Medicinal Activity of Withania somnifera and Otostegia limbata against Selected Bacterial Pathogens


Abstract: Different types of infectious diseases can be treated with the help of plants which are the richest source of medicines. In undertaken study selected medicinal plants was screened for their antimicrobial activity. Chloroform and in vitro crude extracts of Withania somnifera and Otostegia limbata was checked for antibacterial activities against Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, Staphylococcus aureus and Klebsiella spp. Ethanolic extract of Withania somnifera and Otostegia limbata plants showed variable results on the basis of their antibacterial activity against selected microorganisms. Maximum zone of inhibition of Withania somnifera i.e., 21 mm was observed against S. aureus whereas 20 mm inhibitory zone was reported against Salmonella spp, E. coli showed a moderate zone of inhibition of 18 mm, followed by P. aeruginosa i.e., 16 mm. Plant extracts showed least activity against Klebsiella spp (10 mm). After applying ethanolic extract of Otostegia limbata against the S. aureus, a maximum zone of inhibition of 19 mm was reported, Salmonella spp showed 18 mm, while against E. coli and P. aeruginosa a moderate, both showed zone of inhibition of 13 mm respectively, while Klebsiella spp showed a minimum zone of inhibition i.e., 11 mm. From the present study it should be cleared that both of the plants have potent antibacterial activity. Hence further phytochemical analysis and HPLC will be helpful for the future elucidation of novel therapeutic agents.

Keywords: Antibacterial Potential • Withania somnifera • Otostegia limbata • Plant Extracts

INTRODUCTION

Plants are mainly used in traditional medicines which play an essential role in health care. For the primary treatment more than half of the world’s population relying mainly on traditional medicines [1]. The therapeutic value of plants is due to the presence of some chemical constituents within the plant tissues which produce a definite physiological action on the human body; include alkaloids, flavonoids, glucosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals and secondary metabolites as well [2,3]. These compounds are believed to play a role in the plants defense against infection by working in synergy with intrinsic antimicrobials. It has therefore been suggested recently, that such compounds can potentially be used to improve the efficacy of antibiotics against bacterial pathogens [4].

From ancient period medicinal plants have been used to cure different types of diseases. Since they are of great importance and commonly used by the local people, due to which globally a lot of people are engaged in the trade of important medicinal herbs. Approximately three-quarters of the world population relies mainly on plants and plant extracts for health care. In China the allopathic drugs are mostly avoided because they use traditional...
medicine for non-toxicity [5]. In Africa up to 80% of the population depends on traditional herbal medicine for primary health care, accounting for around 20% of the overall drug market according to the World Health Organization (WHO) [6].

Over the past 20 years, as source of new antimicrobial agents there has been a lot of study on plants. But motionless there is an instant need to recognize novel substances lively in the direction of pathogens with high resistance [7]. In diverse organisms numerous drug resistances are becoming common and resistance to antimicrobial agents is rising in a wide diversity of pathogens [8]. Due to lesser side effects, lesser toxicity, cheap and more effective compared to the synthetic drug agent the use of plant for treating infectious diseases has gradually increased in the world [9].

*Withania somnifera* (kutilal) belongs to the family solanaceae. The plant has been found useful in the treatment of burns, wounds and dermatological disorders and gastrointestinal diseases, dysfunctions of the respiratory system, asthma, bronchitis, cancer and geriatric problems. *Withania somnifera* is widely used in Ayurvedic medicine, the traditional medical system of India [10]. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism) and as a general tonic to increase energy, improve overall health and longevity and prevent disease in athletes, the elderly, during pregnancy. Many pharmacological studies have been conducted to examine the properties of *Withania somnifera* in an attempt to authenticate its use as a multi-purpose medicinal agent. The anti-inflammatory properties of linn have been investigated to validate its use in inflammatory arthritis, while animal module studies have been established, its use as an antistress agent [10]. Several studies have examined the antitumor and radiosensitizing effect of WS. Besides, phytochemical screening of the extracts was also carried out to assess the presence of different phytochemical in different extracts [11].

*Otostegia limbata* Benth (Fishkarmily labiatae and leaves are used for gum diseases and curing of wounds. There are reports on the isolation of two new tricyclic clerodane-type diterpenoids (limbatolide D and E) from the roots of *O. limbata*. It is also reported that juice of *O. limbata* plant are used to treat bleeding gums of children and ophthalma [12].

The present study is carried out to evaluate the antibacterial activity of *Withania somnifera* and *Otostegia limbata* plants extracts against human pathogenic bacteria and compare the activity of plants and selected antibiotics against the human pathogenic bacteria.

**MATERIALS AND METHODS**

The antimicrobial activity of *Withania somnifera* and *Otostegia limbata* plants was carried out in the department of Microbiology, Kohat University of Science and Technology, Kohat.

**Selection and Sub-Culturing of Pathogenic Bacteria:**
Human pathogenic bacterial isolates obtained from the department of Microbiology Kohat University of Science and Technology Kohat, included; Gram negative microorganisms i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp*, *Klebsiella spp* and Gram positive microorganism i.e., *Staphylococcus aureus*. All this research work was done under maximum aseptic conditions. For culture refreshment total 5 test tubes of 5ml were taken and for each microorganism separate test tube was used. Prepared the Broth in each test tube and then the colonies were introduced in it from separate culture plate through wire loop and placed in incubator for 24 hours growth. Then on the next day the titter form culture occur in small test tube.

**Collection of Plants and Preparations of Extract:**
Two medicinal plants were collected from the region of Khyber Pakhtunkhwa, Pakistan. *Withania somnifera* was isolated from district Kohat and *Otostegia limbata* from district Malakand. Firstly the plants were collected and then washed with tap water, air dried and cut into small pieces. After cutting and slicing, the collected plants pieces were place in shade for drying and kept until used in experiments. Then plant materials crushed into powder form with the help of grinder and powdered sample were place in clean closed glass container.

