

## ANTIBACTERIAL ACTIVITY IN HERBAL PRODUCTS USED IN PAKISTAN

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### Abstract

Antibacterial activity was examined in three herbal products and from ten selected medicinal plants: *Ziziphus vulgaris*, *Malva sylvestris*, *Onosma bracteatum*, *Hyssopus officinalis*, *Ephedra gerardiana*, *Cordia latifolia*, *Althaea officinalis*, *Mentha piperita*, *Glycyrrhiza glabra*, *Justica adhatoda*. Antibacterial activity was determined by the agar well diffusion method; crude extracts were obtained by using methanol as the extraction solvent. Five concentrations (15 mg/ml, 12.5 mg/ml, 10 mg/ml, 7.5 mg/ml and 5 mg/ml) were used to check the antibacterial activity of plant extracts. Each plant sample was tested against one Gram-positive (*Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. Most of the plant extracts showed antibacterial activity against the Gram-positive bacterium. The order of antibacterial activity was *S. aureus*>*P. aeruginosa*>*E. coli*. Maximum zones of inhibition were seen in *Hyssopus officinalis* ( $3.37 \pm 0.05$  mm) against *S. aureus*, *Glycyrrhiza glabra* ( $3.6 \pm 0.3$  mm) against *E. coli* and *Justica adhatoda* ( $2.67 \pm 0.06$  mm) against *P. aeruginosa*. Herbal product 1 showed antibacterial activity against *P. aeruginosa* ( $2.93 \pm 0.15$  mm), *S. aureus* ( $2.2 \pm 0.1$  mm) and *E. coli* ( $1.33 \pm 0.21$  mm). Herbal product 2 showed antibacterial activity against *S. aureus* ( $2.93 \pm 0.15$  mm), *P. aeruginosa* ( $2.1 \pm 0.1$  mm) and *E. coli* ( $1.33 \pm 0.11$  mm). Herbal product 3 was also effective against *S. aureus* ( $2.6 \pm 0.1$  mm), *P. aeruginosa* ( $2.33 \pm 0.51$  mm) and *E. coli* ( $1.33 \pm 0.15$  mm). All three herbal products show significant antibacterial activity.

### Introduction

Plants have a great importance in our lives because they fulfill our basic needs for food, shelter, clothing, fuel, ornamentals, flavoring and medicine. The study of indigenous plant use by people of a particular culture and region is known as Ethnobotany. Throughout the world plants are used to treat various infectious diseases. They provide natural products that are used against infectious diseases. Plant-derived materials or products with therapeutic properties are known as herbal medicines; they may contain processed or raw ingredients from one or more plants that are beneficial for human health. Medicinal plants are important with respect to new drug and pharmacological research development. They are widely used and accepted as home remedies and raw materials for the pharmaceutical industry. Indigenous knowledge of plants and animals that are used to maintain health is known as Ethnopharmacology. The use of plants as medicines dates back to ancient times. Chinese physicians used *Ephedra* tea for asthma, hay fever and colds in 3,000 BC (Chevallier, 1996). Recently, the use of medicinal plants increased substantially (Khan *et al.*, 2001).

Pakistan has a rich flora, including medicinal plants that are being used for therapeutic purposes. Developing countries like Pakistan depend on plant resources for food, shelter, fodder, agriculture and herbal medicines (Shinwari, 2010). The country's natural resources are becoming contaminated due to increased human population, industrialization, urbanization, and the emission of organic hydrocarbons and inorganic heavy metals into the atmosphere. Medicinal plants play an important role for the management of different microbial infections (Shinwari *et al.*, 2009). Medicinal plants must be tested for microbiological contamination and foreign materials to assure quality (Kruti *et al.*, 2011). In third world countries, including Pakistan, where contagious diseases are common, it is important to search out and promote plant-derived medicines. These medicines can destroy microbes that cause certain contagious diseases and should be used in conjunction with modern medicines

and antibiotics. Plants that are being used in conventional herbal remedies should be investigated for their potential to produce new drugs with antimicrobial properties similar to those of modern medicines. Antimicrobial agents are currently being imported and are limited to those who can afford them, but local medicinal plants can be much more widely available. Medicinal plants have been used for centuries in Pakistan as remedies for human diseases (Haq, 1997).

People use herbs to treat different diseases because they are cheap and effective, but doctors are often reluctant to prescribe them because of knowledge deficiency, real concerns about product safety, concerns about liability, and the presence of pathogens and compounds that are injurious (Ernst, 2002; Hussain *et al.*, 2009). Medicinal plants are very important for the cure of different microbial infections but heavy metals adversely affect bacterial viability (Pennanen *et al.*, 1996) and activity (Diaz-Ravina & Baath, 1996). Experiments on the use of plant compounds against microbes were first documented in the late 19<sup>th</sup> century (Zakia, 1975). Natural products perform various functions and many have interesting and useful biological activities (Galal *et al.*, 1991). Researchers are turning their attention to natural products to develop better anticancer, antiviral and antibacterial drugs (Harvey, 1999; Hoffmann *et al.*, 1993; Srinivasan *et al.*, 2001). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1997; Nimri *et al.*, 1999; Saxena & Sherma, 1999). Many researchers have examined the uses of medicinal plants, but only a few studies have tested these Ethno-botanical findings in a laboratory setting to confirm the real antimicrobial properties of these plants (Bhattarai *et al.*, 2008; Shakya *et al.*, 2008).

