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

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Abstract

Essential oils are composite of biologically active ingredients used as flavoring ingredients and as components for many commercial products for a long time now. Currently, their significance is further highlighted due to their intensifying response for foodstuff, cosmetics and pharmacological productions. Antibiotic, antifungal and antioxidant potential of essential oils has been shown recently by the scientific examination. Given the many uses of essential oils, their characterization is very important on the basis of their chemical profiles. The present study critically review *T. oriental* and thujone which are very active ingredients in preparations used to overcome a number of health issues. Microbial /worm infection can be effectively controlled with Orientalis preparations. The essential oils were hydro distilled and distinguished by their physical and biological activity. The chemical components of the extracted oils were investigated primarily by GC and GC-MS, which revealed the presence of hydrocarbons, monoterpenes and oxygenated sesquiterpenes. The essential oils examined showed good measured antioxidant, cytotoxic and antimicrobial activities of various in-vitro tests and experimental models.

Key words: *Thuja orientalis*, antimicrobial agents, insect repellent, *Thuja* oil derivatives

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1. Introduction

Essential oil is known as a concentrated hydrophobic liquid from plants with a volatile aroma. These are ethereal oils, such as rosemary oil, also known as "oil of" the plant from which it is isolated. The oil is called essential because it has a distinctive plant smell. From the practical viewpoint, these constituents can be demarcated as the odious organisms of an oily natural, which are almost completely derived from vegetable tissues [1]. The name of the source plant is usually identified as an essential. Essential oils are liquid, aromatic generally 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 maquillages and scent industries. Essential oils are secondary metabolites [2] and used as antiseptic, antiviral, antifungal [3], antibacterial [4] and insect repellent [5]. They contain mostly hydrocarbons such as terpenes (isoprene) and terpenoids and designate a small ratio of plant configuration (less than 5 percent of the dry material of the plant). They are the product of hydrocarbon terpenes that are oxygenated like esters, alcohols, phenols, ketones, acids, aldehydes and ethers [6-7]. Some terpenes are effective drugs for diseases such as tumors [8], heart disease and malaria. Others have insecticide 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), which are compounds of essential oils of two categories. They may be acyclic configurations of hydrocarbon. The second ones, also called isoprenoids [9]. 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 [10]. 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 is other procedures used for insulation of essential oils [1].

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 and ice creams and confection and provide baked foodstuffs. Currently about 300 essential oils out of 3,000 are commercially important for the pharmaceutical, agriculture, food and sanitation, cosmetics and perfume industry. 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 [11]. They also act as food additives and are used to consider in several diseases in folk medicine systems [12]. In determining its reliable and accurate compositional data, the complexity of essential oils is an actual challenge. The chemical study image of essential oils has been changed completely by the rapid progress made in chromatographic and spectroscopic technologies. For example, many methods have been 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 UV, IR spectroscopy, NMR spectroscopy and gas chromatography [13]. 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. GC and GC-MS techniques have fulfilled these requirements satisfactorily. Gas chromatography has proven to be an effective way to characterize essential oil [6-14]. The combination of gas chromatography and mass spectrometry (GC - MS) makes it possible to quickly and trustworthily identify essential oil elements [15-17].

Thuja orientalis L. belongs to the ever-green coniferous plants of the Cupressaceae family. *Thuja orientalis* includes quercetin, ado flavone, hinokiflavone, myricetin, carotene, ascorbic acid and xanthophyll. It is a flavorful tree cultivated in moderate areas of Manchuria, Taiwan, India, Korea, Pakistan and Iran. Different parts of *T. Orientalis* in traditional Korean medicine was used as expectorant, hemostatic, hypotensive, bleeding, dermatitis, gout and chronic tracheitis [18]. In addition, *Thuja* is rarely used to treat skin, intestinal tract, blood, brain, spongy tumors, warm outgrowths and kidney infections. The extensive literature shows that diterpenoids, flavonoids, phenolic compounds and essential oil from this plant have shown significant cytotoxicity against cancer cells, neuroprotective, antioxidant, anti-inflammatory, antifungal, antibacterial and anti-diabetic activity [19].

