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ANTIBACTERIAL ACTIVITY OF TWIGS OF THUJA OCCIDENTALIS

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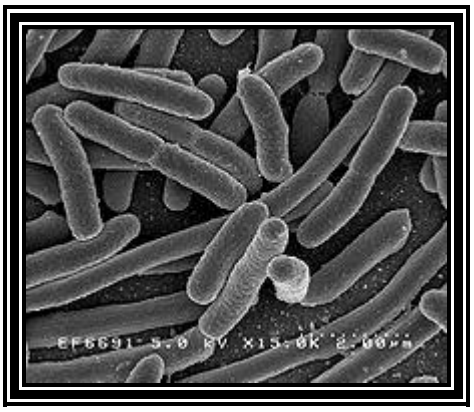
ABSTRACT

Objective: To evaluate the antibacterial activity of twigs of thuja occidentalis. **Methods:** Twigs extract of thuja occidentalis was prepared by the process of Soxhlet apparatus. **Results:** The plant Thuja occidentlis is an indigenous herb which was chosen for the present investigation study. The plant belongs to the family cupress*aceae*. The scanty availability of information on this plant facilitates the study on it. This attempt was made to study the pharmacognostical, phytochemical and antibacterial activities of plant. The study was divided into three major parts viz. Pharmacognostical studies, Phytochemical screening, Antibacterial activities. **Conclusion:** It was concluded that the Thuja occidentlis is antibacterial drug and shows good antibacterial activity against 'Gram Positive' bacteria and 'Gram Negative' bacteria.

KEYWORDS: antibacterial, Thuja occidentlis, extraction.

INTRODUCTION

Thuja occidantelis to family cupressaceae medicinally important plant, commonly grown in some parts of our country and used in the treatment of various disease and disorders of human ailments by tribal and rural people of our country. So, far no any systematic work was carried out to investigate the anti-bacterial activity of twigs of the selected plant therefore, the plant was selected for present investigation and Bacteria are a single-celled organism which can only be seen through microscope.[1,2] Bacteria come in different shapes and the size of bacteria is measured in micro-meter (which is a millionth part of a meter). Bacteria are found everywhere and in all type of environments. Bacteria are grouped as 'Gram Positive' bacteria and 'Gram Negative' bacteria, which is based on the results of Gram Staining.[3,4]



Structure of Bacteria figure No.1

MATERIAL AND METHOD

Material

The twig of the selected plant was collected in the months of august from the botanical gardens of Ujjain district of Madhya Pradesh and all the chemicals and reagents including drug were received from UIPS College Ujjain.

Methods

Selection of plant: Thuja occidantelis to family cupressaceae medicinally important plant, commonly grown in some parts of our country and used in the treatment of various disease and disorders of human ailments by tribal and rural people of our country. So, far no any systematic work was carried out to investigate the anti-bacterial activity of twigs of the selected plant therefore, the plant was selected for present investigation.

Macroscopic characters: Various morphological studies of the twig were studied and result was reported in table

1) Color
2) Odour
3) Taste
4) Shape
5) Size

Physico-chemical evaluation

The dried twigs of thuja occidentalis were subjected to standard procedure for the determination of various physicochemical parameters.

Determination of ash values

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Extraction of Plant Material

The extraction of plant material (twigs) was carried by soxhlet apparatus. 25 gm of dried twig powder was taken and dissolved in 100 ml of water and ethanol and petroleum ether Remain for 24 hours and filtered, concentrated, dried and then filtered.

Preliminary Phytochemical screening

The aqueous extract obtained after extraction of twig was subjected to various Phytochemical screening as per the standard procedure to reveals various active phytoconstituents.

1. Tests for fixed oils and fats

- **Spot test:** A small quantity of extract solution was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

2. Tests for steroids and triterpenoids

- **Libermann-burchard test**: The extract solution was treated with few drops of acetic anhydride, boiled and cooled. Then con. Sulphuric acid was added from the side of test tube.
- **Salkowski test**: The extract solution was treated with few drop of conc. sulphuric acid,

3. Test for proteins and free amino acids: A small quantity of the extract solution was dissolved in few ml of water and treated with following reagents.

- **Million's reagent:** Small quantity of extract solution was taken, added few drops of millions reagent.
- **Ninhydrin reagent:** Small quantity of extract solution was taken, added few drops of ninhydrin reagent (0.1% solution in butanol).
- **Biuret's test:** Small quantity of extract solution was taken, added 5% of sodium hydroxide and 1% of copper.

