25%), and vinegar (50%) were 35.6 ± 1.34 and 32 ± 0.62 , and for a combination of *Cassia angustifolia* (25%), *Lawsonia inermis* (50%), and vinegar (25%) were 18.5 ± 0.31 and 25.3 ± 1.7 , respectively. The inhibitory zones formed by a combination of *Cassia angustifolia* (50%), *Lawsonia inermis* (25%) and vinegar (25%) was 14.8 ± 0.98 and 18.3 ± 0.26 , respectively (Fig. 7).

MIC and MBC values of those plants which showed activity against *S. aureus* were determined. *Azadirachta indica* and *Mentha arvensis* had an MIC of 2.5 mg/ml against *S. aureus* and an MBC of 5mg/ml (Table 2).



Cassia angustifolia (C.A.), *Lawsonia inermis* (L.I.), Vinegar (V).

Fig. 7. Zones of inhibition of plant extract with other plant and vinegar against the selected bacteria (mm)

Discussion

Many angiospermic species are in use as herbal medicine in Pakistan since centuries (Yousaf *et al.*, 2010). But so far, not enough studies have been conducted whether the cure that is claimed when herbs are used, is because of anti bacterial activity or due to nutritious value that the herbs are having (Adnan *et al.*, 2010; Hussain *et al.*, 2009). Especially when plants are used to cure skin abnormalities there is a danger of Phytotoxicity (Gilani *et al.*, 2010) and when used in combination that may have allelopathic effect (Gilani *et al.*, 2007; Khan *et al.*, 2009).

In this study, methanolic extracts of 12 commonly used medicinal plants from 10 different families were tested to determine their antimicrobial potential. Plants are important sources of useful substances that are sources

of new chemotherapeutic agents. In order identify these agents, the first step is the *in vitro* antibacterial activity assay (Tona *et al.*, 1998).

Result showed that out of 12 plants that were studied, six plants produced inhibitory effects against *Staphylococcus aureus* and *Candida albicans*. *Azadirachta indica* and *Mentha arvensis* showed high antibacterial activity against *Staphylococcus aureus*. *Azadirachta indica*, *Cassia angustifolia*, *Phoenix dactylifera* and *Lawsonia inermis* were found to be effective against the fungus *C. albicans*. None of the plants showed antimicrobial activity against *E. coli* and *P. aeruginosa*.

In the present study, *Azadirachta indica* showed antimicrobial activity against *S. aureus* (7.96mm) and *C. albicans* (14.46mm). Grover *et al.*, (2011) reported that the methanolic extract of the leaves of *Azadirachta indica* exhibited activity of 18mm against the Gram-positive *Staphylococcus aureus* and 14mm against Gram-negative *P. aeruginosa* but no activity was observed against *E. coli*. Methanolic extracts of *Azadirachta indica* leaves exhibited high activity (15 mm) against *C. albicans*. This difference in results may be due to using test organisms that were clinical isolates and might have been highly resistant. *Azadirachta* has been used for variety of purposes in Pakistan (Sultana *et al.*, 2011).

Results showed that *S. aureus* was sensitive to *Mentha arvensis*, which produced an inhibitory zone of 15mm. Saxena *et al.*, (2011) reported that *Mentha arvensis* has shown activity against *S. aureus* and produced a zone of inhibition of about 5mm. This difference in results may be due to the difference in climatic conditions or soil nutrients where the plant was grown.

Against the fungus *C. albicans*, *Lawsonia inermis* (5 mg/ml) showed the highest activity. Ahmad & Beg (2001) reported that *Lawsonia inermis* (50 mg/ml) was effective against not only *C. albicans* but also against *S. aureus* and *E. coli*. The difference in results may be due to the fact that the extract was used in a lower concentration that did not inhibit the organism. *Cassia angustifolia* and *Phoenix dactylifera* showed inhibitory activity against *C. albicans* in this study. The observed antibacterial and antifungal properties may be due to the presence of secondary metabolites in them (Cowan, 1999; Draughon, 2004).

Some of the ethnobotanically explored medicinal plants (*Albizia lebbek*, *Saussurea lappa*, *Nigella sativa*, *Lawsonia inermis*, *Punica granatum*, *Dalbergia sissoo*, *Trigonella foenum-graecum* and *Curcuma longa*) did not show any antimicrobial activity. Some combinations were also not effective against any pathogen, however, negative results do not mean that bioactive constituents are absent or that the plant is not effective. The active compound(s) may be present in deficient quantities in the crude extracts and not show antimicrobial activity with the dose levels employed and the strains used. Activity may be increased by large doses (Farnsworth, 1993; Taylor *et al.*, 2001)

The ethnobotanically explored plants showed effects on the Gram-positive bacterium (*S. aureus*) but not on the Gram-negative bacteria. The difference in sensitivity between Gram-positive and Gram-negative may be due to the structural differences between these microorganisms. The Gram-negative bacteria have outer phospholipid membrane with lipopolysaccharide components, which

makes the cell wall resistant to antimicrobial chemical components. On the other hand, the Gram-positive bacteria are more susceptible as they have only an outer peptidoglycan layer which is not a useful permeability barrier. Therefore, the Gram-negative bacteria cell walls are more complex than Gram-positive bacteria so, they are less susceptible to antimicrobial agents (Hodges, 2002; Babita *et al.*, 2008; Costa *et al.*, 2008; Walter *et al.*, 2011).

The combination result showed that the vinegar significantly inhibited the growth of bacterial and fungal strains, but in combination antimicrobial activity was reduced. This showed that vinegar can potentially inhibit pathogenic bacterial growth. The vinegar also had good antifungal potential against *C. albicans*. Results showed that actually it was the vinegar that produced a zone of inhibition, not the plant extract. Plants extracts may be effective if large dose of these plant extract were used in combination form. Vinegar is chemically acetic acid. Acetic acid is produced industrially both synthetically and by bacterial fermentation. In the case of olive oil, the zone of inhibition is formed by active compounds in *Lawsonia inermis*, not by the olive oil. In conclusion, we recommend that efforts be made to not only conserve the natural resources (Shinwari & Qaisar, 2011) but its knowledge also (Qasim *et al.*, 2010), as it can be used for variety of purposes as the case of *Lawsonia* for example (Jan *et al.*, 2011).

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