

Antibacterial activity of the crude extracts from medicinally important *Thuja occidentalis*

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Abstract: The present research was carried out at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, KPK Pakistan. In this study crude methanolic extracts from *Thuja occidentalis* were tested for their antimicrobial activity against five different bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Xanthomonas sp.* and *Staphylococcus aureus*) at two different concentrations (10 and 20 mg/ml) using disc diffusion assay. The results showed that *Klebsiella pneumoniae* was most sensitive to crude extracted sample from leaves at both low and high concentrations measuring 37 and 57% zone of inhibition respectively and *Xanthomonas* and *Staphylococcus aureus* was found to be more resistant to the crude extracted samples from leaves at both concentrations. Similarly, *Klebsiella pneumoniae* was most sensitive to crude extracted samples from seeds at high concentration followed by *E. coli* at both concentrations. *Staphylococcus aureus* on the other hand was found to be more resistant to the crude extracted samples at low and high concentrations. These results suggested that extracts prepared from the leaves and seeds of *Thuja occidentalis* can be used as natural remedy for the treatment of various bacterial infections.

Keywords: Antimicrobial activity, *Thuja occidentalis*, disc diffusion assay, crude extracts.

INTRODUCTION

Indiscriminate use of antibiotics commonly used to cure various infectious diseases has increased the resistance of different microorganism. As a result, researchers are looking for new antimicrobial substances from various sources including medicinal plants (Bauer *et al.*, 1996; Adekunle and Adekunle, 2009). Among 2600 plant species, 700 are used as medicinal herbs (Ali-Shtayeh *et al.*, 2000). It is estimated that about 80% of the world population in the developing countries is still dependent on herbal medicines for their health issues. It is reported that nearly 28% of drugs available in the market are plant-based products (Newman *et al.*, 2003). Herbal products and their bio-molecules have historically been used as an important source of new pharmaceutical agents (Koehn and Carter, 2005). Human beings all over the world have used plants for treating their health issues since long and this trend is continuing to be the main resource for different pharmaceutical products. Plant-based products are effective, simple, and reveal broad spectrum activity (Chin *et al.*, 2006). Herbal plants are the richest resource of traditional and modern medicines systems, food supplements, nutraceuticals and bio-molecules for synthetic medicines (Hammer *et al.*, 1999). Plants contain various bioactive compounds which have shown good antimicrobial activity against different microbes (Bilal *et al.*, 2018; Bakht *et al.*, 2017; 2019). The benefit of herbal medicines usually results from the presence of different secondary metabolites and requires the isolation and identification of secondary metabolites and their use as active bio-molecules in modern medicines (Kubmarawa *et*

al., 2007; Zahin *et al.*, 2010).

Thuja occidentalis, a well-known medicinal plant belongs to the family Cupressaceae. *Thuja occidentalis* is usually found in Eastern North America and is also grown in Europe as an ornamental tree. The plant was first identified as an effective remedy for the treatment of scurvy by native Indians in Canada. *Thuja occidentalis* has been used to cure bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Change *et al.*, 2000). When combined with *Echinacea purpurea*, *Echinacea pallida* and *Baptisia tinctoria*, it is also used to treat acute and chronic infections of the upper respiratory tract and in acute bronchitis, angina and pharyngitis. The present study was conducted to investigate the antimicrobial activity of crude extract obtained from the leaves and seeds of *Thuja occidentalis* against different bacterial strains.

MATERIALS AND METHODS

Experimental material

The present research work was carried out at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, KPK Pakistan. Leaves and seed of the plant (*Thuja occidentalis*) were collected from the Department of Botany, University of Peshawar. Plant materials were washed with water to remove dust and dirt and kept at room temperature for two weeks to be shaded-dried.

Crude extract preparation

The dried plant materials (leaves and seeds) were chopped into small pieces and grinded into fine powder using

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tissue homogenizer (Infinigen™ Tissue Mixer Mill). About 350g of powder was placed into a large container (extraction drum). Methanol was added to the extraction drum in such a way that the whole plant material was completely dipped into methanol. The extraction drum was covered with aluminum foil and placed at room temperature for a week. During this period of time the plant material was regularly shaken thrice a day. After a week, the solution was filtered through Whatman No.1 (Whatman™) and fresh methanol was added to the remaining plant material and the same process was repeated thrice. The filtrate was dried by evaporating methanol using rotary evaporator (Rotavapor^R-R 210/R215; BUCHIL Labortechnik AG) fixed at 45°C. The extracted plant material was isolated and dried in china dish at 45°C.

