Antibacterial Activity of Economically Important Medicinal Plants in Pakistan Against Different Bacterial Strains

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ABSTRACT: The emergence of medication resistance and unfavorable side effects from existing antibiotics has prompted the quest for novel antimicrobial agents over the last 2 decades. Plant extracts have been shown to have antibacterial effects in numerous studies. The objective of this study was the evaluation of the antibacterial effect of economically important medicinal plants found in Pakistan. *Onosma bracteatum* (flowers and leaves), *Viola odorata* (flowers and leaves), *Cuscuta reflexa* (whole plant), *Swertia chirata* (whole plant), and *Fagonia arabica* (whole plant) were used against *Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*. Water and ethanol extracts were obtained from different parts of the plants. To evaluate the antibacterial effect of these plants, qualitative assay agar well diffusion method was performed. The minimum inhibitory concentration (MIC) was determined by the broth micro dilution method. Results revealed that the highest inhibition zone (18 mm) was shown by ethanol extract of *V odorata* flower against *P aeruginosa*. Ethanol extract of *C reflexa* plants is best for all 3 tested microbes (*P aeruginosa, B subtilis*, and *E coli*). The results concluded that all these plants have abilities to fight against these tested bacteria. Ethanol extract of *V odorata* flower has the highest activity against *P aeruginosa*.

KEYWORDS: *Antibacterial, Onosma bracteatum, Viola odorata, Cuscuta reflexa, Swertia chirata, Fagonia arabica, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*

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Introduction

Instead of tremendous progress in antibiotic production, severe infections occurred that are responsible for deaths and health illnesses in patients. Doctors give a second- and third-degree medicine for curing, but these drugs produced dangerous side effects on patient's health. To overcome this problem, there is a need to take serious steps in designing new antibiotics. The major advances in drugs are mostly derived from Unani herbal traditions. A large number of herbal medicines are used for curing human health problems because it contains antibacterial active compounds, such as alkaloids, polyphenols, and volatile oils, that are used in many familiar human drugs.[1] The plant extracts that stop the bacterial growth and kill them with no harmful side effects on host tissue are considered as more powerful drugs for new antibacterial medicine. Many years ago, plants were used as a drug for wound healing, reducing blood pressure, hormone disorders, enzyme production, and stopping blood clotting.[2]

The plant species used in medicine ranged from 35 000 to 70 000 out of 422 127 worldwide.[3] All plant parts are accustoming as medicine, eg, roots, leaves, flowers, seeds, and fruits.[4,5] Human health is maintained by natural plant parts for a longer period. Phytochemical extract of plants has an essential value against bacterial diseases.[6,7] Plants contained nutrients that yield energy and help to overcome food-borne disease.[8,9] Medicinal plants are used in various forms, such as decoction, distillation, extracts, oil, and powders. Extract forms of plants mostly show active action. Tincture or extract form mostly enters the pathogens' cell wall, prevents cell growth, and decreases their cells number.

Approximately, 700 plants show medicinal value in Pakistan. More than 186 vegetables used as medicine against different diseases enlisted in Hamdard pharmacopoeia of Eastern Medicine, while the National Council of Tibb reported that 900 plants are used as a drug in Pakistan.[10] Alkaloids obtained from plants express a greater resistance against microbes, used as anti-inflammatory, anti-oxidants, and antitumor.[11] Herbal drugs demand increases periodically due to pathogens showing resistant against antibodies and the growth of multiple resistant microbes.[12-14] Synthetic medicines cost higher, disease cure incomplete, and more health-related issues occur.

Onosma bracteatum wall is commonly known as Gaozaban. The pharmacologic values of *O bracteatum* are represented by flavonoids, alkannin, shikonin, vanillic acids, and ferulic. It is used as a primary element in a variety of Unani and Ayurvedic formulations to deal with a variety of human health problems.[15] *Viola odorata* is also used to treat health problems including cough, common cold, fever, headache, constipation, dysuria,

Table 1. Plant local, scientific, and family name, and part used.

