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ANTIMICROBIAL ACTIVITY OF LEAVES OF *THUJA COMPACTA* AGAINST PATHOGENIC ORGANISMS COMPARED WITH CONTROL DRUGS

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ABSTRACT
Fresh juice and acetone, chloroform, methanol, petroleum ether and water extracts of leaves of *Thuja compacta* traditionally used in Indian system of medicines were screened against standard strains of the *Escherichia coli* NCIM 2931, *Salmonella typhi* MTCC 734, *Salmonella typhimurium* MTCC 98, *Klebsiella pneumoniae* MTCC432, *Proteus vulgaris* NCIM2857, *Proteus mirabilis* MTCC425, *Pseudomonas aeruginosa* NCIM5029, *Staphylococcus aureus* MTCC 96, *Staphylococcus epidermis* MTCC 435, *Bacillus cereus* NCIM 2155, *Bacillus subtilis* NCIM 2063 and *Bacillus megaterium* NCIM 2087 by using agar well diffusion method. Fresh leaves juice showed significant activity against Gram negative organisms except *E. coli* and *S. typhi*. Similarly active against Gram positive bacteria except *S. aureus*, *S. epidermis.* Acetone, chloroform, methanol, petroleum ether extracts of leaves showed significant activity against only Gram positive bacteria. Zone of inhibition of the extract compared with the standard antibiotics, Am.[30] Amoxycilin; Cf.[30] Ciprofloxacin; Co.[25] Cotrimaxazole; G.[50] Gentamicin and Tetracycline—T.[30] It is concluded that the *Thuja compacta* may serve as valuable source of compounds with therapeutic potential.

KEYWORDS: Antimicrobial activity, *Thuja compacta*, Antibiotics, Acetone, Chloroform, Methanol and Petroleum ether extracts.

INTRODUCTION
The genus Thuja of exotic origin belongs to the Cupressaceae family and species, including *Thuja compacta* commonly known as the tree of life. *Thuja* is antibacterial. A 2017 trial showed that its extract effectively killed both gram-positive and gram-negative bacteria (*Sah*, 2017). The leaves and leaf oil are used as a medicine. Thuja is used for respiratory tract infections such as bronchitis, bacterial skin infections, and cold sores. It is also used for painful conditions including osteoarthritis and a nerve disorder that affects the face called trigeminal neuralgia. Thuja is sometimes applied directly to the skin for joint pain, ostearthritis, and muscle pain. Thuja oil is also used for skin diseases, warts, and cancer; and as an insect repellent.

Thuja occidentalis, commonly known as American Arbor vitae or white cedar, is indigenous to eastern North America and is grown in Europe as an ornamental tree (Chang LC. et al., 2000). The plant was first identified as a remedy by native Indians in Canada during a 16th century expedition and was found to prove effective in the treatment of weakness from scurvy (British Herbal Pharmacopoeia 1983). In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Shimada K, 1956- Baran D, 1991). In combination with other immunomodulating plants, such as Echinacea purpurea, Echinacea pallida and Baptisia tinctoria, this medicinal plant is also used as evidence-based phytotherapy for acute and chronic infections of the upper respiratory tract (Reitz HD.et al., 1990) and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina and pharyngitis. Several reviews and monographs describe the botany, constituents and some pharmacological properties, and the use of this herbal substance in the treatment of the common cold (Bodinet C. et al., 2002).

Several scientists have worked on antimicrobial activities of various medicinal plants and found that these plants are effective against the microorganisms which showed drug resistance to various antibiotics (Gislene, 2000). Numbers of medicinal plants were screened for their antimicrobial activity. Many workers have studied the effect of different plant extracts on bacteria producing diseases in human being (Dulger and Gonuz 2004, Shah and Jadhav).

Present study involves the screening of medicinal plant having potential antimicrobial activity against those bacteria which showed antibiotic resistance against common antibiotics. Our study deals with the crude extracts of leaves of *Thuja compacta* plants. It is always successful to test crude extract.