2. Essential oils of *Thuja*

The essential oil of *Thuja orientalis*, obtained from the leaves and branchlets was used as an anti-rheumatic, astringent, diuretically, emmenagogue, expectorant, insect repellent, rub-facing, stimulant and vermifuge substance [20]. *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. These expensive, synthetic chemicals can be replaced with sprays, fumigants, and vaporizers that repel mosquito and cockroach [21]. 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 theoretically poisonous characteristics and was premeditated for its antagonistic receptor GABA (c-amino butyric acid). *Thuja's* essential oil toxicity kills larvae that might have infested the organization due to the presence of thujone. It can remove worms such as roundworms, tapeworms and hookworms, which can lead to uncomfortable and dangerous health conditions. Biochemical ingredients of *Thuja orientalis* like flavonoids and terpenoids have shown the natural accomplishments. The oil of *Thuja* herbs compels the 5α-reductase activities [22]. In androgenic alopecia, acne, hirsutism etc., its flavonoids and diterpenes may be beneficial. The essential oil extracted from *Thuja orientalis* shrubs remained separated by the hydro - distillation method and assessed by gas chromatography and mass spectroscopy (GC - MS) [23].

3. Methods of extraction

Essential oil separation methods may be classified as enfleurage, steam extraction, solvent extraction, hydrodistilation and supercritical fluid removal. Hydrodistilation or Steam Distillation is the frequently used physical technique for isolating essential oil from botanic substance [24].

3.1. Steam Distillation Method

The principle of steam distillation is that two immiscible liquids, when mixed, each exerts a vapor pressure, as if each liquid were pure. Therefore, the boiling mixture's entire vapor pressure equals that amount of the partial pressure from each mixture component. When the total vapor pressure influences the atmospheric pressure, the mixture begins to boil. The boiling point of the mixture is thus extended at a lesser temperature than the boiling points of the distinct constituents. Thus, by decreasing boiling point and by preventing extreme temperatures, steam distillation can separate volatiles from non-volatile compounds [25]. The displacement of atmospheric oxygen through steam, protecting complexes from corrosion, is another major advantage of this technique. The disadvantage is that hydrolysable complexes such as esters and thermally labile constituents may be broken down through the extraction method. Moreover, more polar petroleum 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 [26].

3.2. Hydrodistilation 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. Cautions must be taken to certify that the oil's most volatile components are not lost in the efficient condensation of steam. The literature gives information on the importance of hydraulic and steam distillation processes. The plants and water are combined in the hydro-distillation and the whole thing is boiled. The hot water draw out the oils just as steam does, and it is carried out to the condenser and cooled into hydrosol and essential oils. This method produces a finer, more complete product, as hot water is collar than steam distillation and shocks the plant material less. The highest yield was obtained with hydrodistilation method at smaller scale [27].

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 very short extraction or distillation. Both hydrodistilation and microwave distillation procedures were used to isolate the essential oil of *Thuja orientalis* [28].

3.4. Supercritical Fluid Extraction

A researcher reported that the extraction of essential oils from *Thuja orientalis* leaves obtained from the GC / MS analysis showed different composition by steam distillation (SD), microwave distillation (MO) and supercritical (SC) CO_2. The authors indicated that the chief constituents of the three essential oils had the similar effective quantities. The period of the distillation procedure is also a significant parameter which affects the performance and configuration of essential oils. Long distillation cycles need to be avoided as at the end of a cycle there is only a minor increment in the oil vintage, contrary to the increase in the aqueous fraction by the increased loss of polar materials [24]. A reduction in production costs in commercial terms is due to the use of shorter distillation cycles. The plant material releases oxygenated compounds from non-oxygenated compounds earlier than the lower point of boiling during the distillation. The boiling water (steam) which figure out the oil glands and dissolved portion of the oil into the gland might be responsible for this. After release from the glands, the oil constituents are vaporized instantly. Polar oxidized complexes are water-soluble rather than non-oxidized compounds so that they spread quicker and distilled first [25]. Various authors have compared the composition of hydro / steam oil and of the product obtained through the extraction of supercritical fluids. They found that hydro-steam oil comprised greater levels of terpenes and hydrocarbons. The super-critical oil, in contrast, contains a superior proportion of oxygenated complexes.

