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Evaluation of Antimicrobial Activity of *Thuja occidentalis* Leaves Extract

[1]Bhatade A. M. and [2]Sandikar B. M.

Department of Microbiology

Maharashtra Udaygiri Mahavidyalya Udgir, Udgir Dist. Latur Maharashtra State, India.

Abstract:

Thuja occidentalis is one of the common ornamental plants cultivated in all parts of India from the cypress family Cupressaceae. Common name for it is White cedar. It has been used to treat various infections in traditional medicine practice due to its medicinal relevance. Present study is focused on the evaluation of antimicrobial activity of leaves of *Thuja occidentalis* against Gram positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*. Ethanol and Petroleum Ether extracts of *Thuja occidentalis* was prepared and tested for its antimicrobial activity by agar well diffusion assay. Significant correlation has been observed between the five different concentrations of these extracts of leaves and diameter of zone of growth inhibition. The promising results suggest that the *Thuja occidentalis* has the effective antimicrobial activity against both the Gram positive and Gram negative bacteria. It can be served as the effective medicinal alternative to treat the human infections.

Keywords: *Thuja occidentalis*, antimicrobial activity, Minimum Inhibitory Concentration

Introduction:

Various plants are known to possess medicinal properties in the ancient medicinal therapies across the world. Plants have been used as the potent antimicrobials even before the knowledge of microbiology as a science. Plants based medicinal system nearly used by 80% of world's inhabitants as their primary health care (Owolabi et al., 2007). As per World Health Organization suggestion plant can be a potent source of variety of drugs, hence plants should be studied on a larger scale to know more about such information (Nascimento et al., 2000). Generally plants are used to treat venereal diseases, skin diseases, and respiratory diseases as part of traditional medicine. Plants produce effective drugs which are effective on human physiology and ultimately on disease (Kumar et al., 2006).

Most active and potent bioactive chemical compounds which are produced by plants include flavanoids, alkaloids, phenolic compounds and tannins (Edeoga et al., 2005). Many pathogenic microorganisms have developed resistance towards variety of antibiotics; this is a critical and alarming sign in the field of medicine

(Duhan et al., 2013). Antibiotics besides their high cost they have adverse impact on hosts like development of ulcers, hypersensitivity and immune compression. Due to such problems attention towards plant based treatment is a need of hour and sustainable approach for human beings. Hence several investigators have developed their interest into this regard to study about phytochemical constituents of plants (Jasuja *et al*., 2012a).

Thuja occidentalis commonly known as white cedar it is native to Eastern North America and Europe. There it has been cultivated as ornamental plant (Chang LC *et al*., 2000). In Hindi this plant is known as 'Morpankhi' and in Nepali it is known as 'Dhupi' (Shah and Qadir, 2013).In India, Nepal, China, Korea and Japan this plant has been cultivated as ornamental plant. In traditional medicine *T. occidentalis* has been used to treat psoriasis, bronchial catarrh, cystitis, uterine carcinomas, enuresis, rheumatism and amenorrhea ((Tsiri*et al*.,2009). From previous research it has been concluded that plant leaves extract possesses antibacterial, antiviral, anti-inflammatory, anticancer and gastro protective activities (Das S and Rani R, 2013).

Abscesses, carbuncles and furuncles like skin infections are caused by variety of organisms among which *Staphylococcus aureus* is most common. Bacillus subtilis cause infections such as endocarditis, septicemia, bacteremia and pneumonia. *Escherichia coli* and *Pseudomonas aeruginosa* causes frequent infections in plants, humans and animals. In current study antimicrobial activity of *Thuja occidentalis* has been studied against potent and well kwon pathogens both from Gram positive and Gram negative groups.

In present study the plants leaves has been collected from local area and leave extracts has been prepared by using Ethanol and Petroleum Ether as solvents in Soxhlet extraction apparatus. Cultures of pathogens were obtained by isolating single colony from pure cultures and suspended in nutrient broth for further study. From Gram positive group we have selected *Staphylococcus aureus* and *Bacillus subtilis* and from Gram negative group we have selected *Escherichia coli* and *Pseudomonas aeruginosa* as test organims.

Materials and Methods:

Collection of Samples

Plant leaves were collected from campus of Maharashtra Udaygiri Mahavidyalaya, Udgir by pricking the leaves from plants and were identified by senior botanist from the department of Botany, Maharashtra Udaygiri Mahavidyalaya, Udgir.

Drying of leaves

The collected leaves were rinsed twice by using distilled water for dust removal and dried in shade at room temperature for about 15 days.

The dried leaves were finely grinded by using mechanical grinder and converted into powder form and sieved to obtain uniform particle size powder. The powder was stored into sterile polythene bags at room temperature until extraction process (Jasuja et al., 2013).

Preparation of leaves extract

Thimble of Soxhlet extraction apparatus was enclosed with 10 gm. of dry powder of Thuja leaves and placed that thimble in Soxhlet extraction apparatus for extraction process. Petroleum Ether and Ethanol solvents were used for the extraction process separately. The siphoned extracts were collected separately when temperature was adjusted at 55°C.

