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Research Article

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Antibacterial and Anatomical Study of *Capparis decidua* L. from Different Localities of District Bhimber Azad Jammu and Kashmir, Pakistan

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Abstract The plant *Capparis decidua* L. belongs to family Capparidaceae and its stem and fruit were extracted successively with four organic solvents viz. petroleum ether, chloroform, ethanol, and distil water. These crude extracts were assessed for antibacterial activity against two gram positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilus* and two gram negative bacteria i.e. *Escherichia coli* and *Pseudomonas aeruginosa* by using disc diffusion method. The antibacterial activity of the crude ethanolic extract of stem of selected plant was more active against *Pseudomonas aeruginosa* (21.33± 0.058) and showed less antibacterial activity against *E. coli* (4.67 ± 0.058). The chloroform extract of stem showed good antibacterial activity against *P. aeruginosa* (14.33 ± 0.115) but represented less activity against E. coli (2.33 ± 0.058mm) The petroleum ether extract of stem of *Capparis decidua* showed antibacterial activity against *P. aeruginosa* (8.67 ± 0.058) and show very less activity against *E. coli* (1.33 ± 0.058). The distill water extract of stem of *Capparis decidua* showed maximum antibacterial activity against *P. aeruginosa* (13.33 ± 0.058) and showed less activity against *E. coli* (8.67 ± 0.058). The Chloroform extract of fruit showed good activity against *P. aeruginosa* (12.69 ± 0.112mm) and minimum activity against *E. coli* that were (3.60 ± 0.058mm), The ethanolic extract of fruit of *Capparis decidua* showed good antibacterial activity against *P. aeruginosa* (19.33 ± 0.058) and show less antibacterial activity against *E. coli* (2.67 ± 0.058). The chloroform and ethanolic extracts showed the significant zones of inhibition for all microorganisms. This set of experiment was conducted to compare the antibacterial activity of stem and fruit of *Capparis decidua.* Anatomical studies were conducted to observe the variation in the cells size of stem of *Capparis decidua* taken from different localities of District Bhimber. There was variation in the size of cells of pith, xylem, phloem, and epidermis of the stem of *Capparis decidua* taken from the different areas of District Bhimber. The size of epidermal cells of stem (1089.54 ± 0.83) was higher that was taken from Kaschanater and size of epidermal cell wasminimum (793.72 ± 0.94) that was taken from Chata. The size of cortex cell wasmaximum (1690.33 ± 0.54) that was taken from Bhimber and wasminimum (1090.32 ± 0.73) that was taken from Chata. It proves that this, medicinal plant is medicinally very important and further research may be conducted to explore its comprehensive pharmaceutical analysis.

Keywords Antibacterial activity; Bhimber; *Capparis decidua;* Anatomy; Zone of Inhibition

Introduction

Medicinal plants are backbone of traditional remedy (Farnsworth, 1994). On our earth lakhs of medicinal plants are available [1]. World over the medicinal plants are used as a main source of traditional and orthodox medicines. The attention has been made towards developing the new antibiotics that reduce the increasing resistance among the

microorganism [2]. Medicinal plants being an effective source of both traditional and modern medicines are openly useful for Primary health care. *Capparis decidua* L. is also an important medicinal plant that is used for the treatment of several diseases [3].

