

Antimicrobial Study of Selected Medicinal Plants (*Datura stramonium* L. and *Hippophae rhamnoides* L.) of Hunza Valley, Gilgit-Baltistan

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Abstract. The adverse environmental and human health impact of chemical use against micro-organism is profoundly increased. For this motive, substitute methods to minimize chemicals are being developed. One of the effective methods is use plant extracts which having natural antimicrobial properties. The aim of this study was to evaluate ethanolic extract of *Datura stramonium* L. (leaves) and *Hippophae rhamnoides* L. (fruits) against the selected micro-organisms. Results revealed that mean zone of inhibition for bacterial species ranged from 23 ± 0.72 to 27 ± 1.24 mm in case of *Hippophae rhamnoides* and from 21 ± 0.79 to 30 ± 1.06 mm in case of *D. stramonium* leaves respectively. For antifungal activity, the inhibition zone ranges from 39.42 ± 1.07 to 47.22 ± 2.11 mm in case of *D. stramonium* and from 38.42 ± 1.19 to 48.46 ± 2.32 mm in case of *H. rhamnoides*. However, sensitivity reaction of bacterial and fungal species against each plant extract was also differential. *D. stramonium* showed highest toxic effect against *E. coli* (30 ± 1.06 mm)) and *Aspergillus flavus* (47.22 ± 2.11 mm). Whereas, *H. rhamnoides* showed highest zone of inhibition against bacterial species (*B. subtilis* 27 ± 1.24 mm) and fungal species (*R. stolonifer* 48.46 ± 2.32 mm). Both plant extract exhibited antimicrobial properties, which could be used against micro-organisms.

Keywords: antimicrobial, inhibition zone, *H. rhamnoides*, *D. stramonium*, Gilgit-Baltistan Pakistan

Introduction

Medicinal plants are the easiest and cheapest mode of medication in rural communities. Ethno botany is the study of the relationship between people and plants with particular emphasis on indigenous cultures. According to World Health Organization (WHO) roughly 65-80% of the world population in developing nations depends mainly on plants for their primary health care due to scarcity and unavailability of modern medicines (Awoyemi *et al.*, 2012). Medicinal plants are important source of antimicrobial compounds. Many infectious diseases are being treated by using medicinally important plant extracts, as they contain a potential microbial activity. Traditionally thousands of medicinal plants are used by people and these are explored for different bioactive chemical compounds as well as their antimicrobial activities (Rice-Evans *et al.*, 1997), and thousands of plants are still unexplored and need to be explored in future (Aberoumand *et al.*, 2010). The

examination of different medicinally important plants for bioactive substances and therapeutically properties were conducted and these studies revealed that most of the plants have antimicrobial properties (Ahsan *et al.*, 2009). Infectious diseases have claimed lives of millions throughout the world, during the past few decades (Sharma *et al.*, 2013). To treat such diseases, modern medicinal drugs are facing challenges to cure perfectly due to side effects and the adaptation of many pathogens for survival in host. Many researches have been conducted in the search for medicinal plants that have improved antimicrobial effects against human and pathogens (Kumar *et al.*, 2007).

Material and Methods

Collection of plants. This study was carried out in the Department of Biological Sciences Karakoram International University Gilgit during the year 2017-18, to determine antimicrobial properties of plants. Two plant *D. stramonium* (Datura) and its fresh leaves

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and *H. rhamnoides* (Sea buckthorn) and its fruits were collected from different localities of district Hunza Gilgit-Baltistan Pakistan. These plants were primarily identified taxonomically with the help of the flora of Pakistan. (Fig. 1).

Preparation of extract. Sea buckthorn fruits were collected and allowed to dry in the shade. Dried berries of sea buckthorn were crushed into fine powder using grinder and stored until use. Whereas, *D. stramonium* leaves were cleaned thoroughly with running tap water and dried in sunlight. Then the leaves were ground into a fine powder. About 195 g of sea buckthorn berries powder and 60 g of *D. stramonium* leaves powder were soaked in 300 mL-500 mL respectively in the 90% methanolic solvent and 10% distilled water (9:1) for 72 h (3 days). This process was repeated two times and finally 100% methanol used for soaking. Then the soaked samples were filtered in different beakers by using filter papers, then the filtrates were evaporated in Rotary Evapourator to obtain the methanolic extract of both samples. This methanolic extract was used to check the antimicrobial bioassay screening.

