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Chapter 57

Potential of *Cupressus sempervirens* (Mediterranean Cypress) in Health

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**ABBREVIATIONS**

| Abbreviation | Description                        |
|--------------|------------------------------------|
| AChE         | acetylcholinesterase               |
| ACHN         | renal adenocarcinoma cell line     |
| ATCC         | American type of culture collection|
| DPPH         | 1,1-diphenyl-2-picrylhydrazyl      |
| HSV          | herpes simplex virus               |
| IC           | inhibitory concentration           |
| LC           | lethal concentration               |
| MIC          | minimum inhibitory concentration   |
| PCL          | luminol-photochemiluminescence     |
| RBC          | red blood cell                     |
| SARS-CoV     | severe acute respiratory syndrome coronavirus |
| SFE          | solid phase extraction             |

**INTRODUCTION**

*Cupressus sempervirens* L., known as Mediterranean cypress, is an ornamental tree and a member of the Cupressaceae family. Among the habitats of the species, northern America, Africa, southeastern Europe, and western Asia can be cited. Using these plants can protect fields from wind damage. Many researchers have reported that the species might demonstrate both fastigiate and horizontal growth habits, and therefore, it has been given subspecific taxonomic ranks. As the fastigiated form has a horticultural origin dating back to early historic or prehistoric times, it is commonly accepted that only the horizontal form precedes human activity.

Cypress species, represented by 25 different taxa in Mediterranean Region, North America, and Asia, are primarily divided into three main groups: Mediterranean cypress, North American cypress, and Asian cypress. Among them, Mediterranean cypress consists of *Cupressus sempervirens* L., *Cupressus atlantica* Gaussen, and *Cupressus dupreziana* A. Camus. Although there are many different subspecies and varieties of this species, the widely accepted varieties by ramification type are branched cypress (*Cupressus sempervirens* L. var. *horizontalis* (Mill.) Cord.) and pyramidal (Ehrami) cypress (*Cupressus sempervirens* L. var. *pyramidalis* (fastigata = stricta) (Pictures 1 and 2)).

The Mediterranean cypress naturally exists on the southern coasts of the Caspian Sea in Iran, Syria, Turkey, Cyprus, Lebanon, Palestine, and some Greek Islands (Crete, Rhodes, Samos, Kos, Symi, and Melos). It is one of the ordinary trees in the Mediterranean region and spreads from the Himalayas to China. All cypresses are quite decorative, especially in their early periods, and used in park and garden landscaping as protective, strip, and live fence trees. The Mediterranean cypress can reach up to 20–30 m in height, and its trunk is in a conical shape (Picture 3). The most important property distinguishing Mediterranean cypress from other species is the oil glands within long cavities on the backs of its dark green scale leaves [1].

The original native distribution of the species is unknown on account of its long horticultural history. In fact, some researchers attribute the native distribution of the species to Greece (some Aegean island), Turkey, Crete, north Iran, Lebanon, Syria, and likely Cyprus (which would only be appropriate) (Picture 4). It might be endemic to Tunisia and northern Libya in north Africa, and at present, the species is grown or found wild throughout the whole Mediterranean region [2].
Various classes of phytochemical compounds have been reported in different parts of *C. sempervirens*, including flavonoid derivatives (rutin, quercetin, quercetin rhamnoside, quercitrin, myricitrin and kaempferol 3-O-rhamnoside, cupressuflavone, amentoflavone, and other biflavonoids) [3,4], diterpenes (neocupressic acid, isocupressic acid, sugiol, communi acid, sandracopimaric acid, imbricatolic acid, acetoxyimbricatolic acid, ferruginol, abita-8,11,13-triene-20-ol) [5], sesquiterpenes (junepediol) [1], catechins and flavonolic oligomers [6], proanthocyanidins [7], essential oils [8–15], phenolic acids (caffeic acid and 3-coumaric acid) [4], and fatty acids [16].

The leaves and cones of *C. sempervirens* have been used as folk remedies in different parts of the world for antiseptic, antipyretic, anthelmintic, astringent, antirheumatic, antihemorrhoidal, antidiarrhoeic, and vasoconstrictive purposes [17–22].

Depending on the phytochemicals found in *C. sempervirens*, it has been reported to possess a number of biological activities desired for human health.

