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GC-MS profiling and anti-bacterial activity of *Thuja orientalis* in reference of plant biodiversity

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Abstract
Thuja orientalis (Commonly-Morpankhi, Family-Cupressaceae) is an evergreen, monoecious trees or shrubs used in various forms of traditional medicines and homeopathy in various ways. In traditional practices *Thuja* is used for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism. The present work was designed to investigate the phytochemical constituents with GC-MS analysis and to establish the antibacterial property of the plant extract against the bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus* for which *Thuja orientalis* plant has been significantly used as a multidrug constituent. It is evident from the results of GC-MS analysis that hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh showed various resolutions of peaks with retention time. The each peak in GC-MS profile of hydroalcoholic extract of *Thuja orientalis* aerial parts represent maximum area under the curve in Tamil Nadu sample followed by Rajasthan and Himachal Pradesh samples. These observations suggest that Tamil Nadu sample of *Thuja orientalis* aerial parts contained higher amount of phytoconstituents than Rajasthan and Himachal Pradesh samples. The results of antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* showed that hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu exhibited higher antimicrobial activity followed by plant collected from Rajasthan and Himachal Pradesh as compared to standard drugs. The exhaustive survey of literature suggested that flavonoids such as quercetin, rutin, luteolin and naringenin have been scientifically claimed as potential antimicrobial activity, so it may be concluded that antimicrobial activity of hydroalcoholic extract of *Thuja orientalis* aerial parts may be due to presence of phenolic and flavonoids.

Keywords: *Thuja orientalis*, GC-MS, biodiversity, anti-bacterial activity

1. Introduction
Natural products are important sources for biologically active drugs. India is a heritable emporium of many medicinal and aromatic plants [1]. *Thuja orientalis* belonging to family Cupressacae is a well-known medicinal plant. *Thuja orientalis*, commonly known as American Arbor vitae or white cedar, is indigenous to eastern North America, Northwestern China and widely naturalized elsewhere in Asia east to Korea and Japan, south to northern India, and west to northern Iran. It is a small, slow growing tree, to 15-20 m tall and 0.5 m trunk diameter (Exceptionally to 30 m tall and 2 m diameter in very old trees) [2-3]. 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 mineral [4], 1.67% free acid and 1.31% tannic acids. The essential oil of the fresh leaves (related to the monoterpene fraction) contains 65% thujone, 8% isothujone, 8% fenchone, 5% sabines and 2% α-pinene as the main monoterpenes [5]. Other monoterpenes, namely carvotanacetone, origanol, origanes, myrcen and camphene, have been described [6-7].
Thuja plants in general have a long history of use for house hold material and buildings as well as in various herbal remedies and aromatherapy preparations. In Western herbal medicine, cedar leaf oil was used as an emmenagogue, abortifacient, vermifuge, diuretic, and digestive aid. It was applied externally to relieve the pains of arthritis and rheumatism, to treat external fungal infections of the skin (Ringworm and thrush), and to remove anal or genital warts. Cedar leaves and twigs are in fact rich in vitamin C, and it was their effectiveness in preventing or treating scurvy that led to the tree's being called arbor vitae or tree of life. In addition, recent research has shown that extracts prepared from the plant do in fact have antiviral, anti-inflammatory, and antibacterial properties. A group of German researchers