SERIAL NO.	SCIENTIFIC NAME	LOCAL NAME	FAMILY	SPECIFIC PLANT PART
1	*O bracteatum*	Gul-e-Gaozaban	Boraginaceae	Flower
2	*C reflexa*	Aftimun	Convolvulaceae	Whole part
3	*O bracteatum*	Berg-e-Gaozaban	Boraginaceae	Leaves
4	*S chirata*	Chirata Talkh	Gentianaceae	Leaves
5	*V odorata*	Gul-e-Banafsha	Asclepiadaceae	Flowers
6	*V odorata*	Berg-e-Banafsha	Asclepiadaceae	Leaves
7	*F arabica*	Badawar	Zygophyllaceae	Flower

insomnia, palpitation, epilepsy, dyspnea, and skin diseases.[16] However, *Cuscuta reflexa* is a parasitic weed plant with therapeutic characteristics and a significant role in Ayurvedic medicine.[17] *Swertia chirata* is a bitter tonic used in traditional medicine to cure fever, digestive problems, diabetes, loss of appetite, skin problems, and a variety of other ailments.[18] *Fagonia cretica* is a plant with unique chemicals that are effective in treating diseases that are currently supposed to be incurable or have severe adverse effects.[19] This study was designed to evaluate the antibacterial activity of Pakistan's economically important medicinal plant extracts *O bracteatum, V odorata, C reflexa, S chirata*, and *Fagonia arabica* against *Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa.*

Materials and Methods

Collection of plants

The fresh herb *C reflexa* was collected from Hamdard chowk, plot number 71, Kotlak pat Lahore, and *V odorata* was collected from Swat, Pakistan. The dry plants *O bracteatum, F arabica*, and *S chirata* were purchased from a local market of Lahore. The authenticity of the plant was confirmed by Hamdard Laboratories, Kotlak pat Lahore, Pakistan. The foreign impurities were removed by the cleaning process and only specific parts of the therapeutic value were used. The local names of plants and their parts used in the study are described in Table 1.

Medicinal plant extract preparation

The plant parts were put in a grinder machine and made into a coarse powder. The plant's coarse powder was dissolved in water and ethanol. The amount of herb, ethanol, and water varies according to the desired plant extract.[20] After filtering the extract with Whatman filter paper, it is stored in an ambient temperature.

Sub-culturing of bacterial strains

The clinically isolated strains of bacteria were obtained from the Department of Life Sciences University of Management and Technology, Johar Town, Lahore, Pakistan. The isolated bacterial strains were pathogenic, namely *E coli, B subtilis*, and *P aeruginosa*. The bacterial strains were sub-cultured and maintained on Nutrient agar, and stored at 4°C in a refrigerator for further use.[21]

Qualitative antibacterial assays

Agar well diffusion method. The plates were prepared with Mueller–Hinton agar at a depth of 4 mm. The media pH ranges from 7.2 to 7.4. The bacterial lawn culture was prepared by swabbing and spreading in aseptic conditions. To produce growth uniformity, we streaked the media plate in one direction and moved the petri plate in 90°, and repeated this procedure for 3 times. Cork borer was sterilized and used to make a hole in media containing agar plate, label the media plate, and add 50 µL of each extract in each well (20 mm apart from one another in each well, 7 mm diameter holes). Positive control (pure solvent) was used in the well. In aerobic condition, the media plate was given an incubation period of 24 hours and incubated at the temperature of 36°C ± 1°C. The next day, after incubation, affluent growth was observed. Bacterial inhibition growth was determined in mm by a ruler. The tests were performed in duplicates.[22]

Quantitative antibacterial assay

Minimum inhibitory concentration (MIC) of medicinal plant extracts was determined using a microtiter plate that contained 96-well. The first step was to took the required amount of Mueller–Hinton broth powder in a bottle and add distilled water to prepare broth media, and take 50 µL media through micropipette in each well of microtitration plate. Adding a 50 µL extract in first well and then diluted serially 2-fold to second last well and leave last well for control. Then, the inoculum suspensions of tested bacteria added in each well of microtitration plate and well mix. All plates of tested herbs were prepared by this method and incubated at 37°C for 24 hours. MIC and bacterial growth were measured after the incubation period; the well without turbidity was taken as MIC.[23,24]

Table 2. Zones of inhibition (mm) by different plant extracts in water and ethanol against bacterial species.