4. Biological effects of *Thuja orientalis*

Essential oil generally show anti-oxidant [29] and anitimicrobial [30] 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 carbohydrate, phenols, alcohols, aldehydes and ketones, depending on the biotic function of aromatic and pharmaceutical vegetation. *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, coolant, and stomach. Their usage is said to enhance the progress of hair. The microbe is sedative, stimulating and lenitive. It is internally used for tremors, sleeplessness, anxious conditions, and constipation of the ageing. Bark is recycled in the action of corrode and scald. The twigs are used to treat toxins, colds, and dysentery, rheumatism and skin parasites. Acid-CoA inhibitor: lysophosphatidylcholine acyltransferase activity compared to psycho-active cannabinoids. Thujone has become an inhibitor of acyl-CoA [31].

4.1. Antibacterial Activity

Thuja orientalis contains large extents of three elements (alpha, beta and gamma thujaplicin) that are intended to serve as low concentrated chelators for *Salmonella typhimurium*. *T. orientalis* was very effectively inhibited the growth of serotype c and d *Salmonella mutans* (MIC fewer than or equivalent to 2.0-7.8 mg / ml) [31].

4.2. Antifungal Activity

In a direct bio autography test by lipophilic with *Thuja* leaf extract, the essential oils indicated antifungal action in the inhibition area for *Alternaria alternata* and *Currularia lunata*. The best bioactive constituent (R_F= 0.80) for antifungal activity had been observed and noted. It formed in a hibition zone with a diameter of thirty and twenty two mm against *A. alternata* and *C. Lunata* respectively. The essential oils from plants, shoots and twigs of large trees and shrub-like trees of *Thuja* were removed by hydrodistilation and supercritical fluid extraction and analyzed by GC and GC-MS. The essential oils shown a certain degree of antifungal activity against the six strains of human pathogenic fungi [32].

4.3. Antiviral Activity

The chemical composition of *Thuja orientalis* essential oils were determined by GC-MS analysis. In order to detect in vitro, the virus-induced cytapathological effect after infection, essential oils were estimated for its inhibitory activities against Sever Acute Respiratory Syndrome Coronavirus (SARSC) and Herpes Simplex Virus Type-1 (HSV-1). Various studies showed allopathic extract of *T. orientalis* might be used as a strong antiviral agents against plant and animal viruses [33].

4.4. Inflammatory

Vascular inflammation was involved in the inhibition and development of cardiovascular infection having atherosclerosis. Anti-vascular inflammatory action of an aqueous extract of *T. orientalis* essential oils and its probable mechanisms were inspected in human umbilical vein endothelial cells (HUVECs). Pre- incubation of *Thuja orientalis* essential oils constrained tumor necrosis factor and also inhibited U937 monocytes adhesion to HUVECs stimulated by tumor necrosis factor (TNF) suggesting that it may inhibit the binding of monocytes to endothelium. Moreover, *T. orientalis* essential oils significantly inhibited TNF-induced production of intracellular reactive oxygen species. Overall, Thuja oil has an anti-inflammatory activity which is at least in part, is due to the decrease in the TNF-induced endothelial adhesion to monocytes by inhibiting intracellular reactive oxygen species production, NF-k_B activation and cell adhesion molecule in HUVECs [34].

