International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html

Techniques for *Thuja* essential oil extraction and production of active chemical derivatives: A review study

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Abstract

Essential oils are complex mixture of biologically active compounds and are used in flavor, fragrance, natural medicine, and several other industries from many centuries. Currently, their significance is further highlighted due to their intensifying response for foodstuff, cosmetics, and pharmacological productions. Anti-biotic, anti-fungal and anti-oxidant potentials of essential oils have been shown recently by several scientific studies. The present study critically reviews, *T. orientalis* and thujone importance as active ingredients in preparations used to overcome many health issues. Microbial/worm infections can be effectively controlled with orientalis preparations. The bioactivities of *T. orientalis* are due to the presence of hydrocarbons, monoterpenes and oxygenated sesquiterpenes. *T. orientalis* essential oil exhibited significant antioxidant, cytotoxic and anti-microbial activities in various *in-vitro* tests and experimental models. Nebulization of *Thuja orientalis* essential oils vapors or smoke of *Thuja orientalis* leaves can be a useful remedy against Coronavirus (COVID-19). It could be quite useful aid in reducing severity of Coronavirus infections and thus help patient immunity to respond better.

Key words: *Thuja orientalis*, anti-microbial agents, insect repellent, *Thuja* oil derivatives

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1. Introduction

Essential oil is known as a concentrated hydrophobic liquid extracted from plants with a volatile aroma [1-19]. The oil is called essential because it has a distinctive plant smell [20]. Essential oils are generally liquid, aromatic and have a congenial smell and tincture. The term "essential oil" is frequently used as a substitute for perfume oil, ingredient, or compound in the scent industries. Essential oils are secondary metabolites [21] and used as anti-septic, anti-viral, anti-fungal [22], anti-bacterial [23] and insect repellent [24]. They contain mostly hydrocarbons such as terpenes (isoprene) and terpenoids and usually present less than 5 percent by weight to the weight of the dry plant material. They are the product of hydrocarbon terpenes that are oxygenated like esters, alcohols, phenols, ketones, acids, aldehydes and ethers [25-26]. Some terpenes are effective drugs for diseases such as tumors [27], heart disease and malaria. Others have insecticidal characteristics. The main complexes are monoterpenes (which have 10 carbon atoms and indicate more than 80% of essential oils), and sesquiterpenes (with 15 carbohydrate carbons). The compounds present in essential oils are of two categories (i) acyclic configurations (ii) isoprenoids [28]. In particular, the extraction of essential oil depends on oil diffusion rate through the tissues of the plant to an exposed surface from which a series of processes can remove the oil. However, composition also vary with different seasons [29]. Depending on the stability of the oil, different methods exist for obtaining oil from herbal material. The most significant procedures for accomplishing essential oils from plants remain steam distillation and hydro-distillation. The procedure of liquefied carbon dioxide or microwaves, low or high pressure extraction via hot water or hot steam are other procedures used for insulation of essential oils [20].

Many herbal plants were tested and used for commercial purposes for their potential essential oil. Essential oils are widespread and varied, for the fragrance and flavoring of consumer finished products in many industries such as: cosmetics and scent, infusions, ice creams, confection, and baked foodstuffs. Currently, about 300 essential oils out of 3,000 are commercially important for the pharmaceutical, agriculture, food, cosmetics, and perfume industries. Some of the essential oils or their bioactive components such as limonene, geranyl acetate and carvone are important ingredients of hygienic products. Essential oils are used in various medicines [30]. They also act as food additives and are used in several diseases in folk medicine systems [31]. The chemical study of essential oils has been changed completely by the rapid progress made in chromatographic and spectroscopic technologies. For example, many methods have influenced for the study of biochemical summaries of essential oil. Many techniques have been used for studying the chemical profiles of essential oils such as ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC) [32]. The increasing significance of essential oils in different areas of social activity, comprising drugstore, perfumery, aromatherapy, greasepaints, foodstuff and beverage manufacturing, has led to the extensive need of consistent procedures for the analysis of essential oils. Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) techniques have fulfilled these requirements satisfactorily. Gas chromatography has proven to be an effective way to characterize essential oils [25-33]. The combination of gas chromatography and mass spectrometry (GC-MS) makes it possible to quickly and trustworthily identify essential oil components [34-36].

