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BIOLOGICAL ACTIVITIES OF COMMONLY USED MEDICINAL PLANTS FROM GHAZI BROTHA, ATTOCK DISTRICT

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Abstract

Medicinal plants are important natural source of possibly secure drugs. They have been playing a significant role in mitigating human miseries by contributing herbal medicines in the primary health care systems of remote areas. About 70% population of rural and remote areas depends on folklore and traditional medicines to cure various ailments. The traditional medicines have gained much popularity due to the high cost and adverse effects of allopathic medicines which encouraged manufacturers of Greco-Arab and Ayurvedic systems of medicines to fuse their orthodox medicines with local traditional medicines in order to spread health coverage at a reasonable rate.

Keeping in view the importance of ethnobotanical survey the current survey was carried out in Attock District, Punjab which comes under the Rawalpindi Division. The region has rural values of old civilizations and customs. The inhabitants of this area have their own trends for a village site, house, family, childbirth, death ceremonies, cultural functions, festivals and socio-religious belief. The ladies are more energetic and laborious as compared to gents. There is a lack of communication with current civilization which has kept them closer to nature from where they fulfill many of their daily needs. The inhabitants of the area are very close to natural flora, both in their habitat and livelihood. People of the area have speculative observations of nature and by communicating with other people of their culture, they discover the inherent knowledge of the local plants. As a result they gain indigenous knowledge, generation after generation. Plants and their derivatives available from the local area are utilized for many purposes such as food, fodder, medicine, veterinary medicines, timbers, households, oilseeds and also for socio-religious and various other purposes. In this way important medicinal plants are collected throughout the year for advertising, personal and entire community use.

Due to random and excessive use of antimicrobial drugs in the treatment of contagious diseases, pathogenic bacteria have developed great resistance against prevailing antibiotics. Multi-drug resistant strains of bacteria generally spread in hospitals and are being isolated from population acquired infections. All this has resulted in severe consequences such as failure of treatment. Hence, it was imperative to explore natural products as an alternative to synthetic antimicrobial agents.

Key words: Biological activities, Medicinal plants, Ghazi Brotha, Pakistan.

Introduction and Background

The local people use traditional knowledge of medicinal plants that reflects the values their elders had, specifically with regard to medicine (Shinwari, & Gilani, 2003; Saqib *et al*., 2011). These people make use of biological resources and conserve them for industrial purposes and world community (Shinwari *et al*., 2011). Medicinal plants practitioners know the effectiveness of medicines, which should not be taken as 'miracle' cures due to chemical compounds, but due to healing potential that draws its medicinal qualities founded on a relationship between the people and the plants (Gilani *et al*., 2007; Hussain *et al*., 2009).

Pakistan holds a distinctive position among the developing countries as it has best prospective with in the variety of medicinal plants due to its diverse climatic and edaphic factors which are replications of variety and valued medicinal plant legacy. A very large number of medicinal plants can be found in northern parts of Pakistan (Shinwari *et al*., 2009; Hussain *et al*., 2009).

The use of medicinal plants is much old. History indicates that the use of plants as healing agents is as old as 4000-5000 B.C. and the first herbal remedy was used by Chinese (Sikar, 1989). On the other hand, in Sub continent plants were used for medicine between 3500-1600 B.C.

Medicinal plant species have significant role in traditional herbal treatments and have made an exceptional position in its origin and evolution. With the passage of time, the indigenous knowledge about the folklore systems have started disappearing due to lack of records, conservation and somewhat due to low incomes in these societies (Shinwari, 2010). Hence, ethnobotanists play crucial roles in rescuing and documentation of this dying knowledge and reinstating it to local communities (Shinwari & Qaiser, 2011). Ways and means are applied for its identification (Shinwari *et al*., 2011, 2014) and ex situ conservation (Hussain *et al*., 2011; Khan *et al*., 2014).