	B SUBTILIS	*E COLI*	*P AERUGINOSA*
C reflexa (water)	No activity	No activity	No activity
C *reflexa* (ethanol)	5 mm	2 mm	4 mm
O bracteatum flower (water)	No activity	No activity	No activity
O bracteatum flower (ethanol)	8 mm	2 mm	0
O bracteatum leaf (water)	No activity	No activity	No activity
O bracteatum leaf (ethanol)	No activity	No activity	2 mm
S chirata (water)	No activity	No activity	No activity
S chirata (ethanol)	6 mm	No activity	8 mm
V odorata flower (water)	3 mm	No activity	No activity
V odorata flower (ethanol)	7 mm	No activity	18 mm
V odorata leaf (water)	No activity	No activity	No activity
V odorata leaf (ethanol)	5 mm	No activity	4 mm
F arabica (water)	7 mm	5 mm	No activity
F arabica (ethanol)	5 mm	No activity	7 mm

Statistical Analysis

Zone of inhibition against *B subtilis, E coli*, and *P aeruginosa* was statistically analyzed by *t*-test ($P < .05$).

Results

Antibacterial screening of plant extracts

The 10 g of powdered extracts of plants were dissolved in 100 mL ethanol and water to determine the antibacterial activity. The ethanol extract of *C reflexa* revealed zones of inhibition range of 5 mm against *B subtilis*, 2 mm for *E coli*, and 4 mm inhibition zone against *P aeruginosa* by agar well diffusion method (Table 2). The water extract exhibited no zone of inhibition against all the 3 tested pathogens. The results of *O bracteatum* flowers ethanol extract manifest inhibition zone of 8 mm against *B subtilis* (Figure 1), 2 mm zone of inhibition for *E coli* (Figure 2), and no activity against *P aeruginosa*. Ethanol extract of *O bracteatum* leaves showed 2 mm inhibition zone for *P aeruginosa* (Figure 3) and none against *B subtilis* and *E coli*. *S chirata* ethanol extract exhibited a 6 and 8 mm inhibition zone against *B subtilis* and *P aeruginosa*, respectively, Figures 2 and 3, and no activity for *E coli*. The water extract of *S chirata* and *O bracteatum* expressed no activity against tested pathogens. Ethanol extract of *V odorata* flowers revealed 7 mm inhibition zone against *B subtilis* and 18 mm against *P aeruginosa* and none for *E coli*. Ethanol extract of *V odorata* leaves revealed inhibition zone measured 5 mm against *B subtilis*, 4 mm against *P aeruginosa*, and none for *E coli* (Table 2). The water extract of *V odorata* leaves expressed no antibacterial activity, while the water extract of *V odorata* flowers exhibited 3 mm inhibition zone against *B subtilis*. Ethanol extract of *F arabica* revealed inhibition zone of 5 mm against *B subtilis*, 7 mm inhibition zone against *P aeruginosa*, and no activity exhibited for *E coli*, and water extract of this plant showed 5 mm inhibition zone