Preparation of Inoculums of Test Microorganisms

Staphylococcus aureus, Bacillus subtilis , Escherichia coli and *Pseudomonas aeruginosa* were used as the test organisms in this present study. The single colony was picked form the pure cultures of test organisms which were stored as the stock cultures in department of Microbiology and inoculated in the peptone broths separately. The inoculated peptone broths were incubated at respective temperatures of organisms for 24 hrs. After completion of incubation the cultures were used as the standard inoculum for further antimicrobial assay in the study.

Evaluation of Antimicrobial Assay

The agar diffusion method was used for the screening of antimicrobial activity of the Thuja leaves extracts against all above listed test organisms (Bauer et al., 1966). The above mentioned bacterial cultures were activated by suspending them into 5ml nutrient broth and incubating at 30°C separately prior the antimicrobial assay.

The sterile nutrient media plates were used in assay and punched the wells in central part of plate by using sterile cork borer of 5 mm diameter. The plates were inoculated with test organisms individually with the spread plate technique and labeled accordingly. The wells were filled with 500 µl leaves extracts of *Thuja occidentalis* of both solvents. The plates were placed for refrigeration for 30 minutes for proper diffusion of leaves extracts. After addition of extracts the plates were incubated at respective temperatures required by test organisms for 24 hrs. for study of antimicrobial activity of *Thuja occidentalis.* That experiment was conducted in triplicates for more clear study.

Evaluation of Minimum Inhibitory Concentration

The MIC was studied by using same microbiological media and agar diffusion assay. The plates were inoculated with the activated test cultures with spread plate method. The wells on plates were filled with the Thuja leaves extracts with serially diluted concentrations such as 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml of both solvents viz. Petroleum Ether and Ethanol. The plates were kept for refrigerator for 30 minutes for proper diffusion of extract. Further plates were incubated at respective temperatures for 24 hrs. The MIC was studied.

Results and Discussion:

Percentage Yields

The percentage yield of extract of any medicinal plant is very important to evaluate the standard extraction efficiency for that particular plant. The percentage yield of both solvents is evaluated such as 68% and 1.5% for Ethanol and Petroleum Ether respectively. This is indicated in Table 1.

Table 1: Percentage Yield of Extracts

Plant Extracts	Extract Yield (g/100g)	Percentage Yield (w/w)
Ethanol (96%)	68	68%
Petroleum Ether	1.5	1.5%

Antimicrobial Activity of Extract on Test Organisms

The present study was carried out to study the antimicrobial property of the *Thuja occidentalis* against the Gram positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa.* It was observed that leaves extract has shown antimicrobial activity against both Gram positive and Gram negative organisms. All test organisms shown the clear zone of growth inhibition around the well filled with 500 µl of leaves extract. The growth of inhibition of respective test organisms is mentioned in the Table 2.

Table 2: Zone of Growth Inhibition of Test Organisms

Test Organisms	**Zone of Growth Inhibition in mm**	
	Petroleum Ether Extract	**Ethanol Extract**
Staphylococcus aureus	20	25
Bacillus subtilis	19	22
Escherichia coli	22.5	24
Pseudomonas aeruginosa	23.5	21

MIC Assay

Minimum inhibitory concentration was studied for five various concentrations of both solvents extracts in various concentrations such as 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. After completion of incubation we have observed that all four isolates shown varying MIC for both solvent extracts which is indicated in Table 3 and Table 4.

Table 3: Zone of Growth Inhibition of Petroleum Ether Extract in MIC Study

Test organism	**Minimum Inhibitory Concentration**				
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**
Staphylococcus aureus	8.7	8	6.3	-	-
Bacillus subtilis	8	7.4	5.7	-	-
Escherichia coli	7	6.5	-	-	-
Pseudomonas aeruginosa	7.6	7	5.4	-	-

Table 4: Zone of Growth Inhibition of Ethanol Extract in MIC Study

Test organism	**Minimum Inhibitory Concentration**				
	100 mg/ml	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**
Staphylococcus aureus	8	7.1	5.8	-	-
Bacillus subtilis	7.5	6	5.5	-	-
Escherichia coli	8.1	6.4	5.3	-	-
Pseudomonas aeruginosa	7.4	6.3	5.2	-	-

Conclusion:

The present study concludes that the *Thuja occidentalis* has antimicrobial activity against both Gram positive and Gram negative bacteria. All four test organisms were sensitive against the leaves extracts of plant.

The solvents used in study shown us the potential yields of leaves extracts and antimicrobial activity in both solvents. MIC study concludes that the 25 mg/ml concentration is almost minimum concentration required to show the antimicrobial activity for all four test organisms in both solvents except petroleum ether extract against *Escherichia coli.*

We conclude that the *Thuja occidentalis* is the useful medicinal plant and can be used to treat the microbial infections instead of the chemical alternatives.

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