Capparidaceae family is generally known as caper family, that belong to the order Brassicales. This family contains 33 genera and about 700 species. The biggest genera are Capparis (about 150 species), Maerua (about 100 species), Boscia (37 species) and Cadaba (30 species). Capparidaceae have long been considered closely related to the family Brassicaceae and have often been included in Brassicaceae, the mustard family in part because both groups produce glucosinolate (mustard oil) compound [4]. All parts of *Capparis decidua* plant have a number of medicinal properties. The plant is usually used to cure toothache, arthritis, asthma, cough, inflammation, intermittent fevers, malaria, rheumatism, and swelling. It is also believed to possess laxative, astringent and vermifuge properties [5-6].The alcoholic extract of fruit pulp and root bark *Capparis decidua* is claimed to have anthelmintic activity. The fruits and the seeds are used to cure cholera, dysentery and urinary purulent discharges and have diuretic and antidiabetic properties [7]. The spicy flavour of fruits serve as an astringent for intestines, a remedy for bad breath and is claimed to cure cardiac troubles. The green immature fruits are considered antihelminthic and laxative and are employed in the treatment of asthma, constipation, coughs, hysteria and other psychological problems. The blanched fruit is used as a vegetable [8]. Green berries are used in food preparations such as pickles. The seeds oil is eatable when processed and also used to cure skin diseases [9-10]. Different parts of *Capparis decidua* exhibit pharmacological properties like antimicrobial, antidiabetic and antioxidant properties [11]. The medicinal plants generally contain number of compounds that may be potential natural antimicrobial agents which may serve as alternative, effective, cheaper and safe antimicrobial agents for the treatment of common microbial infections [12].

Materials and Methods

Collection of plant material

The stem and fruit of *Capparis decidua* was collected freshly from Kaschanater (District Bhimber). The stem and fruit of *Capparis decidua* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

Preparation of extracts

The collected plant material was dried in the shade and ground to a powder. The dried and ground plant material (10 kg) was successively extracted with different solvents like petroleum ether, chloroform, ethanol and Distill water for 72 hours each. The different solvent extracts were concentrated to dryness under reduced pressure. The obtained extracts were stored in a refrigerator at 40 degrees centigrade until use.

Microbial strain

Four strains of bacteria are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli.* All the strains were stored at freeze temperature until use.

Antimicrobial assays

A 24h old culture of each bacterium was used as an inoculum for the test. The slants were prepared in test tube. The nutrient agar medium was used for bacterial growth. *In vitro* antimicrobial screening was performed by disc diffusion method as described by Vander and Vlietnck (1991) [13]. The sterilized nutrient agar medium when temperature reached between 40 degrees centigrade and 45 degrees centigrade was poured in the petri dishes containing bacterial suspension. Three wells or cavities were made in agar containing each Petri dish by a sterilized steel borer. To these cavities standard and test compound solutions were filled. All the work was carried out under aseptic conditions for microbial assay. The plates for the bacteria were incubated at 37 degrees centigrade for 24 hours. After the incubation time, all the plates were examined for the presence of inhibition as a property of antimicrobial activity [13].

Anatomy of Stem

Collection of Plant Material

According to requirement and purposes of the research work, surveys were made to different regions of study area of District Bhimber. Sample of *Capparis decidua* stem were collected to study the anatomy of plant taking from different areas of District Bhimber (Kaschanater).

Procedure Adopted

Few thin green branches of recent growth of *Capparis decidua* were collected. Hold the material between the thumb and index finger in such a way that the tips of the finger and smooth cut surface of the material are in a line, while the tip of the thumb is just a few mm below the upper surface of the material [14]. Wet the surfaces of razor blade/scalpel blade move the blade horizontally over the sample in quick succession in a manner that a very thin and complete slice of the stem sample cut and obtained over the surface of razor blade. After cutting several sections in this manner, transfer all these in to a watch glass containing water then picked the thinnest possible and complete sections from the petri-dish and transfer it into a watch-glass containing safranin and allow these to remain there for about 2 minutes. With the help of a brush gently transfer the section into another watch glass containing water to remove excess of blue safranin stain and kept the material for few minutes and transfer it into a watch glass containing a few drops of dilute acid in water to remove excess of safranin stain. Wash with water and transfer the section on to a clean slide. Placed a cover slip over it avoiding air bubbles and observed it under microscope [14].

Results and Discussion

The plant under study is very important for medicinal purpose. It has been used in traditional healing by local communities of the area. Its different parts were evaluated for their potential as antibacterial agent and then a correlation was discovered that how altitudinal variations have impact on morphology and anatomy of plants, and what will be fluctuations in physiology and chemical profiling of plants to cope with such environment changes.