Thuja orientalis L. belongs to the ever-green coniferous plants of the Cupressaceae family. *Thuja orientalis* includes quercetin, flavones, myricetin, carotene, ascorbic acid, and xanthophyll. It is a flavorful tree cultivated in moderate areas of Taiwan, India, Korea, Pakistan and Iran. Different parts of *T. Orientalis* in traditional Korean medicine [37]. In addition, *Thuja* is used to treat skin, intestinal tract, blood, brain, spongy tumors, warm outgrowths, and kidney infections. The extensive literature survey shows that diterpenoids, flavonoids, phenolic compounds and essential oils from this plant have shown significant cytotoxicity against cancer cells, neuroprotective, anti-oxidant, anti-inflammatory, anti-fungal, anti-bacterial and anti-diabetic activity [38].

2. Essential oils of *Thuja*

The essential oil of *Thuja orientalis* (Figures 1-2), obtained from the leaves and branchlets was used as an anti-rheumatic, astringent, diuretically, emmenagogue, expectorant, insect repellent, rub-facing, stimulant and vermifuge substance [39]. *Thuja's* essential oil toxicity can kill the pests and keep them away from households or areas where it has been used. The inhabited insects are cockroaches, ants, mosquitoes, lice, flea and bed bugs. The expensive, synthetic chemicals can be replaced with *Thuja* sprays, fumigants, and vaporizers that repel mosquito and cockroach [40]. The main components of essential oil of *Thuja* were found to be α-humulene, α-pinene, sabinene, limoneme, α-terpinolene, α-terpinyl acetate and cedrol. *Thuja* oil consists of thujone, which has poisonous characteristics. *Thuja's* essential oil toxicity kills larvae and can remove worms such as roundworms, tapeworms and hookworms, which can lead to uncomfortable and dangerous health conditions [41]. In androgenic alopecia, acne, hirsutism etc., *Thuja* flavonoids and diterpenes may be beneficial. *Thuja orientalis* components were previously assessed by gas chromatography and mass spectroscopy (GC-MS) [42].

3. Methods of extraction

Essential oil separation methods may be classified as enfleurage, steam extraction, solvent extraction, hydrodistilation and supercritical fluid extraction. Hydrodistillation and steam distillation is the frequently used physical techniques for isolating essential oil from plant materials [43].

3.1. Steam distillation method

Steam distillation can separate volatiles from non-volatile compounds by preventing extreme temperatures [44]. The displacement of atmospheric oxygen through steam, protects complexes from corrosion, is another major advantage of this technique. The disadvantage of steam distillation is that hydrolysable complexes such as esters and thermally labile constituents may be broken down through the extraction method. Moreover, more polar components may be partially lost because of their water affinity. Although, steam distillation is popular with commercially isolated essential oils and this process produces 93 percent of the oils, it is not a favored technique in the research laboratory. This is possibly due to unavailability of the steam generator and appropriate distillation vessels [45].

3.2. Hydrodistillation method

During the hydrodistilation process, the material is submerged in a liquid which is heated to the boiling point by an exterior heat resource. In the hydro and steam extraction procedures, the vapors can be condensed and the oil can be separated from the aqueous phase [46].

3.3. Microwave-assisted hydrodistilation extraction

It is the most recent technique used to recover volatile components. In this method, plant material placed in a Clevenger type apparatus is heated inside a microwave oven for a short time to extract the essential oil. Heat is produced by microwave energy. The sample reaches its boiling point very rapidly, leading to truly short extraction or distillation time. Both hydrodistilation and microwave distillation procedures were used to isolate the essential oil of *Thuja orientalis* [47].

3.4. Supercritical fluid extraction

Supercritical fluid extractions involve the use of carbon dioxide for the purpose of essential oil isolation. This technique usually produced higher quality essential oils having aroma close to the material used for the extraction.

4. Biological effects of *Thuja orientalis*

Essential oil generally show anti-oxidant [48] and anti-microbial [49] activities due to the presence of some specific chemical constituents. The chief components of *Thuja orientalis* essential oils are found to be mono and sesquiterpenes, containing phenols, alcohols, aldehydes and ketones. *Thuja orientalis* is consumed internally for treating toxins, bleeding, coughs, asthma, skin infections, skin dysenteries, arthritis, and premature blandness. The leaves are anti-pyretic, astringent, diuretic, emmenagogue, softener, sputum, and coolant. Their usage is said to enhance the progress of hair. The twigs are used to treat toxins, colds, dysentery, rheumatism, and skin parasites [50].

4.1. Anti-bacterial activity

T. orientalis very effectively inhibited the growth of serotype c and d *Salmonella mutans* [50].

4.2. Anti-fungal activity

The essential oil of *Thuja orientalis* have shown anti-fungal activity against human pathogenic fungi [51].

