Effect of different solvent extracted samples from the leaves and fruits of *Datura stramonium* on the growth of bacteria and fungi

Jehan Bakht¹, Maryam Qureshi², Arshad Iqbal² and Mohammad Shafi³

¹Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, KPK, Pakistan
²Department of Botany, Islamia College, Peshawar, Pakistan
³Department of Agronomy, The University of Agriculture, Peshawar, KPK, Pakistan

Abstract: Different solvent extracted samples from the leaves and fruits tissues of *D. stramonium* were tested against five pathogenic microorganisms by disc diffusion susceptibility method using 1, 2 and 3mg disc⁻¹ concentrations. Methanol and chloroform extracted fractions from both leaves and fruits measured good growth inhibition of all the tested microorganisms at all concentrations. *Bacillus subtilis* was very resistant to n-butanol and aqueous extracted fractions of fruits tissues at all the tested three concentrations. The growths of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were efficiently reduced by all the solvent extracted fractions from the fruits while aqueous fraction was unable to restrain the growth of *Bacillus subtilis*. The growth of *Candida albicans* was effectively reduced by aqueous extracted fraction from the leaves tissue at the highest concentration. Maximum growth reduction of (77%) was shown by chloroform extracted fractions from the leaves against *Klebsiella pneumonia* at 3mg disc⁻¹ concentration. Minimum zone of inhibition (35.4%) was measured by n-butanol extracted fractions from the leaves against *Pseudomonas aeruginosa* at the lowest concentrations of 1mg disc⁻¹. In case of leaves the most vulnerable bacteria was *Bacillus subtilis* while in case of fruits *Klebsiella pneumonia* was the most susceptible while *Bacillus subtilis* was the most resistant one.

Keywords: Antimicrobial, disc diffusion assay, Gram negative, Gram positive, *D. stramonium*

INTRODUCTION

Throughout the world antibiotic resistance is of great interest (Westh et al., 2004). In recent years, because of unsystematic use of commercial antimicrobial drugs for the treatment of contagious diseases, increase in multiple resistances in humans is observed from various sources. The search and screening of plants, their extracts and products revealed that higher plants show significant medicinal activity and are a great source of antibiotic prototypes (Afolayan, 2003). Many studies indicated that herbal plants contain many bioactive and antibiotic compounds (Basile et al., 2000). Traditional healing systems all over the globe that utilize herbal medicines are a huge blast for the discovery of novel antibiotics (Okpekon et al., 2004). Various traditional remedies have produced certain compounds that are effective against antibiotic-resistant bacterial species (Kone et al., 2004). However, results obtained from the previous study indicated that traditional health systems require more research (Romero et al., 2005). As a result the scope of pharmacological studies tremendously increased which leads to the synthesis of more powerful drugs with reduced toxicity and side effects (Ebaña et al., 1991).

Infectious diseases are the leading cause of death worldwide. The microbes are getting challenging against known antibiotics which have become a worldwide concern. This antibiotic resistance leads to emergence of multi drug resistant pathogens which is a great threat to the efficiency of many existing antibiotics (Parekh and Chanda, 2007). Research has shown that Gram positive bacteria are more prone to plants extracts compared to Gram negative bacteria (1999; Parekh and Chanda, 2006). Such differences may be due to the fact that Gram negative bacteria contain multiple layer cell wall while the cell wall of Gram positive bacteria is single layered.

Plants possess different bioactive compounds such as alkaloids, flavonoids, saponins, phenols tannins which have shown good antimicrobial activity against different microbes (Bakht et al., 2013 a, b; 2014 a, b, c; 2015; 2017 a,b,c,d; Amjad et al., 2016; Wajid et al., 2016; Bilal et al., 2017). For the identification of therapeutic effects, extracts of different medicinal plants have been tested and these extracts act as sources of therapeutic effects. As a result several natural products have been accepted as new antibacterial drugs, although there is an imperative desire to categorize some new substances that are dynamic towards resistant pathogens (Ayaz et al., 2017; Cragg et al., 1997). Novel sources of antimicrobial agents with accurate mechanism of action are probably obtained from some plants as natural products (Hamil et al., 2003; Machado et al., 2003; Motsei et al., 2003; Barbour et al., 2004). Conversely many infectious diseases are healed by many therapeutic agents which shows that plant origin antimicrobials are not linked with adverse effects (Iwu et al., 1999).