Early studies revealed that *C. sempervirens* had strong antimicrobial and antiviral activity [23]. For instance, the proanthocyanidin-rich fraction of *C. sempervirens* was found to have in vitro antiviral activity against two retroviruses, human immunodeficiency virus and human T-lymphotropic virus III B [24]. The inhibitory activity of the fruit essential oil of *C. sempervirens* subsp. *pyramidalis* was examined using severe acute respiratory syndrome coronavirus (SARS-CoV)
Cupressus sempervirens var. pyramidalis (by M. Karakose).

The fresh cones and leaves of Cupressus sempervirens var. horizontalis (by I. Tumen).

The dry cones and seeds of Cupressus sempervirens var. pyramidalis (by I. Tumen).
and *Herpes simplex* virus type 1 (HSV-1) replication assays, and the oil exerted a mild effect only against SARS-CoV (700 ± 2.3 μg/mL) [11]. *C. sempervirens* essential oil was identified with marked antiviral activity against HSV-1, having virucidal effects of 68.0% and 53.2% at concentrations of 1:32 and 1:64, respectively [12].

The essential oil of the plant totally inhibited the growth of both antibiotic-susceptible and antibiotic-resistant *Helicobacter pylori* strains at a concentration of 0.1% (v/v) [25]. In a similar study, an inhibition zone 1.1 cm in diameter was caused by the ethanol extract of *C. sempervirens* against *H. pylori* [26]. The remarkable antimicrobial activity of the oil was also demonstrated on the survival and growth of three food pathogens: *Escherichia coli*, *Staphylococcus aureus*, and *Listeria innocua* [27]. The antimicrobial activity of the methanol extract from the male and female leaves and fruits of *C. sempervirens* var. horizontalis, *C. sempervirens* var. sempervirens, and *C. sempervirens* cv. cereformis was investigated against *Pseudomonas aeruginosa*, *S. aureus*, *E. coli*, and *Candida albicans*, using a number of methods, including the disc diffusion, hole plate, cylinder agar diffusion, and agar dilution methods, along with minimum inhibitory concentration (MIC) values determined by the agar dilution method [28]. The extracts exerted antimicrobial activity in varying ranges, but they were most effective against *S. aureus*. In a similar study [29], the essential oils obtained from the needles and twigs of *C. sempervirens* were tested against three bacteria (*E. coli*, *Micrococcus luteus*, and *Bifidobacterium lactis*) using the diffusion method. The oils were also tested against seven fungus strains (Aspergillus niger, *A. flavus*, *A. fumigatus*, *Fusarium solani*, *F. oxysporum*, *Penicillium digitatum*, and *Candida utilis*), using flask culture and potato dextrose agar methods, and they were found to have strongest activity against *B. lactis*. On the other hand, the oils were most active against *A. niger* in the flask culture method and against *C. uterus* in the potato dextrose agar method. The leaf essential oil of *C. sempervirens* was also demonstrated to possess significant antimicrobial and antifungal effects against a wide range of bacteria (*Bacillus subtilis*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*) and fungi (*A. niger*, *A. flavus*, *A. fumigatus*, and *C. albicans*) [30].

Nevertheless, the essential oil of the plant displayed insignificant antifungal activity against *Casuarina timber*, *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.*, and *Mucor sp.*, as tested by the agar disk diffusion method [31]. Consistent with this study, the leaf extract of *C. sempervirens*, which was determined to be rich in polyphenols expressed as quercetin glycosides (174 μg/mL) and biflavonoids (1460 μg/mL), did not exert any notable antifungal effect against a wide range of isolated strains of 24 yeast species from different countries (*Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. zeylanoides*, *Pichia guilliermondii*, *Clavispora lusitaniae*, *Issatchenka orientalis*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Cryptococcus laurentii*, *Filobasidiella neoformans*, *Prototheca wickerhamii*, and *P. zopfii*) using an agar diffusion well bioassay [32]. The essential oil of the plant was evaluated another time for its antifungal activity against American type of culture collection strains of five food-spoilage yeasts, including *C. albicans*, *Rhodotorula glutinis*, *Schizosaccharomyces pombe*, *S. cerevisiae*, and *Yarrowia lipolytica*, and four fungus strains were notably inhibited by the essential oil with MIC values of 0.06–0.08 mg/mL, while it possessed moderate effect against *Y. lipolytica* (MIC = 0.23 mg/mL) [33]. The oil was subjected to in vitro assays to establish its antifungal effect toward the human pathogen *Aspergillus niger* through the inhibition of hyphal growth and spore formation in *A. niger* [34]. However, it did not show any antifungal effect in these assays.

