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Medicinal potential of *Thuja occidentalis* and its essential oil

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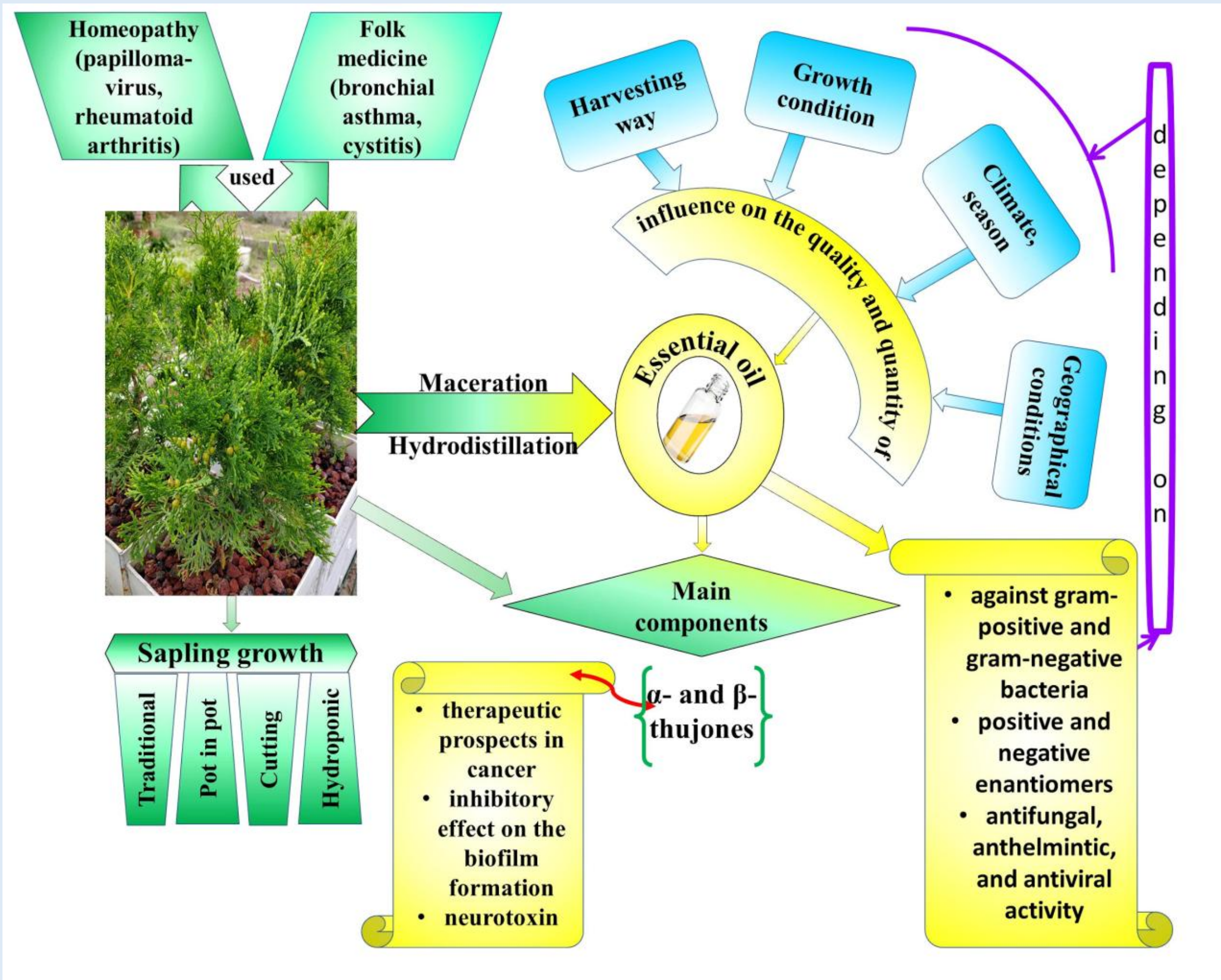
ABSTRACT:

Thuja occidentalis L., which belongs to the *Cupressaceae* family, is commonly used in urban landscaping for its positive effects on microclimate regulation, noise reduction, air humidity maintenance, and air quality improvement. In folk medicine, *Thuja occidentalis* is used to treat bronchial asthma, rheumatism, and cystitis due to its anti-inflammatory, antibacterial, antiviral, antifungal, and anthelmintic properties. The crude extract of the aerial parts and their fractions in the *Thuja occidentalis plant* have been shown to have dose-dependent spasmolytic effects on isolated rabbit intestines.