Datura belongs to the family Solanaceae (the nightshade family) containing about 2,400 species (Siegel, 1989). Some of the most common manes of datura includes crazed stinkweed, thorn apple, angel’s trumpet, Jimson...
weed, Devil’s apple and nightshade (Avery, 1959; Heiser, 1969). *D. stramonium* is a most important medicinal plant. Traditionally it has an important medicinal value throughout the universe. For the treatment of different diseases the leave and seeds of Datura are important. The leaves and stem of *D. stramonium* contain highest content of scopoline and hyoscynine. In many pharmacopeias these compounds were included because of their anticholinergic actions. Variety of alkaloids such as scopoline hydrobromide, atropine and hyoscynine are also present in *D. stramonium* (Ivancheva, 2006) which has shown antimicrobial activity. Darura contains atropine active ingredient of tropane alkaloids which is used as hallucinogen (Banso, 2006). For the treatment of skin disorders leaves of *D. stramonium* are mixed with mustard oil. Juice obtained from the flower petals is used to cure ear pain. Seeds are effective to relieve asthma, fever and cough and for narcotic purposes (Khan et al., 2013). Its roots and shoot extracts show high antimicrobial activities anti-fungal (Gul et al., 2012). Keeping in view the medicinal importance of *D. Stramonium* the present research work was carried out to investigate the antibacterial and antifungal activity of different solvent extracted samples from the leaves and fruits of *D. stramonium* through disc diffusion assay and to compare antimicrobial activity of different solvent extracted samples from the leaves and fruits of *D. stramonium*

**MATERIALS AND METHODS**

**Plant material collection**

The plant parts (leaves and fruits) of *D. stramonium* were collected from the Farms of The University of Agriculture Peshawar, Pakistan. Plant material was washed thoroughly with distilled water in order to make it dirt and dust free. The plant material was dried under shade at room temperature for 15-18 days. Grinding was done by electric grinder in order to make it pulverized.

**Crude extract preparation**

The powdered plant material i.e., leaves and fruits (500 gm each) were soaked in 4 liters of commercial grade methanol for 10 days. Continuous shaking was done for the complete dissolution of the bioactive compounds into methanol. Filtration of the soaked plant material was done by using Whatman No. 1 filter paper. The residue which remained was again soaked in fresh methanol and filtration was done. The whole process was repeated four-five times and pooled together all the methanolic filtrates. Drying of the filtrate was carried out under reduced pressure using rotary evaporator at 45°C. The dried methanolic extract was then divided into two fractions; one fraction was used as crude methanolic extract and the other was fractioned with chloroform, ethyl acetate, n-butanol and water.

**Fractionation of crude methanolic extract**

Hundred grams each of the crude methanolic extract from leaves and fruits was added into 450 ml distilled water in separatory funnel. The mixture was added to chloroform, slowly shaken and allowed to stand for 15-18 min until two separate layers were formed. The upper chloroform layer was collected and the process was repeated four times by adding fresh chloroform to aqueous fraction. Chloroform fractions were combined together, dried under reduced pressure using rotary evaporator at 45°C. The same procedure of fractionation was followed for ethyl acetate and n-butanol. The last fraction of aqueous phase was dried under reduced pressure in rotary evaporator at 45°C (Bakht et al., 2017 a).