MATERIALS AND METHODS

Selection of medicinal plant for this study

Thuja compacta

Family: Cupressaceae

Parts used: Leaf

Traditional uses: Thuja's main action is due to its stimulating and alterative volatile oil. In bronchial catarrh Thuja combines expectoration with a systemic stimulation beneficial if there is also heart weakness. Thuja should be avoided where the cough is due to over stimulation, an in dry irritable coughs. *Thuja occidentalis* has a specific reflex action on the uterus and may help in delayed menstruation, but because of this action is should be avoided in pregnancy. Where ordinary incontinence occurs due to loss of muscle tone, *Thuja occidentalis* may be used. Also used in the treatment of psoriasis and rheumatism. Externally it may be used to treat warts. A marked anti-fungal effect is found if used externally for ringworm and thrush. Thuja or Cedarwood (*Thuja occidentalis*) is a versatile and useful plant and has been used successfully for the treatment of psoriasis, rheumatism, and for warts. Also known as the Tree of Life or Arborvitae, it is useful as a counter-irritant in the relief of muscular aches and pains, including those of rheumatism. It can be applied externally in a salve for warts and other skin problems (Brijesh et al., 2012).

Chemical constituents: 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 (Harnischfeger G. et al., 1983), 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% sabines and 2% α-pinen as the main monoterpenes (Witte l. et al., 1983). Other monoterpenes, namely carvotanacetone, origanol, origanes, myrcen and camphen, have been described (Berlin J. et al., 1984- Hänsel R. et al., 1994).

Identification and Preservation of Plant materials

Fresh plant leaves were collected from the Nagpur area of India. The taxonomic identities of this plant was determined by the expertise of the Post Graduate Department of Botany of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. Specimen was labeled, numbered and noted with date of collection, the locally and their medicinal uses and their approximate dosages of administration were recorded. Plant leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4^{o}C for further use.

Preparation of crude extract (Fresh juice)

Thuja compacta plant leaves were collected from around Nagpur region in the month of August-September. Leaves were cleaned under running potable water and cut into pieces and grounded in pestle and mortar (made up of dolerite stone) till homogenized mass was obtained. Homogenized mass was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was kept in sterilized glass bottle. All crude extract were prepared fresh and used before 2 hours.

Crude extraction

Aqueous extraction

Ten grams of dried powder was extracted in 100 ml distilled water for 6 h. at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume (Shahidi Bonjar GH 2004). It was then autoclaved at 121ºC and 15 lbs pressure and then stored at 4ºC.

Solvent extraction

Ten grams of dried powder was extracted with 100 ml of each solvent (acetone, chloroform, methanol and petroleum ether) and flasks were kept on a rotary shaker at 190-220 rpm for 24h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Shahidi Bonjar, G.H. 2004). It was stored at 4^{o}C in airtight bottles for further studies.

Bacterial cultures

The microbial strains are identified strains and were procured from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains were *Bacillus cereus* NCIM2155, *Bacillus subtilis* NCIM2063, *Bacillus megaterium* NCIM2087, *Escherichia coli* NCIM2931, *Proteus vulgaris* NCIM2857 and *Pseudomonas aeruginosa* NCIM5029. *Staphylococcus aureus* MTCC96, *Staphylococcus epidermis* MTCC 435, *Salmonella typhi* MTCC 734, *Salmonella typhimurium* MTCC 98, *Klebsiella pneumoniae* MTCC432, *Proteus mirabilis* MTCC425, these strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. They were sub-culured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4^{0}C, fresh inoculums were taken for test.

Media

Hi -Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai, India. The media were prepared according to the instructions given (Tumane P.M.et al.2000).

Screening for the antimicrobial potential of the plant leaves extracts

The antimicrobial activity of different solvent extracts was evaluated by agar well diffusion (Perez C, et al., 1990& Parekh, J.et al., 2007) using Hi-sensitivity test agar (M 485).

Preparation of inoculum-A loopful of culture was inoculated from the stock slant culture in 5 ml of Hi-sensitivity test broth and broth was incubated at 35±0.5^{0}C in incubator for 18-20 hours. After incubation a loopful of actively growing culture was inoculated into 10 ml of Hi-sensitivity broth. Broth was incubated at 35±0.5^{0}C for 6-8 hours. This culture was used for the inoculation of Hi-sensitivity test agar plates.