Thuja essential oil has antifungal, anthelmintic, antiviral, and antimicrobial properties. Thujone is the main component of the thuja, while α-thujone displays antibacterial activity against some gram-negative bacteria. This effect creates an inhibitory effect in low concentrations of biofilm formation within different bacterial strains. Therefore, α-thujone may be a valuable tool for antibacterial therapy. The qualitative and quantitative composition of *Thuja occidentalis* essential oil is determined by the geographical conditions, climatic conditions of tree growth, and methods of harvesting the raw plant material. This paper collects various research on the medicinal properties of *Thuja occidentalis* essential oil and investigates the variables that influence oil composition.

Keywords: thuja, thujone, growth, plant extract, enantiomer



Graphical Abstract: Medicinal potential of Thuja occidentalis and its essential oil

INTRODUCTION

Thuja occidentalis L. (white cedar, *TO*) from the *Cupressaceae* family positively influences the microclimate, reduces noise, and maintains high air humidity levels. It decreases temperature oscillations and dust particles and improves air quality [1]. *TO* has around 200 structure variations (tree, bush). The plant thrives in solidarity, or tall columnar (like varieties 'Fastigiata' and 'Columna'), and pyramidal (like variety 'Pyramidalis') shapes in parks and open spaces [2]. The 'Pyramidalis Compacta' variety can be used to landscape cities near nuclear power plants due to the durable leaves and ability to accumulate radionuclides [3].

TO is an essential oil that is also used in cosmetics and medicine. For instance, it is often used in an anti-acne cream [4]. *TO* leaf extract influences the expression of apoptosis-related proteins such as caspase-3 and p53, while inducing apoptosis [5]. This paper aims to summarize the medicinal properties of Thuja essential oil while understanding the variations in growing conditions. The article selection for this paper was primarily based on search phrases such as '*TO* in medicine' and '*TO* essential oil in medicine.' A majority of sources were retrieved from Google Scholar and ResearchGate.

Novelty and Actuality: This article combines studies regarding the growth potential of *TO* and its essential oil content grown in different countries with varying conditions. The goal is to conclude that *TO* essential oils and extracts may have different effects due to variations in composition, depending on the growth location and conditions.

Growth of *TO*: The saplings of TO 'Pyramidalis' growth is difficult. Therefore, methods aside from traditional soil growth were attempted.

"Pot-in-Pot" technology increases root ability while promoting the growth characteristics and development of the root system in woody plants. When the environment's temperature is critically high, this technology maintains advantageous conditions for plant growth while minimizing temperature stress. Optimal root system conditions were created with a 5 L pot shielding 10 and 15 L pots [6].

EBB&FLOW hydroponic system allows for water conservation due to circulation. The nutrient solution moves towards the plants and returns into the solution tank several times daily. The substrate may occupy 90% of the adequate water holding capacity [7]. In Ararat Valley, an EBB&FLOW system was used to grow *TO* 'Pyramidalis'. This process concluded that red slag is the most effective hydroponic substrate for the Thuja ' Pyramidalis ' saplings, providing quick growth and rich foliage [8]. In this environment, hydroponic thujas produce more essential oil than when soil grown [9].

TO planting may be propagated using fractions taken from 10-15-year-old plants. These fractions must be successfully rooted after a 12-hour treatment with the disodium salt of 2-(quinolin-4-ylthio) succinic acid solution, in a concentration of 50 mg/L [10].

The TO leaves contain a well-developed cuticle, with epicuticular wax reflecting light. This protects the photosynthetic apparatus while reducing thermal stress. Parenchyma cells are vital for structural support under adverse conditions. Bioactive compounds, including tannins, are primarily localized around the phloem near the conductive bundles [11].

Essential Oil Of *TO*: The composition of volatile compounds in essential oils varies depending on factors such as the time of day, season, and weather when plant material is collected. Temperature, oxygen levels, and light exposure may also influence the composition of volatile compounds. Different environmental conditions result in different quality, organoleptic properties, and bioactivity [12].