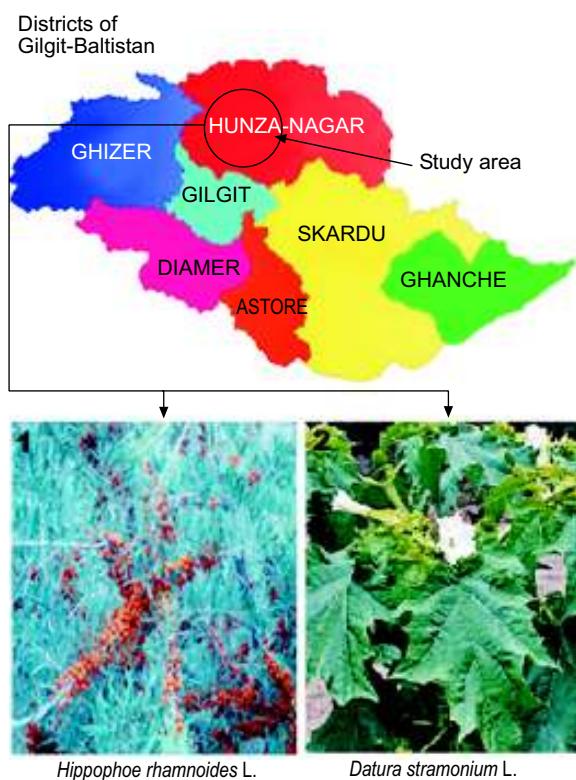


Fig. 1. Map of study area.

Isolation of micro-organisms. Different human faces and soil samples were used to isolate targeted micro-organisms by serial dilution techniques. In serial dilutions 1 g of sample was aseptically transferred into 9 mL of distilled water and homogenized by vortex. Following serial dilutions were made up to 10^4 for isolation of micro-organisms. For isolation of bacterial species nutrient agar was used, whereas for fungal species potato dextrose agar. Identification of bacterial species was identified as described in WHO Manual, 1987 and fungal species (Nagamani et al., 2006; Gilman, 1957). Finally three bacterial species (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*) and three fungal species (*Fusarium* spp., *Aspergillus flavus*, *Penicillium* spp. and *Rhizopus stolonifer*). Antimicrobial bioassays for bacterial were performing by mean of disc diffusion method (Boussaada et al., 2008; Basu et al., 2005; Nostro et al., 2000). For fungal species food poisoned technique was applied (Mishra and Tiwari, 1992). The inhibition zones were calculated in mm (Daniyan and Mahammad, 2008). The efficiency percentage of inhibition zone percentage (mm) was calculated by using this formula:

$$\text{Zone of inhibition \%} = \frac{\text{CGC} - \text{CGT}}{\text{CGC}} \times 100$$

CGC=Colony growth in control; CGT=Colony growth in treatment.

Results and Discussion

Plant wealth is treasure of nature and medicinal plants application for medications are very common among the mountainous communities of the world. The antimicrobial activity of medicinally important plants was determined by measuring to their inhibition zone against different strains of human pathogenic bacteria and fungi. For bacterial species *Datura stramonium* showed mean inhibition zone ranges between 30 ± 1.06 to -30 ± 1.06 mm, while in *Hippophae rhamnoides* inhibition zone varied from 23-27 mm (Fig 2). *D. stramonium* was effective against *E. coli* followed by *S. aureus*, while *H. rhamnoides* showed slightly higher antibacterial properties against *B. subtilis* and *S. aureus* (Table 1).

