Antimicrobial Activity of *Punica granatum* and *Cichorium intybus* Extracts against some Pathogenic Bacteria

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**Abstract:** Humans use various kinds of herbs and shrubs for medicinal purposes since time immemorial. *Punica granatum* (pomegranate) and *Cichorium intybus* (chicory) are example of such plants. The undertaken study of selected medicinal plants was screened for their antibacterial activity. Peel extracts of *Punica granatum* (pomegranate) and leaves of *Cichorium intybus* (chicory) were evaluated for antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Two types of solvents were used to prepare the plant extracts; water and an organic solvent Dimethylesulfoxide (DMSO). The results reveal that DMSO dissolved plants extracts had higher solubility when compared with water. The *Cichorium intybus* showed antimicrobial activity against all the pathogenic strains. We also observed that *Cichorium intybus* is more effective against Gram (+) bacteria than that of Gram (-) bacteria. Whereas *Punica granatum* showed zone of inhibition against *S. epidermidis* only. Finding from the present study showed that both of the plants have potent antibacterial activity. Further study will be helpful for the future elucidation of novel therapeutic agents.

**Key words:** Antibacterial potential · *Punica granatum* · *Cichorium intybus* · Phytochemicals · Plant extracts

**INTRODUCTION**

Humans have used various kinds of herbs and shrubs for medicinal purposes since time immemorial. *Punica granatum* (pomegranate) and *Cichorium intybus* (chicory) are example of such plants [1]. The two plants have been used in different cultures for their nonpoisonous products for a long [2].

*Cichorium intybus* (chicory) is a medically important plant that belongs to the family Asteraceae. It is a medicinal herb and different plant parts especially leaves and roots are used in the treatment of diseases such as gallstones, gastro-enteritis, sinus problems cuts and bruises. Chicory is well known for its toxicity to internal parasites. It is also known to be used for blood purification, antipyretic, ant bilious, laxative and diuretic [3].

Similarly *Punica granatum* is a fruit baring tiny tree which is native to Asia [3]. It belongs to Lythraceae family [4]. *P. granatum* is widely used as a medicine to cure a variety of diseases like dysentery, diarrhea, acidosis and respiratory pathologies in many countries [5]. Different plant parts having therapeutic implication are fruit rind, immature fruits, leaves and bark [6]. Vitamins, alkaloids insulin, coumarins, flavonoids, saponins and tannins are the medicinally important compounds due to which all plant part have great medical importance[7, 8].
Antimicrobial activity in most of the extracts is exhibited by phenolics and polyphenols which are one of the largest groups of secondary metabolites. Phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins are the important sub classes of this group of compound. Tanins are group of substances which are phenolic polymeric in nature and are present in most of the plant parts including bark, wood, leaves, fruits and roots [9]. Alkaloids are nitrogen compounds which are heterocyclic in nature and are one of the oldest isolated compound taken from plant [10]. Terpenes are derived from isoprene subunit and mostly involve in membrane disruption by a lipophilic compound. Flavonoids in higher plant are broadly dispersed and act as antioxidant and give protection to cell from damages caused by free radicals [11].

*P. aeruginosa* as a causative agent of nosocomial infection and causes severe, lethal and untreatable infection due to larger rate of appearance of antibiotics resistance during treatment [15]. In developed and developing countries *E. coli* as present in both acute and chronic forms and causes disease in both in children and adults [12]. *S. aureus* is mostly associated with skin, skin glands and mucous membranes, particularly in a nose of healthy individuals [16]. *S. epidermidis* is also a Gram positive bacteria and mostly involved in hospitalized infections. Most of these bacteria are antibiotic resistant [11].

Now a days the most important problem with the treatment of infectious diseases is the development of resistance to many antibiotics by the pathogenic bacteria. Hence, work is going on the extraction of anti-infectional compounds including bioactive from natural sources like plants and animals. The objective of the current study is to determine the antimicrobial activity of the phytochemicals of the medicinal plants; *Punicagranatum* and *Cichoriumintybus* against common human pathogens *S. aureus*, *S.epidermidis*, *P.aeruginosa* and *E. coli*.

**MATERIALS AND METHODS**

The antimicrobial activity *Punicagranatum* and *Cichoriumintybus* of plants was carried out in the department of Microbiology Kohat University of Science and Technology Kohat.

