



## Carotenoid and Antibacterial Analysis of *Thuja Occidentalis*

### KEYWORDS

Carotenoid, Secondary Metabolite, Biodiversity, Medicinal Plant, *Thuja occidentalis*

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**ABSTRACT** Carotenoid are secondary metabolite included in class of tetraterpenoids. This study conducted for analyze the carotenoid content of methanol and ethanol leaf extract of *Thuja occidentalis*. It revealed to determine the amount of total carotenoid in each leaf extract in addition to this study compared the effectiveness of leaf extract against both gram positive and negative bacteria and related to total carotenoid content to antibacterial activity. *Thuja occidentalis* Leaves shows a good antibacterial activity against these selected bacterial species such as *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloquifaciens*, *Proteus vulgaris* and *Salmonella typhi*. But the more effective against *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus amyloquifaciens*, *Proteus vulgaris* and *Salmonella typhi*. The total carotenoid content of leaf extract was measured using this method of Tao et al. This result revealed that methanol and ethanol leaf extract have 11.25617 ug /gm, 8.9881 ug /gm of total carotenoids respectively. Methanol extract which has the highest total carotenoid content also exhibited highest zone of inhibition observed in *Bacillus amyloquifaciens* and *Salmonella typhi*. These results revealed that there might be some secondary metabolite which are present in the *Thuja occidentalis* plants which have more potent antibacterial activity than other parts of *Thuja occidentalis* plant as compare to the leaf extract. There is no report found on carotenoid and antibacterial analysis of *Thuja occidentalis*.

### Introduction

A large portion of the world population especially in developing countries depends on the traditional systems of medicine to treat a variety of diseases and its cure by several hundred genera of plants are used as medicine value (McGaw et al; 2000). The world health organization (WHO) reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies. Which involve the use of plant extracts or their active constituents (Ahmad & et al; 1998).

The knowledge of the use of plants as a source of medicine is as old as humanity. Vast ethnobotanical knowledge exists in India from ancient time. More than 1500 herbal plants are mentioned in ancient Indian texts. India is one of the 12 megadiversity countries (Nayar, 1996) and it is gifted with enormous wealth of biodiversity. It has diverse climatic zones and wealth of vibrant ethnomedicinal traditions. There are about 45,000 plant species recorded in India so far (MoEF, 1994).

*Thuja occidentalis* L. called the tree of life is an endemic plant in eastern North America and cultivated in northern Europe and in Brazil as ornamental tree. (Chang LC & et al; 2000). The plant is highly used by rural people in curing various disorders. *Thuja occidentalis* has an effective natural origin that has a tremendous future for research as the novelty and applicability of *Thuja occidentalis* are still hidden. Such things can be overcome through modern scientific research. The plant was first identified as a remedy by native Indians during a 16th century expedition and was found to prove effective in the treatment of weakness from scurvy (British Herbal Pharmacopoeia. *Thuja*, British Herbal Medicine Association, West Yorks, UK 1983). In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhoea and rheumatism (Shimada K & et al; 1956)

The most important of the metabolites present in this medicinal plant are the flavonoids (Naser B & et al; 2005), polysaccharides (Naser B & et al; 2005) and constituents of the essential oils, characteristic of the *Thuja* genus (Svajlenka E & et al; 1999) including diterpenes, monoterpenes ( $\alpha$ -thujone,  $\beta$ -thujone, fenchone), and a sesquiterpene (Chang LC & et al; 2000). The polysaccharides as well as flavonoids (kercetin, campherol), tannins and proteins were identified by phytochemical investigations in methanolic and ethanolic fraction of aerial part of *T. occidentalis* used in several pharmacological studies conducted by Dubey & Batra for hepatoprotective, antidiabetic, anti-ulcerative, antioxidant and hypolipidemic activities (Dubey SK & et al; 2009). In addition of flavonoids also are involved with the anti-tumor activity

This study describes various traditional and medicinal utility of the chemical composition and antibacterial activity of the plant and its constituents.

### Material and Method

#### Collection of plant

The plant material was collected from the Ganpat University in *Thuja occidentalis* L. leaves, kherva-384012, Dist: Mehsana, Gujarat. This Plant species was identified by G.L.Shah; Flora of Gujarat. The collected leaves were dried and grind with mechanical grinder and stored at 4° C for further used.