Benli et al. (2007) described that methanol solution of *Artemisia dracunculus* L. revealed superior results as compare to chloroform and acetone extracts. Its methanol extract was found active after being tested with dual strains of *E. coli* and three other bacterial types, however

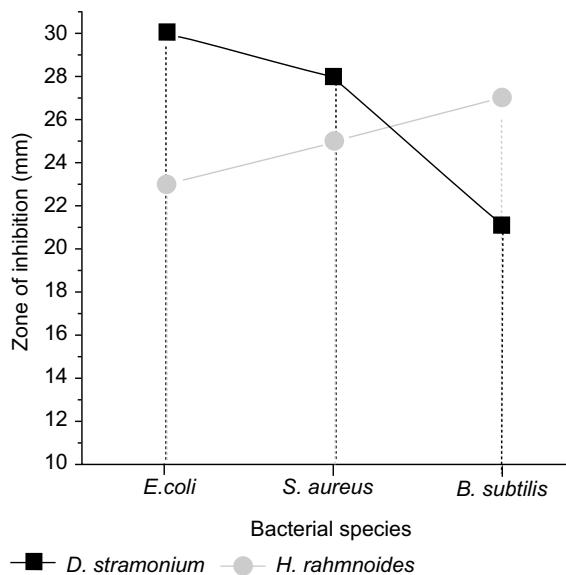


Fig. 2. Shows the inhibition zone of different strains of bacteria.

Table 1. Antibacterial activity of *D. stramonium* and *H. rhamnoides*

Bacteria	Gram morphologies	Inhibition (mm) zone of 5% <i>D. stramonium</i>	Inhibition (mm) zone of 5% <i>H. rhamnoides</i>
<i>E. coli</i>	Gram -ve	30±1.06	23±0.72
<i>S. aureus</i>	Gram +ve	28±1.76	25±1.14
<i>B. subtilis</i>	Gram +ve	21±0.79	27±1.24

it was found ineffective against *S. pyogenes* and *S. aureus*. In the present research study, the methanolic extract of *D. stramonium* leaves showed a significant effect against *E. coli* with inhibition zone of 30 mm, *S. aureus* with zone of inhibition 28 mm and it showed a minimum inhibition zone of 21 mm against *B. subtilis*. Similarly, the methanolic extract of sea buckthorn berries recorded 27 mm with maximum inhibition zone against *B. subtilis*, 25 mm inhibition zone against *S. aureus*, and 23 mm inhibition zone against *E. coli*. In some recent researches conducted to evaluate the efficacy of *Artemisia nilagirica* different extract against 15 bacterial strains and the results were highly promising (Ahameethunisa and Hopper, 2010).

In one more investigation, diverse classes of the genus *Ferula* (Apiaceae) has revealed flexible antibacterial outcomes. The chloroform extract of *Ferula persica* roots was effective against *E. coli*, *K. pneumoniae*, *S. typhi*, *S. aureus* and *S. epidermidis*. The active compound

was identified as umbelliprenin (Shahverdi *et al.*, 2005). *Ferula rigidula* has displayed a wide range of activities (Sener *et al.*, 1998). Root volatile oil of *Ferula hermonis* has also demonstrated the highest antibacterial agent (Hilan *et al.*, 2007). Seddik (2010) stated that ethyl acetate and chloroform extracts of *Artemisia herba-alba* Asso, indicated outstanding activity to counter bacteria. These extracts contain large amount of phenolic compounds. Antifungal activity of extracts against selected fungal species was evaluated by food poison techniques. The data showed that the tested extracts exhibited varied degree of zone of inhibition against the fungi. Mean zone of inhibition percentage of fungal species against extracts have been presented in the Fig. 3 and Table 2.

Range of inhibition zone (mm) was 39.42 ± 1.07 to 44.64 ± 1.90 in *D. stramonium*, 38.42 ± 1.19 to 48.46 ± 2.32 mm in *H. rhamnoides* and *D. stramonium* effectively

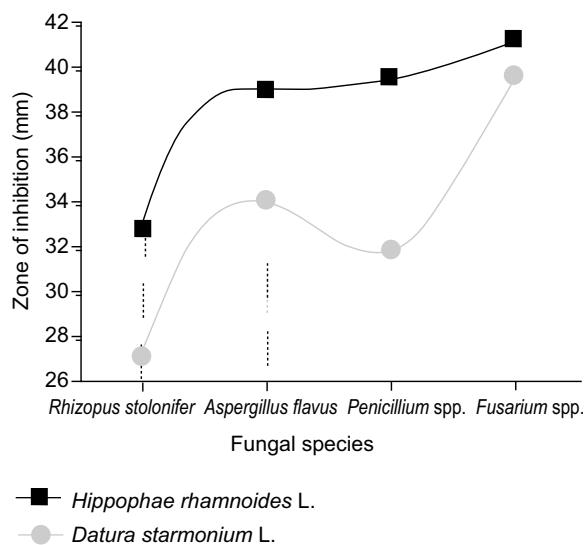


Fig. 3. Shows the efficiency of different strains of fungus.

