



Chemical Constituents of *Cupressus sempervirens* L. cv. *Cereiformis* Rehd. Essential Oils

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Abstract

The essential oils obtained from fresh fruits and terminal branchlets with adherent leaves of *Cupressus sempervirens* L. cv. *Cereiformis* Rehd. growing in Iran, were analyzed by GC-MS, and screened for bacteriostatic and fungistatic activities. Thirteen components were identified in the essential oils. The main constituents of both fruits and leaves were α -pinene, Δ -3-carene, β -terpenyl acetate and terpinolene. The essential oil of leaves and fruits showed no antimicrobial activity against, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. The defatted ethanolic extract of leaves and fruits were examined for the presence of alkaloids, flavonoides, and saponins. The non-volatile compounds in leaves and fruits were quite rich in tannins and flavonoids but the amounts of alkaloids and saponins were not significant.

Keywords: Conifers, Essential oil, *Cupressus sempervirens* L. cv. *Cereiformis* Rehd., Non-volatile components.

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1. Introduction

Cupressus sempervirens L. cv. *Cereiformis* Rehd. [*C. sempervirens* var. *stricta* Aiton, *C. pyramidalis* Targ-Tozz., *C. sempervirens* L. var. *pyramidalis* (Targ-Tozz.) Nyman, *C. sempervirens* L. f. *pyramidalis* (Targ-Tozz.) Holmboe], Cupressaceae

family, is a monoecious and evergreen aromatic tree, up to 30 meters high which grows in different parts of many countries including Iran. Its Persian name is "Sarve Naz" [1-4]. Although there have been some reports on phytochemical and biological studies of *Cupressus sempervirens* L. [5-8] there are no previous reports on phytochemical and biological studies of this cultivar.

As a part of a systematic research on the chemical composition and antimicrobial properties of Iranian conifers, the present

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work reports on *Cupressus sempervirens* L. cv. *Cereiformis* Rehd. essential oils content of fruits and terminal branchlets with adherent leaves. In addition, the defatted ethanolic extracts of these organs were investigated qualitatively for the presence of alkaloids, flavonoids, saponins and tannins.

2. Materials and Methods

2.1. Plant material

The fruits and leaves of *Cupressus sempervirens* L. cv. *Cereiformis* Rehd were collected (October 2002) from Eram Garden of Shiraz, Fars, southern Iran. The collected materials were stored at -20 °C in order to avoid unfavorable changes in the chemical components [9]. The plant was authenticated by the late Prof. Karim Djavanshir, Forestry Department, Faculty of Natural resources, Tehran University.

2.2. Isolation and analysis of non-volatile components

The fruits and leaves of the plant (500 g) were dried at 50 °C and then were powdered separately. Each powder was defatted with petroleum ether (BP 40-60 °C) using soxhlet apparatus for 6 h. The chemical components of defatted powders were extracted by maceration with 70% ethanol for 4 times. The ethanolic extracts were concentrated at reduced pressure and analyzed for the presence of alkaloids, flavonoids, saponins and tannins [10-13].

2.3. Isolation and analysis of volatile components

The volatile oils were isolated from fresh plant material by wet steam distillation for 4 h. The essential oils were separated from the aqueous layer and were dried over anhydrous sodium sulfate.

2.4. Gas chromatography-mass spectrometric analysis

The GC-MS apparatus consisted of a

Varian 3400 gas chromatograph equipped with a fused-silica column (DB-5, 30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific Inc.), and interfaced with a quadruple mass spectrometric detector (Incos 50, Finnigan). The operating conditions were: oven temperature 60-280 °C with the rate of 3 °C/min; injector mode: split injection; with the carrier gas, He; flow rate 2 ml/min; ion source, 70 eV; ionization current, 750 mA; scan range, 40-300 µ.

Identification of the components was performed by comparison of their RRT and mass spectra with those of authentic samples, literature data and computerized MS-data bank (Saturn version 4). The peak area method was followed for quantitative determination of different constituents; the percentages were calculated relatively.

