

# Phytochemical screening and antibacterial activity of *Opuntia dillenii* and *Onosma bracteatum*

## Abstract

Medicinal plants are those plants which contain substances that possess some therapeutic and antimicrobial agent that can be used for treatment of different diseases caused by microbial infections. The medicinal plants are of great importance because these are utilized as medicines. The aim of this research work was to evaluate the antibacterial activity of *Opuntia dillenii* and *Onosma bracteatum* plants against various pathogenic strains of bacteria. The hot and cold water extract of *Opuntia dillenii* and *Onosma bracteatum* were used against four bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus mirabilis* in order to check the antibacterial activity of these plants. Antibacterial activity was conducted by agar well diffusion method. The above mentioned plants showed different levels of antibacterial activity. The hot and cold water extract of *Onosma bracteatum* showed maximum activity against *Staphylococcus aureus* while showed limited activity against other microorganisms. The zone of inhibition of *Opuntia dillenii* was less.

**Keywords:** medicinal plants, *Opuntia dillenii*, *Onosma bracteatum*, antibacterial activity

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## Introduction

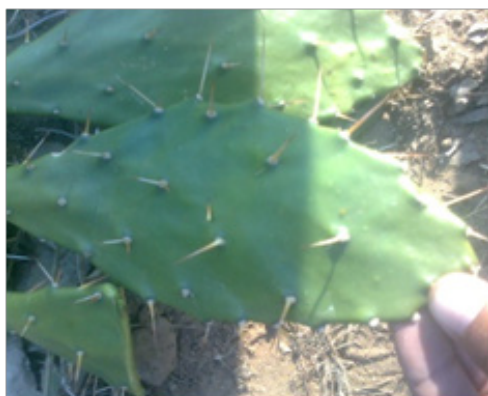
Plants are the beauty of nature and these plants have great medicinal and economic importance throughout the world. Almost all daily human basic and luxurious requirements like feeding, clothing, sheltering, nursing, and hunting are fulfilled by plants. As plants are sources of medicines, they have formed the basis for innovative and traditional systems and continuously providing mankind with new remedies. In the previous few years, the interest in traditional medicine has highly increased. This discipline is gaining the scientific basis for its appropriate application.<sup>1</sup> Herbal medicines have been the main source of primary health care in all nations. The plants as medicine are used in different system of medicines such as ayurveda, allopathy, Unani, Homeopathy and even in other system. The history of plants to be utilized as medicines is thousands of years old.<sup>2</sup> About 80% of the world population is still dependent on traditional medicine. From ancient times, plants have been a rich source of effective and safe medicines. Due to their safe, effective and inexpensive nature, indigenous remedies are popular among the people worldwide. We may call herbal medicine as the medicine in which plant based formulations are used to alleviate diseases. It is also known as botanical medicine or phytomedicine. Latterly phytotherapy has been introduced as more accurate synonym of herbal or botanical medicine. In the early twentieth century herbal medicines were source of prime healthcare system, before the discovery of antibiotics or analgesics. With the advent of allopathic system of medicine, herbal medicine gradually lost its popularity among people, which is based on the fast therapeutic actions of synthetic drugs.<sup>3</sup> The importance of medicinal plants and traditional health system in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the

detriment of natural habitats and mother population in the countries of origin. Most of the developing countries have adopted a traditional medical practice as an integral part of their culture. Historically, all medicine preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.<sup>4</sup> *Opuntia dillenii* (Cactaceae) is commonly known as pear bush, prickly pear, mal rchette or tuna, is a succulent shrub growing in semi-desert regions in the tropics and subtropics regions.<sup>5</sup> The various parts of this plant were used for the treatment of diabetes.<sup>6</sup> Gastric ulcers, anti-inflammatory,<sup>7</sup> analgesics,<sup>8</sup> and anti hyperglycemic.<sup>9</sup> While *Onosma bracteatum*, belongs to the family Boraginaceae. The drugs obtained from this plant is used as a tonic, demulcent, alternative, refrigerant, as well as diuretic, furthermore it is constructive as a spasmolytic. Flowering part of this is useful in rheumatism, alternative, diuretic, syphilis, leprosy along with heart diseases. It is one of such therapeutic plant recognized traditionally in Ayurveda for the dealing of asthma as well as bronchitis.<sup>10-11</sup> The present study recommended that *O. dellenii* and *O. bracteatum* are significant plants from medicinal point of view and Plants based on antimicrobial studies have enormous therapeutically potential as they can serve the purpose without any side effect that already is associated with synthetic antibiotics.

## Material and methods

### Collection of plants materials

*O. dillenii*, wet weight (200g) (Figure 1) was collected from Haripur nursery form and *O. bracteatum*, wet weight (200g) (Figure 2) was collected from Pansar store near Lari Ada Mansehra. These plants were identified by the Department of Botany, Hazara University, Mansehra, Pakistan.