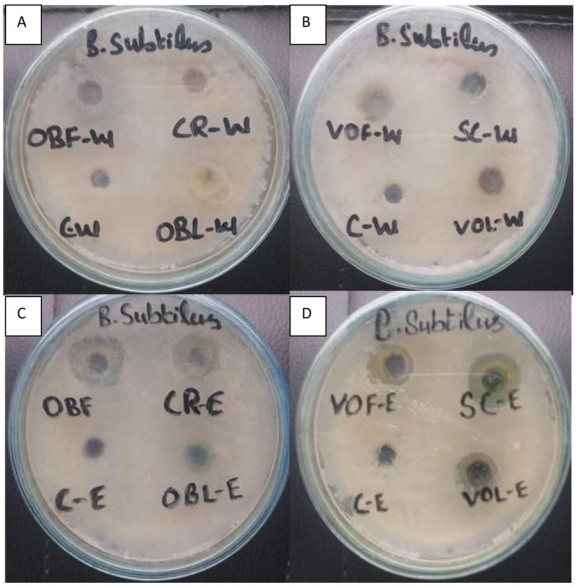

Figure 1. Comparison of water and ethanolic plant extracts against *B subtilis*. (A) OBF-W; *O bracteatum* flower extract in water, CR-W; *C reflexa* in water, C-W; control water, OBL-W; *O bracteatum* leaf extract in water. (B) VOF-W; *V odorata* flower extract in water, SC-W; *S chirata* extract in water, C-W; control water, VOL-W; *V odorata* leaf in water. (C) OBF-E; *O bracteatum* flower extract in ethanol, CR-E; *C reflexa* in ethanol, C-E; control ethanol, OBL-E; *O bracteatum* leaf extract in ethanol. (D) VOF-E; *V odorata* flower extract in ethanol, SC-E; *S chirata* extract in ethanol, C-E; control ethanol, VOL-E; *V odorata* leaf in ethanol.

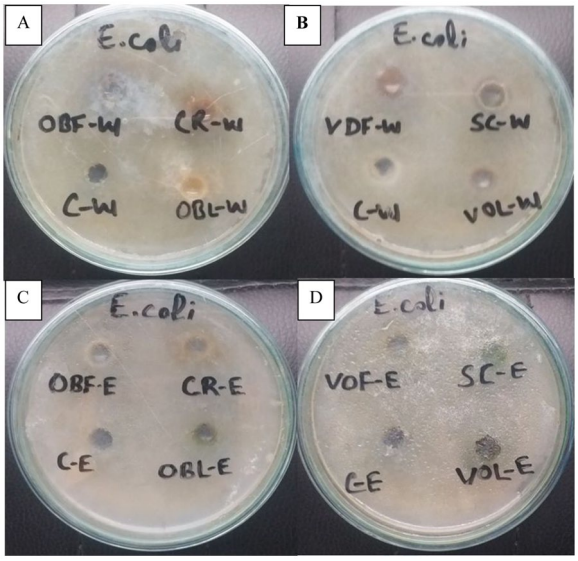

Figure 2. Comparison of water and ethanolic plant extracts against *E coli*. (A) OBF-W; *O bracteatum* flower extract in water, CR-W; *C reflexa* in water, C-W; control water, OBL-W; *O bracteatum* leaf extract in water. (B) VOF-W; *V odorata* flower extract in water, SC-W; *S chirata* extract in water, C-W; control water, VOL-W; *V odorata* leaf in water. (C) OBF-E; *O bracteatum* flower extract in ethanol, CR-E; *C reflexa* in ethanol, C-E; control ethanol, OBL-E; *O bracteatum* leaf extract in ethanol. (D) VOF-E; *V odorata* flower extract in ethanol, SC-E; *S chirata* extract in ethanol, C-E; control ethanol, VOL-E; *V odorata* leaf in ethanol.