Medicinal plants can provide a wealth of antimicrobial agents, and hundreds have been investigated for biological activities. Local people collect raw materials in small quantities and use them to treat diseases. Raw materials are also collected in huge amounts and traded in the marketplace to supply herbal industries (Uniyal *et al.*, 2006). Pathogens and parasites

will remain the greatest threat to humans. Climate change may allow pathogens to establish in new areas. Pathogens and their vectors are evolving resistance to many of the manufactured compounds used to control them. The increasing occurrence of multidrug resistant strains of bacteria and the recent emergence of strains with less susceptibility to antibiotics poses challenges for treating bacterial infections (Sieradzki *et al.*, 1999).

## Materials and Methods

**Collection of samples:** Ten species of plants were collected from different areas of Pakistan including Khyber

Pakhtoonkhwa and Northern Pakistan: *Ziziphus vulgaris*, *Malva sylvestris*, *Onosma bracteatum*, *Hyssopus officinalis*, *Ephedra gerardiana*, *Cordia latifolia* (cultivated), *Althaea officinalis*, *Mentha piperita* (cultivated), *Glycyrrhiza glabra*, *Justica adhatoda* (Table 1). The collected plants were identified with the help of literature (Nasir & Ali, 1970-1989; Ali & Nasir, 1990-1992; Ali & Qaiser, 1992-2009). Three herbal products were purchased from the local market. Their ingredients were listed on the labels and confirmed by the manufacturers. For the sake of intellectual property rights and business ethics, their names are not mentioned in this article.

**Table 1. Common plants in herbal products.**

No.	Botanical name	Common name	Local name	Family	Part used	Collection site
1.	<i>Ziziphus vulgaris</i>	Jujube fruit	Anab	Rhamnaceae	Fruits	Mianwali
2.	<i>Malva sylvestris</i>	Common mallow	Khabazi	Malvaceae	Leaves, roots and flowers	Rawal Dam
3.	<i>Onosma bracteatum</i>	-	Gao zuban	Boraginaceae	Leaves	Kashmir
4.	<i>Hyssopus officinalis</i>	Hyssopus	Zoofa	Lamiaceae	Leaves	Azad Kashmir
6.	<i>Ephedra gerardiana</i>	Ephedra	Soma kalpa	Ephedraceae	Whole plant	Baluchistan
7.	<i>Cordia latifolia</i>	Latifolia	Sebestan	Boraginaceae	Flowers and fruit	Abottabad
8.	<i>Althaea officinalis</i>	Marsh mallow	Khatmi	Malvaceae	Root, leaves and flowers	Muzaffarabad
9.	<i>Mentha piperita</i>	Peppermint	Satpodina	Labiatae	Leaves	Kashmir
10.	<i>Glycyrrhiza glabra</i>	Glycyrrhiza	Rab afsos	Leguminosae	Root	Peshawar
11	<i>Justicia adhatoda</i>	Vasica	Berg bana	Acanthaceae	Leaves or whole plant	Margalla Hills

**Preparation of plant extracts:** Dried samples of *Ziziphus vulgaris*, *Malva sylvestris*, *Onosma bracteatum*, *Hyssopus officinalis*, *Ephedra gerardiana*, *Cordia latifolia*, *Althaea officinalis*, *Mentha piperita*, *Glycyrrhiza glabra*, and *Justica adhatoda*, along with herbal product 1, 2 and 3 were ground into powder using a kitchen blender.

The powders were extracted in methanol and kept for two weeks at room temperature (25°C) in extraction bottles. After two weeks, the mixtures were filtered twice, using Whatman-41 filter paper. Methanol was completely evaporated by rotary evaporator to obtain the extract. The semisolid extracts were kept in open air for two days. After complete evaporation of methanol, the extracts were stored in freezer at -8°C for further dilutions and processing.

**Bacterial Strains:** *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) bacteria were used to test for antibacterial activity in the plant extracts. The bacteria were collected from the Pathology Laboratory of a local hospital (Pakistan Institute of Medical Sciences, Islamabad).