Figure 1 *Opuntia dillenii*.Figure 2 *Onosma bracteatum*.

### Selection of media

During the whole research project, two types of media were used, the Nutrient Agar and Simple Agar. Nutrient agar is the best culturing media for testing micro-organism because it provides nutrients for the growth of all types of bacteria.

### Preparation of media

20 g of nutrient agar was dissolved in 1 liter of distilled water in a conical flask and 4 g of simple agar is also added and plugged in a flask and shaken to mix well. Then it is heated on the hot plate stirrer to dissolve the media completely. The media and all glassware swabs were sterilized by means of autoclaving under the pressure 15psi and temperature 121°C for 15 minutes in an autoclave. After this media was poured aseptically into Petri dishes in a laminar flow cabinet.

### Preparation of plant extracts

The sample was washed properly with de-ionized water for removing dirt, dust and other possible impurities. The plant was dried at room temperature for fifteen days and then crushed into powder using a grinder and then stored in clean sterile plastic bags for further processing. About 120g of powder of both *O. dillenii* and *O. bracteatum* were taken for experimental use to prepare plant extracts, cold and hot weigh 10.7g and 8.8g of *O. dillenii* and cold and hot extracts weigh 10.2g and 9.6g of *O. bracteatum* respectively.

**Cold water extraction:** 60g powder of *O. dillenii* and *O. bracteatum* plants were soaked in cold 300ml distilled water and shaken on an electric rotator at 200rpm for 24 hours. After 24 hours the solution was filtered through a filter paper, then centrifuged at 4400rpm for 7 minutes three times the supernatant appeared at the top was collected which was considered as 100% pure plant extract while the pellet appeared at the bottom of centrifuge tubes was discarded the pure extract were then ready for antimicrobial sensitivity test (Table 1 & Table 2).

Table 1 Cold water extraction of *Opuntia dillenii*

Microorganism	Imipenem (Standard drug)	Zone of inhibition in (mm)			Mean
<i>Escherichia coli</i>	35	-	-	-	-
<i>Bacillus subtilis</i>	36	14	13	13	13.33
<i>Staphylococcus aureus</i>	43	-	-	-	-
<i>Proteus mirabilis</i>	91	-	-	-	-

Table 2 Cold water extraction of *Onosma bracteatum*

Microorganism	Imipenem (Standard drug)	Zone of inhibition in (mm)			Mean
<i>Escherichia coli</i>	35	-	-	-	-
<i>Bacillus subtilis</i>	36	-	-	-	-
<i>Staphylococcus aureus</i>	43	15	15	16	15.33
<i>Proteus mirabilis</i>	91	-	-	-	-

**Hot water extraction:** 60g of *O. dillenii* and *O. bracteatum* powder were soaked in 300ml distilled water in a conical flask and then placed in the incubator at 37 °C for 12 hours, after this they were placed in the hot water bath for 2 hours and then centrifuged for 7 minutes at 4400rpm three times, then filtered through filter paper the

supernatant obtained from 3<sup>rd</sup> time centrifugation collected while pellet were discarded and this was considered as a pure hot extract of these plants and these were ready for sensitivity tests (Table 3&Table4).

**Table 3** Hot water extraction of *Opuntia dillenii*

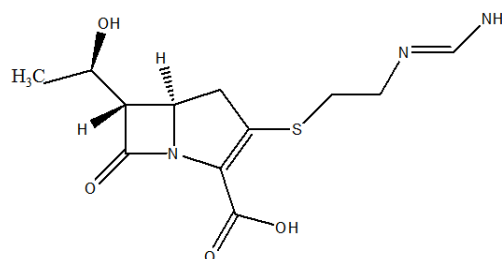
Microorganism	Imipenem (Standard drug)	Zone of inhibition in (mm)			Mean
<i>Escherichia coli</i>	35	-	-	-	-
<i>Bacillus subtilis</i>	36	-	-	-	-
<i>Staphylococcus aureus</i>	43	-	-	-	-
<i>Proteus mirabilis</i>	91	14	13	13	13.33

**Table 4** Hot water extraction of *Onosma bracteatum*

Microorganism	Imipenem (Standard drug)	Zone of inhibition in (mm)			Mean
<i>Escherichia coli</i>	35	-	-	-	-
<i>Bacillus subtilis</i>	36	15	16	16	15.6
<i>Staphylococcus aureus</i>	43	16	15	17	16
<i>Proteus mirabilis</i>	91	-	-	-	-

