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COMPARISON OF THE ESSENTIAL OIL INGREDIENTS FROM A WILD POPULATION OF ST. JOHN'S WORT (*HYPERICUM PERFORATUM*) IN ITS NATURAL ENVIRONMENT VERSUS INTENSIVE CULTIVATION.

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ABSTRACT

Hypericum perforatum, widely recognized as St. John's wort, is a member of the Hypericaceae family. This herb is noted for its therapeutic properties, particularly its potent anti-inflammatory and antidepressant effects. The present study aims to analyze the essential oil content and its characteristics from a natural population of the plant in its native habitat, as well as from an intensively cultivated population in Larissa during the year 2023. The population originates from Ellinopirgos, Karditsa, situated on Agrafa Mountain at an altitude of 650 meters. The harvest was carried out manually on two occasions (the first on 10/6/2023 and the second on 19/7/2023) for the population established in the Larissa region under intensive cultivation, while it was performed once (on 25/6/2023) for the natural population located in the Ellinopirgos area. Following each sampling, the collected samples were sent to the laboratory for drying until a stable weight was reached. A sufficient number of samples from both locations were subjected to hydrodistillation to obtain the essential oil, using a Clevenger apparatus in the Laboratory. The results indicated a variation in the components of the essential oils, with certain substances exhibiting a zero percent content depending the sampling. These substances included: cadinene-δ, cadinene-γ, cadinol-α, dodecanol-n, (E)-β-farnesene, himachalene-β, himachalene-γ, himachalol, limonene, muurolene-γ, myrcene, nonane (N-), pinene-β, and undecane (N-). Additionally, among the other detected substances, which were caryophyllene (E-), caryophyllene-oxide, himachalene-α, pinene-α, selinene-α, selinene-β, spathulenol, and tetradecanol (n-), statistically significant differences were observed between the two cuts in intensive cultivation as well as across the various environments. This control study serves as a basis for further research into other natural populations to determine whether the essential oil content and its characteristics are influenced by the ecological conditions of the area or are inherent to the biotype. The presentation of these findings may provide a crucial cultivation guide for St. John's wort, aiding in the selection of areas and populations with the ultimate goal of optimizing its production (experiments in progress).

INTRODUCTION

Hypericum perforatum, commonly known as St. John's Wort, is a perennial plant belonging to the Hypericaceae family. It is found throughout Central and Eastern Europe, Asia, North Africa, and North America (Russo et al., 2014). This plant is particularly noted for its medicinal benefits, especially its strong anti-inflammatory and antidepressant properties (Butterweck 2003; Nahrstedt and Butterweck 2010; Becker et al., 2016).

There is a long-standing history of traditional applications and ongoing scientific exploration, where *Hypericum perforatum* remains a subject for individuals interested in natural solutions for various health issues. Extensive research has been conducted on the chemical properties and bioactivity of *Hypericum perforatum* L. Since the 1980s, this species has involved from a traditional herb primarily gathered from the wild to a prominent medicinal crop cultivated globally. Products such as pharmaceuticals and dietary supplements derived from *H. perforatum* are ranked among the best-selling herbal products in international sales (Barnes et al., 2019).

The raw material obtained from *H. perforatum* is comprised of the flowering tops of the shoots, which are required to fulfill certain compound content standards (Agapouda et al., 2019). Many studies have analyzed the chemical compositions of both native and commercial samples (Bozin et al., 2013) and investigated how the origin affect the chemical structure of different St. John’s Wort species (Çırak et al., 2010).

However, additional research is required to explore the scenario in which wild populations of the genus are utilized for mother propagation material, and the resulting plants are cultivated in different areas of the growing environment. It is essential to understand how they will adapt to varying climatic conditions and the subsequent effects on their phytochemical profiles. In Europe, the primary production of this crop occurs in Belarus, Germany, Italy, Poland, Romania, Switzerland, and Siberia. A considerable part of the total supply of Hypericum is derived from the harvesting of wild plants, which has resulted in a concerning decline in their natural populations (Lazzara et al., 2017; Lazzara et al., 2021).