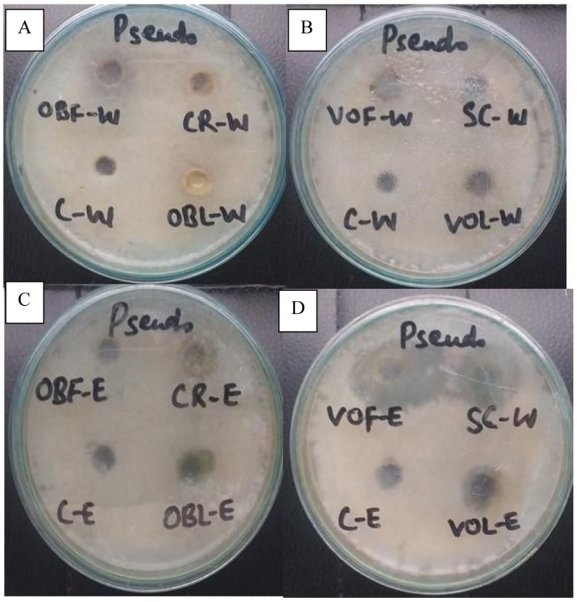

Figure 3. Comparison of water and ethanolic plant extracts against *P aeruginosa*. (A) OBF-W; *O bracteatum* flower extract in water, CR-W; *C reflexa* in water, C-W; control water; OBL-W; *O bracteatum* leaf extract in water. (B) VOF-W; *V odorata* flower extract in water, SC-W; *S chirata* extract in water, C-W; control water, VOL-W; *V odorata* leaf in water. (C) OBF-E; *O bracteatum* flower extract in ethanol, CR-E; *C reflexa* in ethanol, C-E; control ethanol, OBL-E; *O bracteatum* leaf extract in ethanol. (D) VOF-E; *V odorata* flower extract in ethanol, SC-E; *S chirata* extract in ethanol, C-E; control ethanol, VOL-E; *V odorata* leaf in ethanol.

against *B subtilis* and 7 mm for *E coli* (Figure 4). Overall, ethanol extract of *V odorata* flower revealed the highest inhibition zone (18 mm) against *P aeruginosa*. Table 3 showed MIC of different plant extracts in water and ethanol against bacterial species through broth microdilution method.

Discussion

Sudden growth of antibiotic-resistant bacteria caused infection with high morbidity and mortality. *P aeruginosa* caused pneumonia and lung infections. This bacteria form colonies in kidneys, urinary tract, and lungs results in failure of the functions of these body organs. *E coli* caused many diseases in soft tissue and skin infection, bone and joint infection, meningitis, pneumonia, urinary tract infection, and gastroenteritis.[25] The diseases caused by these bacteria lead stimulating researchers to develop a powerful active compound or antibacterial agents from the herbs, plants, and food spices. Plants play a crucial role to fight against pathogens that caused disease and are used as a potent source for modern synthetic drugs in pharmaceutical industries worldwide.[26]

Ethanol extract of *V odorata* flower revealed the highest inhibition zone (18 mm) against *P aeruginosa* as compared with all other plants used in this study. Alkaloid's violin is present in seeds, flowers, roots, and leaves of *V odorata.* It formed salts with acids and has volatile oil.[27,28] Aslam et al. (2018) estimated the antibacterial property of different extracts of *V odorata* against 5 bacterial strains including *E coli, B subtilis, B cereus, M luteus*, and *K pneumonia*, and exposed that the ethanol extract was more efficient against the tested pathogens. Previous studies indicated that methanol and ethanol extract of leaves of this plant showed significance zone of inhibition against 2-gram negative bacteria, eg, *B subtilis, S aureus*, and gram-positive bacteria, eg, *P aeruginosa, E coli.* The aqueous extracts of this herb leaves exhibited no zone of inhibition against selective pathogens due to loss of active compounds during boiling or phytochemicals not soluble in water.[29] Phytochemical's screening of this plant extract revealed various compounds, such as tannins, saponins, terpenes, alkaloids, glycosides, flavonoids, and steroids.[30]

In this study, ethanol extract of *O bracteatum* flower showed maximum inhibition zone (8 mm) against *B subtilis*, then *E coli* and *P aeruginosa.*[31] The study by Yasmin et al also exposed the antibacterial property of *O bracteatum* leaves against *P aeruginosa, S aureus*, and *E coli.*[32] Gaozaban herb is used as an essential ingredient of familiar herbal drug Joshanda. This plant's dry stems and leaves are used to cure asthma and bronchitis.[33] In a study, *O bracteatum* showed maximum activity against *S aureus*.[34]

In this study, ethanol extract of *S chirata* represents a larger zone of inhibition (8 mm) against *P aeruginosa* than *B subtilis and E coli.* Aqueous and ethanol extract of *F arabica* revealed the same zone of inhibition, respectively, against *B subtilis* (7 mm), *P aeruginosa* (7 mm), and no inhibition zone