4.3. Anti-viral activity

Thuja orientalis essential oils were estimated for its inhibitory activities against Sever Acute Respiratory Syndrome Coronavirus (SARSC) and Herpes Simplex Virus Type-1 (HSV-1). Initial studies performed by Dr. Hanif showed that nebulization of *Thuja orientalis* essential oils vapors or smoke of *Thuja orientalis* leaves can be a useful remedy against Coronavirus (COVID-19). It could be quite useful aid in reducing severity of Coronavirus infections and thus help patient immunity to respond better. A previous study showed that *T. orientalis* can be used as a strong anti-viral agent against plant and animal viruses [52].

4.4. Inflammatory

Thuja orientalis has an anti-inflammatory activity which is at least in part, is due to the decrease in the tumor necrosis factor induced endothelial adhesion to monocytes by inhibiting intracellular reactive oxygen species production, nuclear factor kappa B (NF-kB) activation and cell adhesion molecule in Human umbilical vein endothelial cells (HUVECs) [53].

4.5. Anti-cancer activity

Strong 5α-reductase inhibitor was extracted from *Thuja orientalis* and fractionated in isolated form as diterpene. The inhibitors were consumed either alone or as vital components of medicines to treat diseases caused by excessive 5α-reductase or hyper-secretion of androgens, including male baldness, androgen ethic alopecia, hirsutism, acne, and prostate cancer. *Thuja orientalis* anti-cancer potential was identified in malevolent melanoma cell line A375 [54].

4.6. Larvicidal activity

Larvicidal activities of *Thuja orientalis* essential oils against fourth-star larvae *Aedes aegypti* and *Culex pipiens pallens* had been observed previously. Leaf oil activity was greater than stem oil, fruit oil, and grain oils in *Thuja orientalis*. At 400 ppm, *Thuja orientalis* caused a mortality of 100% and 71.6% against *A. aegypti*. The larvicidal activity was observed from various age class (I-III). High levels of mortality were found in age class II [55].

***4.7. Insecticidal* activity**

Thuja orientalis leaf extracts showed a repellant activity against *Chilo partellus* [56].

4.8. Nematicidal activity

Mortality in *Meloidogyne incognita* egg juvenile in three intervals was caused by the ethanol extract from *Thuja orientalis* leaf (20, 40, 60 and 80 percent). The link among the strength of the plant extract and the number of hatched tadpoles was linear. The young deaths were directly related to the extract strength [57].

4.9. Molluscocidal activity

Ethanol extract of *T. Orientalis* leaf (LC50: 32.74 mg/l) and purified column fraction (LC50: 29.25 mg/l) have potent molluscicidal action against *Lymnaea acuminata*. Thujone (LC50: 08.09 mg/l) has been identified as an active molluscicidal component in the *Thuja* essential oils. The molluscicidal actions of *Thuja* leaf or fruit and their active constituents purified fraction by means of synergetic piperonyl butoxide (PB) or N-Octyl bicycloheptene dicarboximide (MGK 264) was examined in binary combination (1:5) against *Lymnaea acuminata*. Mixture of *Thuja* leaf/thujone or fruit/column extract of *Thuja* fruit with PB or MGK-264 showed synergistic toxicity upto 189.02 times. The toxicity of the binary mixture increased hundreds of times because its components show synergistic action [58].

5. Economic value

Thuja has been used in many ways throughout the history. It was used like incense in ancient civilizations in rituals. Cough, cystitis, fever, intestinal parasites and venereal diseases were treated with *Thuja* leaf decoctions. For gout, psoriasis, rheumatism, verrucae, warts and more, ointments made from different parts of *Thuja* were used. *Thuja* oil is still used in a number of industries. It is used as a counter-irritant in pain relief products. It is used in pharmaceutical disinfectants, sprays, in some perfumes, toiletries as a fragrance and in many foods as a flavoring agent [39]. *Thuja* is used for respirational tract toxicities like bronchitis, skin, and cold infections. It is also used in the treatment of painful conditions such as arthritis and nervous disturbances which affect the face. Some people use the Thuja to loosen mucus (as an expectorant), to boost the immune system, and to increase urine flow (as a diuretic). Thuja is occasionally useful directly to the skin for joint agony, osteoarthritis, and muscle pain. *Thuja* oil is also used in warts and cancer. Thuja is used in food and beverages as an additive agent. Thuja is also used in cosmetics and soap manufacturing [40].