Currently many human diseases and infections are cured by a variety of plants or plant derived products (Mahmood *et al*., 2011; Walter *et al*., 2011). Microbial (bacterial and fungal) infections are treated with the available antibiotics. The currently available antibiotics play crucial role in the mitigation of various ailments but it also have some side effects such as appearance of multidrug resistant pathogenic species and the outbreak of the new contagious diseases (Abdalla, 2011; Shaheen *et al*., 2012 and Shinwari *et al*., 2012). However, the condition is more alarming in developing as well as developed countries due to haphazard consumption of antibiotics. Many drug-resistant microorganisms and pathogenic fungal species have further complexed the treatment of contagious

diseases in individuals with compromised (Cancer and AIDS) immune system (Rinaldi, 1991; Diamond, 1993). In the prevailing situation, the appearance of varied drug resistance to human pathogenic organisms, there is a need to search for new antimicrobial agents from various diverse sources like herbs (Gul *et al*., 2012; Nadeem *et al*., 2013).

Traditionally used medicinal plants produce a range of compounds of well-known therapeutic properties. The constituents that can be taken as candidates for developing new antimicrobial drugs should be able to either inhibit the growth of pathogens or kill them and have no or least toxic effects on the host cells. It is predicted that plant extracts displaying target sites other than that used by antibiotics will be active against drug-resistant microbes. For quality assurance medicinal plants should be tested for microbiological impurity and foreign substances (Deeb *et al*., 2013). Microbiologists are investigating some phytochemicals in some higher plant products for their misuse as antimicrobials. Such medicinal plant products would be decomposable and safe to human health (Wang *et al*., 2010; Mahmood *et al*., 2013).

Certain drugs used to cure various ailments were isolated from natural resources including ethnomedicinal plants. Medicinal plants provide several new therapeutic agents that prove to be beneficial against multidrug resistant bacterial infections. Plant-based medicines enjoy a reputable position nowadays, particularly in the developing nations, where up-to-date health service is inadequate. Indigenous remedies that are more efficient, safe and low-cost are attaining reputation among both rural and urban areas. Knowledge from cultural groups has displayed a dynamic role in the discovery of new products from plants as chemotherapeutic agents (Katewa *et al*., 2004; Shinwari *et al*., 2013). The world health organization (WHO) has highlighted the significance of conventional indigenous medicines, since a lot of rural people in the developing states still use these medications for health care (Goleniowski *et al*., 2006). Worldwide, about 85% of all medicines for primary health care are isolated from plants (Farnsworth, 1988).

These associations can be social, economic, commercial, symbolic, religious and artistic (Aumeeruddy-Thomas & Pei, 2003). District Attock is a well-known historical region and is situated in the north of Punjab. It is a gateway for the Khyber Pakhtoonkhwa (KP) province (Pakistan). District Attock is situated between 33º-7' and 34º-0 North latitude, 71º-45' and 73º-0' East longitude. It has very useful resources of medicinal plants due to its unique location. The Indus River bound it on North and West. District Haripur of the KP province and Rawalpindi district of Punjab province lies in its east. District Chakwal is situated in the Southern side of Punjab. The average annual rainfall is calculated to be 783 mm. The mean maximum and minimum temperature in January oscillates between 17.9°C and 5.2°C respectively, whereas in July it is 42°C and 26.45°C respectively. The total area on which this district stretches is 6856.703 sq. km (Anon., 1998). The ethnobotanical information of uninhabited and cultivated plants use are interrelated to local culture and history. Not much work has been carried out in this area except Mahmood *et al*. (2004) who collected ethobotanical information on 40 species (21 families) of the Kala Chitta Hills (Salt Range) of District Attock.

In this part of Pakistan, traditional healers "Hakims" use ethnomedicinal plants at their medical centers locally known as "Tibbi Dawakhanas". Regrettably, "Hukama" have given very less importance to the ethnobotanical feature of plants as they are only concerned with the floral and vegetative portion of plants without bearing in mind their botanical characteristics or distribution in the different ecological zones of Pakistan (Hamayun *et al.,* 2005). A large number of traditional healers (Hakims) are produced by North-West Punjab of Pakistan. Some of these traditional healers (Hakims) are extremely famous not just locally but also at international level, where these local healers even provide expertise free of charge.

The ethno botanical research based on ethno pharmacological knowledge is usually considered as effective method in the discovery of new anti-infective agents from higher plants (Kloucek *et al*., 2005). With the trend of coming back to nature in the past years, medicinal plants have become the focus of intense study regarding their potential pharmacological values. Herbal medicines are based on the principle that plants consist of natural ingredients that support health and cure illness. Plants used in traditional medicine produce a number of compounds that have proven therapeutic properties. Many diseases caused by pathogens can be cured by using these compounds found in higher plants and herbs. According to a survey on infectious diseases infections caused by pathogenic microorganisms results in a high mortality rate. According to statistics, the numbers of patients affected by these infections are increasing in the hospitals (Santos *et al.,* 2009). Throughout mankind's history, infectious disease has resulted in many disability and death, contributing 22% of the global disease burden (Murray & Lopez, 1997). Recent surveys have shown that an astonishing 50% of the deaths in children in Sub-Saharan Africa are due to infectious causes (Lopez *et al.,* 2006).