reported in 2002 that an extract prepared from cedar leaf, alcohol, and water inhibits the reproduction of influenza virus type A, while a team of researchers in Japan found that an extract of Western red cedar was effective in treating eczema [8]. The homeopathic preparation known as Thuja is made from the leaves of *Thuja orientalis*, and is given to treat soft or bleeding rats on the genitals or anus. The most widely used homeopathic materia medica, or reference book, also recommends Thuja for headaches that feel like a nail is being driven into the head; vertigo brought on by standing up; emotional depression and restlessness; pain or itching in the scalp; painful swallowing or a feeling of obstruction in the throat; intense thirst at night or early in the morning; stomach cramps that are worse in the evening; difficulty in breathing combined with a violent thirst for cold water; frequent need to urinate, with frothy or cloudy urine; insomnia or restless sleep; or fever and chills that grow worse toward evening [9]. Many individuals today are affected with various distinguish disorders for which general medical practitioners treat the patients with antibiotics, whereas Ayurvedic practitioners deal individuals with several herbal formulations. Pharmacological industries developing medicines against most dreadful disease pathogens are becoming unsuccessful due to multi-resistance of the pathogens to many drugs. The herbal drugs sold in the market for various diseases do not explain the content of medicines properly like, correct plant names, plant parts, quantity of bio-compounds or active parts, etc. So, there is a need to understand the proper working principle of the herbal drugs for their efficient outcome in light of their biological/therapeutic activities. In the present study, an attempt has been made to investigate the antimicrobial activity of *Thuja orientalis* extract against two test microorganisms, one gram positive bacterium *Staphylococcus aureus* and One gram negative bacteria *Escherichia coli*. Further, these extracts were subjected to GC–MS analysis for the presence of various components that are responsible for their antimicrobial properties.

2. Materials and Methods

2.1 Collection and Identification of plant material

The dried aerial parts of *Thuja orientalis* were collected from wild regions of different states such as Himachal Pradesh, Rajasthan and Tamil Nadu. The identity of collected dried aerial parts of *Thuja orientalis* was confirmed by Plant Anatomy Research Centre (Prof. P. Jayaraman, PhD, Director, Retd. Prof. Presidency College Chennai-5) with registration number of certificate PARC/2019/4034, dated 15/03/2019. The identity of collected dried aerial parts of *Thuja orientalis* was also confirmed from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR with reference no. – NISCAIR/RHMD/Consult/ 2018/3203-04-02, dated 27/04/ 2018).

2.2 Extraction and Phytochemical analysis

The powdered plant material of whole part was taken separately and successively in each of different solvents (Petroleum Ether, Benzene, Chloroform, Ethyl Acetate, Ethanol, Methanol & Water) and subjected to Soxhlet extraction procedure. Then finally dried powdered aerial parts of different places collected *Thuja orientalis* (250 gm each) were separately extracting exhaustively with mixture of ethanol and water (1: 1) in a Soxhlet apparatus to obtain hydroalcoholic extracts. The hydroalcoholic extracts were concentrated using rotary vacuum evaporator and all hydroalcoholic extracts were preserved for antimicrobial and GCMS Profile in a vacuum desiccator [10].

2.3 Gas chromatography-mass spectrometry (GCMS) analysis: The GC-MS investigations of hydroalcoholic extract of aerial parts of *Thuja orientalis* was completed utilizing GC-MS instrument which comprises of a Shimadzu QP2010 gas chromatographic framework outfitted with a fire ionization finder combined with particle mass spectrometer. Chromatographic division was performed on DB-FFAP (nitroterephthalic corrosive altered polyethylene glycol) capillary column (30 m long, 0.32 mm i.d., film thickness 0.25 µm). GC-MS location was performed by contrasting mass spectra with National Institute of Standards and Technology 02 library [11].

2.4 Gas Chromatography-mass spectrometry conditions

The each hydroalcoholic extract of aerial parts of *Thuja orientalis* was dissolved separately in isopropyl liquor to acquire a last grouping of 30 mg/ml and went through Whatman polytetrafluoroethylene syringe channel of pore size 0.22 µ for GC-MS investigation. Helium was utilized as bearer gas and all out run time was of 24 min. Splitless infusion mode was chosen and infusion volume was set at 1 µl. Chamber temperature was set at 120 ºC for 7 min and expanded to 200 ºC at 20 ºC/min and held for 17 min. The injector temperature was 230 ºC.

3. Anti-bacterial analysis

3.1 Collection of micro-organisms

The organisms used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213). The strains were maintained on nutrient agar slants at 4 ºC. The strains of *Staphylococcus aureus* and *Escherichia coli* were inoculated into conical flask containing 100 ml of sterile nutrient broth. These conical flasks were incubated at 37 °C for 24 hours. This was referred to as seeded broth.

