Research Article

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Determination of essential oil and chemical composition of St. John's Wort

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Abstract: Considering it contains a variety of physiologically active compounds, including flavonoids, common phenols, and essential oils (EOs), St. John's wort (*Hypericum perforatum* L.) is a common plant in Bulgaria that is predominantly used in folk medicine to cure various disorders. Determining the chemical makeup of St. John's wort inflorescences that were gathered from northern Bulgaria was the purpose of this investigation. The antioxidant activity of *H. perforatum* L. extracts was assessed using 1,1-diphenyl-2-picrilhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) tests on methanol extract. The amount of EO obtained by water distillation was 0.08%, with its main components (over 3%) being *n*-nonane (27.46%), *β*-sesquiphellandrene (11.17%), heptanal dimethyl acetal (5.94%), ethyl hexyl ketone (5.93%), undecane (3.75%), sabinene (3.3%), and tridecyl alcohol (3.1%). Methanol extracts were obtained from the inflorescences, with the total flavonoid content determined as 8.66 mg quercetin equivalents (QE)/mg and total phenolic content as 271.33 mg Gallic acid equivalent/g. The FRAP assay yielded 493.07 μmol/L of antioxidant activity, while the TEAC assay yielded 106.39 μmol/L, respectively. Our findings enable a comprehensive characterization of *H. perforatum* from Bulgaria and an assessment of its oil suitability for potential industrial applications. Additionally, the results could guide the selection of specimens for future targeted breeding efforts.

Keywords: St. John's wort, biologically active substances, antioxidant activity

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1 Introduction

Herbal remedies and aromatic plants have been utilized for sustenance, warmth, protection, and shelter throughout human history. Archaeological discoveries have also shown that these plants were formerly utilized by people as a remedy for a variety of illnesses. Modern science has made significant strides, expanding the use of aromatic and therapeutic plants. Nowadays, the primary industries that use these plants are the food, cosmetics, pharmacy, medicine, dyeing, perfumes, and agriculture [1–5].

The genus *Hypericum* L. comprises approximately 480 species worldwide. *Hypericum* L., also known as St. John's wort, is represented by 61 species in 17 divisions of the European flora. There are 22 species of this genus found in Bulgaria, five of which are native to the country. The fact that St. John's wort is widely distributed across the nation has led to a great deal of research on the plant [6]. The flowers of this species are profuse and bloom from June until September [7]. *Hypericum perforatum* L. is among the most commonly used medicinal and aromatic plants in folk medicine [8]. Extracts of *Hypericum perforatum* L.

are known to reduce oxidative stress and exhibit neuroprotective, anti-inflammatory, and anti-gastrointestinal properties. Moreover, *Hypericum perforatum* L. is used in the treatment of depression [9]. Preventing oxidative damage by the use of naturally occurring antioxidants, including phenolic compounds, has received a lot of attention. Oxidative stress represents an imbalance in the body's antioxidant defense system, as a result of cells producing more reactive oxygen species, which can harm DNA, lipids, and proteins. Numerous chronic degenerative illnesses, including cancer, neurological disorders, and cardiovascular diseases, are linked to the pathophysiology of aging and mutagenesis. Prooxidant agents, such as hydroxide radicals, peroxide radicals, singlet oxygen, and superoxide anions, are among them. Depression is increasingly associated with oxidative stress in the body [10]. Free radicals generated during normal bodily functions have detrimental effects on the immune system and cells, leading to diseases and premature aging. Antioxidants act as scavengers for these free radicals, thereby preventing diseases by neutralizing them [11,12].

Essential oils (EOs) obtained from plants are colorless or light yellow, have a distinctive odor, and are liquid at room temperature, consisting of many components. Their many qualities, including antiseptic, antifungal, antioxidant, antimicrobial, and antiviral, have been utilized in traditional medicine since ancient times. While EOs are generally found in large quantities in the roots, leaves, and flower parts of the plant, they are found in smaller quantities in other plant organs such as bark and stems. The most commonly detected EO components in *H. perforatum* L. are monoterpenes and sesquiterpenes. With 600 of the 3,500 plant species known to have therapeutic qualities, Bulgaria enjoys a varied flora rich in medicinal plants. Despite this abundance, Chimshirova et al. [7] pointed out that little is known about the antioxidant potential and modern therapeutic uses of numerous Bulgarian medicinal herbs. The purpose of this study is to identify the EO and chemical makeup of St. John's wort aerial parts collected in northern Bulgaria.