Medicinal plant species are very rich source of antimicrobial compounds and due to this reason the WHO promoted that both developed and underdeveloped countries use the traditional medicine with a view of safe and effective remedies of various ailments. Plants have substantial medicinal activities and are usually used as a source of medicine worldwide. These plant species is chief source of various valuable and prevailing crude drugs (Srivastava *et al.,* 1996). A huge number of medicinal plants have gained recognition due to their biological and antimicrobial potential (Walter *et al*., 2011).

Medicinal plants produce a large diversity of secondary metabolites which are either used as precursors or lead compounds in the pharmaceutical industry (Shokeen *et al.,* 2009). Secondary metabolites generally play vital role in plant defence against herbivory and other interspecies defences. Humans use secondary metabolites as medicines, additives and amusing drugs. Bioactive compounds are normally present in all plant cells as secondary metabolites but the concentration of these plant metabolites depends on season, environment and specific growth period. Discovering new secondary metabolites is a requirement for the development of novel pharmaceuticals. This is particularly a vital task in the case of antibiotics due to the rapid emergence of bacterial resistances and the occurrence of multi drug resistant pathogenic strains, which poses severe clinical complications in the treatment of infectious diseases. Such compounds are mostly accumulated by roots and leaves so people give preference to utilize them for

therapeutic purposes. Some of the active compounds impede the growth of disease causing microorganisms either individually or in combination (Cowan, 1999). These active compounds delay the growth of microbes by attaching to their surface proteins, breaking the peptide bonds, changing their biochemical systematics or by stopping the ingestion of available nutrients to the microorganisms. Lyses of microbial cells are also caused by some compounds (Cowan, 1999).

Raw drugs are generally extracted from various parts of medicinal plants due to their different medicinal properties. The diverse plant organs include leaves, root, stem, flower, fruit and various other modified parts of plant are used. These different parts of plants are gathered in small amount for local use and in bulk quantity they are collected for trading as well as for manufacturing of herbal products in herbal industries (Uniyal *et al.,* 2006).

Objectives: The present study was conducted with following objectives

a. To discover the flora of the study area for medicinally importance species.
b. To understand the native people's dependence on natural therapies.
c. To find the antimicrobial activities of these ethnobotanically discovered medicinal plants which are used to treat different diseases.
d. To find out the MIC and MBC count of the plant extracts that proves to be prospective in preliminary screening.
e. To evaluate antioxidant activities of ethnobotanically important medicinal plants.
f. To support national scientists in the studies of drug innovation from medicinal plants.

Materials and Methods

Plants were collected from Ghazi Brotha area of Attock district. Literature survey and general observations adds some more information to the data provided by the local people. Ethnobotanical data, plant parts used along with their vernacular name, their botanical name and family are given in Table 1. The voucher specimens were deposited in the Herbarium of Molecular Systematics and applied Ethnobotany Lab.

Ten species were chosen for further analyses. Whole parts of these plants were used in this study. These plant species were identified and voucher specimens were deposited in the herbarium, Quaid-i-Azam University, Islamabad (ISL). The parts of plants used (barks, leaves and roots) were washed with clean distilled water. The parts of plants used were shade dried for 14 days at temperature of 28ºC to 30ºC.

Table 1. Ethnobotanical data of some selected medicinal plants.