4. Test for tannins.

- **Ferric chloride solution:** Treated the extract solution with ferric chloride solution.
- **Lead acetate solution:** Treated the extract solution with 10% lead acetate solution white precipitate was obtained.

5. Test for Flavonoides.

- **Alkaline reagent test**

To the extract solution added few drops of magnesium hydroxide solution.

- **Shinoda test**

To the extract solution added few magnesium turnings and concentrated hydrochloride drop wise pink.

6. Test for mucilage's and gums: Small quantities of extract solution was added separately to 25 ml of absolute alcohol with constant stirring and filtered

7. Test for waxes: To the extract solution added alcoholic alkali solution, wax got saponified.

8. Test for Alkaloids

- **Dragendorff's reagent:**-To the extract solution added few drops of Dragendorff's reagent (potassium bismuth iodide solution).
- **Mayer's reagent:** - To the extract solution added few drops of Mayer's reagent (potassium mercuric iodide solution)
- **Wagner's reagent:** - To the extract solution added few drops of Wagner's reagent (iodine- potassium iodide solution)
- **Hager's reagent:** - To the extract solution added few drops of Hager's reagent (saturated solution of picric acid)

9. Test for Carbohydrates

- **Molisch's test:**-To the extract solution added few drops of α-naphthol, and then added few drops of sulphuric acid through the side of test tube.

10. Test for Glycosides: To the extract solution added the solution of a-naphthol and sulphuric acid.

11. Test for Starch: To the aqueous extract added weak aqueous iodide.

1) Procedure for Antibacterial Activity: Collection of bacteria: - Two bacterial strains i.e. Escherichia-coli (gram –ve) and Pseudomonas (gram –ve) was collected from Ujjain institute of pharmaceutical and sciences.

2) Drug Entrapped disc were Prepared: - Whatman filter paper was pieces into small disc one quarter inch diameter. All dilutions were applied to autoclaved filter paper disc using micro pipette with sterile pipette tip.

3) Culture media plate was prepared

Table 1: Nutrient agar media for bacteria.

S. No.	Ingredients	Quantity Prescribed	Quantity Taken
1	Beef Extract	10 gm	2.5 gm
2	Peptone	10 gm	2.5 gm
3	Sodium chloride	5.0 gm	1.25 gm
4	Agar	20 gm	5 gm
5	Distilled Water	1000 ml	250 ml

- **Procedure for bacteria media**

1. 5 gm of agar was dissolved in 250 ml of water.
2. Then beef extract, peptone and sodium chloride was added in above agar solution with continuous stirring.
3. The media was heated to dissolve the agar to form the clear liquid.
4. Then pH was maintained at 7.2-7.4 by pH meter.
5. Sterilized by autoclave at 115^{o}c at 15 lb pressure for 15 minutes.

4) Culture for bacteria: - The identified organism was applied to media for sensitivity test by streaking of medium with help of swab.

5) Application of disc: - All the disc of sample, standard, control with different dilution was placed on the inoculation plate with the help of flame sterilized forceps. After application of disc lead of petri-plate was closed, petri-plate was inoculated at 37^{o}c for 24 hours for bacteria.

6) Procedure for dilution

1) 100 mg drug was dissolved in 100 ml of distilled water to prepared stock solution (1000µg /ml).

2) From the above solution 10 ml was taken in volumetric flask and diluted upto 100 ml to prepared sub-stock solution (100µg/ml).
3) From the above solution the adequate concentration 20, 40, 60 and 80 µg/ml was prepared and in the same way standard drug concentration 60µg/ml was prepared.

7) Zone Of Inhibition: - After incubation the plate were inspected to identify zone of inhibition. The diameter of zone of inhibition of each compound and the diameter of disc was recorded.

The result of zone of inhibition was reported in table 10.The structure of Initial Anti bacterial plate was reported in fig.26 and structure of anti fungal growth was reported in fig.27.
Zone of inhibition= Diameter of sample/ slandered/ control - diameter of disc
Zone of inhibition= Diameter of sample/ slandered/ control – 0.5 mm

RESULTS AND DISCUSSION

The plant Thuja occidentlis is an indigenous herb which was chosen for the present investigation study. The plant belongs to the family cupress*aceae*. The scanty availability of information on this plant facilitates the study on it. This attempt was made to study the pharmacognostical, phytochemical and antibacterial activities of plant. The study was divided into three major parts viz.