Therefore, the current study aims to serve as preliminary research by analyzing the essential oil content and composition from a natural population of the plant thriving in its native environment, as well as from plants of the same population that are under intensive cultivation in Larissa in the year 2023.

MATERIALS AND METHODS

The natural population being studied originates from the Ellinopirgos of Karditsa, situated in Agrafa at an altitude of 650 meters, where the plants thrived. In contrast, the propagated plants (from the same population) were cultivated in the Larissa region, specifically on the University of Thessaly's farm (Giaopolis), under an intensive cultivation system.

The harvest was carried out manually at the ideal harvesting stage, which corresponds to full flowering, a stage known to maximize essential oil content, on two occasions (the first on 10/6/2023 and the second on 19/7/2023) for the population established in the Larissa region, while it was performed once (on 25/6/2023) for the natural population located in the Ellinopirgos area.

The aerial parts (flowers and upper leaves) were promptly transported to the laboratory, where they were air-dried in a shaded, well-ventilated space at room temperature until a stable weight was reached. A sufficient number of samples from

both locations was processed using hydrodistillation to obtain the essential oil, utilizing a Clevenger apparatus in the Laboratory. A total of 10 g of dried plant material was subjected to hydrodistillation with 250 mL of distilled water for a duration of 2.5 hours.

The essential oils that were extracted were measured volumetrically and subsequently analyzed using gas chromatography coupled with mass spectrometry (GC–MS) on a fused silica DB-5 column. The relative content of each compound was calculated as a percentage of the total chromatographic area, and the findings are presented as the mean percentage of three replicates (Sarrou et al., 2017; Tsivelika et al., 2018).

Identification of the compounds was achieved by comparing retention indices (RIs) relative to n-alkanes (C7–C22) with existing literature data and by matching spectra against mass spectrometry libraries (NIST98, Willey; Adams, 1995).

RESULTS AND DISCUSSION

The results indicated a variation in the components of the essential oils, with certain substances exhibiting a zero percent content depending the sampling. These substances included: cadinene-δ, cadinene-γ, cadinol-α, dodecanol-n, (E)-β-farnesene, himachalene-β, himachalene-γ, himachalol, limonene, muurolene-γ, myrcene, nonane (N-), pinene-β, and undecane (N-). Additionally, among the other detected substances, which were caryophyllene (E-), caryophyllene-oxide, himachalene-α, pinene-α, selinene-α, selinene-β, spathulenol, and tetradecanol (n-), statistically significant differences were observed between the two cuts in intensive cultivation as well as across the various environments.

Table 1.

Essential oil characteristics (% components found using GC–MS; Caryophyllene (E-), Caryophyllene- oxide, Himachelene-α, Pinene-α, Selinene-α, Selinene-β, Spathulenol, Tetradecanol (n-)) between the different harvests and the different environments.

	Caryophyllene (E-)	**Caryophyllene-oxide**	**Himachalene-α**	**Pinene-α**	**Selinene-α**	**Selinene-β**	**Spathulenol**	**Tetradecanol (n-)**
Larissa 1st	3,73	2,8	2,29	38,06	3,279	3,558	0,81	3,15
Larissa 2nd	3,21	2,18	1,16	35,28	2,124	2,442	0,23	1,54
Ellinopirgos	15,17	10,93	6,12	9,89	3,72	3,475	3,24	7,11
$LSD_{,05}$	1,882	1,335	0,765	2,544	0,3945	0,4938	0,75	0,891
CV (%)	11,3	11,1	10,6	4	5,7	6,9	23,2	10

*LSD: least significant difference; ns: non-significant

As shown in Table 1, the plants from the natural population that thrived in their environment (Ellinopirgos) exhibited a significantly higher percentage of the following components: Caryophyllene (E-), Caryophyllene-oxide, Himachelene-α, Spathulenol, and Tetradecanol (n-). However, in the case of Pinene-α, the propagated plants from the same population that were cultivated in the Larissa region demonstrated a statistically significant higher content.

Table 2.
Essential oil characteristics (% components found using GC–MS) between the different harvests and the different environments in certain instances, which characteristics were subject to zeroing.