Figure 1: *Thuja orientalis* mature plant

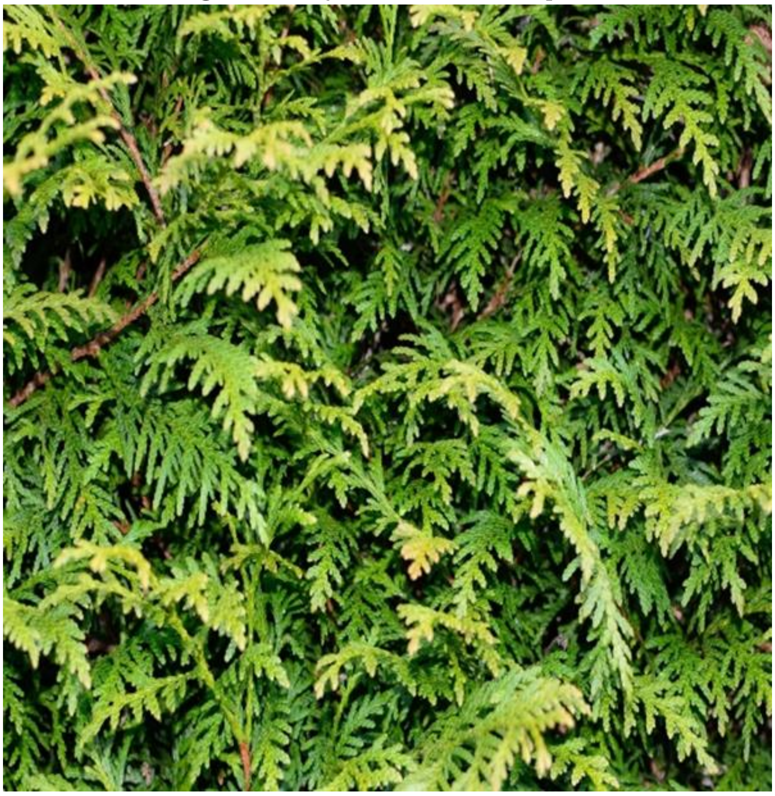

Figure 2: Thuja plant leaves

6. Biological actions of *Thuja* oil

The chief components of *Thuja orientalis* fresh leaves essential oil extracted through hydrodistillation were investigated by gas chromatographic analysis. About twenty two compounds representing 94.0% of the total oil had been identified. The main components of leaf oil were α-pinene

(29.2%), alpha-cedrol (9.8%), caryophyllene (7.5%), al-humulene (5.6%), limonene (5.4%), alpha-terpinolene (3.8%) and alpha-terpinyl acetate (3.5%). Anti-fungal activity was demonstrated by essential oil against *Alternaria alternata* in a direct biological autographic test. Two main bioactive compounds b_1 (RF=0.54) and b_2 (RF=0.80) were observed and tested for anti-fungal actions against *Alternaria alternata* in a direct bioautographic assay. They produced an inhibition zone with the diameter of 5 mm and 10 mm, respectively. The preparatory thin layer chromatography was used to further purify components b_1 and b_2 and their anti-fungal efficacy was re-tested. The minimum inhibitory amount of b_1 and b_2 against *A. alternata* was determined as 30.5 and 4.5µg, respectively, using a bioautography test. The bioactive constituent corresponding to b1 was determined as α-cedrol by using GC/MS analysis [59].

The essential oil of *Thuja orientalis* from seed coats obtained in a yield of 1% had been studied for its anti-microbial actions against six bacteria and five fungi using filter paper disc agar diffusion technique. The oil had shown good to moderate action of the oil against all the six test bacteria. The observations for the fungal organisms revealed that the pure essential oil of *T. orientalis* had exhibited exceptionally good activity against *C. lunata*, *A. niger* and *A. fumigatus*. The oil had shown poor activity against *R. oryzae* and *F. psidi.* The oil had good activity in 1:100 and 1:1000 dilutions against *A. niger*, *C. lunata*. Detailed future studies are required to explore anti-microbial activities of *T. orientalis* [60].

7. Biological actions of *Thuja* derivatives

The reaction of α-and β-thujones to dansyl hydrazine (DNSH) has been investigated using reverse phase liquid chromatography (LC) with fluorescence detection. These derivatives have shown neurotoxic effects on worms [61].

8. Conclusions

The results of previously done research have shown that *Thuja* leaves are used ethno-botanically and have considerable prospective against several diseases. *Thuja* oil and its derivatives could also be used anti-microbial agents after further thorough investigations. The most abundant compounds in *Thuja* can be extracted by the hydrodistillation and steam distillation methods.

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