S.No.	Local name	Botanical name	Family	Part of plant used	Ethnobotanical use
1.	Booh	*Aerva javanica*	Amaranthaceae	Whole plant	The decoction of *Aerva javanica* is used as a gargle for toothache. It is also used in skin infection, inflammation and abdominal worms.
2.	Tarkha	*Artemisia brevifolia*	Zingiberaceae	Flower heads and leaves	The leaves and inflorescence are ground to form powder (phaki) which is used for gastric problems
3	Paleeet	*Conyza bonariensis*	Asteraceae	Whole plant	The herb is used as homeostatic, stimulant, astringent and diuretic. It is also used in dysentery and haemorrhage.
4.	Lunduri	*Cynoglossum lanceolatum*	Boraginaceae	Leaves, roots	It is used in the treatment of acute nephritis, periodontitis, acute snake bite etc. It also eliminates toxic heat and inducing diuresis to reduce edema.
5.	Itsit	*Boerhaavia procumbens*	Nyctaginaceae	Leaves, roots	The leaves are cooked as potherb and are given in edema and dropsy. 50 ml juice of the plant is given thrice a day in dysmenorrhea. The powder of the dried roots is snuffed in flue. The powder of the roots along with honey is given in cough & asthma.
6.	Lassi Bhattar	*Launaea procumbens*	Amaranthaceae	Whole plant	Used as coolent, diuretic, demulcent, allergic infections. The plant is grinded in water along with candy (Misri) and is given orally for painful micturation.
7.	Saunchal	*Malva neglecta*	Malvaceae	Leaves and stem	The plant is known as cooling, emollient, and demulcent. The leaves are recommended in piles, and scurvy. The seeds are used in bronchitis, cough inflammation, ulcera-tion of bladder, and in haemorrhoids; externally applied in skin diseases.In digestive problem , for food poisoning,as fodder, but excess amount cause loose motion.
8.	Shatera/papra	*Fumaria indica*	Fumariaceae	Whole plant	The whole plant is boiled in water and is used in itching, pimples and boils of skin.
9.	Gulahi booti	*Silene conoidea*	Caryophylaceae	Whole plant	The plant is known as emollient and is used in bath or as fumigant.
10.	Cheridana	*Stellaria media*	Leguminosea	Whole plant	It is known as cooling, astringent, and vulnerary, used in plasters to be employed on broken bones.

Antimicrobial Bioassays

Grinding of samples: The shade dried samples of *Aerva javanica, Artemisia brevifolia, Boerhavia procumbens, Conyza bonariensis, Cynoglossum lanceolatum, Fumaria indica, Launaea procumbens, Malva neglecta, Stellaria media, Silene conoidea* were grounded separately by using electric grinder to obtain a fine powder. In solvent extraction (5 g) of air dried and powdered plant materials were mixed with 500 ml of different solvents like methanol, n-Hexane and ethylacetate. The supernatant was filtered using Whatman No.4 filter paper and was then concentrated to dryness at low temperature (40ºC) by using rotary evaporator and finally the extracts were dissolved in dimethyl sulfoxide (DMSO) to yield 20 mg/ml of the extracts. The extracts were stored at 4 ºC in sterile airtight vials for further studies.

Antimicrobial assays: The well diffusion method (Parekh & Chanda. 2007; Chanda & Baravalia, 2010) was used to assess the inhibition activities of different bacterial strains by plant extracts.

Test microorganisms: For *in vitro* antibacterial activities, six Gram-negative bacteria: *Klebsiella pneumonia, Escherichia coli*, *Shigella sonnei, Yersinia pestis, Pseudomonas aeroginosa*; four Gram-positive bacteria: *Staphylococcus Aureus, Staphylococcus Epidermidis*, *Listeria monocytogenes, Micrococcus luteus* and one fungal strain *Candida albicans* were procured from Pakistan Institute of Medical Sciences (PIMS), Islamabad. Each organism was preserved on an agar slope at 4ºC and sub-cultured before use.

Media preparation for bacteria (nutrient agar and Mueller-Hinton agar): To determine the *in vitro* antimicrobial activities, Muller Hinton agar (MHA) and Nutrient agar was used. Nutrient agar (2 g) was suspended in distilled water (100 ml) for the preparation of Nutrient agar medium. Likewise, Mueller Hinton agar (3.8 g) was dissolved in distilled water (100 ml) for the preparation of Mueller Hinton agar. In order to check sterility both the media were autoclaved and kept in an incubator at 37°C for 24 h.

McFarland 0.5 barium sulphate turbidity standard: McFarland standards can be used to visually approximate the concentration of cells in a suspension. 0.5 McFarland standard was prepared by adding 0.05 mL of barium chloride dihydrate (1.175% BaCl2. 2H2O) to 9.95 mL of sulfuric acid (1% H2SO4) with constant mixing. The correct density of the suspension was confirmed by determining the absorbance using spectrophotometer at 625 nm wavelength. The absorbance should be between 0.08 and 0.1. McFarland standard was transferred in to the screw cap tube. It can be stored for up to 6 months at ambient temperature and with caps tightened to prevent evaporation of the solution. To aid easy assessment, comparison of McFarland standard with inoculum was performed against a white background with harmonizing black lines.