**Antiprotozoal Activity**

The antiprotozoal effect of the hydroalcoholic extract of *C. sempervirens* was found to be mildly active against *Leishmania amazonensis* [35]. In consistency with this data, the methanol extract of *C. sempervirens* was ineffective in antiprotozoal assays against the erythrocytic schizonts of *Plasmodium falciparum*, the intracellular amastigotes of *Leishmania infantum*, and the *Trypanosoma cruzi* and free trypomastigotes of *T. brucet* [36].

**Insecticidal Activity**

The essential oil obtained from *C. sempervirens* was reported to exert repellent activity against the codling moth, *Cydia pomonella* [37]. The flagellate populations of the termite *Kalotermes flavicollis* were reduced by the essential oil of *C. sempervirens* on the second (31%) and fourth days (100%). With the hindgut spirochetes of this termite, the essential oil was again effective (46 and 100%) [31]. The repellent and toxic potentials of the essential oil of *C. sempervirens* and its main constituent (cymol) were evaluated against *Sitophilus zeamais* and *Tribolium confusum* using impregnation on filter paper discs, as well as coating onto maize grains, and these potentials led to the diminishment of grain weight loss [38]. Moreover, the essential oil had a higher repellent effect than that of cymol. Nevertheless, the cypress essential oil was shown to possess low repellent activity against the mosquito *Aedes aegypti* [39].

In a study of five *Cupressus* species (*C. arizonica*, *C. benthamii*, *C. macrocarpa*, *C. sempervirens*, and *C. torulosa*), the essential oil from *C. benthamii* exerted the highest larvicidal activity (LC$_{50}$ = 37.5 mg/L) against the mosquito *Aedes*
albopictus, while the rest of the essential oils displayed only moderate toxicity against the larvae (LC$_{50}$ = 47.9–70.6 mg/L) [14]. In addition, *C. sempervirens* essential oil provided a mild repellant effect against this mosquito species.

**Antihyperlipidemic Effect**

The cone hydroalcoholic extract of *C. sempervirens* was tested on two groups of rats, one of which was the control group, for its antihyperlipidemic activity on parameters of serum lipid, muscle and liver enzymes, red and white blood count, platelets, and serum concentrations of uric acid and creatinine [40]. The extract displayed a significant reducing effect on serum cholesterol, whereas it did not cause any changes in triglyceride and high density lipoprotein cholesterol.

**Anticancer and Cytotoxic Effect**

The cytotoxic effect of the hydroalcoholic fruit extract of *C. sempervirens* var. *horizontalis* was tested against three human tumor cell lines by MTT assay. However, the extract did not exhibit any cytotoxic effect in this assay [41]. The cytotoxic effect of *C. sempervirens* subsp. *pyramidalis* leaf and cone essential oils was evaluated on several human amelanotic melanoma (C32) and renal adenocarcinoma (ACHN) cells [42]. The leaf oil led to a decrease in ACHN cell viability at 100 µg/mL (53%) and 400 µg/mL (40%), while it displayed cytotoxicity against C32 cells with an IC$_{50}$ value of 104.90 µg/mL. However, the cone oil was found to be completely inactive in cytotoxicity assays.

In a screening study that tested the plants used in Yemeni traditional medicine on three human cancer cell lines, *C. sempervirens* was among the most active plant species, having IC$_{50}$ values below 50 µg/mL [19]. In another study [12], the CHCl$_3$ fraction of *C. sempervirens* demonstrated a significant cytotoxicity against HeLa cells. The cytotoxicity of the essential oil of the plant was tested on HepG2, Hep3B, A549, MCF-7, and MDA-MB-231 cancer cells, and it showed much less cytotoxicity against these cell lines [42].

**Antioxidant Effect**

The essential oil of *C. sempervirens* was evaluated in three antioxidant test systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, β-carotene bleaching test, and luminol-photochemiluminescence (PCL) assay. It showed a mild scavenging effect (below 30%) against DPPH and a marked lipid peroxidation inhibitory effect in the β-carotene bleaching test (around 60%), while it was ineffective in the PCL assay [33].