2 Material and methods

2.1 Plant material

The aerial portions of *H. perforatum* L. were gathered in June 2020 from Dulovo, Bulgaria (43°50′26.5″N 27°07′51.4″E), at a height of about 230 meters above sea level, during the blossoming season. After being gathered, the samples were dried at room temperature (25 ± 2°C). Plant samples were kept in storage prior to analysis. The plant sample was identified botanically at Yozgat Bozok University's (YBOZ) Department of Botany. By drying the plant to a constant weight at 105°C, the moisture content of the plant was determined. According to AOAC [13], the chemical analysis findings were given on a dry weight basis. The voucher specimen, identified by voucher number YBOZ-2020-45, was stored in the Herbarium of the Biology Department, Faculty of Science and Letters, YBOZ, for future use.

2.2 Chemical composition

2.2.1 EO isolation

Using a Clevenger-style equipment, 100 g of dried aerial portions of the plant sample was hydrodistilled for 3 hours. Based on dry matter, the EO (%, v/w) contents of the samples were computed. After being extracted, the EOs were put into bottles with a dark tint and kept in a refrigerator at +4°C until analysis.

The QP2010 ULTRA mass spectrometer, a Shimadzu gas chromatography–mass spectrometric (GC/MS) instrument, was used to perform a GC/MS study. A GC analysis was performed using an Agilent 7890 A gas chromatograph equipped with an HP-5 ms column (60 m × 0.25 mm, 0.10 µm). The software set the oven temperature in 3°C increments from 60°C to 200°C, and it maintained this setting for 4 min. The injector temperature was set to 260°C, and the scan range was set between 35 and 600 m/z. Helium (1.00 ml/min, split 1:30) was the carrier gas. By comparing the relative retention durations with the National institute of standards and technology 08 database (library data), the chemical components were identified.

2.2.2 Extract

Methanol (40 mL) was combined with the plant sample (4 g) at a ratio of 1:10 w/v. The prepared samples were placed in an oven (Electo-mag M 5040 P) and incubated for 24 h at 40°C. Then, it was filtered using balloon flasks filled with Whatman No. 1 filter paper. The samples were placed in a rotary evaporator (Heating Bath B-491, BUCHI) to extract the methanol. The balloon bottles were blown up and then dried completely in the oven for a whole day. The extracts were put into falcon tubes, covered with parafilm, and stored at +4°C in order to be employed in the analysis.

The methanol extract's total phenolic contents: The extract's total phenolic content was ascertained using the