Preparation of saline: To prepare normal saline, sodium chloride (0.9 g) was dissolved in distilled water (100 ml), filtered and then autoclaved. It can also be stored for up to 6 months at ambient temperature and with caps tightened to prevent evaporation of the solution.

Preparation of inoculum: Before 24 hrs of work, the microbial strains to be used were streaked onto nutrient agar medium in order to obtain isolated colonies of the respective pathogenic strains. After incubating for 24 hrs. at 37°C, 3-5 well isolated colonies were selected using sterilized wire loop, and this microbial growth was transferred to physiological normal sterile saline solution (3-4 ml). This inoculum and 0.5 McFarland standard were then matched. If the density of inoculum and 0.5 McFarland did not match, the turbidity can be adjusted by adding more bacterial growth (to increase turbidity) or by adding normal sterile saline (to decrease turbidity).

Preparation of seeded agar plates: Once the turbidity was adjusted, a sterile cotton swab was dipped in the inoculum. The cotton swab was rotated many times to remove the excess fluid by pressing the swab against the inside walls of the test tube above the level of inoculum. The Mueller Hinton Agar was inoculated by streaking the cotton swab over the entire surface of the agar. The process of streaking was carried out three times by rotating the plate in three dimensions to ensure equal distribution of inoculum. After five minutes, wells of 6 mm diameter were dug in the inoculated plates with the help of sterilized metallic borer.

Pouring of test solutions, incubation and measurement of zone of inhibitions: With the help of sterile micropipette, plant extract (50 µl) and negative control (DMSO) was supplemented in to each well. About 15 mm from the edge of the plate antibiotics disc gentamicin was placed with the help of sterilized needle. The disc was pressed down lightly with the help of sterilized syringe to guarantee its contact with the agar surface. After 15 min. the plates were inverted and placed in an incubator at 37ºC for 24 hrs.

After 18 to 20 hours of incubation, the diameter of the zones of inhibition was measured in mm using ruler and the results were documented. The experiment was performed in triplicate to get the average. Formation of inhibition zones was an indication that the antibacterial activity was present. Mean diameter of inhibition zones and standard deviation of the documented values were also calculated.

Determination of minimum inhibitory concentration (MIC): The method described by Akinpelu & Kolawale (2004) was used to determine the MIC of the extracts. The reconstituted extracts were serially diluted two fold in nutrient broth in order to make numerous concentrations of the stock, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml and were assayed against the tested microorganisms. The minimum inhibitory concentration (MIC) was defined as "the lowest concentration able to inhibit any visible bacterial growth."

Determination of minimum bactericidal concentration (MBC): The method described by (Spencer and Spencer method, 2004) was used to assess the minimum bactericidal concentration (MBC) of the plant extracts. Using micropipette a drop of inoculum was taken from the tubes of the MIC that exhibited no growth of the microorganisms, and dropped on nutrient agar plates. The plates were incubated at 37^{o}C for 24 hrs. The lowest concentration of the extract that yielded no growth of bacterial colony on the agar plates was taken as MBC.

Results and Discussion

Antimicrobial activities

Antifungal activities against *Candida albicans*: The results revealed that the methanol, hexane and ethyl acetate extracts of the selected plant species were inactive activity against the selected strain using concentration of 20 mg/ml.

Antibacterial activities against *Escherichia coli*: All the extracts of the selected plant species exhibited no antibacterial activity against the clinical isolate of *E. coli*.

Antibacterial activities against *Staphylococcus aureus*: All the extracts of the selected plant species did not show any activity against the selected strain at 20 mg/ml concentration.

Antibacterial activities against *Staphylococcus epidermidis*: Seven methanolic plant extracts showed promising results against *S. epidermidis.* The methanolic extract *of Artemisia brevifolia* (14±37.1) showed the highest zone of inhibition followed by *Stellaria media* (13.3) and *Cynoglossum lanceolatum* (12.6±0.57) respectively. *Fumaria indica, Malva neglecta and Silene conoidea* methanolic extracts also produced almost similar zones of inhibition of (10.6±0.57) respectively.