**Disc diffusion susceptibility assay for antibacterial activity**

For culturing and growth of bacteria to carry out disc diffusion assay, nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used. Nutrient broth was used for shaking, incubation and standardization of different microorganisms. Media was prepared as described by Bakht et al. (2011 a). Antibacterial activity of the extracts was evaluated by the methods of Bauer et al. (1966) and antifungal by Ramadas et al. (1998). Stock solutions of the different extracts were prepared in sterile DMSO. The plates were inoculated using microbial inoculums (a standardized inoculums 1-2 × 107 CFU ml⁻¹ 0.5 McFarland Standard) under sterile conditions. Three discs of Whatman No. 1 filter paper (6 mm diameter) were placed on the petriplates using sterilized forceps. Three volumes i.e., 6, 12 and 18 μl from each stock solution were applied on discs respectively corresponding to three concentrations of the extracts i.e. 1, 2 and 3 mg disc⁻¹. The plates were labeled, properly sealed by parafilm and incubated at 37 °C for 24 hours. Antibiotics were used as positive control while DMSO as negative control. After incubation, zone of inhibition was measured in millimeters in comparison with positive control. The same procedure was repeated for each micro-organism. The percent (%) inhibition was finally calculated by the following formula

\[
\text{% inhibition} = \frac{\text{Zone of inhibition of sample (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100 \text{Posi}
\]

**Positive controls**

For Gram-positive bacteria; Ciprofloxacin 50μg per 6μl For Gram negative-bacteria; Ciprofloxacin 50μg per 6μl

**Negative control**

DMSO was used as negative control.

**Microorganisms tested**

Antibacterial activity of different solvent extracted samples was tested against different bacterial and fungal strains (table 1).

**STATISTICAL ANALYSIS**

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Least Significant Difference (LSD) test was employed upon obtaining significant difference at p<0.05 (Steel et al., 1997).
Table 1: Microbial strains used during the experiment

<table>
<thead>
<tr>
<th>Microbial Species</th>
<th>Gram strain type</th>
<th>Details of the Microbial strains used</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumonia</td>
<td>Negative</td>
<td>Clinical isolate obtained The Department of Microbiology, Microbiology, Quaid-I-Azam University Islamabad Pakistan</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>Negative</td>
<td>ATCC # 9721</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>Positive</td>
<td>Clinical isolate obtained The Department of Microbiology, Microbiology, Quaid-I-Azam University Islamabad Pakistan</td>
</tr>
<tr>
<td>E. coli</td>
<td>Negative</td>
<td>ATCC # 25922</td>
</tr>
<tr>
<td>C. albicans</td>
<td></td>
<td>ATCC # 10231. Plant Pathology Department, The University of Agriculture Peshawar KPK Pakistan</td>
</tr>
</tbody>
</table>

**RESULTS**

**Antibacterial and antifungal activity of different solvent extracted samples from the leaves of D. stramonium**

The anti-bacterial activity of crude methanolic, chloroform, ethyl acetate, n-butanol and aqueous extracts from the leaves of *D. stramonium* against *B. subtilis* by disc diffusion assay is shown in fig. 1. The data revealed that all solvents extracted samples decreased the growth of *B. subtilis* at higher concentration with significant zone of inhibition. The data further suggested that *B. subtilis* showed highest susceptibility to crude methanol, chloroform, ethyl acetate and aqueous extracted fractions measuring highest inhibitory activity of 68% at concentration of 3 mg disc\(^{-1}\) followed by n-butanol extracted samples at concentration of 3 mg disc\(^{-1}\) measuring 63% ZI. These extracts were also active at 1 and 2 mg disc\(^{-1}\) against the tested microbe when compared with other samples and controls. The antibacterial activity of different solvent extracted samples from the leaves of *D. stramonium* against *Escherichia coli* is shown in fig. 2. The data indicated that chloroform extracted fractions showed highest growth reduction of 64.5% against *E. coli* at the highest concentration of 3 mg disc\(^{-1}\) followed by 58% and 42% ZI at 2 and 1 mg discs\(^{-1}\) respectively compared to other samples and controls. Good activity was also shown by n-butanol extracted fractions measuring 62% ZI at 3 mg disc\(^{-1}\). Analysis of the data also revealed that reasonable activity was shown by methanol and aqueous extracts showed average activity of 55% at 3 mg disc\(^{-1}\), while lowest activity was recorded for ethyl acetate extracted samples (35.4% ZI) at 1 mg disc\(^{-1}\).