Preparation of Hi-sensitivity test agar medium

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at 45-50^{0}C in constant temperature water bath.

Inoculation of medium with test organism

0.5 ml of 6-8 hours old test organism is transferred to petridish of 100mm size (Sterilized in oven at 180^{0}C for 1 hr.) using sterile micropipette. Hi-sensitivity test agar medium maintained at 45-50^{0}C was poured and mixed properly to ensure uniform distribution of organism with medium. Seeded plates are allowed to set at room temperature.

Preparation of agar well for fresh leaves juice

10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 100 µl crude extract (fresh leaves juice) was transferred by micropipette per well. Plates were immediately kept at 4^{0}C in refrigerator for 1 hr. for the diffusion of extract and then shifted to 35±0.5^{0}C in incubator. Zone of inhibition was measured after 24 hours. of incubation by zone scale.

Preparation of agar wells for different solvent extracts

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made.

A 50 ul solvent extract was transferred by micropipette per well. Plates were immediately kept at 4^{0}C in refrigerator for 1 hr. and them shifted to 35^{0}C±0.5^{0}C in incubator. Zone of inhibition was measured after 24 hrs. of incubation. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were sustracted from the test zones and the resulting zone diameter is obtained.

RESULTS AND DISCUSSION

Table 1: Results of antimicrobial activities of fresh leaves juice and solvent extracts of *Thuja compacta* leaves and compared with standard antibiotics.

Sr. No. Microorganisms	Zone of inhibition in millimeter										
	Leaves extracts						Standard antibiotics				
	FJ	WE	AE	CE	ME	PE	Am^{30}	Cf^{30}	Co^{25}	G^{50}	T^{30}
1. *Escherichia coli*	---	---	---	---	---	---	32	29	24	17	22
2. *Salmonella typhi*	---	ND	ND	ND	ND	ND	38	28	25	18	19
3. *Salmonell typhimurium*	20	ND	ND	ND	ND	ND	32	22	24	17	17
4. *Klebsiella pneumoniae*	20	ND	ND	ND	ND	ND	15	16	19	15	12
5. *Proteus vulgaris*	11	---	---	---	---	---	---	23	31	20	24
6. *Proteus mirabilis*	15	ND	ND	ND	ND	ND	20	20	20	14	12
7. *Pseudomonas aeruginosa*	13	---	---	13	---	---	14	36	---	34	22
8. *Staphylococcus aureus*	---	---	16	---	12	12	31	23	20	16	17
9. *Staphylococcus epidermis*	---	ND	ND	ND	ND	ND	36	27	15	26	21
10. *Bacillus cereus*	16	---	17	13	12	13	15	27	---	23	24
11. *Bacillus subtilis*	14	---	16	11	14	13	31	50	36	40	32
12. *Bacillus megaterium*	11	---	12	11	11	13	29	46	24	23	33

Key: FJ—Fresh juice of leaves; WE—Water extract; AE—Acetone extract; ME—Methanol extract; CE- Chloroform extract; PE— Pertroleum ether extract; Am[30]--Amoxycilin; Cf[30] –Ciprofloxacin; Co[25] –Cotrimaxazole; G[50]--Gentamicin; Tetracycline—T,[30] ND—Not determined; --- Negative.

Antibacterial activity of different solvent extracts of leaves of *Thuja compacta* (TC) zone of inhibition in millimetre (mm).