### Antimicrobial Assay

Media which was prepared and autoclaved was spread on the Petri dishes in a laminar flow cabinet. The Electric fan of laminar flow cabinet was turned on to solidify the media and the pores are made in Petri dishes containing media by tips in a laminar flow cabinet. Then the sterilized cotton swab was dipped in the distilled water and then dipped in the bacterial culture placed it on the Petri dish containing media in order to streak culture on the surface of nutrient agar media of Petri dish uniformly. One cotton swab is used for only once streaking of one Petri dish, then discarded (cotton swab). Poured the hot and cold water extracts of plants in the well in media of Petri dish by micro pipette of 100 ml. After pouring all plates were incubated in an incubator for about 24 hours at 37 °C, and then antibacterial activity was checked. The zone of inhibition was measured by scale in mm and the antibacterial activities were assigned according to the zone of inhibition produced by the plant extracts after 24hours. Imipenem was used as standard antibiotics (Figure 3).

**Figure 3** Chemical structure of imipenem antibiotic.

### Phytochemical screenings

Phytochemical screening was performed using standard procedure (Table 5)

**Table 5** Results of phytochemical screening of *Opuntia dillenii* and *Onosma bracteatum*

Plants Names	Tests	Reducing Sugar	Saponin	Tannin	Terpenoides	Flavonoides	Alkaloids
<i>Opuntia dillenii</i>	-	-	+	+	+	+	+
<i>Onosma bracteatum</i>	-	-	+	+	-	-	+

**Test for reducing sugars (FEHLINGS TEST):** The aqueous extracts of both plants (0.5g in 5ml of water) were added to boiling Fehling's solution (A and B) in a test tube. The solutions were observed for color reactions.

**Test for terpenoides (SALKOWSKI TEST):** To 0.5g each of the extract of both plants were added to 2ml of chloroform separately. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown colorations of the interface indicate the presence of terpenoides.

**Test for flavonoides:** 4ml of extract solutions of both the plants were treated with 1.5ml of 50% methanol solution separately. The solutions were warmed and metal magnesium was added. To these solutions, 5-6 drops of concentrated Hydrochloride acid were added and the red color was observed for flavonoides.

**Test for tannins:** About 0.5g of the extract of both the plants were

boiled in 10ml of water in test tubes and then filtered. A few drops of 0.1% ferric chloride was added to both the test tubes and observed for brownish green or a blue-black coloration.

**Test for saponins:** To 0.5g of extract of both the plants were added 5 ml of distilled water in test tubes. The solutions were shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Test for alkaloids:** Alkaloids solutions produce a white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extracts of both the plants were heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixtures were filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the turbidity or yellow precipitation.

## Results

In the present research work the antibacterial activity of the hot and cold water extract of *O. dillenii* and *O. bracteatum* were checked against four Gram positive and Gram negative bacterial strains. These strains included *Staphylococcus aureus*, *Escherichia Coli*, *Proteus mirabilis* and *Bacillus subtilis*.

## Discussion

Plants are more significance source of potentially useful structures for the development of new chemotherapeutic agents. The first face towards this goal is the *in vitro* antibacterial assay.<sup>12</sup> Many reports are available on the anthelmintic, antibacterial, antiviral, antifungal, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in recognizing the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. In the current investigation the antibacterial activity of *O. dillenii* and *O. bracteatum* were checked against four pathogenic bacterial strains among them two were Gram positive such as *S. aureus* and *P. mirabilis* and two were Gram negative such as *E. coli* and *B. subtilis*. The cold water extract of *O. dillenii* showed moderate antibacterial activity against the *B. subtilis* and no activity against the *E. coli*, *S. aureus* and *P. mirabilis*. The hot water extract of *O. dillenii* showed moderate antibacterial activity against the *P. mirabilis* and no antibacterial activity against the *E. coli*, *B. subtilis* and *S. aureus*. On the other hand cold water extract of *O. bracteatum* was found to exhibits significant antibacterial activity against *S. aureus*. The zone of inhibition measured was 15.3mm while no activity against *E. coli*, *B. subtilis* and *P. mirabilis*. The hot water extract of *O. bracteatum* showed the significant antibacterial activity against the *S. aureus* and *B. subtilis* the zone of inhibition measured were 16mm and 15.6mm, respectively, while no activity against *E. coli* and *P. mirabilis*. According to literature survey, 50% aqueous extract of *O. bracteatum* was found to exhibits significant activity against *S. aureus*. The zone of inhibition measured, reported in the literature was 25mm.<sup>13</sup>

## Conclusion

The present work has shown that *O. dillenii* and *O. bracteatum* is potentially a good source of antibacterial agents which can be used in the future for supporting primary health care.

## Acknowledgments

None.

## Conflicts of interest

Authors declare that there is no conflict of interest.

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