**Minimum Inhibitory Concentrations (MICs):**
The aim of broth and agar dilution methods is to determine the lowest concentration of the analyzed antimicrobial agent (Minimal Inhibitory Concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being examined. MIC values are used to determine susceptibilities of bacteria to plant extract. For dilution of broth, bacteria were inoculated into a liquid growth medium in the presence of different concentrations of plant extract. Growth was evaluated after incubation for a defined period of time (15–19 h) and the MIC value was read and noted.
Minimum Bactericidal Concentrations (MBCs):
The Minimum Bactericidal Concentration (MBCs) is the lowest concentration of an antibacterial agent required to kill a specific bacterium [13]. By sub culturing to agar plates it can be determined from broth dilution minimum inhibitory concentration (MICs) tests.

RESULTS

In the present study, antibacterial activity of Withania somnifera and Otostegia limbata was observed. Ethanolic extracts of these plants showed variable results on the basis of their antibacterial activity against selected microorganisms. In overall process, positive control (Imipenem) and negative control (DMSO) were used. In positive control, zone of inhibition was reported more than 30mm and on negative control there was no zone of inhibition reported.

Withania somnifera: The extract of Withania somnifera plants was evaluated in total three experiments in vitro against E. coli, P. aeruginosa, Salmonella spp, Klebsiella spp and S. aureus.

The different zones of inhibition recorded are given in table 1. The ethanolic extract of Withania somnifera leaves was significantly active against all bacterial isolates. Maximum zone of inhibition of 21 mm was observed against S.aureus followed by Salmonella spp with a zone of inhibition of 20 mm, E. coli showed a moderate zone of inhibition of 18 mm, followed by P. aeruginosa (16 mm). The extract of Withania somnifera showed least activity against Klebsiella spp (10 mm).

Otostegia Limbata: The extract of Otostegia limbata plants was evaluated in total three experiments in vitro against the E. coli, P. aeruginosa, Salmonella spp, Klebsiella spp and S. aureus.

The different zones of inhibition recorded are given in table 2. It was observed that in ethanolic extract of Otostegia limbata against the S. aureus the maximum zone of inhibition of 19 mm was reported, Salmonella spp also showed increased zone of inhibition of 18 mm, while against E. coli and P. aeruginosa, the activity of otostega limbata was moderate with both showing zone of inhibition of 13 mm each, while Klebsiella spp showed the minimum zone of inhibition i.e., 11 mm.

DISCUSSION

There are many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions and post-harvest processing which effect type and level of biological activity exhibited by any plant material. For example, during grinding the relatively high temperatures can denature chemical constituents and the extraction solvent, secondary metabolites extracted from plants tissue can be affected by temperature and time period [14].

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>*Average zone of inhibition (mm) of ethanol extract relative to DMSO control</th>
<th>Average zone of inhibition (mm) Ampicillin</th>
<th>Average zone of inhibition (mm) Cephradine</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>18</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>20</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>16</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>21</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

*Average of three independent biological replicates. The zone of inhibition of DMSO was substracted from test.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>*Average zone of inhibition (mm) of ethanol extract relative to DMSO control</th>
<th>Average zone of inhibition (mm) Ampicillin</th>
<th>Average zone of inhibition (mm) Cephradine</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>13</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>18</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>13</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>19</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>11</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

*Average of three independent biological replicates. The zone of inhibition of DMSO was substracted from test.
Natural material as a source of new antibacterial agents has been much interest over the past few decades. Different traditional medicinal plant's extracts have been tested. According to many reports, against microorganisms traditional herbs show effectiveness, so the base of modern medicine is plants [15].

The current study was conducted to investigate the antimicrobial properties of two medicinal plants i.e., *Withania somnifera* and *Otostegia limbata* against common human pathogenic bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp* and *Staphylococcus aureus* while moderate activity was exhibited against *Klebsiella spp*.

Among all the selected plants *Withania somnifera* showed increased zone of inhibition against *S. aureus*. Against other tested microorganisms we also observed that the *Withania somnifera* activity was optimal but minimum activity was observed against *Klebsiella spp*. Previous work conducted by Rinku et al., (2012) which reported that ethanolic extract of *Withania Somnifera* leaves showed antibacterial activities against bacterial strains including the *S. aureus* our findings are in line with his observation. *Withania somnifera* showed higher antimicrobial activity. Our finding is supported by these results and evaluates the traditional use of the plant in therapeutic use against microbial infection.

The ethanolic extract of *Otostegia limbata* showed the zone of inhibition of 13 mm against the *E. coli*, whereas an increased zone of inhibition of 18 mm was observed against *Salmonella spp*. Against the *S. aureus*, the maximum zone of inhibition of 19 mm was observed. A recent study by Anwar et al. [16] showed that the *otostegia limbata* plant showed moderate activity of 11.5 mm against *E. coli*. A minimum of 8 mm zone of inhibition was observed against *salmonella spp*, while against *S. aureus* an increased activity of 18 mm was observed (Table 1 and Table 2).

**CONCLUSIONS**

The present study demonstrated that *Withania somnifera* and *Otostegia limbata* can serve as potential source of antibacterial agent. The argument about the activity against human pathogenic bacteria about biomolecules can also clarify that it may be helpful for therapeutic intervention in future against human pathogenic bacteria and different types of diseases can also be treated. It was concluded that MIC and MBC against *E. coli* were positive and both plant extracts showed antimicrobial activity against *E. coli*, because *E. coli* shows more resistant than other human pathogenic bacteria. It shows that it is feasible to evaluate the antimicrobial activity against *E. coli*.

**REFERENCES**