



Research Article

Antibacterial Effects of *Thuja* Leaves Extract

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Abstract

Various medicinal plants are common in use in Nepal for the treatment of different diseases. Nowadays, drug resistance has emerged as a major problem for various infections, in such case plants can be used as alternative for the production of new antimicrobial agents. *Thuja* (in Nepali: **dhupi**) is a small evergreen genus of the Cupressaceae family. This species is widely cultivated as a common ornamental plant in Nepal and India. This study examined the antibacterial activity of *Thuja* leaves extract on gram positive (*Staphylococcus aureus* and *Streptococcus* spp.) and gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*). *Thuja* leaves were collected from different localities of Dharan, and dried under shade for 10 days. They were then grinded using mechanical grinder. Leaf extract (oleoresin) was obtained by soxhlet extraction technique using mixture of Ethyl acetate, Ethanol and chloroform in the ratio 40:30:30 as the solvent. The antibacterial activity of *Thuja* oleoresin was tested using both Agar well diffusion as well as disc diffusion technique. Minimum Inhibitory Concentration (MIC) was determined by agar well diffusion on MHA plates. *Thuja* oleoresin showed distinct antibacterial activity towards all four isolates on both agar well and disc diffusion methods. MIC for *Pseudomonas aeruginosa* and *Streptococcus* spp. was found to be 12.5 μ l whereas for *E. coli* and *Staphylococcus aureus*, MIC was 25 μ l. Thus from our results, it is concluded that *Thuja* leaves have antibacterial effects and can be a potential source for production of antibacterial drugs.

Keywords: *Thuja*; Antibacterial effect; Extraction; Minimum inhibitory concentration; oleoresin

Introduction

Various plants have been used as traditional medicine from the ancient time of human civilization. Many ancient documents revealed that plants were used for medicinal purposes in china, India, Egypt and Greece long before the beginning of Christian era. Plants were used as antimicrobials before microbiological study had even been started. (Gyawali, 2013b). Plant derived bioactive substances are considered a very good and cheap source of medicines that play a significant role for human health improvement as well as used against different types of microbial disease (Kumar et al., 2006; Mathabe et al., 2006).

Plants have great medicinal relevance because infections caused by drug resistant microorganisms have become a major therapeutic problem nowadays (Venkatesan and Karrunakaran, 2010). In addition, plant extract and their phytochemicals are getting more importance as they have the great potential sources for microbial and viral inhibitors. Therefore, thousands of researchers have focused their

interest to investigate phytochemical constituents of plant for human health (Jasuja et al., 2012a). The bioactive constituents of plants such as tannins, flavonoids (Mandalari et al., 2007), saponins (Avato et al., 2006), terpenoids (Funatogawa et al., 2004) and alkaloids (Navarro and Delgado, 1999) have great antimicrobial activity.

Thuja is a small evergreen genus of the Cupressaceae family comprising five extant species. (Tsiri et al., 2009). It is a genus of coniferous plant called 'Dhupi' in Nepali and 'Morpankhi' in Hindi. It grows naturally in china, Korea, Japan and Iran. (Shah and Qadir, 2013). Also this species is widely cultivated as a common ornamental plant in Nepal and India.

Essential oils derived from many aromatic plants are well known to possess cytotoxic, antioxidant, antifungal, insecticidal and antimicrobial activities. (Shah and Qadir, 2013) In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism. (Tsiri et al., 2009). Bacteria nowadays due to improper use of drugs are

getting antibiotics resistant day by day. Emerging of new disease as a new threat has become common. To solve these types of problems new drugs are to be examined and prepared. Plants are rich source of antibacterial components. Different plant extracts are being used in daily life to combat bacterial and fungal infections. (Duhan et al., 2013).

The search of natural products has revolutionized the drug discovery programs in which plant origin have provided numerous crucial molecules.

In this study, oleoresin extracted from *Thuja* leaves by a mixture of ethyl acetate: ethanol: acetone (40:30:30) was explored for their antibacterial activity against various bacteria. The target of the present study is to unravel the effect of *Thuja* species on some common gram positive (*Staphylococcus aureus*, *Streptococcus* spp.) and gram negative bacteria (*E. coli*, *Pseudomonas aeruginosa*).

Materials and Methods

Collection of Samples

The *Thuja* leaves were collected from different places of Dharan, a town in eastern Nepal.

Drying of Thuja Leaves

The plant leaves were dried under shade at room temperature for about 10 days. The dried plant samples were then grinded into powder by and sieved to make 0.5 – 1.5 cm particle size. The powder was stored in polythene bags at room temperature before extraction (Jasuja et al., 2013).