3.2 Standardization of seeded broth (Viable count)

One ml of 24 hours seeded broth of each strain was diluted with 99 ml of sterile water containing 0.05% Tween 80 (8 drops of Tween 80 in 100 ml of normal saline). From that 1 ml was further diluted to 10ml with sterile water. This was continued till 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} up to 10^{-10} dilutions of the seeded broth were obtained. The dilutions were studied by inoculating 0.2 ml of each dilution on to the solidified nutrient Agar medium by spreading method. After incubation at 37±1 °C for 24 hours, the numbers of well-formed colonies on the petridishes were counted. The seeded broth was then suitably diluted to contain between 10^{-6} to 10^{-7} microorganisms or cfu (Colony forming unit) per ml. This was designated as the working stock which was used for *in vitro* anti-bacterial studies (Seeded broth).

3.3 Preparation of solution of test drug

The drug solution was prepared by dissolving in dimethyl sulphoxide (DMSO) in a specific gravity bottle (Stoppered). The DMSO was removed from the refrigerator 1 hr prior to its use and allowed to warm up to room temperature. The solution of the test drug (Hydroalcoholic extract at the concentration of 20 mg/ml in DMSO) and standard drug (Streptomycin sulphate) 100 mg/ml in DMSO were prepared. Solvent control of DMSO was maintained throughout the experiment.

3.4 Determination of antibacterial susceptibility of the test by cup-plate method: This method depend on the diffusion of the various drugs from a cavity through the solidified agar layer of petridish, to an extent such that growth of the added microorganism is prevented entirely in a circular area or zone around a cavity containing the drugs. Using a micropipette, 0.2 ml of each of the seeded broth containing 10^{-6} to 10^{-7} cfu per ml test organisms were inoculated on the solidified Agar plate and speeded uniformly with a glass spreader. Then four wells were made in the Agar layer of each plate with an aluminium borer. To the two wells, 0.2 ml of the solution of the test drugs at the concentration of 20 mg/ml was added. All the work was carried out under aseptic conditions. The plates were left at room temperature for one hr after addition to allow the diffusion of the solution into the medium and then incubated at 37±1 °C for 24 hours. After the incubation period, the mean diameter of the zone of inhibition in millimetre obtained around the well was measured.

3.5 Determination of minimum inhibitory concentration (MIC): As appreciable zone of inhibition was found out from the study, so further study was carried out for the determination of minimum inhibitory concentration (MIC) of the test drugs. The hydroalcoholic extracts of aerial parts of *Thuja orientalis* were studied for their anti-bacterial activity by determining the MIC using Two-fold serial dilution technique.

The hydroalcoholic extracts were separately dissolved in DMSO to obtain a 10 mg/ml solution. A series of six assay tubes were used against each strain. To the first assay tube, 1.8 ml of seeded broth was transferred and then 0.2 ml of the test drug was added and mixed thoroughly and forms the first dilution. To the remaining five assay tubes, 1 ml of seeded broth was transferred. From the first assay tube, 1 ml of the content was pipetted out into the second assay tubes and mixed thoroughly to obtain the second dilution and so on till six such dilution were obtained (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml and 31.25 µg/ml). The experiment was performed in aseptic condition.

Similarly the solvent control (DMSO) solutions were prepared in seeded broth such that the dilution contains the amount of DMSO same as that of the previous dilutions. The racks of assay tubes were put into incubator at 37±1 °C for 24 hours. The observations were made at the end of 24 hours. The assay tubes were removed, observed for any deposits, shaken to aerate the solution and to suspend bacteria that might have settled at the bottom of the assay tubes. The lowest concentration of the test drug and the standard drug which caused apparently a complete inhibition of growth of organism was taken as minimum inhibitory concentration (MIC expressed in µg/ml). The solvent control tubes were also observed for any inhibitory action of DMSO [12].

