# TRESURE OF ENDEMIC PLANTS – VOLATILE COMPOSITION AND BIOLOGICAL ACTIVITY OF *CHAEROPHYLLUM COLORATUM* L.

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## INTRODUCTION

*Chaerophyllum coloratum* L. is a plant belonging to the Apiaceae family, whose members have long been used in traditional medicine to treat various ailments, but are also commercially important. *C. coloratum*, a species endemic to the Dinarides, is widespread in Croatia, Bosnia and Herzegovina, Montenegro, Kosovo (Prokletije Mountains) and Albania. In Croatia, it can be found along the coast and on the islands, from the Paklenica National Park (South Velebit) in the north to the Privlaka peninsula in the south (Nikolić 2015). Despite the frequent occurrence of *C. coloratum* in the sub-Mediterranean part of the Dinaric Mountains, its phytochemical composition and biologically active compounds have been insufficiently studied. Stešević et al. (2016) investigated the composition of essential oils and phenolic compounds, while Vajs et al. (1995) reported on the analysis of essential oils from ripe fruits and umbels, both analysing *C. coloratum* from Montenegro. The main compounds previously described in the composition of the essential oil of *C. coloratum* were monoterpene hydrocarbons, especially in the roots and stems. A high content of myrcene was described in the root while *β*-(E)-ocimene, *β*-(Z)-ocimene and terpinolene were dominant compounds in the stem. In the oil of leaves spathulenol, *p*-cymene-8-ol and *p*-cymene were predominant compounds. In addition, monoterpenes terpinolene and *p*-(Z)-(E)-ocimene were identified as the main constituents of the flower oil, while in the fruits, the most abundant constituents were caryophyllene oxide, (Z)-*β*-farnesene, (E)-pinocarveol and myrtenol (Stešević et al. 2016). Several studies conducted with different species of the genus *Chaerophyllum* have confirmed its significant pharmacological potential, but there are no data on the biological activity of *C. coloratum*.

The study of the phytochemical composition of plant volatiles and biological potential of *C. coloratum* points us to new plant species and their potential, to which we must devote our attention in the future if we want to avoid the environmental and health consequences of the excessive use of synthetic compounds to which we are exposed on a daily basis.

## AIM OF THE STUDY

- **Determine chemical composition of volatile organic compounds (VOCs) from hydrosol, essential oil and fresh plant material**
- **Investigate the cytotoxic activity od *C. coloratum***
- **Investigate the antiphytoviral potential of *C. coloratum* on plant hosts infected with tobacco mosaic virus**

## MATERIAL AND METHODS

**1. Plant material**

The plant material (leaves of *Cheaerophyllum coloratum* L.) was collected in May 2019 in Majdan, Municipality of Klis, Croatia. Methanol extract was prepared from dried plant material was freeze-dried and homogenized in 80% methanol-water followed by extraction in an ultrasonic bath

**2. Hydrodistillation**

Dried plant material was mixed with water in the flask of the Clevenger apparatus; water and pentane were added to the inner tube of the Clevenger apparatus. After hydrodistillation for 3 h, the fractions of hydrophobic-essential oil and hydrophilic volatile compounds were removed from the apparatus separately and stored at -20 °C and +4 °C.

**3. Headspace solid-phase microextraction (HS-SPME)**

Plant or hydrosol samples were added to the glass vials. The equilibration of the sample lasted 15 minutes at 60 °C. The extraction was then continued for 45 minutes. Thermal desorption lasted 6 minutes at an injection temperature of 250 °C directly into the GC column.

**4. Gas chromatography-mass spectrometry analysis (GC-MS)**

The VOCs isolated from *C. coloratum* were analysed using a gas chromatograph and a tandem mass spectrometer detector. The conditions for GC-MS analysis and the procedure for compound identification were described in detail by Radman et al. (2022).

**5. Cytotoxic activity**

The cytotoxic activity of the methanolic extract of *C. coloratum* on three cancer cell lines, the human cervical cancer cell line (HeLa), the human colon cancer cell line (HCT116) and the human osteosarcoma (U2OS) as well as on a healthy cell line, the retinal pigmented epithelial cells (RPE1) was performed using the MTS-based CellTiter 96® Aqueous Assay (Fredotović et al., 2021).