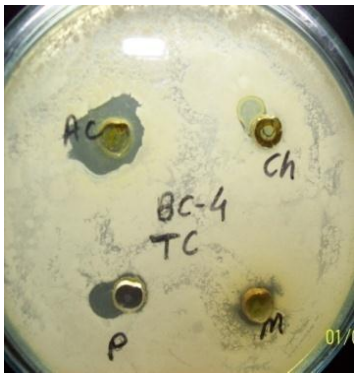

Figure-1.
Activity against *Bacillus cereus*
Acetone extract (A)-14 mm
Chloroform extract (C) – 13 mm
Methanol extract (M) – 12 mm
Petroleum ether extract (P)-10 mm

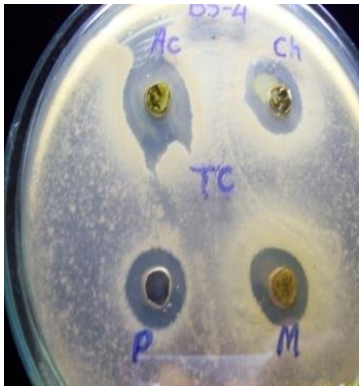

Figure 2.
Activity against *Bacillus subtilis*
Acetone extract (A)-17 mm
Chloroform extract (C)-13 mm
Methanol extract (M)-12 mm
Petroleum ether extract (P)-13 mm

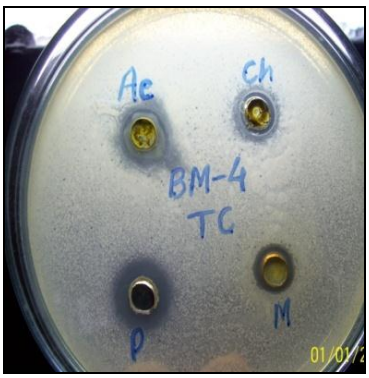

Figure 3.
Activity against *Bacillus megaterium*
Acetone extract (A)-12 mm
Chloroform extract (C)-11 mm
Methanol extract (M)-11 mm
Petroleum ether extract (P)-13 mm

The extracts prepared from *Thuja compacta* leaves using different solvents showed varying degree of antimicrobial activity against organisms selected for the study. Fresh leaves juice showed significant activity against Gram negative organisms except *Escherichia coli* and *Salmonella typhi*. Similarly active against Gram positive bacteria except *Staphylococcus aureus, Staphylococcus epidermis.* Acetone, chloroform, methanol, petroleum ether extracts of leaves showed significant activity against only Gram positive bacteria *Bacillus cereus* (Fig-1), *Bacillus subtilis* (Fig-2) and *Bacillus megaterium* (Fig-3). Only *Pseudomonas aeruginosa* shows activity against chloroform extract. All the organisms are susceptible to Am[30]--Amoxycilin; Cf[30]–Ciprofloxacin; Co[25]–Cotrimaxazole; G[50]—Gentamicin and Tetracycline—T[30] *Proteus vulgaris* is found to be resistant to Amoxycillin Am,[30] *Pseudomonas aerugenosa* and *Bacillus cereus* found to be resistant to Cotrimaxozole Co.[25] It is concluded that the *Thuja compacta* may serve as valuable source of compounds with therapeutic potential.

The alcoholic extract of twigs of *Thuja occidentalis* was established for Antibacterial activity against both gram negative and gram positive organisms i.e., *Pseudomonas aeruginosa*, *Yersinia aldovae, Citrobacter, Shigella flexneri, E. coli* and *Staphylococcus aureu, Vernoniaanthelmintica, Dryopteris chrysocoma* and *Trachyspermum ammi* were tested In vitro for their antibacterial and antifungal activities. Antibacterial study performed against six bacteria viz., *Escherichia coli, Citrobacter, Shigella flexenari, Yersinia aldovae, Staphylococcus aureus* and *Pseudomonas aeruginosa* indicated that had potent activity against all microorganisms. The antifungal activity of these extracts was performed against six fungi, viz., Saccharomyces cereviciae, Aspergillus parasiticus, Trichophyton rubrum, Macrophomina, Fusarium solani and Candida albicans. The extracts showed significant results against different fungal strains (Jahan N. et al., 2010).

CONCLUSION

The present review reveals that the plant *Thuja compacta* is found to have therapeutic uses in treating various ailments. A detailed research work in the characterization and standardization is strongly required for this potential plant in developing its various formulations, which can ultimetly be beneficial for humans as well as animals. Further studies are warranted to explore much depth about this plant known by the name "The tree of life".

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