- Pharmacognostical studies
- Phytochemical screening
- Antibacterial activities

Pharmacognostic studies

Macroscopy: The morphological features of the leaves of the selected plant Thuja occidentalis was presented below

Table 2: Macroscopic Characters.

S. No.	Character	Appearance
1	Color	Green
2	Odour	Strongly camphoraceous
3	Taste	Strongly camphoraceous
4	Shape	Lanceolate
5	Size	3mm long
7	Apex	Acute
8	Margin of Lamina	Entire Margin
9	Venation	Parallel Venation

10	Mid Rib	Present

Physico-chemical evaluation: - The physicochemical analysis of leaves powder of Thuja occidentalis was carried out. In this study ash values (total ash, acid insoluble ash and water soluble ash) were determined, Swelling index, LOD, Foaming index, foreign organic matter (F.O.M.) was determined and the results are given below:

Table 3: Physico-chemical evaluation.

S. No.	Physicochemical Parameters	Result (w/v)
1	Ash Values	5.6%
1.1	Total Ash Value	4.8%
1.2	Acid Insoluble Ash Value	2.52%
1.3	Water Soluble ash Value	0.5%
2	Moisture Content (Loss On Drying)	0.9%

Table 4: Foreign organic matter.

S. No.	Drug	Weight of drug before	Weight of drug after	% of FOM
1	Twigs	100gm	96.2gm	4.2%

Table.5. Swelling Index.

S. No.	Drug	Drug	Inference
1	Before (cm)	After(cm)	
2	50cm	58.1cm	8.1

Phytochemical Screening

The various extract of the plant of Thuja occidentalis were subjected to phytochemical screening which reveal the presence of various pharmacological active components. Phytochemical Screening of sample thuja occidentalis was done as follows.

Table 6: Preliminary Phytochemical Screening.

S. No.	Tests	Aqueous extract
1	Fixed oil and fats Spot test	-
2	Tannins Ferric chloride test Alkaline reagent	+ -
3	Proteins Millions Reagent Ninhydrin Reagent Biuret Test	+ + + -
4	Flavonoides Alkaline reagent test Shinoda test	+ +
5	Steroids and triterpenoids Lieberman burchard test Salkowski test	- +
6	Mucilage and gum Reaction with 90% alcohol	-

7	Waxes Reaction with alcoholic KOH	-
8	Alkaloids Dragendorff's reagent Mayer's reagent Wagner's reagent Hager's reagent	+ + + +
9	Carbohydrates Molish test	-
10	Glycosides Borntrager's test	+
11	Starch (Amylum)	-

Antibactial Activity: The antibacterial activity of Thuja occidentalis was determined.

Table 7: Zone Of Inhibition Petry Plate.

Strain	**Escherichia-coli**				
Concentration (µg/ml)	20	40	60	80	100
Sample Thuja occidentalis	–	4	5	7	9
Standard					
Penicillin(60 µg/ml)	28				
Control (distilled water)	Nil				

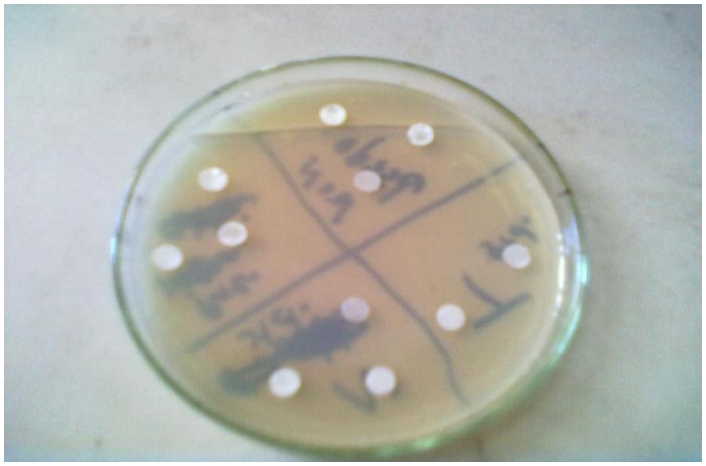


Figure 2: Zone Of Inhibition.

CONCLUSION

The antibacterial activity of Thuja occidentalis was determined on various bacteria.

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