**Isolation of Bacterial Cultures:** Four pathogenic bacterial species were used to study the antimicrobial effects of the two plant extracts. These four pathogenic bacteria isolates used in our study were obtained from the Departmental of Microbiology Kohat University of Science & Technology (KUST) Kohat, Khyber Pukhtunkhwa. The species used in this research are common human pathogens. These are; *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. For culture refreshment total 5 test tubes of 5ml were taken and for each 5 groups of microorganisms there was a separate test tube. Broth were prepared in each test tube and then the colonies were depth in it from separate culture plate through wire loop and placed it in incubator for 24 hours growth. Then on the next day the titter form culture appeared in small test tube [13, 14].

**Preparation of Plant Extracts:** The plant extracts were prepared according to method suggested by Daud *et al.*, 2014. The plant material was carefully examined for any stones, unwanted plant material and then thoroughly washed with distilled water to remove any soil impurities. Both the plant materials were left to shade dry after washing and were ground separately into a fine powder in a mortar and pestle [13]. A 250g of each powdered material of the two plants was dissolved in 2 liters of distilled water in a flask. This dissolved plant material in water was shaken regularly on a shaker for next 21 days. After that both the solutions were filtered and evaporated by rotary evaporator at 44 °C. The plant extracts were dried until all the moisture was evaporated and a solid residue was left behind. Two types of solvents were used to prepare the extract; water and DimethyleSulph oxide (DMSO). For DMSO extract a 250mg of each plant extract was dissolved in 10ml of DMSO, whereas for the aqueous extract 250 mg of plant extract was also dissolved in 10ml of distilled water. The extract was ready for antibacterial activity [14].

**RESULTS**

In the present study, antibacterial activity of *Punicagranatum* and *Cichoriumintybus* was observed. Extracts of these plants showed variable results on the basis of their antibacterial activity against selected microorganisms. In overall process Imepenem was used as positive control and DMSO was used as negative control. The DMSO dissolved extract of *Cichoriumintybus* L showed remarkable antimicrobial activity against all the pathogenic strains used in our study. The positive control showed zone of inhibition more than 30mm while negative control showed no zone of inhibition.
Table 1: List of plant extracts used in the study

<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Common Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cichoriumintybus</td>
<td>Chicory</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>Pomegranate</td>
</tr>
</tbody>
</table>

Table 2: List of Bacterial isolates used in study area

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogenic Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>2.</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>3.</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>4.</td>
<td>Staphylococcus epidermidis</td>
</tr>
</tbody>
</table>

Table 3: Inhibition zones by plant extracts dissolved in distilled water against pathogenic isolates

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogenic Isolate</th>
<th>Cichoriumintybus L.</th>
<th>Punica granatum</th>
<th>(-)Control Distill water</th>
<th>(+)Control Imipinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. aureus</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.7 cm</td>
</tr>
<tr>
<td>2</td>
<td>S. epidermidis</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.6 cm</td>
</tr>
<tr>
<td>3</td>
<td>E. coli</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.9 cm</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas aeruginosa</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.7 cm</td>
</tr>
</tbody>
</table>

Table 4: Inhibition zones by plant extracts dissolved in DMSO against different strains of pathogenic bacteria.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Pathogenic isolate</th>
<th>Cichoriumintybus L.</th>
<th>Punica granatum</th>
<th>(-) Control DMSO</th>
<th>(+) Control Imipinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. aureus</td>
<td>1.6 cm zone</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.7 cm zone</td>
</tr>
<tr>
<td>2</td>
<td>S. epidermidis</td>
<td>1.2 cm</td>
<td>1.3 cm</td>
<td>No clear zone</td>
<td>1.5 cm</td>
</tr>
<tr>
<td>3</td>
<td>E. coli</td>
<td>1.5 cm</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>2.0 cm</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas aeruginosa</td>
<td>1.5 cm</td>
<td>No clear zone</td>
<td>No clear zone</td>
<td>1.7 cm</td>
</tr>
</tbody>
</table>

**Plant Extracts Dissolved in Water:** In case of aqueous plant extracts there were no inhibition zones of any of the two plants against any one of the pathogenic isolate. The only zones produced in the plates were that of positive control, imipinum as shown in Table 3.