**Preparation of sample dilutions:** Extracts (15 mg) were completely dissolved in 1 ml of DMSO. The stock solution of 15 mg/ml was diluted to prepare five concentrations of the extract: 15 mg/ml, 12.5 mg/ml, 10 mg/ml, 7.5 mg/ml, 5 mg/ml. Solutions of a standard antibiotic (2 mg/ml of ampicillin) were also prepared. The standard antibiotic and pure DMSO solutions were used for positive and negative controls. Dilutions of plant extracts with DMSO are presented in Table 2.

**Table 2. Concentration of plant extracts tested for antimicrobial activity.**

No.	Final Conc. (mg/ml)	Plant extract (ml)	DMSO (ml)	Final vol. (ml)
1.	15.0	1.00	0.00	1
2.	12.5	0.833	0.167	1
3.	10.0	0.666	0.334	1
4.	7.5	0.500	0.500	1
5.	5.0	0.344	0.666	1

**Preparation of media (nutrient broth and nutrient agar):** Nutrient broth medium was prepared by dissolving 0.65 g of nutrient broth in 50 ml of distilled water for the growth of bacterial inocula; pH was adjusted 7.0 and the solution was autoclaved. Nutrient agar medium was prepared by dissolving 2.0 g of nutrient agar in 100 ml of distilled water; pH was adjusted 7.0 and the solution was autoclaved.

**McFarland 0.5 barium sulfate turbidity standard:** The standard was prepared by adding 0.5 ml 0.04 M barium

chloride to 99.5 ml 0.36 N sulfuric acid (Koneman, 1988). The bacterial inocula were diluted with saline solution until their color matched a tube with 4-6 ml of the turbidity standard.

**Preparation of inocula:** Centrifuged plates of bacteria from 24-hour old culture in nutrient broth (SIGMA) of selected bacterial strains were mixed with physiological normal saline solution until a McFarland turbidity standard [ $10^6$  colony forming unit (CFU)  $\text{ml}^{-1}$ ] was obtained. Then this inoculum was used to seed nutrient agar.

**Preparation of seeded agar plates:** Nutrient agar was prepared by suspending 2.0 g nutrient agar (MERCK) in 100 ml of distilled water; pH was adjusted to 7.0 and the agar was autoclaved. Petri plates were prepared by pouring 75 ml of nutrient agar and allowing it to solidify. Seven wells were made per plate using a sterile cork borer under aseptic conditions (Kivack *et al.*, 2001).

**Pouring of test solutions; incubation and measurement of zone of inhibitions:** Using a micropipette, 100  $\mu$ l of test solution was poured in respective wells. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of clear zones of inhibition was measured with a ruler. Antibacterial activity of five dilutions of each plant extract was determined against three bacterial strains.

## Results

The antibacterial activity of selected plant species against selected bacterial strains was analyzed by the

presence and diameter (mm) of inhibition zones. Antibacterial activity at the different concentrations of extract is represented in Figs. 1 to 5. All plants except *Mentha piperita* had an antibacterial effect. However, the plants differed considerably in their activity against bacterial strains. The most active plants were *Hyssopus officinalis* (3.37 mm) against *S. aureus*, *Glycyrrhiza glabra* (3.6 mm) against *E. coli* and *Justica adhatoda* (2.67 mm) against *P. aeruginosa*. The herbal products had similar antibacterial activities. Herbal product 1 was most effective against *P. aeruginosa* (2.93 mm), followed by *S. aureus* (2.2 mm) and *E. coli* (1.33 mm). Herbal product 2 was most effective against *S. aureus* (2.93 mm), followed by *P. aeruginosa* (2.1 mm) and *E. coli* (1.33 mm). Herbal product 3 was less effective against *S. aureus* (2.6 mm), but more effective against *P. aeruginosa* (2.33 mm) and the same for *E. coli* (1.33 mm). The sensitivity of Gram-positive and Gram-negative was *S. aureus*>*P. aeruginosa*>*E. coli*.