	Cadinene-δ	Cadinene-γ	Cadinol-α	Dodecanol-n	(E)-β-Farnesene	Himachalene-β	Himachalene-γ	Himachalol	Limonene	Muurolene-γ	Myrcene	Nonane (N-)	Pinene-β	Undecane (N-)
Larissa 1st	0,00	0,00	0,00	0,00	2,11	0,00	1,75	0,00	0,00	1,49	0,59	2,56	6,96	0,00
Larissa 2nd	0,00	0,00	0,00	0,00	0,51	0,00	0,00	0,00	0,75	0,00	0,93	4,40	5,22	1,27
Ellinopirgos	3,17	1,14	1,82	4,60	0,00	2,25	2,50	2,90	0,00	6,56	0,00	0,00	0,00	0,00

Additionally, Table 2 illustrates the percentage components across various harvests and environments in specific cases, where certain characteristics were recorded as zero. In particular, it was observed that the propagated plants (originating from the examined population) cultivated in the Larissa region exhibited a zero percent content for the compounds: Cadinene-δ, Cadinene-γ, Cadinol-α, Dodecanol-n, Himachalene-β, and Himachalol, in both the first and second harvests. Conversely, the plants from the natural population that thrived in their environment (Ellinopirgos, Karditsa), were found to have a 0% content for the compounds: (E)-β-Farnesene, Limonene, Myrcene, Nonane (N-), Pinene-β, and Undecane (N-). Lastly, a notable difference was also identified where the percentage content was zero (Himachalene-γ, Limonene, Undecane (N-), Muurolene-γ) between the two harvests for the plants from the population that cultivated in Larissa.

There are few studies that have documented the chemical variability of H. perforatum, both between different chemotypes and between different cultivation practices, indicating a lack of certain compounds (Avato and Gugliemi, 2004, Tegou et al., 2024), which is consistent with the results of the present study.

Furthermore, this study highlights the influence of cultivation practices and environmental factors on the essential oil composition of wild plant populations in the broader Thessaly region, which includes the adjacent prefectures of Larissa and Karditsa, as well as the timing of plant harvests within this area (first and second harvests).

The research conducted by Crockett et al. (2010) and Schepetkin et al. (2021) indicates that the essential oil derived from H. perforatum comprises various compounds in notable concentrations, such as β-pinene (18.32 %), α-pinene (5.56–30.92 %), δ-cadinene (0.0–22.58 %), and caryophyllene (15.26 %). However, these

findings do not entirely correspond with the current study, particularly regarding wild plants where β-pinene is found at 0%, and in cultivated plants from Larisa, where the levels of β-pinene (5.22-6.96 %), α-pinene (35.28-38.06 %), and caryophyllene (approximately 3 %) differ significantly.

CONCLUSIONS

The observed variability in the essential oil composition of *Hypericum perforatum* across different sampling conditions and cultivation environments demonstrates the intricate interplay between genetic factors and ecological influences. The absence of certain compounds in specific samples, together with statistically significant variations in key constituents, suggests that both environmental conditions and inherent biotypic characteristics contribute to the chemical diversity of the species. Moreover, in the sample collected from the natural population located in the area of Ellinopyrgos (Karditsa), a particularly high proportion of sesquiterpene hydrocarbons (E-caryophyllene, α-, γ-, and β-himachalene, as well as γ-muurolene) and oxygenated sesquiterpenes (spathulenol, caryophyllene oxide, himachalol, and α-cadinol) was recorded compared to the population cultivated under intensive conditions in the region of Larissa. These compounds are well-documented for their strong antimicrobial and anti-inflammatory properties; therefore, their increased presence in the essential oil is not interpreted as an indication of toxicity or harmfulness to humans but rather as evidence of enhanced pharmacological activity. These findings provide a valuable foundation for future investigations aimed at elucidating the extent to which ecological parameters modulate essential oil profiles. Ultimately, such research will inform more precise selection of cultivation areas and populations, thereby supporting the optimization of *H. perforatum* production and its associated phytochemical quality.

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