Culture media and its preparation

Nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used for culturing and growth and nutrient broth was used for shaking incubation and standardization of different microorganisms. Media was prepared as described in Bakht *et al.* (2014).

Microbial strains

The antimicrobial potential of the crude methanolic extracts *Thuja occidentalis* was determined against different bacterial and fungal species (table 1).

The different microbial strains, from the stock culture, were freshened on the nutrient agar medium using streak method. The microorganisms were then inoculated in agar broth medium and placed in shaking water bath (GLSC-SBR-04-28) at 37°C and 200 rpm for 24 hr.

Disc diffusion susceptibility method

The antibacterial activity of crude extracts from the leaves and seeds of *Thuja occidentalis* were tested by disc diffusion assay according to the methods of Bauer *et al.* (1996). Two concentrations of the extracts (10 and 20 mg/disc) in volume of 6 and 12µl were applied to the disc. Antibiotic drugs used as positive control for Gram positive and Gram negative bacteria were Erythromycin and Ciprofloxacin respectively.

For Gram positive bacteria: Erythromycin 50µg 6 µl⁻¹
For Gram negative bacteria: Ciprofloxacin 50µg 6 µl⁻¹

STATISTICAL ANALYSIS

MSTAT (Version 5.4) computer software was used for the data analysis. Least Significant Difference (LSD) test was used to compare treatment means upon obtaining significant differences at p<0.05 (Steel *et al.*, 1997).

RESULTS

Data regarding antimicrobial activity of crude extracted samples from the leaves of *Thuja occidentalis* against different bacterial strains is shown in fig. 1. The results revealed that *K. pneumoniae* was more sensitive to crude extracts at low and high concentrations. The data indicated that crude extracts from the leaves of *Thuja occidentalis* reduced the growth of *K. pneumoniae* by 37% and 51% at 10mg and 20mg/disc respectively. The results also showed that *S. aureus* and *Xanthomonas Sp* were more resistant to crude extracted samples from leaves measuring 0% ZI at both concentrations. *E. coli* and *B. subtilis* showed resistance at low concentrations (10mg/disc) recording 0% ZI, however, its growth was reduced by 7.3% and 5.3% at high concentration when compared with controls (fig. 1).

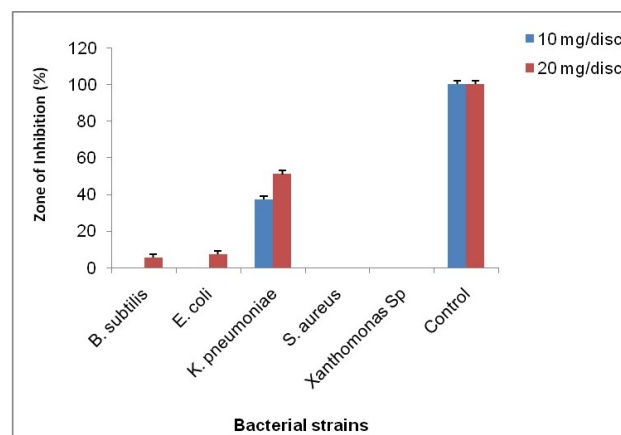


Fig. 1: Antibacterial activity of crude (methanol) extracted samples from the leaves of *Thuja occidentalis* against *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. aureus* and *Xanthomonas Sp* by disc diffusion assay (Bar represent ±LSD value at p< 0.05).