4.5. Anticancer Activity

Strong 5α-reductase inhibitor was extracted from *Thuja orientalis* and fractionated in isolated form as diterpenes. The inhibitors were consumed either alone or as vital components to treat diseases caused by excessive 5α-reductase or hyper-secretion of androgens, like male baldness, androgen ethic alopecia, hirsutism, acne, prostate galaxy and prostate cancer. Many researchers reported the pathological and antioxidant activities of *Thuja orientalis*. The thujone-rich fraction (TRF) properties including Anti-proliferative and apoptosis were separated from *Thuja orientalis*. The anti-cancer potential was identified in malevolent melanoma cell line A375 [35]. The potential of their anti-cancer had been identified in the malicious melanoma cell line A375. Inflammation caused by cancer by a polysaccharide or long chain sugar derived from *Thuja* shrubs extract was concluded. It also prevented the metastasization or spread of cancer throughout the body.

4.6. Larvicidal Activity

Larvicidal activities of *Thuja orientalis* essential oils against fourth-star larvae *Aedes aegypti* and *Culex pipiens pallens* had been observed by a researcher. 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) and found high levels of mortality in age class of II of *Thuja orientalis* against *Aedes aegypti* and *Culex pipiens pallens* larvae. Leaf Part and age class of class II of Thuja has strong larvicidal actions against *Aedes aegypti* and *Culex pipiens pallens*. The *Thuja* oil leaf shows natural larva ides against the *Aedes aegypti* and *Culex pipiens pallens* [36].

***4.7. Insecticidal* Activity**

Thuja orientalis leaf extracts shows a repellant activity against *Chilo partellus*. *Thuja* ether extract (68.63%), acetone extracts (67.51%) was found to adequate repellent action. Semi-compact, solid crude foliar application of *Thuja* extract was very efficient on maize against *Chilo partellus* [37].

4.8. Nematicidal Activity

Mortality in *Meloidogyne incognita* egg juvenile in three intervals was caused by the ethanol extract from *Thuja orientalis* leaf meditations (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 meditation and interval of plant excerpts [38].

4.9. Molluscicidal Activity

A researcher reported that ethanol extract of *T. Orientalis* leaf (24 h LC50-32.74 mg / l) and purified column fraction (24 h LC50-29.25 mg / l) have potent molluscicidal action against *Lymnaea acuminata*. Thujone (24 h 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 or MGK-264 was examined in binary combination (1:5) against *L acuminates*. Mixture of *Thuja* leaf /thujone or fruit /column extract of *Thuja* fruit with PB or MGK-264 shown synergistic toxicity up to 189.02 times. The toxicity of the binary mixture had been increased hundreds of times, because its components show synergistic action. The activity in *Lymnaea acuminates* nerve tissue of acetyl cholinesterase, acid and alkaline phosphatase in vivo column sanitized section of *Thuja orientalis* and its dynamic molluscicidal compound Thujone were considerably inhibited by sub lethal therapy (40 and 80 per cent LC50). It can be determined from the previous literature that *Thuja orientalis* is particularly susceptible to several medical problems viz. microbial, fungal and worm infection [39].

5. Economic value

Thuja has been used in many ways throughout history. It was like an 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 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 [20]. Leaves and oil are used as a medicines. *Thuja* is recycled for respirational tract toxicities like bronchitis, microbial skin contagions and cold infections. It is also used in the treatment of painful conditions such as arthritis and nervous disturbances which affect the face called trigeminal neuralgia. 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 skin diseases, warts and cancer as a repellent for insects. Thuja is used in food and beverages as an additive agent. Thuja is also used in maquillages and soap manufacturing [21].