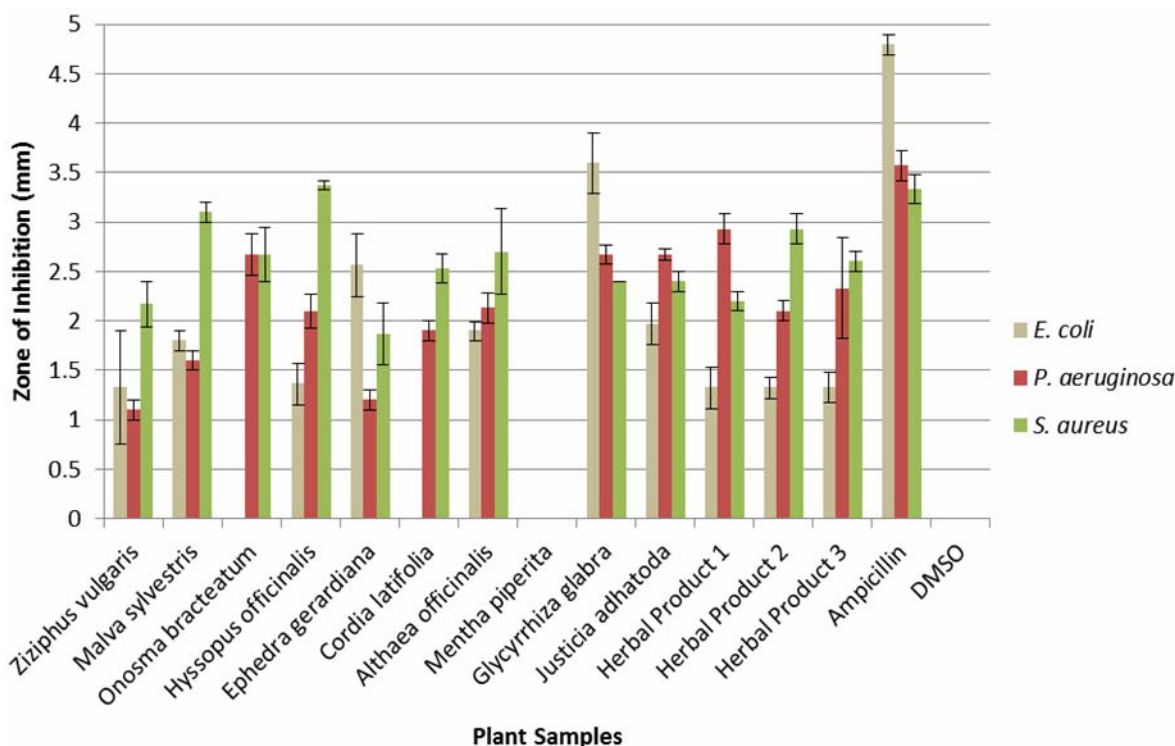


Fig. 1. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 15 mg/ml. (Ampicillin = Positive control; DMSO = Negative control).

Extracts from the fruit of *Ziziphus vulgaris* exhibited the strongest antibacterial activity against *S. aureus* (2.17 mm) at 15 mg/ml. The ranking of antibacterial activity of *Z. vulgaris* against the three bacterial strains was *S. aureus*>*P. aeruginosa*>*E. coli*. Dried leaves, roots and flowers of *Malva sylvestris* were used to prepare extracts. *Malva sylvestris* exhibited maximum antibacterial activity against *S. aureus* (3.1 mm) at 15 mg/ml. The ranking of antibacterial activity of *M. sylvestris* against the three bacterial strains was *S. aureus*>*E. coli*>*P. aeruginosa*. An extract from the leaves of *Onosma bracteatum* were most

effective against *S. aureus* (2.67 mm). The ranking of antibacterial activity of *Onosma bracteatum* against the three bacterial strains was *S. aureus*>*P. aeruginosa*>*E. coli*.

Leaves of *Hyssopus officinalis* were used to prepare an extract. *Hyssopus officinalis* exhibited maximum antibacterial activity against *S. aureus* (3.37 mm) at 15 mg/ml. The order of antibacterial activity of *H. officinalis* against the three bacterial strains was *S. aureus*>*P. aeruginosa*>*E. coli*.