Against *S. epidermidis*, the methanolic extract of *Artemisia brevifolia* and *Malva neglecta* show MIC and MBC value of 10 and 20 mg/ml respectively. *Cynoglossum lanceolatum* and *Fumaria indica* showed an analogous MIC and MBC values ranging from 5-10 mg/ml. *Stellaria media* and *Silene conoidea* methanolic extracts also showed comparable MIC and MBC values ranging from 5-2.5 mg/ml respectively.

Antimicrobial screening of hexane extracts against *S. epidermidis* revealed that the microbe was sensitive to only one plant *Fumaria indica* and produced zone of inhibition of (17.3±0.57) with MIC and MBC values ranged from 10-20 mg/ml.

Ethylacetate extracts of five plants showed promising results against *S. epidermidis*. The ethylacetate extract of *Conyza bonariensis* showed (16±3.21) highest zone of inhibition while *Boerhavia procumbens* (14.6±0.57), *Silene conoidea* (13±0), *Fumaria indica* (12.6±0.57) and *Cynoglossum lanceolatum* (12±0) showed zone of inhibition at a concentration of 20 mg/ml. *Boerhavia procumbens, Conyza bonariensis, Fumaria indica and Silene conoidea* showed an analogous MIC and MBC values of 10-20 mg/ml respectively. The MIC and MBC value of *Cynoglossum lanceolatum* was *5 and 10* mg/ml respectively.

Antibacterial activities against *Shigella sonnei*: The growth of *Sh.sonnei* was only inhibited by the methanolic extract of *Fumaria indica* and showed (13±0) zone of inhibition. *Fumaria indica* has MIC and MBC value of 5 and 10 mg/ml.

Antibacterial activities against *Klebsiella pneumonia*: *K. pneumonia* was found to be resistant to all the plant extracts tested.

Antibacterial activities against *Salmonella paratyphi*: The methanolic extract of *Fumaria indica* produced highest zone of inhibition (11±0) followed by *Stellaria media* (10.6±0.57) and Sil*ene conoidea* (9.6±1.15). The MIC and MBC value of *Fumaria indica* was 10 and 20 mg/ml, while for *Stellaria media* MIC and the MBC value was 2.5 and 5 mg/ml respectively. *Silene conoidea* was found to have MIC and MBC value of 5 and 10 mg/ml.

The n-hexane plant extracts were found to be resistant against *S. paratyphi*. Against *S. paratyphi* the ethylacetate extract of *Cynoglossum lanceolatum* showed (12±0) highest zone of inhibition while *Artemisia brevifolia* showed (9.3±0.28) zone of inhibition at 20 mg/ml. *Cynoglossum lanceolatum* and *Artemisia brevifolia* extracts showed comparable MIC and MBC values ranging from 10-20 mg/ml respectively.

Antibacterial activities against *Salmonella typhimurium*: The methanolic extracts of all the medicinal plant extracts did not show any activity against the clinical isolate of *S. typhimurium*.

Only the hexane extract of *Silene conoidea* was found susceptible to *S. typhimurium* and produced zone of inhibition of (9±0) mg/ml. *Silene conoidea* has MIC and MBC value of 10 and 20 mg/ml.

Antimicrobial screening assay revealed that microbe was sensitive to extracts of 6 plants ethylacetate. It was most sensitive to *Conyza bonariensis* (13.3±0.57) followed by *Boerhavia procumbens* (13±0), *Malva neglecta* (12±0), *Fumaria indica (*11.6±1.52), *Silene conoidea (11±0)* and *Cynoglossum lanceolatum* (10±0).

Boerhavia procumbens, Conyza bonariensis, Fumaria indica and Malva neglecta showed an analogous MIC and MBC values of 10-20 mg/ml. The MIC and MBC value of *Cynoglossum lanceolatum* was 5 and 10 mg/ml while *Silene conoidea* was found to have low MIC and MBC value of 2.5 and 5 mg/ml.

Antibacterial activities against *Listeria monocytogenes*: The methanolic extracts of all plants were inactive against the tested bacterial strain. Only n-hexane extracts of four plants were sensitive to *L. monocytogenes*. It was most sensitive to *Conyza bonariensis* (12.6±0.57), *Artemisia brevifolia* (11.3±2.08), *Malva neglecta* (10.6±0.57), and *Fumaria indica* (10±0).