![Fig. 1: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted samples from the leaves of *D. stramonium* against *B. subtilis* by disc diffusion assay (Bar shows LSD at p<0.05).](image)

Fig. 3 reveals the antibacterial activity of different solvent extracted samples from the leaves of *Datura* plant against *K. pneumonia*. Analysis of the data indicated that maximum inhibitory activity was shown by chloroform extracted fractions measuring 77% ZI at concentration of 3 mg disc\(^{-1}\) followed by 52.5% and 46% ZI at 2 mg and 1 mg disc\(^{-1}\) respectively of the same sample when compared with controls. It is also clear from the data that 76% and 72% inhibitory zone was shown by ethyl acetate and n-butanol extracted fractions at the highest concentration of 3 mg disc\(^{-1}\) compared to controls and other samples. Crude methanolic and aqueous extracts showed moderate activities of 69% and 68.1% ZI respectively at 3 mg disc\(^{-1}\). Results indicated that these samples also showed good results at 1 and 2 mg disc\(^{-1}\). The antibacterial activity of different solvent extracted samples obtained from the leaves against *P. aeruginosa* is shown in fig. 4. The data indicated the maximum activity of 82% was measured by chloroform extracted fraction at concentration of 3 mg disc\(^{-1}\) followed by ethyl acetate extracted fraction having 79% inhibitory zone at the same concentration against *P. aeruginosa*. The data also suggested that n-butanol extracted samples showed moderate activities at 3 mg disc\(^{-1}\) (75% ZI).when compared with other samples and control. On the other hand, aqueous extracts and crude methanol showed average activity of 64.5% ZI at 3 mg disc\(^{-1}\) concentration. These results clearly indicate that good results were recorded against all extracted samples at 2 and 3 mg disc\(^{-1}\) against *P. aeruginosa*.

The antifungal activity of crude methanolic, n-hexane, n-butanol, ethyl acetate and aqueous extracts from the leaves of *D. stramonium* against *C. albicans* is presented in fig. 5. The data implied that *C. albicans* showed highest susceptibility to aqueous extracted fraction having maximum ZI of 74% followed by 72% and 57.1% inhibitory zones at 2 and 1 mg disc\(^{-1}\) respectively compared with other samples and controls. The data also revealed that good inhibition activities were shown by crude methanol and chloroform extracted samples measuring 69% and 65.4% at 3 mg disc\(^{-1}\) concentrations respectively. Maximum activity was also revealed by n-butanol extracts (63% ZI) followed by ethyl acetate extracted samples which showed 60.3% ZI at 3 mg disc\(^{-1}\). fig. 6 presents the antibacterial activity of crude methanolic, n-hexane, n-butanol, ethyl acetate and
aqueous extracts obtained from the fruits of *D. stramonium* against *B. subtilis*. The data indicated that *B. subtilis* was highly susceptible to crude methanolic extract measuring 41.3% zone of inhibition at the highest concentration of 3 mg disc⁻¹ concentration followed by 32% and 22.5% ZI at 2 and 1 mg disc⁻¹ respectively. The data also indicated that good activity was also measured by ethyl acetate (40% ZI) at 3 mg disc⁻¹ concentration. Lower inhibition activities were measured by aqueous extracted fractions measuring 20.6% ZI each at 1 mg disc⁻¹ concentration. Moderate activity was shown by chloroform extracted samples (36.5% ZI). N-butanol and aqueous extracts showed zero activity at 1, 2 and 3 mg disc⁻¹ concentrations when compared with other samples and controls.

![Fig. 2: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted samples from the leaves of *D. stramonium* against *E. coli* by disc diffusion assay (Bar shows LSD at p<0.05).](image1)