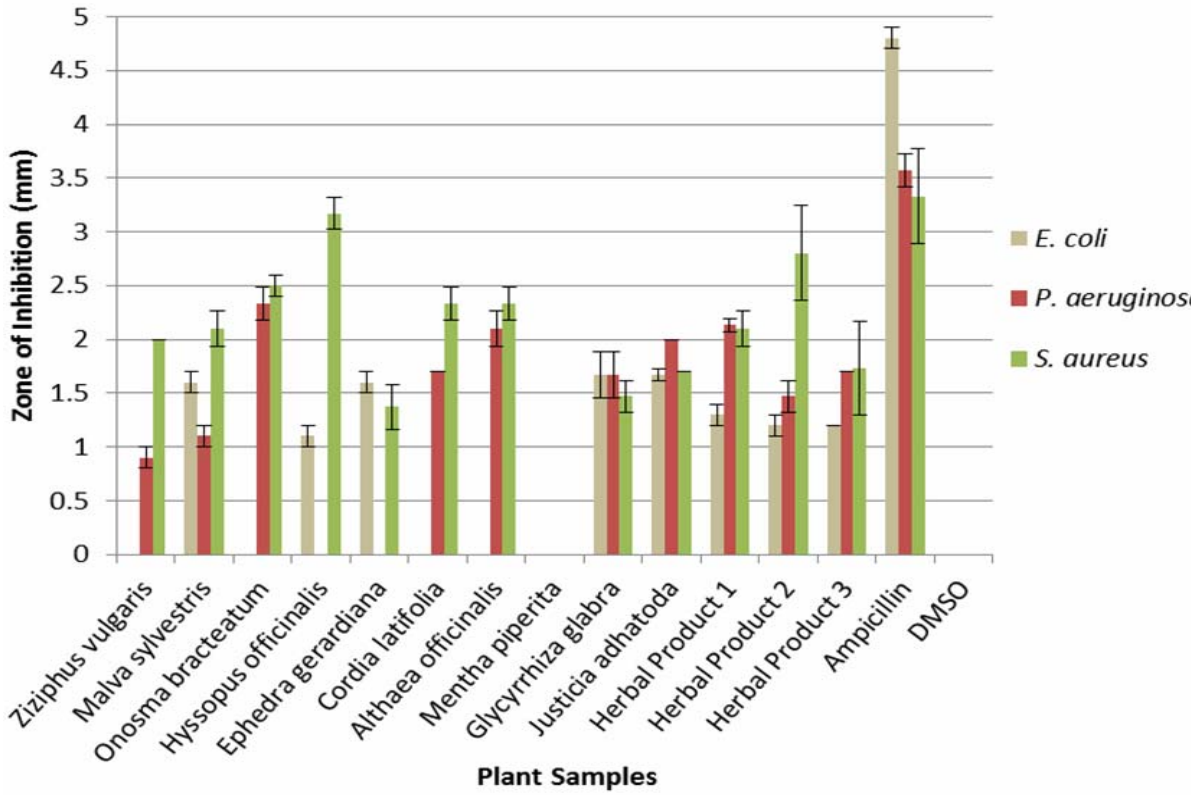


Fig. 2. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 12.5 mg/ml. (Ampicillin = Positive control; DMSO = Negative control).

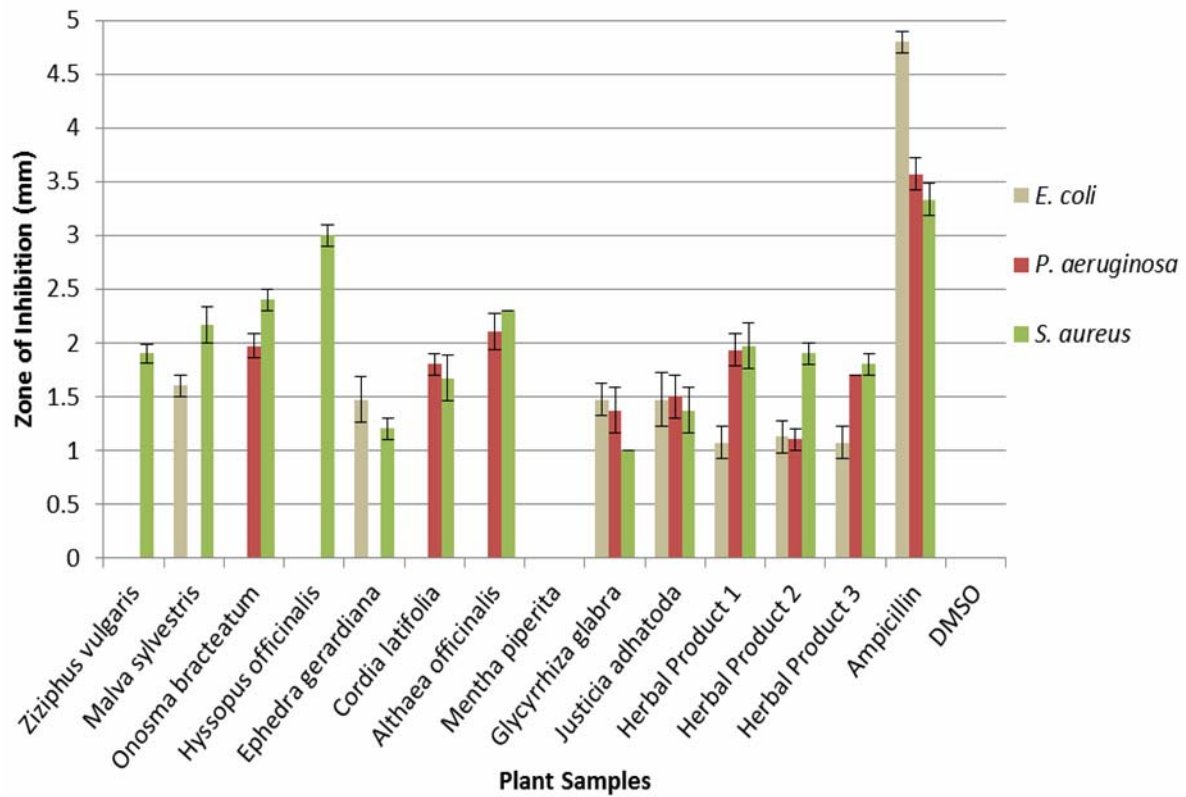


Fig. 3. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 10 mg/ml. (Ampicillin = Positive control; DMSO = Negative control).