6. Biological actions of *Thuja* oil

Research on the *Thuja orientalis* assay had been conducted in the previous studies. The essential oil used for the insect fumigation by chromatography with a phase gas had shown that there are 22 compounds, comprising alpha-thujone (49.64 %), fenchone (14.06 %) and β-thujone (8.98 %). Oil fumigation for adult bruchids alone resulted in 1.1, 0.7, 0.5 and 0.2 µL / insect mortality after 3, 6, 9 and 12 hours. The fumigation was reported in adulthoods. Significant differences between treatment and control had been found in the activities of insects with flavored powder of *Thuja*. The 100 mg powder flavored at 3 µL of essential oil G-1 was applied to bruchid pairs, leading to 95% of females and 100% of male with 0% of control death after a period of 6 hours of exposure. Egg hatching was 1.2% (treated with kaolin-flavored powder with essential oil of *Thuja*), 41% (alone) and 44% (kaolin-free control) five days after deposition. 80% of adults (in treatments involving kaolin only) were recorded at 100% (without kaolin) and 0% (with kaolin aromatized with *T. orientalis* essential oil) 30 days after treatment in the same experiment. The germination of cowpea seeds was not affected by treatments substantially. Five days after seeding, germination was 88, 97 and 97%, with the unrepresented and no exposed treatment, actions and exposure of cowpea grains and 15% germination of cowpea exposure [40]. The chief components of oil of *Thuja orientalis* fresh leaves were investigated by some researchers. The GC and GC-MS analysis was used to analyze and isolate the *Thuja* components by means of hydrodistilation in the northwest of Himalaya's, 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 %). Antifungal activity was demonstrated against *Alternaria alternata* by essential oil 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 antifungal actions against *Alternaria alternata* in a direct bio autographic assay; they produced an inhibition zone with the diameter of 5 mm and 10 mm respectively. The chromatography of the preparatory thin layer further purified the components b_1 and b_2 and their antifungal efficacy was re-tested. The minimum amount of b_1 and b_2 inhibitory against *A. alternata* was determined as 30.5 and 4.5µg respectively, using a bio autography test. The bioactive constituent corresponding to b1 was determined as α-cedrol by using GC/MS analysis [41].

The essential oil of *Thuja orientalis* from seed coats obtained in a yield of 1% had been studied for its antimicrobial 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 neat essential oil of *T. orientalis* had exhibited very 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 Nigam* and *Rao* had also reported the antifungal efficacy of the neat essential oil against 8 other fungal organisms suggesting its potential as an antifungal agent. A detailed in vivo study would being to fore the practical efficacy of the oil against these microorganisms [42].

7. Biological actions of *Thuja* derivatives

The reaction of α-and β-thujones to dansyl hydrazine (DNSH) had been investigated using reverse phase liquid chromatography (LC) with fluorescence detection. Although the derivative procedure was slow, the C_{18} solid phase extraction column for purification was used to determine white vermouth α-thujones at concentrations of 0.45-4.5 mg / L; recovery of α-thujone were in the range of 60-77 percent. The dansyl α-and β-thujones were separated by the LC and their identity was confirmed by the LC-tandem mass spectrometry. Determination of thujone was reported on GC-MS reversed fluorescence detection and chromatography. Liquid chromatography is a technique used for separating a sample into its various parts. In fact, the detections of GC-flame and GC-MS have been extensively used for analysis of α-and β-thujones plant materials. Toxic ingredients are thujones in absinthe. The neurotoxic effect of absinthe resulting from chronic absinthe use, an alcoholic drink containing wormwood extract, has been suggested to Thujone. Other alcoholic drinks, including red and white vermouth, which can contain wormwood and flavorings also include thujones. For its stability and to avoid their evaporation, the byproducts α and β thujones were used. The derivatization rate was not changed by changing the temperature and the catalyst [43].

8. Conclusion

The results of previously done research have shown that Thuja leaves are used ethno-botanically and have considerable prospective in the improvement of bacterial medications syndrome. This study also showed that the Thuja oil and its derivatives could be used an antibacterial agent after a thorough investigation. The most abundant compounds in Thuja are extracted by the hydrodistilation method. The vital components α-pinene, β-pinene, limonene and terpinolene essential oil were obtained. The results from previous research showed that Thuja is used for medicinal purposes. Furthermore, these results form an excellent basis for the plant selection for further phytochemical and pharmaceutical research.

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