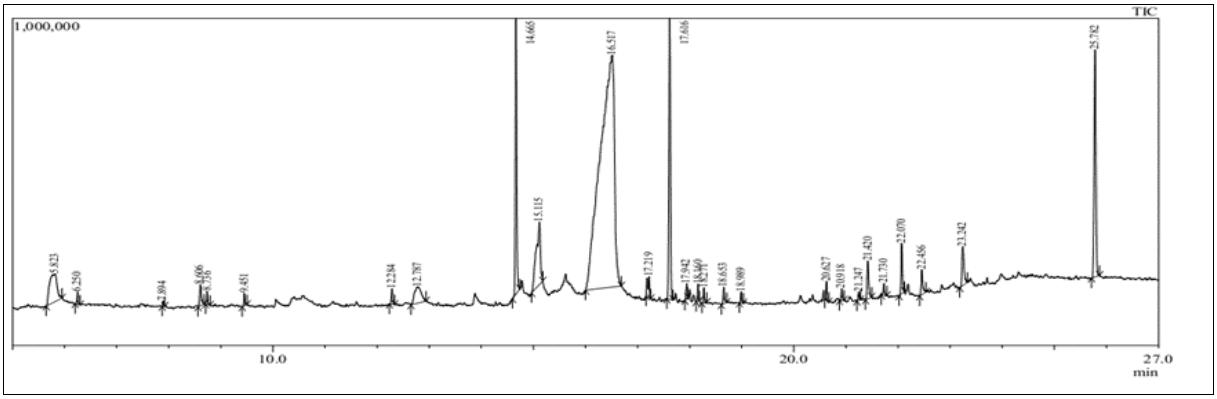
4. Results

4.1 Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh were was subjected to GC-MS analysis for qualitative characterization. GC-MS is important strategy over other accessible liquid chromatographic and spectroscopic strategies for characterization of phytoconstituents as a result of its straight forwardness in technique improvement for division of phytoconstituents and distinguishing proof of such constituents dependent on their mass number. The chromatogram of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh showed twenty seven peaks each representing a different phytoconstituents. The results of the GC-MS analysis of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh are presented in Table & Figure 1. It is evident from Figure 1, *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh showed similar resolution of peaks with same retention time. The identity of these phytoconstituents was confirmed by comparing their mass spectra with NIST 02 (National Institute of Standards and Technology) library. The each peak in GC-MS profile of hydroalcoholic extract of *Thuja orientalis* aerial parts represent maximum area under the curve in Tamil Nadu sample followed by Rajasthan and Himachal Pradesh samples. These observations suggest that Tamil Nadu sample of *Thuja orientalis* aerial parts contained higher amount of phytoconstituents than Rajasthan and Himachal Pradesh samples.

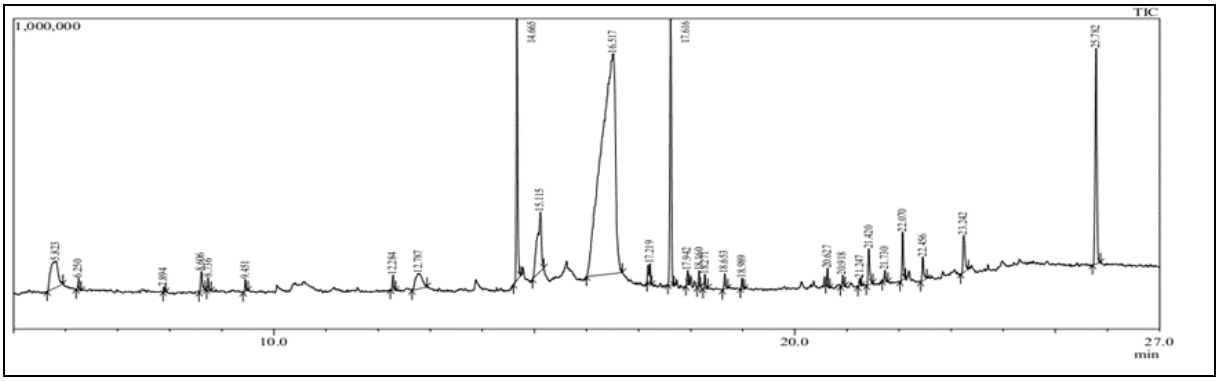
Table 1: Phytoconstituents present in hydroalcoholic extract of *Thuja orientalis* aerial parts (A) Tamil Nadu; (B) Rajasthan and (C) Himachal Pradesh identified by GC-MS