**Plant Extracts Dissolved in DMSO:** After 24 hours of incubation the plates with plant extracts dissolved in DMSO were examined for inhibition zones. Cichoriumintybus showed inhibition zone of about 1.6 cm against S. aureus, 1.2 cm against S. epidermidis, 1.5 cm against E. coli and 1.5 cm against P. aeruginosa. On the other hand the Punica granatum showed zone of inhibition only against S. epidermidis i.e., 1.3 cm. (Table 4).

**DISCUSSION**

After 24 hours of incubation the two plant extracts dissolved in distilled water had no clear growth inhibition zones against any of the bacterial strains including E. coli, S. aureus, S. epidermidis and P. aeruginosa. The negative control also did not show any zone. But the (+) control imipenem showed zone against all the four strains of bacteria i.e., 17 mm against S. aureus and 16 mm against S. epidermidis, 19 mm against E. coli and 17 mm against P. aeruginosa (Fig. 1 & 3).

However when the two plant extracts of Cichoriumintybus and Punica granatum were dissolved in DMSO, they showed clear growth inhibition zones against some of these bacterial strain (Fig. 4 & 6). The Cichoriumintybus extract showed zone of 16 mm against S. aureus, 12 mm against S. epidermidis, 15 mm against E. coli and 15 mm against P. aeruginosa. Our results also revealed that compared to Cichoriumintybus the Punica granatum showed resistance only against S. epidermidis with an inhibition zone of 13 mm (Fig. 4). The negative control had no zone against all of the four strains of bacteria examined. Besides this the positive control imipenem showed zone of 17 mm against S. aureus and 15 mm against S. epidermidis, 20 mm against E. coli and 17 mm against P. aeruginosa (Fig. 4 & 6).
Fig 01: Plates with extracts of PG and CI dissolved in distilled water showing no inhibition zones against S. aureus. The only zone in the plate is that of (+) control.

Fig 02: Plant extracts dissolved in distilled water, showing no antimicrobial activity against PG (Punica granatum) and CI (Cichorium intybus).

Fig 03: Plates with plant extracts of PG and CI dissolved in distill water showing no zone against pseudomonas aeruginosa. Whereas zone of (+) control is clearly seen.
Fig4: Plates with Cichorium intybus and Punica granatum showing zones against S. Epidermidis

Fig5: Plant extracts dissolved in DMSO showing zone against S. aureus
Cl (Cichorium intybus), PG (Punica granatum)

Fig6: Zone formation of plant extracts of Cichorium intybus and Punica granatum against E. coli on MHA agar
We also observed in the present study that the extract dissolved in DMSO had considerably higher activity then that of the extract dissolved in distilled water (Table 3 & 4) proving that these plant extracts are more soluble in organic solvents.

Our results also indicate that the Gram negative bacteria *P. aeruginosa* and *E.coli* showed resistance to both the plant extracts which is in accordance with the results reported by Dulger and Gonas 2004 [15]. Whereas *Cichorium intybus* was found more effective against Gram positive than Gram negative bacteria. [16] Mathabe reported similar results that most of the Gram positive have less inhibitory zones compared to Gram negative bacteria.

We observed that *Cichorium intybus* are more reliable than that of *Punica granatum* as *Cichorium intybus* which are effective against the entire test microorganism but the *Punicagranatum* was effective only against *S. epidermidis*. Similar results were reported by Mathabe et al. [16] in which an aqueous leaf extract was moderately effective against *B. cereus* and *E. coli*. The water dissolved extract was not effective. Daud et al. [13] and Dulger et al. [16] also reported in their study that plant extracts are more soluble in organic solvents like DMSO or acetone etc compared to water similar to our results. Whereas unlike our results McCarrell et al. [17] found that aqueous macerated extract of pomegranate rind inhibits growth of *S. aureus* and *P. aeruginosa*. The reason for this contradiction could be that our study was conducted in mid-June and therefore the high temperatures might have affected the solubility of the extracts.

### CONCLUSION

The results of our study proves that the two plant extracts *Punica granatum* and *Cichorium intybus* consists of phytochemicals with antimicrobial activity against *S. aureus*, *E. coli* and *S. epidermidis* and *P. aeruginosa*. Plant extracts dissolved in organic solvent i.e. DMSO showed better results compared to water. We conclude that the use of these plant extracts could be effective in the treatment of the diseases caused by these pathogens. Therefore in the next step the chemical nature of these phytochemicals which are responsible for the antimicrobial activity against the pathogenic strains needs to be investigated.

### REFERENCES