Table 1: Chemical composition of *H. perforatum* L. EOs

No	Retention time, min	Retention index[a]	Compounds	Content (%)
1	8.598	916	*n*-Nonane	27.46 ± 0.3
2	9.828	953	Butyl isobutyrate	1.31 ± 0.0
3	10.991	972	Sabinene	3.3 ± 0.0
4	12.333	1001	Decane	2.60 ± 0.0
5	12.513	1004	Pseudolimonene	0.26 ± 0.0
6	14.445	1069	Sabinene hydrate <*cis*>	0.78 ± 0.0
7	14.562	1070	Heptanal, dimethyl acetal	5.94 ± 0.2
8	15.365	1088	Isobutyl tiglate	0.75 ± 0.0
9	15.548	1093	Ethyl hexyl ketone	5.93 ± 0.1
10	15.787	1094	Nonan-2-ol	0.52 ± 0.0
11	15.947	1095	Linalool oxide <*cis*->	0.60 ± 0.0
12	16.749	1115	Solustrerol	1.58 ± 0.0
13	16.933	1124	*Trans*-*p*-2-menthen-1-ol	1.12 ± 0.0
14	17.720	1146	*Trans*-limonene oxide	0.44 ± 0.0
15	18.310	1149	Camphor	1.46 ± 0.0
16	18.437	1163	Isoisopulegol	2.06 ± 0.0
17	18.570	1175	*p*-Menth-1-en-9-al	0.61 ± 0.0
18	19.466	1180	Terpinen-4-ol	1.04 ± 0.0
19	19.958	1198	*α*-Terpineol	1.18 ± 0.0
20	20.107	1202	Myrtenol	0.16 ± 0.0
21	20.439	1208	Verbenone	2.98 ± 0.0
22	21.019	1223	Carveol <*trans*>	0.47 ± 0.0
23	21.892	1249	Isobutyrate <heptyl->	1.85 ± 0.0
24	22.248	1273	Diamyl ketone	0.75 ± 0.0
25	22.735	1284	Lavandulyl acetate	0.72 ± 0.0
26	26.590	1390	*β*-Elemene	0.45 ± 0.0
27	28.095	1423	*β*-Cedrene	0.73 ± 0.0
28	28.432	1438	Aromadendrene	0.62 ± 0.0
29	30.175	1487	*β*-Selinene	0.94 ± 0.0
30	30.859	1508	β-Bisabolene	2.48 ± 0.0
31	30.962	1523	*β*-Sesquiphellandrene	11.17 ± 0.0
32	31.109	1530	Epiglobulol	0.42 ± 0.0
33	31.953	1546	*α*-Elemol	1.29 ± 0.0
34	32.324	1562	Nerolidol	1.14 ± 0.0
35	32.808	1576	Spathulenol	1.16 ± 0.0
36	32.982	1580	Tridecyl alcohol	3.10 ± 0.0
37	33.161	1587	Caryophyllene oxide	1.34 ± 0.0
38	36.122	1682	Butyl undec-10-enoate	0.20 ± 0.0
39	36.720	1710	Undecane	3.75 ± 0.0
40	37.527	1733	Heptafluorobutyric acid, hexadecyl ester	1.88 ± 0.0
41	41.855	1900	Nonadecane	0.47 ± 0.0
42	46.462	2106	Phytol	2.99 ± 0.0
Aliphatic hydrocarbons, %				34.28
Oxygenated aliphatics, %				22.23
Sesquiterpene hydrocarbons, %				16.39
Oxygenated monoterpenes, %				13.62
Oxygenated sesquiterpenes, %				6.93
Monoterpene hydrocarbons, %				3.56
Oxygenated diterpenes, %				2.99

[a]RI – retention (Kovat's) index. Results indicate three injections of same sample.

Folin-Ciocalteu reagent technique. The usual method of controlling phenolic substances was to employ gallic acid. The results are presented as the conjugate of gallic acid.

The total flavonoid assay was calculated by refining aluminum chloride colorimetric technique. Consequently, the total flavonoid concentration was estimated as quercetin equivalents (QE)/g of extract.

1,1-Diphenyl-2-picrilhydrazyl (DPPH) free radical, a well-known and often utilized radical, was employed to measure the DPPH free radical scavenging activity [14]. As benchmarks, butyl hydroxytoluene (BHT) and butyl hydroxyanisol (BHA) were utilized. Ferric reducing antioxidant power assay (FRAP) Използван е метод, описан от at 593 nm, Trolox was used as standard, and the results are given as Trolox equivalent.

TEAC assay consists of reducing the absorbance of the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) at 660, 734 and 820 nm.

The hydroxyl radical scavenging test developed in the previous study [15] was used to assess the hydroxyl radical scavenging capability.

As nitro blue tetrazolium to formazan decreased due to superoxide radical suppression, the superoxide scavenging capability was ascertained [16].

2.3 Statistics

The results of all measurements were expressed as mean ± standard deviation (SD) of three parallel measurements and analyzed using MS-Excel software.

3 Results and discussion

Table 1 presents the chemical components of the EOs. Forty-two constituents representing 100% of the total EO content were identified in *H. perforatum* L. The main EO compounds (over 3%) were *n*-nonane (27.46%), *β*-sesquiphellandrene (11.17%), heptanal dimethyl acetal (5.94%), ethyl hexyl ketone (5.93%), undecane (3.75%), sabinene (3.3%), and tridecyl alcohol (3.1%). The distribution of major groups of aroma substances in the EOs is shown in Table 1. Aliphatic hydrocarbons (34.28%) are the predominant group in the EOs, followed by sesquiterpene hydrocarbons (16.39%), oxygenated monoterpenes (13.62%), oxygenated sesquiterpenes (6.93%), and oxygenated diterpenes (2.99%).

The composition of EO may vary between different parts of the plants [17]. Previous studies have shown variations in the chemical compounds of St. John's wort EOs obtained from the aerial parts of the plant depending on the collection period and the location of the collected plants [18]. According to previous studies, *H. perforatum* was characterized by major components such as *α*-pinene (3.7–36.5%), 2-methyloctane (1.1–15.5%), caryophyllene oxide (3.3–17.7%), *β*-caryophyllene (1.2–12.4%), and *n*-tetradecanol (3.6–10.4%) [19]. According to the study by Crockett [20], the most regularly reported EO components from *Hypericum* L. species include *n*-nonane, *α*-pinene, *β*-caryophyllene, *β*-pinene, and caryophyllene oxide.