Peak	Retention Time	Area (A)	Area (B)	Area (C)	Name
1.	5.823	868447	865125	863114	Glycerin
2.	6.250	60041	58547	56444	Oxalic acid, isobutyl pentyl ester
3.	7.894	23838	22458	21145	Oxalic acid, isobutyl pentyl ester
4.	8.606	153983	151458	150789	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
5.	8.736	65920	63658	62558	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl
6.	9.451	60122	59001	57001	Tridecane
7.	12.284	88250	86365	85569	1,3-Dioxolane, 2-ethyl-2-isopropyl-4,5-dimethyl
8.	12.787	448937	445987	444125	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro
9.	14.665	1732803	1730789	1728490	Diethyl Phthalate
10.	15.115	1060339	1058115	1056120	Bicyclo[2.2.1]heptane-2,3-diol, 1,7,7-trimethy
11.	16.517	13474549	13470788	13468111	4-O-Methylmannose
12.	17.219	185136	183005	180400	Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1
13.	17.616	1836730	1834987	1832879	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexa
14.	17.942	75154	73458	71470	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexa
15.	18.160	81651	79547	78126	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexa
16.	18.271	74232	72636	71659	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexa
17.	18.653	95801	93408	92980	Undecanoic acid
18.	18.989	58414	56102	54560	Ethyl tridecanoate
19.	20.627	87591	86258	85777	cis,cis,cis-7,10,13-Hexadecatrienal

20.	20.918	61058	59200	57358	(1R,4aR,4bS,7S,10aR)-1,4a,7-Trimethyl-7-vinyl
21.	21.247	47004	45444	44190	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha)
22.	21.420	260363	258658	256006	Andrographolide
23.	21.730	66252	65111	63778	Dibenzo[a,h]cyclotetradecene, 2,3,11,12-tetra
24.	22.070	291470	289004	286987	Ferruginol
25.	22.456	173921	172650	171478	(1S,4aR,5S,8aR)-1,4a-Dimethyl-6-methylene
26.	23.242	321196	320007	318985	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alph
27.	25.782	1677160	1675668	1674879	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo



A



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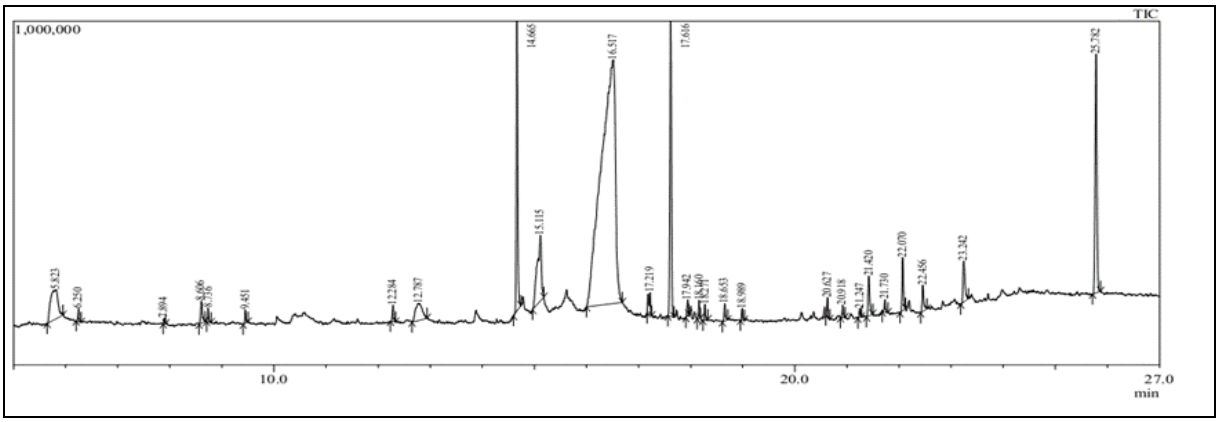

C

Fig 1: GC-MS chromatogram of hydroalcoholic extract of *Thuja orientalis* aerial parts (A) Tamil Nadu; (B) Rajasthan and (C) Himachal Pradesh