The qualitative and quantitative composition of thuja essential oil is influenced by geographic conditions, climatic conditions, and harvesting methods [13,14]. These factors may impact their treating abilities. Thujone (16.58%), b-myrcene (14.62%), and bornyl acetate (9.31%) were the main components found in the essential oil extracted from TO leaves grown in Korea. Some other components were α-pinene (9.21%), limonene (5.05%), camphene (2.52%), and β-phellandrene (1.64%) [15]. The essential oil from trees in Belarus contained over 26 components, with the dominant components being: α-thujone (~55–60 %), β-thujone (~10–12 %), and fenchone (~8–16 %) [13]. Almaty plant essential oil has the highest composition of 3-O-methyl-D-glucose (17.33%), followed by thujone

(16.42%), 19-hydroxy-3(α)-,5-cyclo-5(α)-androstan-17-one (14.53%), and pimaric acid (8.48%) [11]. Among *TO* grown in the Czech Republic, the predominant terpene in the leaves was beyerene (38.4%), α-pinene (64.9%) in the trunk, and β-phellandrene (25.5%) in female cones [16]. Therefore, TO essential oil grown in different countries varies in composition and main component ratios (Table 1).

Caruntu et al. documented that a fresh plant has about 0.6% essential oil, with the main components being monoterpenes such as thujone (65%), isothujone (8%), fenchone (8%), sabines (5%), and α-pinene (2%). Essential oil comprises 1.4–4% of the dry plant, consisting of borneol, camphene, fenchone, limonene, myrcene, α-terpineol, terpinolene, thujone, and thujyl alcohol. Thujone is only about 0.76–2.4%, with 85% being α-thujone and 15% being β-thujone [17]. This may be due to the evaporation of some compounds during the drying process.

TO essential oil composition changes with environmental conditions and plant health. Under drought conditions, thuja slows the secretion of α-thujone (about 1.7 times), β-thujone (about 1.5 times), terpinen-4-ol (about 4.3 times), camphor (about 3.3 times), and fenchone (about 1.5 times). However, the plant increases the secretion of α-pinene and α-thujene (together about 124.2 times), camphene (about 59 times), fenchene (about 89 times), β-pinene (about 25.8 times), myrcene (about 4 times), and limonene (about 2.8 times). Infection caused by *Phloeosinus aubei also* influences the composition of the oil. The oil may contain greater amounts of: α-thujone (about 16.3 times), β-thujone (about 31.5 times), terpinen-4-ol (about 4.3 times) and fenchone (about 3.6 times) decreases, and the amount of α-pinene and α-thujene (together about 1040 times), camphor (about 1.4 times), camphene (about 33 times), fenchene (about 138 times), β-pinene (about 123.6 times), myrcene (about 28.1 times) and limonene (about 55 times), compared to healthy plants [18].

Leaf extract contains the highest concentration of monoterpenes and monoterpenoids during the spring months, determining the essential oil's antimicrobial and antioxidant activities. Therefore, the spring is the most effective time to obtain terpenoids from thuja leaves. Increased sunlight, temperature, and photosynthesis boost extract yield up to 9.9%, compared to 3.3% in winter. However, essential oil contains high levels of monoterpenes in both seasons (from 51.7% in winter to 67.3% in spring). Diterpenes are at their greatest amount in autumn and winter (37.6% and 37.7%, respectively), but lower in spring and summer (about 25% of the whole extract) [12].

TO leaf extract can be obtained through maceration in an organic solvent or hydrodistillation. Maceration must be done in an organic solvent, such as dichloromethane**.** Chajduk et al. identified 70 compounds in the essential oil collected through maceration. The TO leaf extract was a thin, dark yellow liquid [12]. The essential oil obtained by hydrodistillation identified thirty-one components in the case of *TO* 'Globosa' and twenty-seven components in the case of *TO* 'Aurea'. The major component in both variants was α-thujone (50.14 and 51.60%, respectively), followed by beyerene (8.54% and 11.28%, respectively), sabinene (4.55% and 3.43%, respectively), and camphor (4.47 and 3.09%, respectively) (Table 1). *TO* 'Globosa' was rich in β-thujone and fenchone, while *TO* 'Aurea' was rich in diterpene rimuene [19]. Moreover, maceration provides a richer essential oil composition than the hydrodistillation method.

Table 1. *TO* essential oil composition in different studies (different countries and different oil extraction methods).