Preparation of Extracts

10 gm of sample was weighed and placed in a thimble and enclosed in it. Solvent (mixture of ethyl acetate: chloroform: ethyl alcohol in the ratio 40:30:30) was prepared. The thimble was placed on the soxhlet apparatus for solvent extraction. The solvent was then placed on the soxhlet apparatus. The solvent was left to siphoned single time. Then again the solvent was added, so as to just cover the thimble. The temperature was adjusted at around 50-55 °C, just around the boiling point of the mixture and the process of siphoning was started. Siphoning was done until the extraction becomes transparent. The extracts were poured on a beaker and concentrated to dryness using rotary evaporator. This process was repeated when more plant extract was required. The extracts were ready for testing antibacterial activity (Jasuja et al., 2013).

Preparation of Standard Inoculums of Test Organisms

The antibacterial activity of *Thuja* leaf extract was tested against four bacterial species: *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli* and *Pseudomonas aeruginosa*. For this, each isolated colonies from pure culture were taken and inoculated on 2 ml nutrient broth. The tubes were then incubated at 37°C for 24 hours and the culture was maintained.

Screening of Antibacterial Activity (Evaluation Methods)

The antimicrobial activity was screened by both agar well diffusion (Basri and Nor, 2014) and agar disc diffusion method (Valgas et al., 2007) on Mueller-Hinton agar (MHA) against some common gram positive (*Staphylococcus aureus*, *Streptococcus* spp.) and gram negative (*E. coli* and *Pseudomonas aeruginosa*) test bacteria.

Minimum Inhibitory Concentration (MIC)

MIC was determined using well diffusion method. The prepared MHA plates were inoculated with respective test organisms, (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus* spp.). Four wells of 5 mm diameter were made at least 1.5cm from edge of the plate. Each well was labeled for the amount of oleoresin to be kept on. *Thuja* leaf extracts of 50, 25, 12.5 and 6.25 µl of oleoresins were respectively poured on the wells and were allowed to dry for few minutes. The plates were then incubated at 37°C for 24 hours for the determination of MIC.

Results

Effect of Leaves Extract (Oleoresin) on the Bacterial Isolates

The study was carried out for the investigation of antimicrobial activity of *Thuja* leaves extract (oleoresin) against some common gram positive (*Staphylococcus aureus*, *Streptococcus* spp.) and gram negative bacteria (*E. coli* and *Pseudomonas aeruginosa*). Well diffusion method as well as disc diffusion methods was performed to observe the effect of oleoresin on the isolates. Well diffusion method, with four wells on each plate was used to determine the MIC values.

Effect of Oleoresin on the Isolates on Well and Agar Diffusion

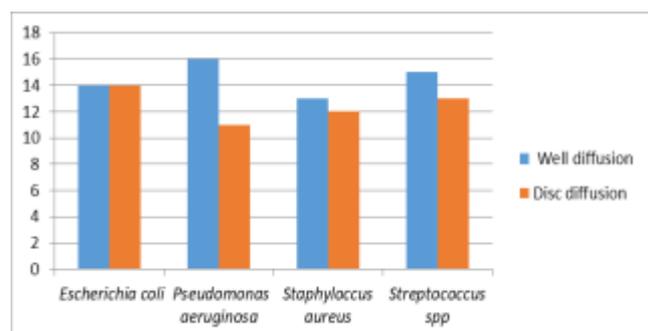
It was observed that *Thuja* oleoresin has inhibitory effect on both gram-positive as well as on gram-negative bacteria. All the four isolates were inhibited by the oleoresin whereas, none was resistant to it. 50µl Oleoresin was poured in the well with 5mm diameter and after overnight incubation zone of inhibition was observed. In disc diffusion 25µl oil was applied on the sterile 5mm disc and was allowed to evaporate. The zone of inhibition was observed as given in Table 1. Bar diagram for zone of inhibition (mm) on well and agar medium is shown in Fig. 1. Effect of oleoresin on *Pseudomonas* spp. & *Pseudomonas* spp. on well diffusion are shown in Fig. 2 and Fig. 3 respectively.