Table 2. Antifungal activity of *D. stramonium* and *H. rhamnoides*

Fungus	Control	Average inhibition (mm) zone of 5% <i>D. stramonium</i>	Average inhibition (mm) zone of 5% <i>H. rhamnoides</i>
<i>R. stolonifer</i>	66.42 ± 2.01	44.64 ± 1.90	48.46 ± 2.32
<i>P. spp.</i>	68.44 ± 1.09	41.42 ± 1.03	46.66 ± 1.90
<i>A. flavus</i>	71.56 ± 4.31	47.22 ± 2.11	43.66 ± 1.99
<i>Fusarium spp.</i>	65.22 ± 3.17	39.42 ± 1.07	38.42 ± 1.19

inhibited mycelial growth of *A. flavus* (47.22 ± 2.11) followed *R. stolonifer* (44.64 ± 1.90 mm) whereas, least zone of inhibition was recorded in *Fusarium* spp. (39.42 ± 1.07 mm). The range of zone of inhibition of *H. rhamnoides* was recorded 23 ± 0.72 to 27 ± 1.24 . Highest zone of inhibition was found in fungal species *R. stolonifer* (48.46 ± 2.32 mm) followed by *Penecillium* spp. (46.66 ± 1.90 mm), while least in *Fusarium* spp. (38.42 ± 1.19 mm). The methanol leaf extracts of numerous plants exhibited improved antifungal activity later treated with *Aspergillus flavus*, *Dreschlera turcica* and *Fusarium verticillioides* have been described (Mahesh and Satish, 2008). Reports of antimicrobial activity or earlier investigations have verified antifungal properties of diverse varieties of extracts (Zhu et al., 2005; Guo et al., 1997; Wilson et al., 1997).

Mann et al. (2008) studied *Anogeissus leiocarpus* and *Terminalia avicennioides*, ethanolic extracts were more efficient than methanolic and all other extracts against fungi. In a current research the methanol extract of *D. stramonium* leaf demonstrated that it has highest antifungal activity against *Penicillium* spp. with 39.47% zone of inhibition, 39.55% inhibition zone against *fusarium*, 34.79% inhibition against *A. flavus* and with minimum zone of inhibition against *R. stolonifera*. Similarly, the methanolic extract of sea buckthorn berries recorded 41.11% with maximum inhibition zone against *fusarium*, 38.99% inhibition zone against *A. flavus*, 31.82% zone of inhibition against *P. spp.*, and 27.04% (minimum) zone of inhibition against *R. stolonifer*. Similar results were found by Manoorkar and Gachande (2014) and Saeed and Tariq (2005). Another supported our results that the maximum antifungal activity of *Mentha* flowers in contrast with that of the further particular plant portions is credited to the most effective acetone, methanol and ethanol extracted metabolites when treated with *F. moniliforme* (Yazgi et al., 2015).

Conclusion

In this review, extracts of two plants were tested for antimicrobial activities against three strains of bacteria i.e. *E. coli*, *S. aureus*, *B. subtilis* and four strains of fungus i.e. *Fusarium*, *A. flavus*, *P. spp.*, *R. stolonifera*. The results of the above investigation clearly indicated that the antimicrobial activities of *D. stramonium* showed a significant inhibition of all tested bacterial and fungal pathogens as compare to sea buckthorn. It was concluded that *D. stramonium* leaves and *H. rhamnoides* berries exhibited antibacterial and antifungal properties against

different strains of bacteria and fungi. Further studies are required to isolate the targeted secondary metabolites exist in these potential plants.

Conflict of Interest. The authors declare no conflict of interest.

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