#### Sample Preparation

The collected 10gm leaves powder and dissolved in respective solvent methanol and ethanol. After the extract put on the magnetic stirrer for 4 hours. After this 4 hours extract filtrate by using Whatman filter paper no.1 and centrifuge 5000-6000rpm for 15 min in 25°C and supernatant are collected for further analysis of Carotenoid and antibacterial activity. (N.Ramman; 2006).

**Carotenoid analysis**

0.5gm plant powder dissolved in 10 ml respective solvent methanol and ethanol using 50ml centrifuge tube and this extract repeated for three times vortex in 1min. after this supernatant was collected and makeup measure volume and adjust 100ml in volumetric flask in respective solvent. It measured O.D at 450nm by using U.V spectrophotometer

$$Total\ Carotinizd = \frac{\frac{Visible(V)}{Absorbance(A)} - 0.00051}{0.175}$$

**Antibacterial Activity**

**Organism used for antibacterial activity**

Ten organism used for antibacterial analysis. This strain collected from Mehsana urban institute of sciences, Ganpat university-384012. All strain identified by microscopic and biochemical identification using berger's manual of systematic of bacteriological. All Strain was preserve in nutrient agar slant and stored at 4°C at our laboratory.

**Sample Preparation for Antibacterial analysis**

For Sample preparation 10gm leaf extract powder dissolved in 100 ml respective solvent methanol and ethanol. For antibacterial analysis sample was taken 10µl to 50µl respective organism. each concentration of sample extract contain 0.00001mg/ml to 0.00005 mg/ml

**Antibacterial Analysis using disc diffusion method**

The disc diffusion method was used for analysis of antibacterial activity of the plant extract against four gram positive bacteria; Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Bacillus amyloquifaciens and six gram negative bacteria; Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi-A, Salmonella paratyphi-B collected as pure cultures from the our MUIS laboratory; Ganpat university.

For analysis disc containing 10µl to 50µl concentration of the plant extract used and residual solvents were completely evaporated. Absolute methanol and ethanol used as control. Further plates were incubated at 37°C for 24hours. To allow maximum growth of the organism. This antibacterial activity of test sample was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken. (Rakesh patel ;2008)

**Result**

This plant was collected from the ganpat university in Thuja occidentalis L. leaves, kherva-384012, Dist: mehsana, Gujarat. This Plant species was identified by G.L.Shah; Flora of Gujarat.



Fig.1 : Thuja occidentalis L.

**Determination of MIC Antibacterial activity on Thuja occidentalis:**

Name of organism	Concentration of plant extract in µl				
	10	20	30	40	50
E.coli	16mm	17mm	20mm	21mm	14mm
Bacillus subtilis	10mm	12mm	15mm	15mm	13mm
Bacillus cerus	-	-	-	-	-
Bacillus amyloliquifaciens	11mm	12mm	13mm	17mm	27mm
Bacillus megaterium	10mm	10mm	10mm	14mm	15mm
Pseudomonas aeruginosa	-	-	-	-	10mm
Proteus vulgaris	10mm	12mm	14mm	15mm	13mm
Salmonella typhi	18mm	21mm	21mm	23mm	26mm
Salmonella paratyphi A	16mm	23mm	24mm	25mm	22mm
Salmonella paratyphi B	12mm	15mm	15mm	18mm	20mm

Table:1 Zone of inhibitory activity (in millimetre) of Thuja occidentalis leaf extracts for methanol against microorganisms.

**Table: 2 Zone of inhibitory activity (in millimetre) of Thuja occidentalis leaf extracts for ethanol against microorganisms.**

Name of organism	Concentration of plant extract in $\mu$ l				
	10	20	30	40	50
E.coli	10mm	13mm	13mm	14mm	27mm
Bacillus subtilis	12mm	11mm	14mm	12mm	14mm
Bacillus cerus	-	-	-	-	-
Bacillus amyloliquifaciens	10mm	12mm	15mm	17mm	13mm
Bacillus megaterium	10mm	12mm	14mm	15mm	15mm
Pseudomonas aeruginosa	-	-	-	-	10mm
Proteus vulgaris	10mm	12mm	13mm	14mm	15mm
Salmonella typhi	-	-	-	-	10mm
Salmonella paratyphi A	-	-	-	-	-
Salmonella paratyphi B	-	-	-	-	10mm

**Total Carotenoids analysis:**

Different solvent	O.D at 450nm	$\mu$ g/ml
Methanol	0.826	11.25617 $\mu$ g/ml
Ethanol	1.001	8.9881 $\mu$ g/ml