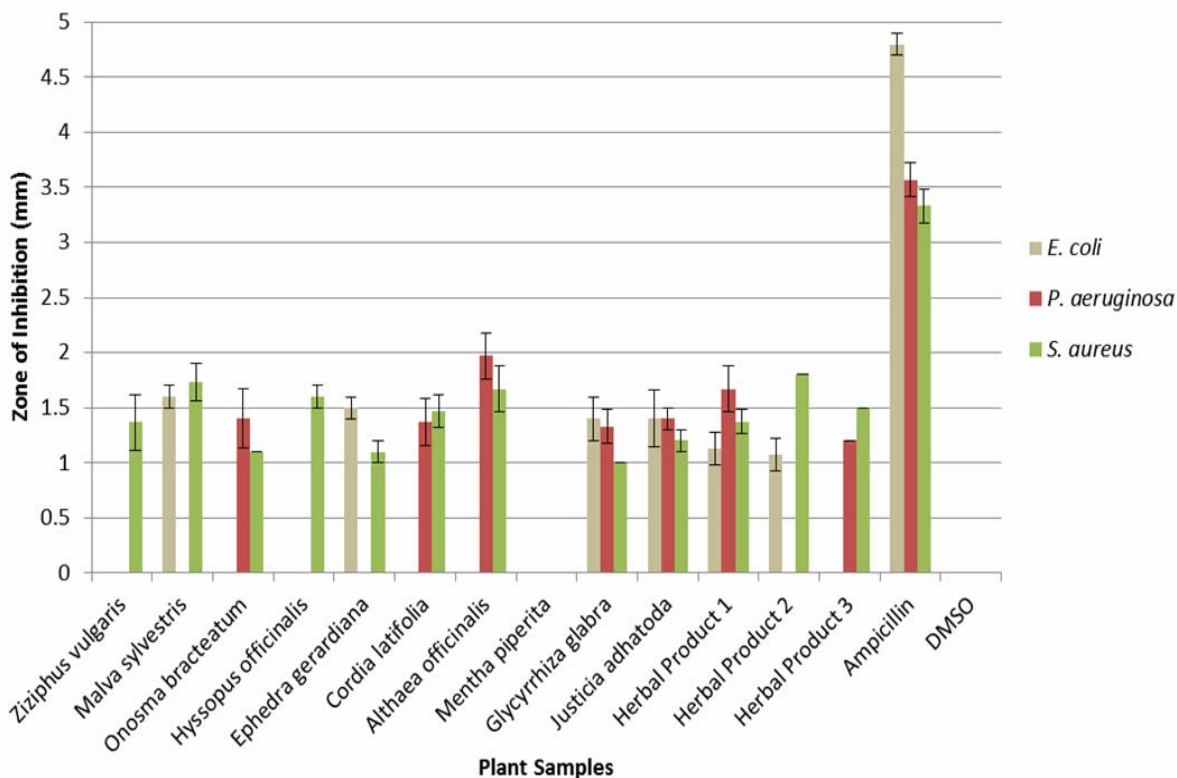


Fig. 4. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 7.5 mg/ml. (Ampicillin = Positive control; DMSO = Negative control).