4.2 Antimicrobial activity

The hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh were investigated for *in vitro* antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* by cup plate method using standard antimicrobial drug streptomycin. The results of antimicrobial activity against *Staphylococcus aureus* showed that hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu (MIC 250 µg/ml; Zone of inhibition reading 16.58 mm) exhibited higher antimicrobial activity followed by plant collected from Rajasthan (MIC 500 µg/ml; Zone of inhibition reading 15.65 mm) and Himachal Pradesh (MIC 500 µg/ml; Zone of inhibition reading 13.25 mm) as compared to streptomycin (MIC 125 µg/ml; Zone of inhibition reading 27.54 mm). The results hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh were investigated for *in vitro* antimicrobial activity against *Staphylococcus aureus* are presented in Table 2 &

Figure no. 2. The results of antimicrobial activity against *Escherichia coli* showed that hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu (MIC 250 µg/ml; Zone of inhibition reading 9.21 mm) exhibited higher antimicrobial activity followed by plant collected from Rajasthan (MIC 250 µg/ml; Zone of inhibition reading 7.12 mm) and Himachal Pradesh (MIC 250 µg/ml; Zone of inhibition reading 6.54 mm) as compared to streptomycin (MIC 125 µg/ml; Zone of inhibition reading 16.25 mm). The results hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh were investigated for *in vitro* antimicrobial activity against *Escherichia coli* are presented in Table 3 & Figure no. 3.

Table 2: The results of antimicrobial activity hydroalcoholic extract of *Thuja orientalis* aerial parts against *Staphylococcus aureus*

Name of Bacteria	Microbial activity / Zone of inhibition reading (mm)					
	31.25 (µg/ml)	62.5 (µg/ml)	125 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)
Control (Negative)	---	---	---	---	---	---
Streptomycin (Positive)	22.45	24.58	27.54	27.89	27.99	27.90
Thuja orientalis (Tamil Nadu)	10.12	12.25	14.25	16.58	16.80	16.90
Thuja orientalis (Rajasthan)	8.98	10.25	12.58	14.25	15.65	15.90
Thuja orientalis (Himachal Pradesh)	6.89	8.25	10.25	12.25	13.25	13.80

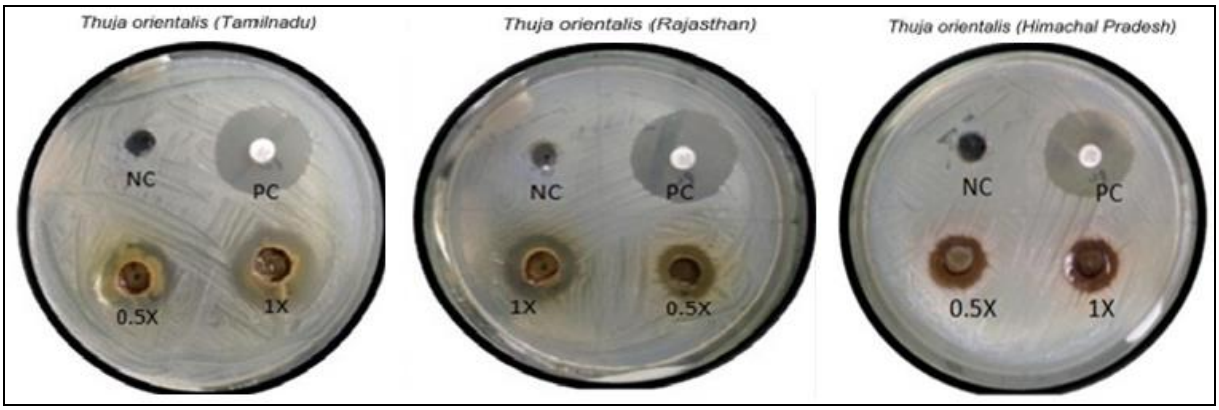

Fig 2: The results of antimicrobial activity hydroalcoholic extract of *Thuja orientalis* aerial parts against *Staphylococcus aureus*

Table 3: The results of antimicrobial activity hydroalcoholic extract of *Thuja orientalis* aerial parts against *Escherichia coli.*