**Table:3 Total carotenoid in O.D at 450nm****Discussion**

The Thuja occidentalis L. antibacterial activity was observed against both gram negative and gram positive bacteria. Thuja occidentalis leaves shows a good antibacterial activity against these selected bacterial species such as Bacillus subtilis, Bacillus megaterium, Bacillus amyloliquifaciens, Proteus vulgaris and Salmonellatyphi. the more effective against Bacillus subtilis, Bacillus megaterium, Bacillus amyloliquifaciens, Proteus vulgaris and Salmonellatyphi and 27mm Zone of inhibition observed in Bacillus amyloliquifaciens and 26mm Zone of inhibition observed in Salmonellatyphi by using Methanolic extract of Thuja occidentalis which are showing better activity than using other solvent like ethanol.

From these results we concluded that there might be some secondary metabolite which had present in the Thuja occidentalis plants which had more potent antibacterial activity. Thuja occidentalis leaves shows a high antibacterial activity against both gram negative and gram positive bacteria especially Salmonellatyphi and Bacillus amyloliquifaciens compared to other selected bacteria such as Bacillus subtilis, Bacillus megaterium, Proteus vulgaris etc. From this above results proved that thuja occidentalis contain various phytochemical constituents like flavonoids, terpenoids, alkaloids, tannins, glycosides etc. due to flavanoid carotenoid content of the thuja occidentalis extraction in ethanol has the lowest content of total carotenoid compared to methanolic extraction of thuja occidentalis leaves. This methanol and ethanol leaves extract less zone of inhibition observed against various bacterial strain like Bacillus subtilis, Bacillus megaterium, Bacillus amyloliquifaciens, Proteus vulgaris and Salmonella typhi.

Above result concluded that Thuja occidentalis L. leaves used as a great source of carotenoid content Kritchevsky et al. suggested that Carotenoid content used as a health beneficial secondary metabolite compound present in thuja occidentalis leaves. It could be effective inhibitor of various disease like heart disease and cancer (Kotikova, Z & et al; 2009). However this studies have shown that carotenoids have potential antibacterial activity.

**REFERENCE**

- Ahmad, I. and Mehmood, Z. and Mohammad, F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethno. 62:183-193;1998 | British Herbal Pharmacopoeia. Thuja, British Herbal Medicine Association, West Yorks, UK; 1983 | Chang LC, Song LL, Park EJ, Luyengi L, Lee KJ, Farnsworth NR, Pezzuto JM, Kinghorn A D: Bioactive constituents of Thuja occidentalis. Journal of Natural Products ; 63 (9): 1235-1238;2000. | Dubey S.K. and Batra, A. Antioxidant activity of Thuja occidentalis linn. Asian Journal of Pharmaceutical and Clinical Research, 2: 73-76;2009 | Kotikova, Z., Hejtmankova, A., and Lachman, J., Determination of the influence of variety and level of maturity on the content and development of carotenoids in tomatoes, Czech J. Food Sci., 27, 200 - 204;2009. | K.R.ANEJA, An introduction to microbiology; 2007. | Krinsky NI. Carotenoid protection against oxidation. Pure Appl Chem 51:649-660;1979. | McGaw, L.J., Jager, A.K. and Staden, J.V. Antibacterial, anthelmintic and Anti-amoebic activity in south Africa medicinal plants. J. Ethno. 72: 247-263;2000. | MoEF. Biodiversity country studies, strategies & action plan. Ministry of Environment & Forest, Government of India. p 48;1994. | Nayar, M.P. Ho! Spots of Endemic Plants in India, Nepal and Bhutan. SB Press, Trivandram, India;1996 | Naser B, Bodinet C, Tegmeier M, Lindequist U: Thuja occidentalis (Arbor vitae): A Review of its pharmaceutical, pharmacological and clinical properties. Evidence-based complementary and alternative medicine ; 2 (1): 69-78;2005 | N. Raaman, Phytochemical Techniques; 2006. | Svajdlenka E, Mártonfi P, Tomasko I, Grancai D, Nagy M: Essential oil composition of Thuja occidentalis L. samples from Slovakia. Journal of Essential Oil Research ; 11: 532-536;1997. | Tao N., Gao Y., Liu Y., and Ge F., Carotenoids from the Peel of Shatian Pummelo (Citrus grandis Osbeck) and its antimicrobial activity, American - Eurasian J. Agric. And Environ Sci., 7(1), 110 - 115;2010. |