2.5. Antimicrobial activity determination

The antimicrobial activity of each essential oil obtained from different parts of the plant was determined using five standard strains of microorganisms from the Persian Type Culture Collection (PTCC) [14]. The following microbial strains were used: *Bacillus subtilis* (PTCC 1023), *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1038), *Pseudomonas aeruginosa* (PTCC 1074) and *Candida albicans* (PTCC 5027). Minimum inhibitory concentrations (MICs) were determined using the agar dilution method [15]. Muller Hinton agar medium (Oxoid, France) was prepared and sterilized by autoclaving for 20 min at 121 °C. The medium was cooled to 50 °C and two-fold serial dilutions of the parent solution (essential oil plus 30% Dimethyl Sulfoxide, v/v) in adequate melted (50 °C) agar medium (1 ml) were added to 24-well plates (Greiner, France). The medium was allowed to solidify and then it was inoculated with the previously prepared microorganism suspension using the quadrant streak method. For each tested

Table1. Major non-volatile components of fruits and leaves of *Cupressus sempervirens* L. cv. Cereiformis Rehd.

| Chemical components | Average content* | |
|---------------------|------------------|--------|
| | Fruits | Leaves |
| Alkaloids | - | - |
| Flavonoids | ++ | +++ |
| Saponins | + | + |
| Tannins | ++++ | +++ |

* Average content was rated from - to ++++

strain, the adequacy of growth conditions, the effect of positive controls (gentamicin for bacteria and clotrimazole for fungus), and the sterility of the medium were tested in two wells. Plates were incubated for 24 h at 37 °C for bacteria and 48 h at 25 °C for *C. albicans*.

3. Results

The amount of non-volatile components (from defatted ethanolic extracts) of the fruits and leaves of *Cupressus sempervirens* L. cv. Cereiformis Rehd are summarized in Table 1. The essential oils, isolated separately from fruits and leaves of *Cupressus sempervirens* L. cv. Cereiformis Rehd, were pale yellow with a strong odor. The fruits and leaves of the plant yielded 0.12% and 0.2%

(v/w) of volatile oils, respectively. Identification of the components of the essential oils by GC-MS revealed the existence of thirteen components the oils of leaves and fruits (Table 2).

The essential oils of both leaves and fruits were tested for their possible antimicrobial activity against five strains of pathogens, as mentioned in materials and methods. However, we did not see any significant antimicrobial effect of the essential oils, and the MIC for all of the tested microorganisms were higher than 100 mg/ml (data not shown).

4. Discussion

The fruits and leaves of *Cupressus sempervirens* L. cv. Cereiformis Rehd were investigated for the presence of non-volatile components (from defatted ethanolic extracts). As it can be seen in Table 1, the leaves and fruits of this plant are quite rich in tannins and flavonoids but they are free from alkaloids and low in saponins.

The analysis of the essential oils isolated separately from fruits and leaves of *Cupressus sempervirens* L. cv. Cereiformis

Table2. Chemical composition of the volatile oil of *Cupressus sempervirens* L. cv. Cereiformis Rehd. fruits and leaves.

| Components | Kovats Index | Leaves (%) | Fruits (%) |
|----------------------------------|--------------|------------|------------|
| α-Pinene | 939 | 30.0 | 39.0 |
| Sabinene | 975 | 2.0 | 3.0 |
| β-Pinene | 981 | 2.6 | 2.2 |
| Myrcene | 998 | 4.1 | 3.9 |
| Δ-3-Carene | 1018 | 24.0 | 24.0 |
| Limonene | 1036 | 4.0 | 3.0 |
| Terpinolene | 1091 | 6.6 | 4.3 |
| Bronyl acetate | 1288 | t | 1.7 |
| α-Terpenyl acetate | 1356 | 6.6 | 5.6 |
| β-Caryophyllene | 1415 | 1.2 | t |
| α-Humulene | 1455 | 1.3 | t |
| Germacrene D | 1483 | 4.0 | 1.7 |
| Grouped compounds: | | | |
| Monoterpene hydrocarbons | | 73.3 | 79.4 |
| Oxygen-containing monerpenes | | 6.6 | 7.3 |
| Sesquiterpene hydrocarbons | | 10.5 | 1.7 |
| Oxygen-containing sesquiterpenes | | 4.0 | t |

t = trace (<0.1 %)

Rehd by GC-MS lead to the identification of thirteen components which were fairly similar in the leaves and the fruits. Monoterpene hydrocarbons represented the most abundant constituents of the oil of the fruits and leaves (79.4% and 73.3%, respectively). The leaves are rich in sesquiterpene hydrocarbons (10.5%), while the fruits contain only 1.7% of sesquiterpene hydrocarbons. Oxygen-containing monoterpenoids were at relatively low levels in both oils (6.6% for leaves and 7.3% for fruits). Although there is some oxygenated sesquiterpenoids in the leaves (4%), there is not a significant amount of these compounds in the fruit oil. No nonterpenic constituents were detected in the essential oils. The main components of both fruits and leaves essential oils were α -pinene (39%, 30%), Δ -3-carene (24%, 24%), β -terpenyl acetate (5.6%, 6.6%) and terpinolene (4.3%, 6.6 %) respectively.

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