Name of Bacteria	Microbial activity / Zone of inhibition reading (mm)					
	31.25 (µg/ml)	62.5 (µg/ml)	125 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)
Control (Negative)	---	---	---	---	---	---
Streptomycin (Positive)	14.25	15.25	16.25	16.50	16.80	16.90
Thuja orientalis (Tamil Nadu)	6.05	7.11	8.25	9.21	9.30	9.25
Thuja orientalis (Rajasthan)	4.32	5.20	6.12	7.12	7.20	7.25
Thuja orientalis (Himachal Pradesh)	3.25	4.12	5.52	6.54	6.67	6.50

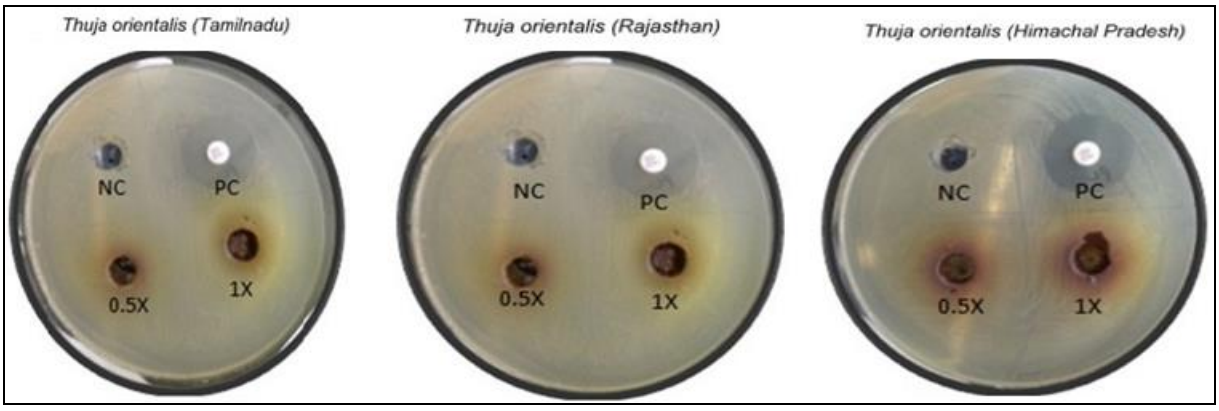

Fig 3: The results of antimicrobial activity hydroalcoholic extract of *Thuja orientalis* aerial parts against *Escherichia coli.*

5. Conclusion

The results of the GC-MS analysis of hydroalcoholic extract of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh are presented with the help of various Figures. It is evident from Figures, *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh showed similar resolution of peaks with same retention time. The each peak in GC-MS profile of hydroalcoholic extract of *Thuja orientalis* aerial parts represent maximum area under the curve in Tamil Nadu sample followed by Rajasthan and Himachal Pradesh samples. These observations suggest that Tamil Nadu sample of *Thuja orientalis* aerial parts contained higher amount of phytoconstituents than Rajasthan and Himachal Pradesh samples. The geographical variation in quercetin content was

determined in various samples of *Thuja orientalis* aerial parts obtained from wild areas of different states Tamil Nadu, Rajasthan and Himachal Pradesh. The sample collected from Tamil Nadu region was found to contain maximum content of quercetin followed by Rajasthan and Himachal Pradesh.

The results of antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* showed that hydroalcoholic extract of *Thuja orientalis* aerial parts collected from Tamil Nadu exhibited higher antimicrobial activity followed by plant collected from Rajasthan and Himachal Pradesh as compared to standard drugs. The exhaustive survey of literature suggested that flavonoids such as quercetin, rutin, luteolin and naringenin have been scientifically claimed as potential antimicrobial activity. Thus, finally it can be concluded that antimicrobial activity of hydroalcoholic extract of *Thuja orientalis* aerial parts may be due to presence of phenolic and flavonoids. Medicinal and aromatic plant species are widely distributed due to a variety of climatic factors and altitudinal variations coupled with varied ecological habitats. On basis of results it may be concluded that plants grown in different biosphere may have variation in their chemical constituent as well as activity as was observed in case of *Thuja orientalis*, collected from various places. However, further detailed studies are needed for establishing this fact. Dunal more rationally and further opened the scope for development of novel phytoconstituents therapeutic drugs from the plant, which may serve as improved therapeutic agents and can create an awareness of the need of in situ conservation of this most wanted medicinal plant.

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