MIC Assay

MIC was determined for all four isolates among which *Pseudomonas aeruginosa* and *Streptococcus* spp. have MIC of 12.5 µl whereas *E. coli* and *Staphylococcus aureus* have a MIC of 25 µl

Table 1: Zone of inhibition against test bacteria for MIC determination

| Organism | Zone of inhibition in | | | |
|-------------------------------|-----------------------|------------|--------------|--------------|
| | 50 μ l | 25 μ l | 12.5 μ l | 6.25 μ l |
| <i>E. coli</i> | 1.4 | 0.7 | - | - |
| <i>Pseudomonas aeruginosa</i> | 1.6 | 1.3 | 1.0 | - |
| <i>Staphylococcus aureus</i> | 1.3 | 0.8 | - | - |
| <i>Streptococcus</i> spp | 1.5 | 1.1 | 0.8 | - |

**Fig. 1:** Bar diagram for zone of inhibition (mm) on well and agar medium.**Fig. 2:** Effect of oleoresin on *Pseudomonas* sp. on well diffusion**Fig. 3:** Effect of oleoresin on *Staphylococcus aureus* in well diffusion

Discussion

Emergence of new diseases resistant to several antibiotics has been one of the major problems. Despite the access to large chemical drugs for the treatment of different diseases, use of herbs as the natural drugs used to remain the alternative to treat deformities made in the normal physiological system by foreign organisms or by any malfunctioning of the body. This study was performed to evaluate the antibacterial activity of *Thuja* leaf extracts on some common gram positive and gram negative bacteria. A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the economic feasibility and suitability of the process to the particular situation. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. (Duhan *et al.*, 2013).

Thuja leaves in our study were collected from different localities of Dharan. They were subsequently dried under shade. According to Gyawali, (2013a) immediate drying prevents microbial fermentation and degradation of metabolites. And protection from direct sunlight is essential to minimize chemical reactions induced by ultraviolet rays. Then the sample was sieved using mechanical grinder. Grinding of leaves is needed to break the cell wall and reduce its size which facilitates subsequent extraction process by increasing the surface area and by facilitating the penetration of solvents into cells. As the plant material constitute different bioactive compounds of different polarities a mixture of solvent i.e. ethyl acetate: ethanol: chloroform in the ratio 4:3:3 was used (Jasuja *et al.*, 2013), among which chloroform has low polarity, ethyl acetate has medium and ethanol has high polarity. Soxhlet extraction was performed for the extraction of essential oil as fresh solvent can continually extract the herbal material efficiently with minimum solvent.

Thuja leaves oleoresin was found to be effective against all four isolates. On both disc as well as well diffusion technique, oleoresin showed a distinctive zone of inhibition on tested bacteria, having zone of inhibition between 11 and 16 mm diameter. MIC of *E. coli* and *Staphylococcus aureus* was found to be 25 μ l and that of *Pseudomonas aeruginosa* and *Streptococcus* spp. was found to be 12.5 μ l.

The present work reveals that the *Thuja* plant is found to have potential in therapeutic uses in treating various diseases. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimately be beneficial for humans as well as animals. The extracted essential oils have been assayed for their antimicrobial activity. Antibacterial activity of *Thuja* leaf

extract was observed against both gram negative and gram positive bacteria. *Thuja* leaves showed a good antibacterial activity against these selected bacterial species i.e. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus* spp. The *Thuja* plants has more potent antibacterial activity because of presence of some secondary metabolites. Thus, this study reveals its use as a medicinal plant.

According to Kumar et al. (2012) a critical factor for *Thuja occidentalis* use as a medicinal herb is its content of essential oil. The fresh plant (related to the dry substance) contains 0.6% essential oil, 2.07% reducing sugar, 4.9% water-soluble polysaccharides, 2.11% water-soluble minerals, 1.67% free acid and 1.31% tannic agents. The essential oil of the fresh leaves (related to the monoterpene fraction) contains 65% thujone, 8% isothujone, 8% fenchone, 5% sabinens and 2% α -pinene as the main monoterpenes. According to Alves et al. (2014), the content of the essential oils, especially thujone, is a critical factor for the use of *T. occidentalis* as a medicinal plant. A similar work was done by the oil of *Thuja* leaves showed appreciable antibacterial effect against all Gram-positive and Gram negative bacteria tested with MIC values between 12.8-25.6 mg/ml (Shah and Qadir, 2013) which also supports the validity of this work.

Conclusion

Our results conclude that the oleoresin extracted from *Thuja* leaves is inhibitory to *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus* spp. Therefore, the leaves of *Thuja* are ethno-botanically used and have great potential in the development of medicines for bacterial diseases. This study also revealed that the *Thuja* leaves extract might be useful as an antibacterial agent following extensive investigation. The results obtained from our investigation confirm the use of *Thuja* as medicinal plant. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation.