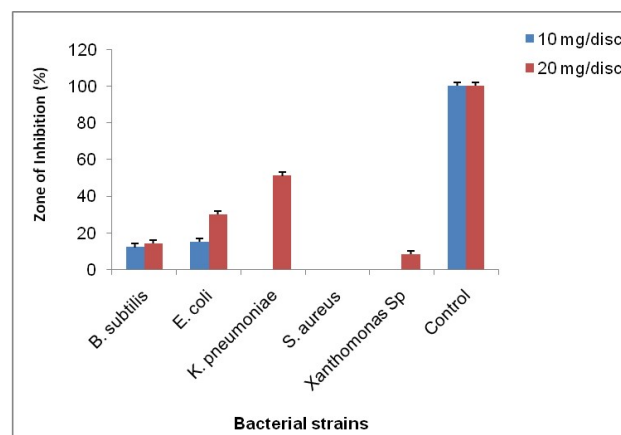


Fig. 2: Antibacterial activity of crude (methanol) extracted samples from the seeds of *Thuja occidentalis* against *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. aureus* and *Xanthomonas Sp* by disc diffusion assay (Bar represent ±LSD value at p<0.05).

Table 1: Microbial strains tested for susceptibility to *Thuja occidentalis* extracts

Microbial species	Gram strain type	Details of the microbial strains used
<i>Bacillus subtilis</i>	Positive	Clinical isolate obtained from the Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
<i>Escherichia coli</i>	Negative	ATCC # 25922
<i>Klebsiella pneumoniae</i>	Negative	Clinical isolate obtained from the Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
<i>Staphylococcus aureus</i>	Positive	ATCC # 6538
<i>Xanthomonas Sp</i>	Negative	Department of Plant Pathology, The University of Agriculture Peshawar, KPK Pakistan

Fig. 2 presents data concerning antimicrobial activity of crude extracted samples from the seeds of *Thuja occidentalis*. Analysis of the data indicated that *K. pneumoniae* was more resistant to crude extracted samples from seeds at low concentration measuring 0% ZI. However, crude extracted samples from seeds reduced the growth of *K. pneumoniae* by 51% when compared with controls. The data also showed that *S. aureus* revealed complete resistance at both concentrations recording 0% ZI compared with controls. Moderate antimicrobial activity was revealed by *E. coli* and *B. subtilis* at both concentrations. The data indicated that crude extracted samples from seeds reduced the growth of *E. coli* by 15% and 30% at low and high concentrations respectively compared with controls. Similarly, the growth of *B. subtilis* was inhibited by crude extracted samples from seeds by 12% and 14% at 10 and 20mg/disc concentrations respectively. The results also revealed that *Xanthomonas* showed complete resistance at low concentrations measuring 0% ZI, however, at high concentration, the growth of *Xanthomonas* was reduced by 8.31% compared with controls (fig. 2).

DISCUSSION

The results showed that crude extracts from *Thuja occidentalis* was effective to control the growth of *K. pneumoniae* at high and low concentrations. Similarly, *S. aureus* and *Xanthomonas Sp* were also resistant to crude extracted samples from the leaves of *Thuja occidentalis* at both concentrations. *E. coli* and *B. subtilis* revealed resistance at low concentrations of 10 mg/disc, however, was sensitive at high concentration when compared with controls. *K. pneumoniae* showed resistance to crude samples from seeds at low concentration, however, reduced the growth of the same microbe at higher concentration. *S. aureus* showed complete resistance at both concentrations compared with controls. Moderate activity was measured in the growth of *E. coli* and *B. subtilis* at both concentrations. Similarly, the activity of *B. subtilis* was reduced by crude samples from seeds at both 10 and 20m/disc concentrations. The results also revealed that *Xanthomonas* showed complete resistance at low concentrations, however, reduced the growth at high

concentration. *Thuja* contains sufficient quantity of α , β and thujaplicin that in low quantity can inhibit *S. typhimurium* and serotype a, c and d of *S. mutans* (Aker et al., 1980; Chen et al., 1989) Tsiri et al. (2009) reported that *Thuja* species contain monoterpene ketones α - and β -thujone, fenchone and sabinene, as well as the diterpenes beyerene and rimuene. Beyerene and the mixture of α - and β -thujone isolated from the oils revealed significant antimicrobial activity. Similar results are also reported by Jahan et al. (2010), Hudson et al. (2011) and Ikram and Abdeen (2017).

CONCLUSION

It can be concluded from these results that crude methanolic extracts of *Thuja occidentalis* was effective to control the growth of both Gram positive and Gram negative bacteria in dose-dependent manner.

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