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Research Article

Chemical composition of endemic *Hypericum bilgehan-bilgilii* Basköse & Savran essential oil and its α-glucosidase antidiabetic, anti-inflammatory, cytotoxic and antioxidant potentials

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Abstract

Hypericum L. is a significantly important genus for flora of Türkiye due to its richness in phytochemicals possessing medicinal and cosmetic benefits. The essential oil composition and biological activities of *Hypericum bilgehan-bilgilii* Basköse & Savran (HEO) were analysed for the first time in the present study. The volatile oil of the whole parts of *H. bilgehan-bilgilii* was obtained by hydrodistillation with Clevenger-type apparatus. The chemical composition was analyzed by GC-MS using non-polar column. α-glucosidase and 5-lipoxygenase inhibitory, cytotoxic, and DPPH radical scavenging activities were investigated. Forty-eight components were identified and represented 97.3% of the whole constituents. Interestingly, the major volatiles: 1-(2,4,5-trimethoxyphenyl)butan-1-one (27.7%) and 3-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (11.2%) were detected for the first time in *Hypericum* essential oils. The oil exhibited a significant activity against the 5-lipoxygenase enzyme with an IC_{50} value of 39 µg/mL. Cytotoxicity potential of HEO was investigated, at different concentrations, towards four cell lines and, IC_{50} values underlies mild cytotoxicity. These results indicated that *H. bilgehan-bilgilii* essential oil may be considered as a valuable source for bioactive ingredients and anti-inflammatory agents.

Özet

Hypericum cinsi, tıbbi ve kozmetik faydaları olan fitokimyasallar bakımından zengin olması nedeniyle Türkiye florası için önemli bir cinstir. *Hypericum bilgehan-bilgilii* Basköse & Savran'ın (HEO)'nin uçucu yağ bileşimi ve biyolojik aktiviteleri bu çalışmada ilk kez analiz edilmiştir. *Hypericum bilgehan-bilgilii* tüm kısımlarının uçucu yağı, Clevenger tipi aparatla hidrodistilasyon yoluyla elde edilmiştir. Kimyasal bileşim, polar olmayan kolon kullanılarak GC-MS ile analiz edilmiştir. α-glukozidaz ve 5-lipoksijenaz inhibitör, sitotoksik ve DPPH radikal süpürücü aktiviteleri araştırılmıştır. Kırk sekiz bileşen tanımlanmış ve tüm bileşenlerin %97,3'ünü temsil etmiştir. İlginç bir şekilde, majör uçucular: 1-(2,4,5-trimetoksifenil)bütan-1-on (%27,7) ve 3-Metil-1-(2,4,6-trihidroksifenil)bütan-1-on (%11,2) *Hypericum* uçucu yağlarında ilk kez tespit edilmiştir. Yağ, 39 µg/mL'lik bir IC_{50} değeriyle 5-lipoksijenaz enzimine karşı önemli bir aktivite göstermiştir. HEO'nun sitotoksisite potansiyeli, farklı konsantrasyonlarda, dört hücre hattına karşı araştırılmış ve IC_{50} değerleri hafif sitotoksisitenin varlığını göstermiştir. Bu sonuçlar, *H. bilgehan-bilgilii* uçucu yağının biyoaktif bileşenler ve anti-enflamatuvar ajanlar için değerli bir kaynak olarak kabul edilebileceğini göstermiştir.

Keywords: aromatherapy, gas chromatography-mass spectrometry, GC-FID, bioactivity, *Hypericum bilgehan-bilgilii*

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Introduction

The genus *Hypericum* L. is a member of the Hypericaceae family and consists of more than 500 species all over the world. There are 109 taxa in 20 sections in the flora of Türkiye and 48 taxa of which are endemic (Duman & Cakir-Dündar, 2020). *Hypericum* species have been used as antidepressants, sedatives, diuretics, antiphlogistics, analgesics, astringents, and antipyretics in various parts of the world (Doğan et al., 2019; Ersoy et al., 2019; Galeotti, 2017; Kandilarov et al., 2018; Kurt-Celep et al., 2020; Marrelli et al., 2016; Velingkar et al., 2017; Wise et al., 2019; Zhang et al., 2020). The genus is a rich source of various secondary metabolites as naphthodianthrones, flavonoids, phloroglucinols, bioflavonoids, proanthocyanidins, and terpenes (Khorshidi et al. 2020; Semerdjieva et al., 2023). The results of extensive studies on phytochemical compositions and biological activities of *Hypericum* species have been reported. Notably, *H. perforatum* L., is prominent for many beneficial pharmacological properties such as antidepressant, antibacterial, antiviral, anti-inflammatory, antinociceptive, and analgesic (Galeotti, 2017).