The antibacterial activity of crude methanolic, n-hexane, n-butanol, ethyl acetate and aqueous extracts from the fruits of *D. stramonium* against *E. coli* is illustrated in fig. 7. The data indicated that almost all solvent extracted samples were effective against *E. coli* and showed inhibition at all the three concentrations i.e. 1, 2 and 3 mg disc⁻¹. Chloroform extracted samples showed highest inhibition zone (40%) at 3 mg disc⁻¹ followed by n-butanol extracts having inhibition zone of 39.2% at the same concentration when compared with other samples and controls. The data also indicated that moderate activities were shown by aqueous and crude methanolic extracted samples measuring 38.1% and 38% ZI at 3 mg disc⁻¹ concentration respectively compared to controls. Ethyl acetate extracted samples showed minimum inhibition zone of 37.5% at 3mg disc⁻¹. fig. 8 demonstrates the antibacterial activity of different solvent extracted samples from the fruit against Gram negative bacteria *K. pneumonia*. Analysis of the data revealed that n-butanol extracted fractions showed maximum inhibition zone of 72% at the highest concentrations of 3 mg disc⁻¹. The data further suggested that ethyl acetate and crude methanolic extracts also showed good activity of 48% and 41% ZI respectively at the highest concentration when compared with controls and other samples. Aqueous extracts showed moderate activity of 37% at 3 mg disc⁻¹. Poor activity was recorded by chloroform extracted samples (33.2% ZI) at 3 mg disc⁻¹ as compared to other samples and controls.

![Fig. 3: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted samples from the leaves of *D. stramonium* against *Klebsiella pneumonia* by disc diffusion assay (Bar shows LSD at p<0.05).](image2)

![Fig. 4: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted samples from the leaves of *D. stramonium* against *Pseudomonas aeruginosa* by disc diffusion assay (Bar shows LSD at p<0.05).](image3)

![Fig. 5: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted](image4)
samples from the leaves of *D. stramonium* against *C. albicans* by disc diffusion assay (Bar shows LSD at p<0.05).

Data regarding antibacterial activity of different solvent extracted samples from the fruits of *D. stramonium* against *P. aeruginosa* is shown in fig. 9. The data revealed that *Pseudomonas aeruginosa* showed highest susceptibility to crude methanolic extracts and its growth was constrained at 3 mg disc⁻¹ measuring 53.5% inhibition zones followed by 49% ZI of the same fraction at 2 mg disc⁻¹. It can be also seen from the data shown in table 10 that chloroform, aqueous and n-butanol extracted samples also effectively controlled the growth of the tested microbe. The data also suggested that ethyl acetate extracted fraction at the lowest concentration measured minimum zone of inhibition (25.1%) when compared with other samples and controls. Data illustrated in fig. 10 reveals the antifungal activity of different solvent extracted samples obtained from the fruits of *D. stramonium* against *C. albicans*. The data suggested that maximum growth inhibition of 54% was shown by crude methanolic extract at concentration of 3 mg disc⁻¹, while ethyl acetate extracted fractions showed 41.5% ZI at concentration of 3 mg disc⁻¹. The data further revealed that these extracts also effectively inhibited the growth of *C. albicans* at 1 and 2 mg disc⁻¹ concentrations. N-butanol extracts also slowed down the growth of the tested microbe recoding 39.5% zone of inhibition at 3 mg disc⁻¹. Moderate activity was shown by aqueous extracted samples (38.4% ZI) while chloroform showed 33% activity at the highest concentration when compared with other extracts and controls.