**6. Antiphytoviral activity**

The antiphytoviral activity was performed according to Vuko et al. 2019.

## RESULTS

### Composition of VOCs – essential oil, hydrosol and fresh plant

The percentage of compounds identified in the essential oil (EO) obtained by hydrodistillation from the air-dried leaves was 92.75% of the total compounds detected, with the sesquiterpenes group dominating at 41.75% (**Fig. 1a**). The oxigenated sesquiterpene spathulenol (14.65%) was the most abundant of all constituents. In HS-P (plant) 92.85% and in HS-H (hydrosol) 93.07% of the total VOCs detected were identified. (**Fig. 1b**). Most dominant compounds present in plant were β-caryophyllene and germacrene D while oxygenated monoterpene *p*-cymen-8-ol was most abundant compound present in hydrosol.

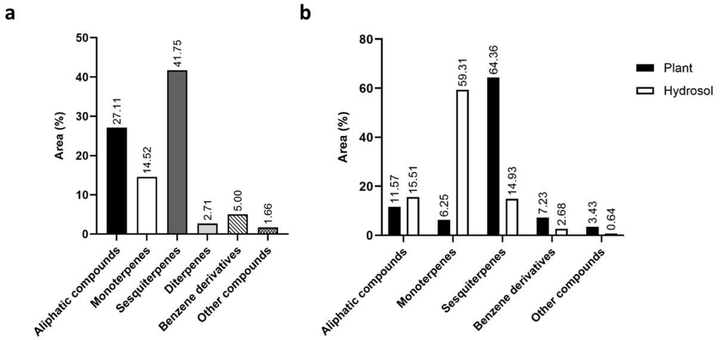


**Figure 1.** Volatile organic compounds (VOCs) of *Chaerophyllum coloratum* essential oil **(a),** plant and hydrosol **(b)**

### Cytotoxic activity

The results showed that this plant extract has significant cytotoxic activity on cancer cell lines and that it is significantly less toxic to the healthy cell line RPE1. *C. coloratum* showed higher toxicity on HeLa cells ($IC_{50}$ = 151, 35 µg/mL) than on U2OS and HCT116 cells ($IC_{50}$ = 256.45 µg/mL; $IC_{50}$ = 298.34 µg/mL) (**Fig. 2**). It is important to emphasize that the extract has low toxicity to the healthy cell line RPE1 ($IC_{50}$ = 677.40 µg/mL) compared with cancer cells.

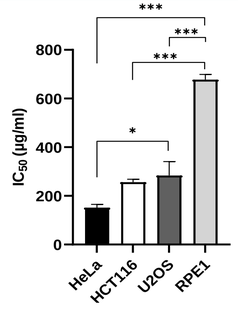


**Figure 2.** Cytotoxic activity of the methanolic extract of *C. coloratum* on HeLa, HCT116, U2OS and RPE1 cells using the MTS cell proliferation assay.

### Antiphytoviral activity

A promising inhibition of local infection of 46.40% confirmed our hypothesis about the antiphytoviral potential of *C. coloratum* volatiles (**Fig. 3**). Based on the results presented, we concluded that hydrosol has antiphytoviral activity and may activate the plant defense response and increase plant resistance to viral pathogens.

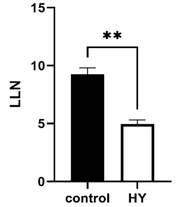


**Figure 3.** Inhibition of number of local lesions (LLN) on the plants treated with hydrosol (HY) of *C. coloratum*

## CONCLUSION

**For the species *Chaerophyllum coloratum* L. (Apiaceae), new data on volatile composition, and biological potential are presented. The essential oil is dominated by oxygenated sesquiterpenes, the hydrosol by monoterpenes and the fresh plant material by non-oxygenated sesquiterpenes. Initial data on the bioactivity of *C. coloratum* show that the plant is a promising source of compounds with selective cytotoxic activity that could be used in the future for the development of natural chemotherapeutics. Its volatile compounds are effective as natural antiphytoviral agents, as plants treated with hydrosol prior to virus inoculation developed a reduced number of local lesions.**

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