Name of compound	Content (%) in essential oil received by hydrodistillation in Poland [19]	Content (%) in essential oil received by maceration in Poland [12]	Content (%) in essential oil received by hydrodistillation in Belarus [13]
α-Thujene	0.27-0.28	0.2-0.4	0.03-0.22
α-Pinene	1.1-1.45	0.7-1.3	0.15-0.53
Camphene	1.0-2.44	<0.1-0.5	0.25-0.59
Sabinene	3.43-4.55	3.3-6.2	0.96-5.1
Myrcene	0.0-1.19	0.6-1.7	0.51-1.57
α-Terpinene	0.29-0.55	<0.1-0.1	0.18-0.25
p-Cymene	0.0-0.18	0.1-0.2	0.19-0.67
Limonene	1.45-3.17	0.7-1.3	0.52-0.93
γ-Terpinene	0.95-1.0	0.1	0.33-0.48
Fenchone	0.17-4.24	4.7-6.4	8.57-15.46
α-Thujone (cis)	**50.14-51.6**	**19.7-36.4**	**55.26-60.93**
β-Thujone (trans)	**2.7-5.58**	**6.8-10.6**	**10.45-11.83**
Camphor	3.09-4.47	0.1-0.2	0.47-3.57
Borneol	0.0-0.3	0.0-0.1	0.37-0.58
Sabine ketone	0.0-0.21	0.1-0.2	0.0
Terpinen-4-ol	2.28-3.28	0.2-0.3	1.64-2.84
p-Cymen-8-ol	0.0-0.25	0.1	0.0
α-Terpineol	0.37-1.38	0.1	0.26-0.34
trans-Piperitol	0.0-0.15	1.2-2.0	0.0
endo-Fenchyl acetate	0.23-0.99	0.3-0.4	0.0
Couminalaldehyde	0.0-0.07	0.0	0.0
Carvone	0.0-0.25	0.0	0.13-0.25
Carvacrol methyl ether	0.0	0.1	0.0
Piperitone	0.0-0.12	0.0	0.0
Cyclofenchone	0.0-0.37	0.0	0.0
(-)-Bornyl acetate	1.32-2.48	3.3-4.5	0.85-2.5
Sabinyl acetate	0.0-0.29	0.0	0.01-0.28
Carvacrol	0.14-0.23	0.1	0.1-0.21
Geranyl acetate	0.12-0.47	0.2-0.3	0.0
trans-Cinnamyl acetate	0.0-0.08	0.4-1.1	0.0
δ-Cadinene	0.0-0.1	0.0	0.0
(-)-Caryophyllene oxide	0.31-1.35	0.0	0.0
t-Muurolol	0.0-0.08	0.0	0.0
Rimuene	0.07-5.61	2.6-4.2	0.0
Beyerene	8.54-11.18	5.5-8.4	0.0
(+)-Beyerene-19-ol	0.0-1.48	0.0	0.0
Kaur-15-en	0.0-0.1	0.0	0.0
trans-Totarol	0.26-1.38	0.0	0.0

TO IN TREATMENT

TO can treat various disorders such as bronchial catarrh, psoriasis, uterine carcinomas, amenorrhea, and rheumatism. TO is also commonly used in phytotherapy. In homeopathy, the oil may treat or manage rheumatoid arthritis or warts caused by human papillomavirus (HPV) infection. *TO* stimulates blood circulation and the secretion of hormones, enzymes, gastric juices, acids, and bile [20, 21]. In folk medicine, *TO* preparations treat bronchial asthma, rheumatism, and cystitis through their anti-inflammatory, fungicidal, antibacterial, antiviral, and anthelmintic properties [13, 22].

The crude extract of *TO's* aerial parts and fractions (ethyl acetate, chloroform, butanol, and water) have dose-dependent spasmolytic effects on isolated rabbit intestines, with the prominent being chloroform [23]. The mother tincture of *TO*, rich in flavonoids and phenolic compounds, may inhibit inflammation in the intestines when administered daily at 25-50 mg /kg for 7 days. The tincture was prepared through cold maceration of the plant's fresh, young, non-woody branches and leaves in a 90% ethanol solution [24]. Plant flavonoids and phenolic compounds have been proven effective when treating intestinal infections [25].