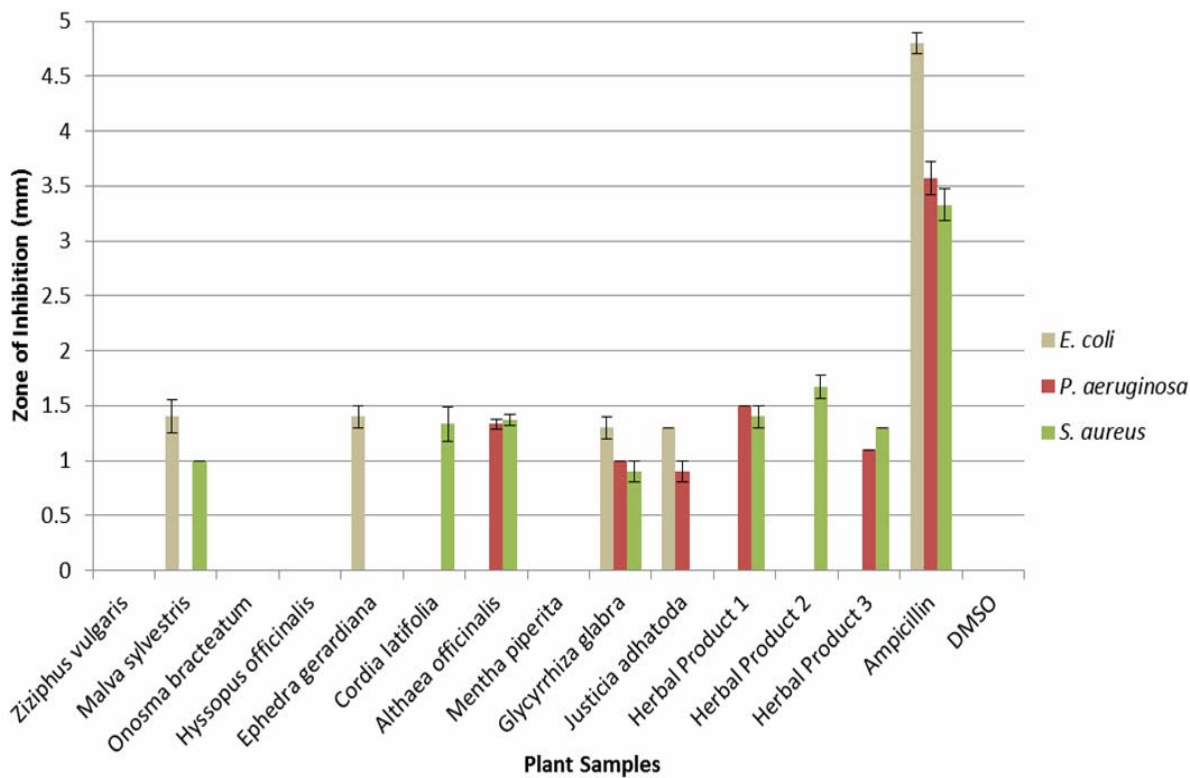


Fig. 5. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 5 mg/ml. (Ampicillin = Positive control; DMSO = Negative control).

The whole plant of *Ephedra gerardiana* was used for the study. *Ephedra gerardiana* exhibited maximum antibacterial activity against *E. coli* (2.57 mm) at 15 mg/ml. The ranking of antibacterial activity of *E. gerardiana* against the three bacterial strains was *E. coli*>*S. aureus*>*P. aeruginosa*. Flowers and fruits of *Cordia latifolia* were used to prepare an extract, which was most effective against *S. aureus* (2.53 mm) at 15 mg/ml. The ranking of antibacterial activity of *C. latifolia* against the three bacterial strains was *S. aureus*>*P. aeruginosa*>*E. coli*. *Althaea officinalis* roots, leaves and flowers were used. *Althaea officinalis* exhibited maximum antibacterial activity against *S. aureus* (2.7 mm) at 15 mg/ml. The ranking of antibacterial activity of *Althaea officinalis* against the three bacterial strains was *S. aureus*>*P. aeruginosa*>*E. coli*. An extract from the roots of *Glycyrrhiza glabra* exhibited maximum antibacterial activity against *E. coli* (3.6 mm) at 15 mg/ml. The ranking of antibacterial activity of *G. glabra* against the three bacterial strains was *E. coli*>*P. aeruginosa*>*S. aureus*.

A whole plant of *Justica adhatoda* was used to prepare an extract that exhibited maximum antibacterial activity against *P. aeruginosa* (2.67±0.06) at 15 mg/ml. The ranking of antibacterial activity of *Justica adhatoda* against the three bacterial strains was *P. aeruginosa*>*S. aureus*>*E. coli*.

## Discussion

Two Gram-negative bacterial strains, *Escherichia coli* and *Pseudomonas aeruginosa*, and one Gram-positive strain, *Staphylococcus aureus*, were selected for this study. Bacterial infections caused by the genus *Staphylococcus* are a great threat to both humans and animals. *Staphylococcus aureus* spreads pneumonia at slow rates (Simor *et al.*, 2001). *Pseudomonas aeruginosa* is a common bacterium that can cause diseases in humans; it is most notorious for causing lung infections or pneumonia. If *P. aeruginosa* colonies occur in critical body organs, such as the lungs, urinary tract and kidneys, the results can be fatal (Balcht & Raymond, 1994). *Escherichia coli* causes many infections including gastroenteritis and urinary tract infections, pneumonia, meningitis, bone and joint infections, and skin and soft tissue infections (Todar, 2007).

*Ziziphus vulgaris* is commonly called jujube fruit and its local name is Anab. In Pakistan, the fruit is eaten both fresh and dried and is known as Ber (Qasim *et al.*, 2010). The fruits are used in Chinese and Korean traditional medicine, where they are believed to alleviate stress (Mill, 2009) and used for colds and coughs; they also have antifungal, antibacterial, antiulcer, anti-inflammatory, sedative and antiseptic properties (Jiang *et al.*, 2007; Mahajan & Chopda, 2009). *Ziziphus vulgaris* exhibited maximum antibacterial activity against *S. aureus* (2.17 mm) at 15mg/ml.

The common name for *Malva sylvestris* is common mallow and its local name is Khabazi. The active ingredients are found in the flowers and leaves, which are rich in mucilage, used for their expectorant properties (Yeole *et al.*, 2010). The plant is largely used to soothe mucous membrane inflammations. *Malva sylvestris* exhibited maximum antibacterial activity against *S. aureus* (3.1 mm) at 15mg/ml. The study conducted by

Pirbalouti *et al.*, (2009) showed *M. sylvestris* is good for skin disorders, as well as having good antimicrobial and anti-inflammatory activity.