Table 1: Antibacterial Activity of Stem of *Capparis deciduas* zone of inhibition (mm)

Number of Bacterial Strains	Chloroform	Ethanol	Petroleum Ether	Distill Water
P. aeruginosa	14.33 ± 0.115	21.33 ± 0.058	8.67 ± 0.058	13.33 ± 0.058
B. subtilus	9.33 ± 0.058	6.67 ± 0.152	7.0 ± 0.058	11.69 ± 0.058
S. aureus	5.69 ± 0.052	8.33 ± 0.058	2.67 ± 0.057	9.33 ± 0.058
E. coli	2.33 ± 0.058	4.67 ± 0.058	1.33 ± 0.058	8.67 ± 0.058

The chloroform extract of stem showed good antibacterial activity against *P. aeruginosa* (14.33 ± 0.115mm) but represented less activity against E. coli (2.33 ± 0.058mm). The ethanolic extract of Stem of *Capparis decidua* showed antibacterial activity against *P. aeruginosa* (21.33 ± 0.058) and show less antibacterial activity against *B. subtilus* (6.67 ± 1.152) but showed very less antibacterial activity against *E. coli* (4.67 ± 0.058) (Table 1.) that were comparable with previous work of Kumar *et al*., (2006) [15].

Table 2: Antibacterial activity of fruit of *Capparis deciduas* zone of inhibition (mm)

Names of Bacterial strains	Chloroform	Ethanol	Petroleum Ether	Distill Water
P. aeruginosa	12.69 ± 0.112	19.33 ± 0.058	9.87 ± 0.056	11.33 ± 0.058
B. subtilus	8.33 ± 0.058	7.67 ± 0.052	10.69 ± 0.058	13.69 ± 0.058
S. aureus	5.38 ± 0.058	10.33 ± 0.060	4.67 ± 0.058	12.33 ± 0.053
E. coli	3.60 ± 0.058	2.67 ± 0.058	1.50 ± 0.57	9.67 ± 0.058

The ethanolic extract of fruit of *Capparis decidua* showed good antibacterial activity against *P. aeruginosa* (19.33 ± 0.058) and show less antibacterial activity against *B. subtilus* and *E. coli* (Table 2.) These results were comparable with previous results of Rathee *et al*., (2010) [16] that ethanol extract shows maximum activity against *P. aeruginosa, S. aureus* and *B. subtilus*. The Distill water extract of fruit of *Capparis decidua* showed maximum antibacterial activity against *B. subtilus* (13.69 ± 0.058) and showed less antibacterial activity against *E. coli* that is 9.67 ± 0.058 mentioned in the Table 2 and showed antibacterial activity against *P. aeruginosa* (11.33 ± 0.058). All the values are given in the Table 1; Fig 1. These results were comparable with previous results of Rathee *et al*., (2010) [16].

Table 3: Anatomy of *Capparis decidua* taking from different localities of District Bhimber

Area	Size of epidermal cells	Size of cortex cells	Size of pith cells	Size of phloem cells	Size of xylem cells
Bhimber	1058.20±0.26	1690.33±0.54	792.74± 0.83	1850.60±0.59	952.12 ± 0.98
Kaschanater	1089.54±0.83	1432.23±0.90	831.63± 1.01	1772.95±0.65	952.15 ± 0.86
Chata	793.72 ± 0.94	1090.32±0.73	527.74± 0.63	1311.06±0.28	1321.14±1.07
Dhok	834.79 ± 0.96	1181.42±0.89	795.03± 0.56	1347.17±0.23	1182.12±0.91

The size of epidermal cells of stem (1089.54 ± 0.83) is higher that is taking from Kaschanater and size of epidermal cells is minimum (793.72 ± 0.94) that are taking from Chata (Fig 2). The size of cortex cells is maximum (1690.33 ± 0.54) that is taking from Bhimber and is minimum (1090.32 ± 0.73) that is taking from Chata. The size of pith cells is maximum (831.63 ± 1.01) that is taking from Kaschanater and is minimum (527.74 ± 0.63) that is taking from Chata. The size of phloem cells of stem is maximum (1850.60 ± 0.59) that is taken from Bhimber and is minimum (1311.06 ± 0.28) that is taken from Chata. The size of xylem cells of stem is maximum (1321.14 ± 1.07) that is taken from Chata and is minimum (952.12 ± 0.98) that is taken from Bhimber. These all values are given in the Table 3. This set of experiment was conducted to observe the variations in the anatomy of stem of *Capparis decidua* taking from different localities of District Bhimber. All the values are given in Table 3 and depicted in graphical form (Fig.1).