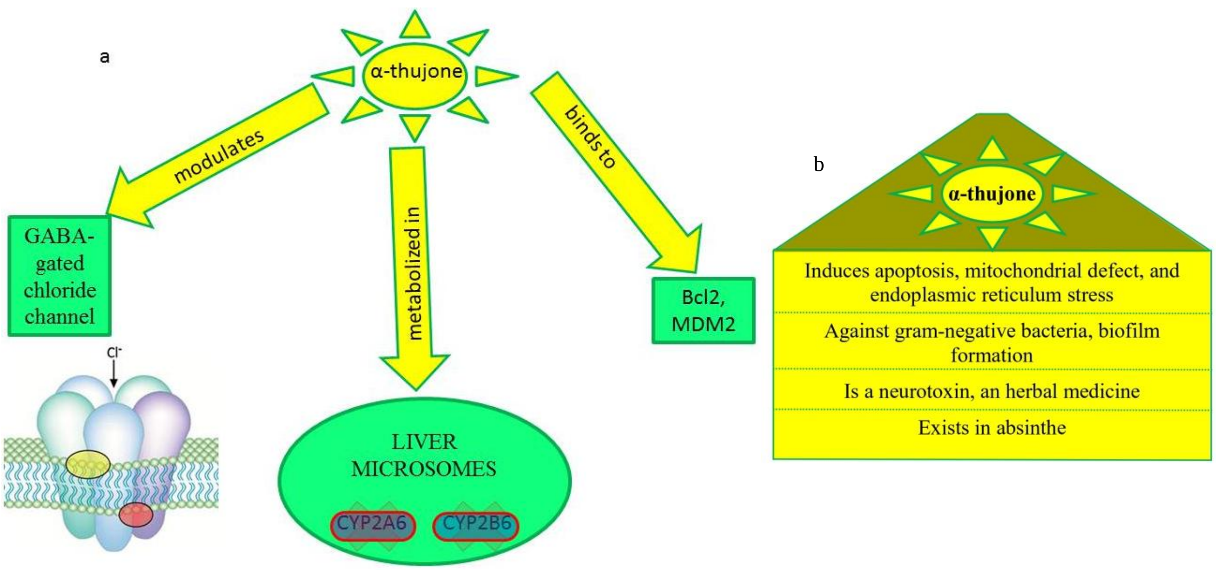


Figure 1. α-thujone function in the human organism and general (a and b).

TO exhibits antioxidant, anticarcinogenic, antiproliferative, pro-apoptotic, and radioprotective properties. Alone or combined with photodynamic therapy (660 nm laser irradiation), plant extracts induce morphological changes in A549 lung cancer cells. These changes include increased lactate dehydrogenase release and decreased ATP levels. These changes ultimately lead to cell death. However, combined therapy is an effective lung cancer treatment option [26]. Plant extracts rich in natural antioxidants protect human cells from free radicals without adverse effects [27].

In rats, the co-administration of phenytoin and methanolic extract from *TO* leaves and branches restored liver and kidney function indices to near-normal levels. This finding indicates a potential protective effect of the extract. Phenytoin has a harmful impact on the liver and kidneys of patients [28]. The ethanolic extract of *TO* may be used in convulsion therapy since it delays the onset of convulsions and reduces their duration [29]. An aqueous extract of *TO* aerial parts could be a potent anxiolytic, nootropic, anticonvulsant, and CNS depressant. Mice treated with this extract did not experience convulsions induced by pentylenetetrazole, possibly due to its influence on the GABA system [30].

Hydroalcoholic *TO* extract possesses antioxidants, anti-inflammatory, anti-hyperalgesia, and hypoglycemic properties because it inhibits the development of diabetic neuropathy [31]. Albino mice fed a diet supplemented with TO experienced faster sciatic nerve healing [32].

The water extract and polysaccharide fraction of *TO* in mice reduced paw edema caused by carrageenan, neutrophil infiltration, cyclooxygenase-2, and inducible nitric oxide synthase immunostaining in paw tissue, without causing gastric toxicity [33].

Al-Roubai et al. demonstrated that water and alcohol TO extracts inhibit *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria [34]. The 70% *TO* ethanolic extract inhibits gram-positive bacteria (Streptococcus pyogenes, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, and Clostridium perfringens). The efficacy varies with TO variety, as TO 'Golden Smaragd' was concluded to be the most effective [35].

α-Thujone, the primary bioactive compound in TO, displays therapeutic potential against glioblastoma, melanoma, and ovarian cancer. This component binds to Bcl-2 and MDM2, promoting apoptosis (Fig. 1). The Thuja drug may inhibit cervical cancer cell proliferation by inducing both apoptotic and autophagic cell death [20]. α- and β-thujones are natural pharmacological compounds that serve as a treatment option for human placental choriocarcinoma. These compounds induce a global mitochondrial defect and stress the endoplasmic reticulum in choriocarcinoma, leading to energy deprivation. This leads to increased apoptosis within choriocarcinoma cells [36]. α-thujone displays antibacterial activity against some gram-negative bacteria, with low concentrations experiencing an inhibitory effect on the biofilm formation of different bacterial strains [37]. Therefore, this could be a valuable antibacterial therapy method. α-thujone is also a neurotoxin in a liqueur absinthe. In absinthe and herbal medicines, α-thujone is a rapid-acting, easily detoxified modulator of the GABA-gated chloride channel. In an *in vitro* study, α-thujone was quickly metabolized by mouse liver microsomes [38], while inhibiting CYP2A6 and CYP2B6 enzymes. This was an interesting finding since CYP2A6 is the most active enzyme in human liver microsomes, responsible for 70-80% of the metabolism [39]. However, the European Parliament and the Council have restricted the use of α- and β-thujone in food and alcohol products since 2008 [40].