*Onosma bracteatum* is known locally as Gao zuban. Dried leaves and stems of *O. bracteatum* are used to treat bronchitis and asthma (Kirtikar & Basu, 1999) and relieve palpitations and urinary complaints. Ahmad *et al.*, (2009) studied *Onosma griffithii* and made different fractions (chloroform, n-butanol, crude extract, ethyl acetate and aqueous). They concluded that the aqueous fraction showed moderate antibacterial activity against *S. aureus*. *Onosma bracteatum* exhibited maximum antibacterial activity against *S. aureus* (2.67 mm).

*Hyssopus officinalis* is an herbaceous plant locally known as Zoofa. Due to its properties as an antiseptic, cough reliever, and expectorant, it is commonly used as an aromatic herb. As a medicinal herb, hyssop has soothing, expectorant, and cough suppressant properties (Van Wyk & Wink, 2004). The plant contains thujone and phenol, which give it antiseptic properties. *Hyssopus officinalis* exhibited maximum antibacterial activity against *S. aureus* (3.37 mm) at 15 mg/ml.

*Ephedra gerardiana* represents the provincial flower of Balochistan, Pakistan. It is locally known as Soma kalpa. Plants of the genus *Ephedra*, including *E. sinica* and others, have traditionally been used by indigenous people for a variety of medicinal purposes, including treatment of asthma, hay fever, and the common cold (Abourashed *et al.*, 2003). *Ephedra gerardiana* exhibited maximum antibacterial activity against *E. coli* (2.57 mm) at 15 mg/ml.

*Cordia latifolia* is locally known as Sebestan and its common name is Latifolia. Hernandez *et al.*, (2007) studied *Cordia curassavica*, which is used to treat gastrointestinal, respiratory and dermatological disorders. *Cordia latifolia* exhibited maximum antibacterial activity against *S. aureus* (2.53 mm) at 15 mg/ml.

The leaves, flowers and the root of *Althaea officinalis* (marshmallow) all have medicinal properties. The leaves and flower have demulcent, expectorant, diuretic, and emollient properties. It is generally used to treat ailments of the lungs and the urinary system (Petkewich & Peter, 2006). *Althaea officinalis* exhibited maximum antibacterial activity against *S. aureus* (2.7 mm) at 15 mg/ml.

Peppermint has a long tradition of medicinal use, with archaeological evidence placing its use at least as far back as ten thousand years ago. Peppermint has a high menthol content and is often used for tea and for its soothing effect, as well as for flavoring ice cream, confectionery, chewing gum, and toothpaste. However, *Mentha piperita* did not show antibacterial activity against any of the bacterial strains.

In traditional Chinese medicine, *Glycyrrhiza glabra* is commonly used in herbal formulae to "harmonize" the other ingredients in the formula and to carry the formula to the twelve "regular meridians" and to relieve a spasmodic cough (Bensky, 2004). Among different plant extracts that were used to treat malaria, *G. glabra* showed antiplasmodic activity when tested on mammalian cells (Esmaeili *et al.*, 2009). *Glycyrrhiza glabra* exhibited maximum antibacterial activity against *E. coli* (3.6 mm) at 15 mg/ml.

*Justicia adhatoda* is a shrub that has a number of traditional medicinal uses. It has been used as an antispasmodic, bronchodilator, and mucolytic agent in asthma and other respiratory conditions (Roja *et al.*, 2011). *Justicia adhatoda* exhibited maximum antibacterial activity against *P. aeruginosa* ( $2.67 \pm 0.06$  mm) at 15 mg/ml.

Herbal products are of great importance because they can be active against infectious diseases. Herbal product 1, Herbal product 2 and Herbal product 3 showed higher antibacterial activity against *S. aureus* than against *P. aeruginosa*, and less antibacterial activity against *E. coli*. According to Keskin *et al.*, (2010) who tested antimicrobial activity in 10 Turkish medicinal plants against different bacterial strains, the most resistant microorganism was *Escherichia coli*. El-Kamali and El-Amir, (2010) who screened the antibacterial activity and phytochemical properties of eight selected species, reported all extracts showed good antibacterial activity against four strains of bacteria.

Sensitivity of bacterial strains was in decreasing order *S. aureus*>*P. aeruginosa*>*E. coli*. The Gram-positive bacterium was more sensitive than the Gram-negative bacteria, which may be due to the difference in their cell wall composition (Ahmad & Beg, 2001).

## Conclusion

In third world countries, including Pakistan, where contagious diseases are common, it is important to search out and promote medicines that are plant-based. This work will help to identify active ingredients for the treatment of bacterial diseases. Additional studies are needed to assess the effects of the selected plants on other pathogenic organisms.

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