Several articles have previously reported on the chemical components of EOs derived from St. John's wort (Table 2). Furthermore, although it is well known that changes in EO composition can result from cultivar differences, cultivar is rarely mentioned in the literature. The main components in the St. John's wort EOs collected in Serbia were 3-methylnonane (4.5%), *p*-cymene (4.8%), and nonane (63.8%) [21]. Samples of *Hypericum perforatum* L. from Portugal were characterized by *α*-pinene (39-64%), *n*-nonane (12-24%), and *n*-undecane (3-9%) [22]. The *H. perforatum* L. plant samples from Uzbekistan, initially investigated by Baser et al. [23], were especially characterized by *α*-pinene (5.0%), spathulenol (6.0%), caryophyllene oxide (6.3%), and *β*-caryophyllene (11.7%) (Table 2). *α*-Pinene and caryophyllene oxide are abundant in the EOs extracted from plants growing in Kosovo and Albania, but not in those from Turkiye. Also, *β*-caryophyllene and germacrene D are the most represented compounds in the EOs of St. John's wort from the Odrinci and Svirachi regions of Bulgaria (Table 2). They consist mostly of mono- and sesquiterpenes, especially methyl-2-octane, *n*-nonane, *α*- and *β*-pinene, *α*-terpineol, geranyl, and trace amounts of myrcene, limonene, and caryophyllene. According to the research, several differences were found in the EO composition of St. John's Wort evaluated from different geographical regions.

Studies on St. John's Wort showed that the EOs contained in the plant have high levels of germacrene D, *α*-pinene, *β*-pinene, nonane, *β*-caryophyllene, *γ*-cadinene, and *α*-selinene. The total flavonoid and total phenolic content of the extracts prepared from the aerial parts of St. John's wort were identified. As illustrated in Table 3, the total phenolic content is higher than their total flavonoid contents. Much research has been conducted on the total phenolic content of St. John's wort [30,31]. The content of EOs in *H. perforatum* plants is highest at the full bloom stage compared to the pre-flowering stage. It is crucial to apply multiple antioxidant methods, considering various oxidation aspects in systems, in order to assess the antioxidant activity [31].

In this sense, three complimentary techniques were used to examine the antioxidant qualities of St. John's

Table 2: Review of the major components of St. John's Wort Eos (aerial parts)

Origin	Main compounds (%)	EO yields	Reference
Serbia	Nonane (63.8%), *β*-selinene (2.1%), 2-methyloctane (2%), *p*-cymene (4.8%), 3-methylnonane (4.5%)	0.15% v/w	[21]
Portugal	*α*-Pinene (39–64%), *n*-nonane (12–24%), *n*-undecane (3–9%), *β*-pinene (2–3%)	0.15% v/w	[22]
Uzbekistan	*β*-Caryophyllene (11.7%), α-pinene (5.0%), caryophyllene oxide (6.3%), spathulenol (6.0%)	0.1% v/w	[23]
Lithuania	Caryophyllene oxide (6.1–35.8%), *β*-caryophyllene (5.1–19.1%), germacrene D (4.5–31.5%),	0.1–0.4% v/w	[24]
Italy	Germacrene D (19.5–20.8%), *e*-caryophyllene (21.6–23.0%), *α*-pinene (15.8%)	—	[25]
Greece	*α*-Pinene (21.0%), 2-methyl-octane (12.6%)	0.28% v/w	[26]
Kosovo	*α*-Pinene (3.7–36.5%), *n*-tetradecanol (3.6–10.4%), caryophyllene oxide (3.3–17.7%), *β*-caryophyllene (1.2–12.4%), 2-methyl-octane (1.1–15.5%)	0.04–0.26% v/w	[19]
Albania	*β*-Pinene (0.36–6.89%), α-pinene (2.03–36.74%), 2-methyl-decane (0.82–3.14%), carvacrol (0.14–5.60%), *α*-selinene (1.34–13.86%), *trans*-(*E*)-caryophyllene (0.5–19.27%), *β*-caryophyllene oxide (1.15–12.35%)	0.11 v/w	[27]
Macedonia	Germacrene D (17.77–39.03%), *β*-selinene (0.69–4.77%), *E*-caryophyllene (11.37–25.71%)	—	[28]
Turkiye	*δ*-Cadinene (3.02–4.94%), spathulenol (2.34–5.14%), *β*-caryophyllene (4.08–5.93%), *α*-selinene (4.1–10.42%), γ-muurolene (5.00–9.56%), caryophyllene oxide (6.01–12.18%), *β*-selinene (5.08–19.63%)	0.04–0.61% v/w	[29]
Bulgaria (Odrinci)	*β*-Caryophyllene (16.08%), germacrene-D (12.87%), *α*-pinene (6.76%), *trans*-*β*-ocimene (5.28%), *γ*-cadinene (3.72%), *δ*-cadinene (3.51%), spathulenol (3.95), caryophyllene oxide (5.12%)	—	[9]
Bulgaria (Svirachi)	Trans-β-ocimene (5.81%), germacrene D (16.8%), *β*-pinene (3.5%), *n*-hexadecanoic acid (4.95%), *β*-caryophyllene (6.19%), *α*-pinene (6.2%), spathulenol (3.5%), caryophyllene oxide (3.35%), *β*-myrcene (2.99%), *γ*-cadinene (3.13%), *δ*-cadinene (3.54%)	—	[9]