TO ESSENTIAL OIL IN MEDICINE

Thuja essential oil has high antifungal, anthelmintic, antiviral, and antimicrobial activity [13, 19, 41]. *TO* essential oil, especially α-thujone, may treat polycystic ovary syndrome without inducing osteoporosis [42].

Monoterpenes, the primary components of essential oil, display notable bioactivity. β-pinene and limonene demonstrate inhibitory effects against herpes simplex virus type 1, with IC_{50} values of 3.5 μg/mL and 5.9 μg/mL, respectively [43]. α-pinene, which is similar to β-pinene, has hypoglycemic and anti-inflammatory effects. The maximum anti-inflammatory activity was observed at 50 mg/kg in alloxan-induced diabetic mice samples [44]. It is also a major component (20–50%) of juniper berry oil [45]. Pinenes exist as optical isomers, each with distinct bioactivities: (–)-α- and (–)-β-pinene possess antiviral properties against infectious bronchitis virus, while (+)-enantiomers exhibit antimicrobial activity against *Candida albicans*, *Cryptococcus neoformans*, *Rhizopus oryzae*, and methicillin-resistant *Staphylococcus aureus* [46]. Monoterpene camphene is commonly found in many conifers. It was effective against many proteins responsible for SARS-CoV-2, specifically against SARS-CoV-2 protease 9 [47]. Wister albino rats experienced a strong cardioprotective effect against myocardial infarction due to doxorubicin [48].

Wang et al study found that *TO* α-pinene is the highest in content, with limonene in second place. Both have an (-)-enantiomer in a minor position compared to (+) one. Camphene experiences the opposite [49].

Thuja essential oil inhibits pathogenic bacteria, especially gram-positive strains, but does not affect Salmonella typhimurium. The key active components in this oil include α-pinene, 3-carene, terpinolene, limonene, β-myrcene, and camphene [50].

It was shown that the major components of the extract from Tunisian *TO* were: α-pinene (34.4%), cedrol (13.17%), and β-phellandrene (8.04%). The major components of the cone essential oil were α-pinene (58.55%) and 3-carene (24.08%). The antioxidant activity in both extracts was higher than that of Trolox, against the 2,2'-diphenyl-1-picryl hydrazyl (DPPH) radical. The highest activity for leaf extract was: *Listeria* monocytogenes ATCC 7644, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium* NCTC 6017. These extracts may be natural preservatives against foodborne pathogens [51].

Kovalenko et al. found that Thuja essential oil's antibacterial and antioxidant properties vary with crown shape. In their study, the (–)-enantiomers of α-pinene, camphene, and limonene comprised 20%, 50%, and 30%, respectively. Essential oils from columnar *TO* cultivars contained higher proportions of (–)-enantiomers than spherical forms. Camphor enantiomer distribution varies with crown shape. Features of the component composition and the nature of the distribution of enantiomers of *TO* essential oils determine their antimicrobial activity. (–)-α-pinene and (+)-camphene have higher antimicrobial activity than their optical isomers. All samples in the range of 0.05 to 5.0% were found to suppress the growth of bacteria. Reducing essential oil concentration decreased its overall antimicrobial activity. Sensitivity varied among microorganisms, with *Clostridium* spp. being the most susceptible, and *E. coli* the least affected. Additionally, *TO* ethanolic extracts showed weaker antimicrobial effects than essential oil solutions [13].

TO IN TEA

Many websites offer dried *TO* leaves in tea form, suggesting a high demand for Thuja as a raw material. Many use Thuja tea as a remedy for certain illnesses, which is reasonable considering that teas contain the essential oils of their plant ingredients. In the case of *TO* tea, its essential oil may be beneficial against various diseases.

CONCLUSION

The quality and composition of *TO* essential oil and other extracts vary with environmental factors such as climate, growing location, harvesting conditions, and extraction methods. The shape of the plant crown may affect the composition of the essential oil, increasing the amount of (+) or (-) enantiomers within the product. Different essential oil compositions provide different therapeutic and medicinal effects.

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Abbreviation: CNS - central nervous system, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2, *TO* – *Thuja occidentalis*.

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