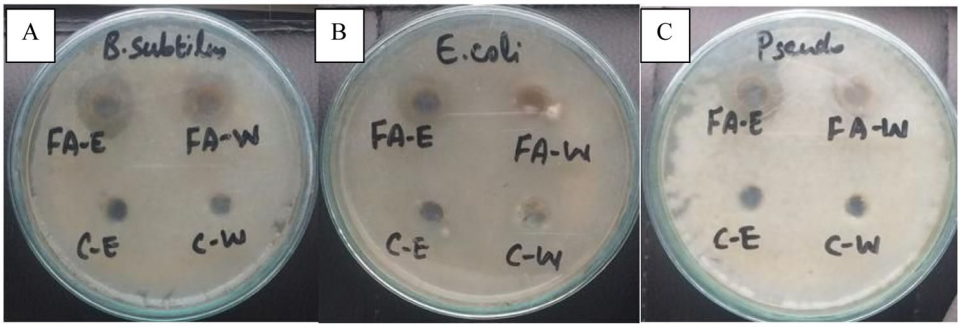

Figure 4. (A) Comparison of *F arabica* extract against *B subtilis*. (B) Comparison of *F arabica* extract against *E.coli*. (C) Comparison of *F arabica* extract against *P aeruginosa*.
C-E indicates control ethanol; C-W, control water; FA-E, *F arabica* extract in ethanol; FA-W, *F arabica* in water.

Table 3. MIC (µg/mL) of different plant extracts in water and ethanol against bacterial species through broth microdilution method.

	B SUBTILIS	*E COLI*	*P AERUGINOSA*
C reflexa (water)	No activity	No activity	No activity
C reflexa (ethanol)	0.25 µg/mL	0.5 µg/mL	0.25 µg/mL
O bracteatum flower (water)	No activity	No activity	No activity
O bracteatum flower (ethanol)	0.5 µg/mL	0.125 µg/mL	No activity
O bracteatum leaf (water)	No activity	No activity	No activity
O bracteatum leaf (ethanol)	No activity	No activity	0.25 µg/mL
S chirata (water)	No activity	No activity	No activity
S chirata (ethanol)	0.062 µg/mL	0.125 µg/mL	0.125 µg/mL
V odorata flower (water)	0.062 µg/mL	No activity	No activity
V odorata flower (ethanol)	0.015 µg/mL	No activity	0.031 µg/mL
V odorata leaf (water)	No activity	No activity	No activity
V odorata leaf (ethanol)	0.125 µg/mL	No activity	0.125 µg/mL
F arabica (water)	0.125 µg/mL	0.25 µg/mL	No activity
F arabica (ethanol)	0.125 µg/mL	No activity	0.125 µg/mL

Abbreviation: MIC, minimum inhibitory concentration.

observed for *E coli*. However,[35] Syed et al documented that dichloromethane extract of *F arabica* possessed antibacterial properties against *E coli*. Previous studies indicated that *S chirata* promisingly exhibited antibacterial properties.[36,37] *S chirata* showed maximum inhibition against the *P aeruginosa, K pneumonia, S aureus*, and *E faecalis*.[38]

In our study, *C reflexa* showed maximum inhibition zone (5 mm) against *B subtilis* as compared with others.[39] Mishra and Dixit also indicated that ethanol extract of *C reflexa* has antibacterial property against *B subtilis* and *E coli*. In another study, *C reflexa* also showed antibacterial property against *Xanthomonas campestris, K pneumonia, Proteus vulgaris, Paracoccus denitrificans*, and *E coli*.[40] *C reflexa* also exhibited antibacterial property against *Salmonella typhimurium*.[41]

Conclusions

In this study, ethanol extract of *C reflexa* plants is best for all 3 tested microbes (*P aeruginosa, B subtilis*, and *E coli*). The ethanol extract of *V odorata* flower revealed highest inhibition zone (18 mm) against *P aeruginosa*. Aqueous extract of *V odorata* revealed potential against *B subtilis*, and *F arabica* showed activity against *B subtilis* and *E coli* while other aqueous extracts of plants shows no inhibition zone. This study succeeded to demonstrate scientific justifications for these plants' use in traditional medicine for the treatment of infections. Hence, it could be concluded that these plants may be a good source of antimicrobials that could be an alternative to antibiotics.

Author Contributions

All authors have equal contributions.

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