The antioxidant properties of the dichloromethane, acetone, ethyl acetate, and methanol extracts obtained from the cones and leaves of *C. sempervirens* var. *horizontalis* and var. *pyramidalis* were demonstrated in our study using DPPH and N,N-dimethyl-p-phenylenediamine radical-scavenging activity and metal-chelation capacity with ferric- and phosphomolibdenum-reducing antioxidant power tests, and a variable level of activity according to the methods was observed [43]. In another study on the antioxidant activity of the essential oils obtained from *C. sempervirens* by supercritical fluid extraction (SFE) with CO$_2$ and hydrodistillation (HD) methods, the essential oil obtained using SFE displayed higher activity in 2,20-azinobis-3-ethylbenzo-thiazoline-6-sulfonate and DPPH radical-scavenging activity tests [15].

The essential oils obtained from the branchlets and fruits of *C. sempervirens* var. *horizontalis* were evaluated using a linoleic acid peroxidation test and peroxyl radical-mediated hemolysis of red blood cells (RBC) assays, and RBC hemolysis and lipid peroxidation were observed to be inhibited by both of the oils in a concentration-dependent manner [13].

**Antiplatelet and Anticoagulant Activity**

The essential oil of *C. sempervirens* was subjected to in vivo assays in order to determine its antiplatelet activity using clot retraction and platelet aggregation effects in guinea pig and rat plasma [9]. Nonetheless, the oil exerted a deprived effect against arachidonic acid-induced aggregation, as well as in a clot retraction assay at up to 300 µg/mL concentration. In accordance with this study, the authors concluded that the essential oil of the plant was ineffective in a thrombin- and collagen-induced antiplatelet aggregation assay. In contrary, Ulusal et al. [44] reported that the fresh cone water extract of *C. sempervirens* was shown to have a strong anticoagulant effect in an in vitro assay.

**Hepatoprotective Activity**

The leaf methanol extract prepared from *C. sempervirens* was tested against CCl$_4$-induced hepatotoxicity in rats by oral administration [4]. According to the findings, the extract caused a substantial reduction in glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, cholesterol level, and triglycerides, but a significant increase in the total protein level was observed with the extract of *C. sempervirens*. 
Wound Healing and Anti-inflammatory Activity

An earlier study revealed that the proanthocyanidin oligomeric fraction of *C. sempervirens* was able to block pancreatic elastase via inhibition of esterolytic activity of the enzyme, which is related to wound healing [16].

The probable wound-healing effects of the cone essential oils of *C. sempervirens* var. *horizontalis* and var. *pyramidalis* were examined in linear incision and circular excision experimental wound models, along with their anti-inflammatory activity in an assay involving acetic acid-induced increases in capillary permeability [45]. However, the essential oils were not active in these experiments.

Neurobiological Activity

The possible neuroprotective properties of the dichloromethane, acetone, ethyl acetate, and methanol extracts obtained from the cones and leaves of *C. sempervirens* var. *horizontalis* and var. *pyramidalis* were evaluated by our group through the inhibition of acetyl- (AChE) and butyrylcholinesterase (BChE) enzymes, which are related to Alzheimer’s disease, as well as tyrosinase, an enzyme associated with Parkinson’s disease [43]. The extracts exerted mild to moderate levels of inhibition against cholinesterases and a low level of inhibition against tyrosinase at 200 μg/mL (Figures 1 and 2).

![Inhibition of acetylcholinesterase (A) and butyrylcholinesterase (B) (inhibition ± S.E.M.%) by the dichloromethane (DCM), acetone (Ace), ethyl acetate (EtOAc), and methanol (MeOH) extracts of the Cupressus species and the reference (galanthamine) at 200 μg/mL. CSH: Cupressus sempervirens var. horizontalis, CSP: Cupressus sempervirens var. pyramidalis.](image-url)
In a previous study [46], the essential oil of the plant was consistently shown to have moderate level of AChE inhibition, with approximately 70% inhibition at 2.5 mg/mL.

CONCLUSION

* Cupressus sempervirens* is an aromatic plant widely used in traditional medicine because it has important biological activities related to human health. However, more research on this species is definitely needed in order to extend its use in health care.

SUMMARY POINTS

- *Cupressus sempervirens* L., known as Mediterranean cypress, is a medicinal and aromatic plant.
- The most common varieties are *Cupressus sempervirens* var. *horizantalis* and *Cupressus sempervirens* L. var. *pyramidalis*.
- Mediterranean cypress has a long history of use in traditional medicine.
- Cypress is rich in polyphenols, terpenes, and essential oils.
- Cypress oil has strong antimicrobial and insecticidal effects.
- Cypress possesses many bioactivities desirable for human health.

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