MIC and MBC values of those plants which were sensitive against *L. monocytogenes* were determined. MIC and MBC of all sensitive plants against *L. monocytogenes* were similar. *Conyza bonariensis, Artemisia brevifolia,*

Malva neglecta, *Fumaria indica* showed MIC and MBC value of 5 and 10 mg/ml respectively. *Malva neglecta* was found to have MIC and MBC value of 2.5 and 5 mg/ml respectively.

Ethylacetate extracts of three plants showed significant inhibition against *L. monocytogenes.* The ethylacetate extracts of *Silene conoidea* showed (11±0) and *Stellaria media* showed (11±0) zone of inhibition at 20 mg/ml concentration against *L. monocytogenes* while *Fumaria indica* showed (10±0) zone of inhibition.

MIC and MBC values of those plants which were sensitive against *L. monocytogenes* were determined. MIC and MBC of *Fumaria indica* was 10 and 20 mg/ml respectively while *Silene conoidea* show MIC and MBC value of 5 and 10 mg/ml respectively. *Stellaria media* has MIC and MBC value of a 2.5 and 5 mg/ml respectively.

Antibacterial activities against *Yersinia pestis*: The results revealed that methanolic extracts of all the plants did not show any activity against the selected clinical isolate.

The hexane extracts of six plants showed activities against *Y. pestis*. The ethylacetate extract of *Conyza bonariensis* showed highest zone of inhibition (16.3±3.21) followed by *Stellaria media* (13±0), *Malva neglecta* (12.3±0.57*), Artemisia brevifolia* (11.3±1.15) and *Fumaria indica* (10±0).

Ethylacetate extracts of only four plants showed antimicrobial activity against *Y. pestis*. *Conyza bonariensis* showed highest zone of inhibition (14±0) followed by *Silene conoidea* (13.3±0.57), *Stellaria media* (11±0) and *Fumaria indica* (10±0).

The hexane extracts of *Artemisia brevifolia*, *Conyza bonariensis* and *Fumaria indica* showed an analogous MIC and MBC values of 10-20 mg/ml while *Malva neglecta* showed MIC and MBC value of 5 and 10 mg/ml respectively. The MIC and MBC value of *Stellaria media* was found to be 2.5 and 5 mg/ml respectively.

The ethylacetate extracts of *Conyza bonariensis, Silene conoidea* and *Stellaria media* showed comparable MIC and MBC value ranging from 10-20 mg/ml. *Fumaria indica* was found to have MIC and MBC value of 5 and 10 mg/ml respectively.

Antibacterial activities against *Pseudomonas aeruginosa*: The methanolic extract of *Conyza bonariensis* was found to have significant zone of inhibition (13±0) mm which showed high concentration of phenolic contents. While the n-hexane extract of *Aerva javanica* was also active against the selected strain and produced zone of inhibition of (20±0) mm. MIC and MBC value of *Aerva javanica* was found to be 5 and 10 mg/ml, while *Conyza bonariensis* showed MIC and MBC value of 10, 11nd 20 mg/ml.

Discussions

Medicinal plants always had a vital role in the therapeutic armory of mankind. Medicinal plants all over the world have been playing a significant part in the efforts of drug discovery efforts. Despite the extraordinary advancement in synthetic organic chemistry of the 20th century, above 25% of prescribed medicines in industrialized countries descend directly or indirectly from plants (Newman *et al*., 2000). Scientific investigation of plant constituents follows a rational path. Plants are collected either in random manner or by following leads provided by local traditional healers in geographical regions where the medicinal plants are found. In order to assess antimicrobial potential of medicinal plants, preliminary screening is done by means of crude aqueous or alcoholic extraction followed by several other organic extraction methods. Approximately all of the compounds isolated from plants are active against microbes are aromatic or saturated organic compounds, they are often obtained through preliminary ethanol or methanol extraction (Vilegs *et al*., 1997).