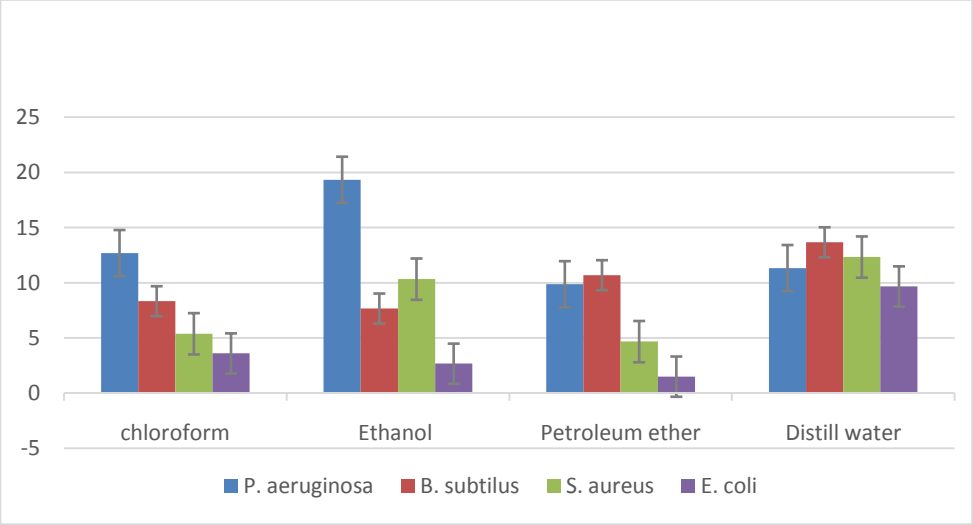

Figure 1: Graph showing Antibacterial Activity of Fruit of Capparis decidua

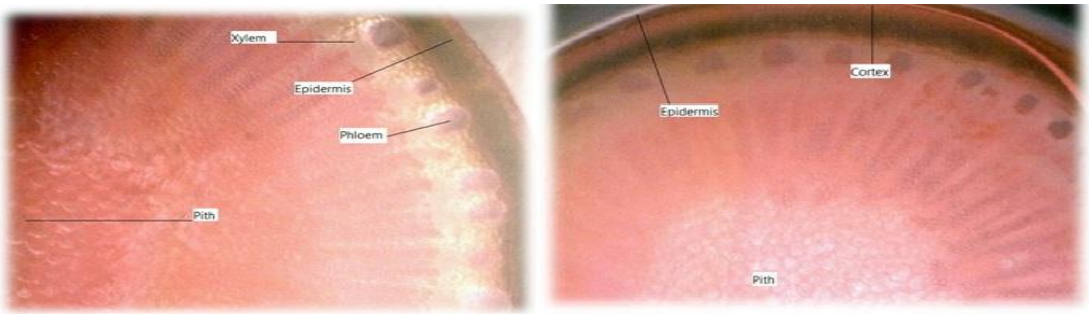

Figure 2: Anatomical Features of Capparis decidua L. from Different Localities of Dist. Bhimber Azad Kashmir

Conclusion

On the basis of present investigations, it is concluded that there exists a great potential in the search of new and more potent antimicrobial substances from the natural sources. The organic solvents extract of stem and fruit of *C.*

decidua showed excellent antibacterial activity against tested bacteria. So it can be concluded that stem and fruit of the selected plant can be regarded as good natural antibiotics with considerable degree of antibacterial activity. As a consequence of this study, we will try to isolate pure compound, which is present in fractions showing large inhibitory activity to bacteria as well as pharmacological and toxicological properties that such compounds might have. On the basis of present studies, it is concluded that variations occur in the size of cells of cortex, pith, epidermis, xylem and phloem of stem taking from different localities of District Bhimber. These variations are due to environmental changes.

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