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References

- Alves LDS, Figueirêdo CBM, Silva CCAR, Marques GS, Ferreira PA, Soares MFR, Silva RMF and Rolim-neto PJ (2014) *Thuja occidentalis* L. (cupressaceae): Review of botanical, phytochemical, pharmacological and toxicological aspects 5: 1163-1176.
- Basri DF and Nor NHM (2014) Phytoconstituent Screening and Antibacterial Activity of the Leaf Extracts from *Canarium odontophyllum* Miq. *American Journal of Plant Sciences* 5: 2878-2886. DOI: [10.4236/ajps.2014.519303](https://doi.org/10.4236/ajps.2014.519303).
- Duhan JS, Saharan P & Surekha (2013) Phytochemical analysis and Antimicrobial potential of leaf extracts of *Thuja orientalis* 6: 291-294.
- Greenwood D, Slack R, Peutherer J & Barer M (2007) Bacterial pathogens and associated disease. *Medical Microbiology*. 17 ed.: Churchill livingstone Elsevier limited.
- Gyawali R (2013a) General steps for the development of new drugs from herbs. *Handbook of pharmacognosy*. 1st ed. Bagbazar, Kathmandu, Nepal: Nabodit Hamro pustakk bhandar.
- Gyawali R (2013b) History of pharmacognosy. *Handbook of pharmaognosy*. 1st ed. Bagbazar, Kathmandu, Nepal: Nabodit hamro pustak bhandar.
- Hugo WB & Russel AD (2013)a Antibiotic development, past and present. In: Denyer, S. P., Hodges, N., Gorman, S. P. & Gilmore, B. F. (eds.) *Hugo and Russell's pharmaceutical microbiology*. 8th ed. Delhi: Lalit printer and Binder. 169-170
- Hugo WB & Russel AD (2013)b In: Denyer, S. P., Hodges, N., Gorman, S. P. & Gilmore, B. F. (eds.) *Hugo and Russell's pharmaceutical microbiology*. 8th ed. Delhi: Lalit printer and Binder.
- Jasuja ND, Choudhary J, Sharma P, Sharma N, Joshi SC (2012b) A review on bioactive compounds and medicinal uses of *Commiphora mukul*. *J. Plant Sci.* 4:113-137. DOI: [10.3923/jps.2012.113.137](https://doi.org/10.3923/jps.2012.113.137)
- Jasuja ND, Saxena R, Chandra S, Sharma R (2012)a Pharmacological characterization and beneficial uses of *Punicagranatum*. *Asain J. Plant Sci.* 11:251-267. DOI: [10.3923/ajps.2012.251.267](https://doi.org/10.3923/ajps.2012.251.267)
- Jasuja, ND, Sharma, SK, Saxena R, Choudhary J, Sharma, R & Joshi, S. C. (2013) Antibacterial, antioxidant and phytochemical investigation of *Thuja orientalis* leaves. *Journal of Medicinal Plants Research* 7(25):1886-1893.
- Kumar B, Rani R, Das S & Das S (2012) Phytoconstituents and Therapeutic potential of *Thuja occidentalis*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 3(2): 354-362. DOI: [10.1016/j.jep.2006.03.013](https://doi.org/10.1016/j.jep.2006.03.013)
- Kumar PV, Chauhan SN, Padh H and Rajani M (2006) Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J. Ethnopharmacol.* 107:182-188
- Mathabe MC, Nikolova RV, Laly N, Nyazema NZ (2006) Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *J. Ethnopharmacol.* 107: 286-293. DOI: [10.1016/j.jep.2006.01.029](https://doi.org/10.1016/j.jep.2006.01.029)
- Shah WA & Qadir M (2013) Chemical composition, Antioxidant and Antibacterial activity of *Thuja orientalis* essential oil. 2, 56-60.
- Sodha S, Lynch M, Wannemuehler K, Leeper M, Malavet M & Schaffzin J. (2006) *Multistate outbreak of Escherichia coli O157:H7 infections associated with a national fast-food chain* [Online]. Available: <http://dx.doi.org/10.1017/S0950268810000920>.

Tsiri D, Graikou K, Poblócka-olech L, Krauzebaranowska M, Spyropoulos C & Chinou I (2009) Chemosystematic Value of the Essential Oil Composition of *Thuja* species Cultivated in Poland—*Antimicrobial Activity*. 14, 4707-4714. DOI: [10.3390/molecules14114707](https://doi.org/10.3390/molecules14114707)

Valgas C, Souza, SMD, Smânia EFA & Artur Smânia Jr. (2007) Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* **38**(2): 369-380. DOI: [10.1590/S1517-83822007000200034](https://doi.org/10.1590/S1517-83822007000200034) VonBaum H and Marre R (2005) Antimicrobial resistance of *Escherichia coli* and therapeutic implications *Int J Med Microbiol* **295**: 503-511. DOI: [10.1016/j.ijmm.2005.07.002](https://doi.org/10.1016/j.ijmm.2005.07.002)