**DISCUSSION**

Analysis of the data revealed that methanolic extracts samples from the leaves of dried samples of *D. stramonium* against *B. subtilis* showed that all the solvents extracted samples were effective to reduce the growth of the tested microbe. However, crude methanol, chloroform, ethyl acetate and aqueous extracts effectively reduced the growth of *B. subtilis* at higher and lower concentrations. Moderate activity was shown by n-butanol extracted samples at 3 mg disc⁻¹ and aqueous extract showed lowest activity at 2 mg disc⁻¹ and 1 mg disc⁻¹ against the tested microbe. These results agree with Gachande and Khiilare (2013). Analysis of the data of different solvent extracted samples from datura leaves indicated that chloroform and n-butanol extracted samples were very effective to control the activity of *E. coli*. Ethyl acetate extracted samples showed poor activity at 1mg disc⁻¹. Similar inhibitory zones were also recorded by n-butanol and methanol extracted samples at higher concentrations at 3mg disc⁻¹ against the tested microbe. Similar results were also obtained by Reddy et al. (2009). Good activity was shown by chloroform and ethyl acetate extracted samples from the leaves, followed by n-butanol at 3mg disc⁻¹ against *K. pneumonia*. The data also presented that n-butanol reduced the growth of the tested microbe at 2 and 1mg disc⁻¹. Shobha et al. (2014)
reported that *D. stramonium* showed significant activity against bacterial isolates such as *E. coli*, *B. subtilis*, *P. aeruginosa* and *K. pneumonia*. Antibacterial activity of different solvents extracted samples from the leaves against *P. aeruginosa* showed that all concentrations were effective to control the growth of tested microbe. Chloroform extracted fraction was more effective to control the growth of tested microbe at 3mg disc\(^{-1}\) concentration. Extracts obtained from ethyl acetate, n-butanol also reduced the activity of the tested microbe at 1 and 2mg disc\(^{-1}\). Lowest activity was shown by methanol and aqueous extracts at 1mg disc\(^{-1}\). Data showing the antifungal activity of methanol, chloroform, ethyl acetate, n-butanol and aqueous extracted samples from the leaves of datura plant against *C. albicans* suggested that aqueous extract showed highest antifungal activity at concentration of 3mg disc\(^{-1}\), followed by methanol and chloroform extracted fractions at the highest concentrations. The lowest inhibitory action was observed by chloroform extracted fraction against the same fungus at 1mg disc\(^{-1}\). Sharma *et al.* (2014) also reported similar antifungal activity.

Antibacterial activity of different solvent extracted samples from the fruits of *D. stramonium* against *Pseudomonas aeruginosa* by disc diffusion assay (Bar shows LSD at p<0.05).

![Figure 9](image)

**Fig. 9**: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and water extracted samples from the fruits of *D. stramonium* against *P. aeruginosa* by disc diffusion assay (Bar shows LSD at p<0.05).

**Fig. 10**: Antibacterial activity of crude methanol, chloroform, ethyl acetate, butanol and watere extracted samples from the fruits of *D. stramonium* against *C. albicans* by disc diffusion assay (Bar shows LSD at p<0.05).

Results concerning the antimicrobial activity of different solvents extracted fractions against *K. pneumonia* indicated that crude methanolic extract and chloroform extracted fractions showed poor activity at 1 and 2mg disc\(^{-1}\). Good zone of inhibition was shown by n-butanol extracted samples at 3mg disc\(^{-1}\) followed by crude methanolic extract and ethyl acetate extracted fraction at higher concentrations. Data suggested that crude methanolic extract effectively controlled the growth of *P. aeruginosa* compared with other fractions and controls. Ethyl acetate extracted samples also reduced the growth of the tested microbe at concentrations of 3 and 2mg disc\(^{-1}\). Chloroform extracted fraction showed good inhibitory zones against the tested microbe when compared with controls. Poor activity was measured by aqueous extracted samples at 1 and 2mg disc\(^{-1}\). Crude methanolic extract obtained from the fruits of datura showed good antifungal activity of different against *C. albicans*. The data also indicated that all the extracted samples from the fruit of the tested plant showed moderate antifungal activity against *C. albicans* at highest concentrations of 3mg disc\(^{-1}\). Lowest activity was shown by chloroform extracted samples at 1 and 2mg disc\(^{-1}\). Similar results are also reported by Sharma *et al.* (2011) who concluded that datura is a naturally grown plant having significant antibacterial and antifungal properties.

**CONCLUSION**

Among all extracted samples, crude methanolic extract and n-butanol extracted fractions showed maximum inhibitory activity against all the test microorganisms in

![Graph](image)
case of fruits while chloroform extracted samples showed maximum inhibition in case of leaves. Aqueous extracted fraction form the fruit tissues showed highest antifungal activity against *C. albicans* at highest concentration. *K. pneumonia* was highly resistant to crude methanolic, chloroform, ethyl acetate and n-butanol extracts obtained from leaves. In comparison to fruits extracts, leaves showed good inhibition activities against all test microorganisms.

**REFERENCES**


Effect of different solvent extracted samples from the leaves and fruits of datura stramonium on the growth