Table 3: Chemical and antioxidant characteristics of *H. perforatum* L. extract

Parameters	Flowers
FRAP assay (μmol/L)	493.07 ± 0.36
TEAC assay (μmol/L)	106.39 ± 1.05
Total flavonoid content (mg QE/g)	8.66 ± 0.41
Total phenolic content (mg Gallic acid equivalent/g)	271.33 ± 0.40
Superoxide scavenging (unit SOD/mL)	20.8 ± 3.2
Hydroxyl radical scavenging (mM ethanol/mL)	19.8 ± 3.9

wort. Table 3 presents the plant's chemical composition and antioxidant characteristic results. BHA and BHT, two well-known conventional antioxidants, were contrasted using one of these techniques, the DPPH free radical scavenging approach (Table 4).

According to our results, the IC_{50} value of *Hypericum perforatum* L. (87.025 ± 0.21 μg/mL) indicates a stronger DPPH scavenging activity than those of BHA and BHT (Table 4). According to the literature, the IC_{50} value in *H. perforatum* was found to be 29.35 μg/mL [31]. In the current study, the obtained results indicate the antioxidant capacity with a TEAC value of 106.39 ± 1.05 μmol/L Trolox and an FRAP assay value of 493.07 ± 36 μmol/L Trolox (Table 3).

In previous studies, St. John's wort has been determined to be a potent inhibitor of the superoxide radical in a cell-free system, and this antioxidant activity has been ascribed to hypericin [32]. Our results are consistent with the view that *H. perforatum* extracts have strong scavenging properties on superoxide capacity.

Phenolic compounds have also been shown to have positive effects on human health, mostly responsible for antioxidant activity [33]. Due to their rich phenolic content, *Hypericum perforatum* L. species is known as a good source of antioxidants [34,31]. According to the results, the yield of EOs was 0.08 mL per 100 g of dry matter for *H. perforatum* L.

Hypericum species generally contain very low amounts of EOs (0.05–0.9%) [35]. Ghasemi Pirbalouti et al. [36] reported the EO yield of *H. perforatum* L. as 0.21 mL/100 g of dry matter. According to another study, the EO yield of *H. perforatum* collected from Serbia was 0.32% (w/w) (Gudzic et al., 2001).

Table 4: Antioxidant activity of *Hypericum perforatum* L. extract by DPPH

	IC_{50} value (μg/mL)
DPPH free radical	87.025 ± 0.21
BHA	19.662 ± 0.34
BHT	13.818 ± 0.50

Mean ± SD of three parallel measurements.

4 Conclusions

The chemical composition of St. John's wort (*H. perforatum*) aerial parts originating in northern Bulgaria was determined. The composition of the EO obtained by water distillation was identified, and the content of flavonoids and total phenols in methanol extracts was determined. The antioxidant activity of the extracts was assessed using different methods. The obtained results provide a basis for future research aimed at determining other biological activities, such as antimicrobial properties, which will contribute to the more comprehensive utilization of this plant species.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on a reasonable request.

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