Therefore, in the present research work, for the preparation of the entire crude extracts methanol, n- hexane and ethylacetate were used as the solvent for extraction. The pathogenic strains used for the study were six gram negative bacteria namely *Klebsiella pneumonia, Escherichia coli*, *Shigella sonnei, Yersinia pestis, Pseudomonas aeroginosa*; four gram positive bacteria namely *Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes* and *Micrococcus luteus* along with one fungal strain *Candida albicans. Aerva javanica* of family Amaranthaceae is a perennial herb, native to Africa, Asia and extensively dispersed in the distant areas of the world (Judd *et al*., 2008). This plant has got a lot of therapeutic uses. This herb is used as diabetic demulcent, diuretic and the subsequent liquid of plant is used to cure of swellings. Plant powder is used to get rid of ulcers of domestic animals. Moreover, the seeds are used to alleviate headache and used in rheumatism too.

Reddy & Reddy (2009) have investigated the occurrence of carbohydrates, steroids, flavonoids and triterpenoids. Mufti *et al*. (2012) reported that *Aerva javanica* showed good activity against the tested pathogens *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Staphylococcus epidermidis* and *Methicillin Resistant Staphylococcus Aureus* (MRSA) except the *Salmonella typhi* which resisted all the fractions(n-hexane, ethylacetate, chloroform, aqueous and crude). In our present study, the n-hexane fraction showed significant activity against *Micrococcus luteus*. *Launaea procumbens* belongs to family Asteraceae. It is commonly known as Lassi Bhattar.

It is used as coolent, demulcent, diuretic and in the management of allergic infections (Reddy & Reddy, 2009). *Launea procumbens* (n-Hexane extract) only showed antibacterial activity against *Yersinia pestis* in the present study by producing zone of inhibition (8 mm) at 20 mg/ml probably due to higher phenolic compounds in the plant extracts.

Stellaria media is commonly known as Cheri dana. It belongs to family Leguminosae. It is also medicinally important and is used for the treatment of various diseases such as skin inflammation and conjunctivitis. Our studies results showed that methanolic extract of *Stellaria media* inhibited *Micrococcus luteus, Salmonella paratyphi* and *Staphylococcus epidermidis* by producing zone of inhibition of 9.6 mm, 10.6 mm and 13.3 mm respectively

at 20 mg/ml. The n-hexane extract showed significant activity against *Micrococcus luteus* and Y*ersinia pestis* by producing zone of inhibition 11 mm and 13 mm respectively. However, ethyl acetate extracts was active against *Listeria monocytogenes* and *Yersinia pestis* by producing zone of inhibition (11 mm). The negative results do not specify the absence of bioactive compounds, nor that the plant is not active. Active compound/s may exist in insufficient amounts in the crude extracts to show antimicrobial activity with the dose levels used. Absence of activity can therefore only be confirmed by using large doses (Parekh & Chanda, 2008).

Malva neglecta is commonly known as Sauncal. All parts of this plant are astringent, urine inducer and have constituents that neutralize inflammation, make the skin softer when applied locally and induce the elimination of mucous secretions from the lungs, the roots have been extensively utilized as a toothbrush. Zare *et al*. (2012) have reported that *Malva neglecta* (aqueous, chloroform, ethanol) have good activity against *Stapylococcus aureus, Pseudomonas aeruginosa and Candida albicans*. Our result showed that *Malva neglecta* (methanolic extract) inhibited *Micrococcus luteus* and *Staphylococcus epidermidis* by producing zone of inhibition of 10.6 mm. The n-hexane extract was also found active against *Listeria monocytogenes* and *Yersinia pestis* and produced zone of inhibition of 10.6 mm and 12.3 mm. However, ethylacetate extract inhibited only S*almonella typhimurium* by producing zone of inhibition of 12mm probably due to poor solubility of various compounds present within plant.

The common name of *Conyza bonariensis* is Paleet and belongs to the family Asteraceae. Some extracts, like the alcoholic extracts of *Conyza bonariensis* exhibited a higher activity spectrum. The alcoholic extract of *Conyza bonariensis* inhibited *Pseudomonas aeruginosa, Micrococcus luteus*, *Salmonella typhimurium, Eschericia coli, Klebsiella pneumonia and Staphylococcus aureus* (Olano *et al*., 1996). Our studies revealed that methanolic extract of *Conyza bonariensis* is active against *Pseudomonas aeruginosa, Micrococcus luteus and Staphylococcus epidermidis*. It was also observed that n-hexane extract of this palnt is active against *Listeria monocytogenes* and *Yersinia pestis* while ethylacetate extract inhibited *Salmonella typhimurium, Yersinia pestis* and *Staphylococcus epidermidis.*

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