As noteworthy utilities of *H. perforatum* in diminishing depressive symptoms have been highlighted, there has been an increasing attention to evaluate whether other *Hypericum* species have similar features. There are many reports on the essential oil composition of *Hypericum* species in the literature with significant amount of variation in their secondary metabolite profiles. The variation in essential oil composition of the *Hypericum* genus may be related to essential oil extraction type, phenological cycle, seasonal variation, plant part, and geographical area. Additionally, gland types (translucent and dark glands) may affect the essential oil composition of *Hypericum* species. Translucent and dark glands are found in different parts of the plants. For example, *H. androsaemum* had translucent glands, which were found on the leaf margin and lamina. (*E*)-2-hexenal (15.5%), hexadecanoic acid (14.7%), β-caryophyllene (11.2%), germacrene B (11.0%) and γ-himachalene (9.8%) were the major components of the lamina glands, whereas β-pinene (22.0%), limonene (17.6%), (*E*)-β-ocimene (6.1%), methyl linoleate (5.7%), terpinolene (5.4%), (*E*)-2-hexenal (4.9%) and α-pinene (4.1%) were the main compounds of the margin glands (Guedes et al., 2012). Due to the diversity of essential oils, *Hypericum* species display different biological activities such as antioxidant, antibacterial, antifungal, cytotoxic, insecticidal, neuroprotective, enzyme inhibitory, hepatoprotective, wound healing, etc. (Bertoli et al., 2011; Grafakou et al., 2022). This wide range of bioactivities of *Hypericum* essencial oils (EOs) has increased interest in novel candidates in the same genus. Although the genus *Hypericum* has many species, the biological activities and chemical composition of essential oils are known only in a few species, with the exception of *H. perforatum*. Due to this reason, studying the chemical composition and biological activities of the essential oils of *H. bilgehan-bilgilii*, which is an endemic perennial plant and was described as a new species in 2018, growing on calcareous rocks, chasmophyte, is of great importance. In light of this information, the *in vitro* α-glucosidase inhibitory, 5-lipoxygenase inhibitory, and DPPH radical scavenging activities of the essential oil of *H. bilgehan-bilgilii* (HEO) were investigated for the first time in the present study. Moreover, cytotoxic properties of HEO were evaluated towards four different cell lines (L929, A549, MCF-7 and CHO) for three different concentrations in order to determine possible toxic effects prior to medical utilizations.

Materials and Methods

Plant Material

Hypericum bilgehan-bilgilii was collected in the calcareous rocks (1600–2200 m, 37º 29’ 21.66’’N, 31º 19’ 35.81’’E) in Beyşehir district of Konya province, Türkiye, on August 2020 by Huseyin Turker and identified by Dr. Ahmet Savran (Baskose & Savran, 2018). Herbarium specimens of *H. bilgehan-bilgilii* was deposited in the Herbarium of Ankara University (Herbarium number: Savran-4600).

Hydrodistillation

The essential oil of dry whole parts (aerial parts and roots) of *Hypericum bilgehan-bilgilii* (HEO) was obtained by hydrodistillation method for 3 hours with Clevenger-type apparatus (Ertosun et al., 2023).

Gas Chromatography (GC-FID)

Gas chromatography analysis was performed on capillary column Innowax FSC (60 m × 0.25 mm, 0.25 m film thickness). Chromatographic conditions were as follows: helium was used as carrier gas at 1.0 mL min^{-1}; injector and detector temperature was 250ºC. Oven temperature was isothermal at 60ºC for 10 min, then increased to 220ºC, at a rate of 4ºC min^{-1}, isothermal at 220ºC for 10 min and increased to 240ºC, at a rate of 1ºC min^{-1}. Split ratio 1:25. The injection volume was 1 µL (Servi et al., 2023).

Gas Chromatography-mass Spectrometry (GC-MS)

The essential oil composition was determined by briefly used method of Barak et al. (2023b). The column type was DP-5 (5% diphenyl, 95% dimethyl polysiloxane; 30 m × 0.25 mm, 0.25 m film thickness). The GC-MS analysis parameters were: the oven temperature was 60ºC for 1 min, then raised at a rate of 3ºC min^{-1} to 246ºC. It was held at that temperature for 30 min. Helium was used as the carrier gas, and a flow rate of 0.9 mL min^{-1} was used. The essential oil components were determined by a comparison of relative retention indices obtained from a series of *n*-alkanes (C5 to C30) to the literature as the method used by Barak et al. (2025) and with mass spectra comparison to the in-house libraries (NIST14 and Wiley7).

In vitro Antioxidant Activity of *Hypericum bilgehan-bilgilii* Essential Oil

DPPH radical scavenging activity of essential oil was specified by briefly used method of Zou et al. (2011). 10 µL of essential oils (5000–9.77 µg/mL) or standard ascorbic acid (250–7.81 µg/mL) in dimethylsulfoxide (DMSO) at different concentrations were mixed with 190 µL of 0.1 mM DPPH solution in MeOH in a well of the 96-well plate. The mixture was kept in the dark at room

temperature for 30 min. The absorbance value was detected at 517 nm. The results are expressed as IC_{50} (mg/mL), the concentration of the sample that scavenges the radical by 50%. Each experiment was applied in triplicate.

In vitro Anti-inflammatory Activity Assay

The anti-inflammatory activity was determined with minor changes according to the method described by Phosrithong & Nuchtavorn (2016) was performed according to Yıldırım et al. (2019), with slight modifications, adapted to the 96-well microplate format. 10 µL of essential oils (250–9.77 µg/mL) or standard indomethacin (100–0.02 µg/mL) were added to 20 µL ethanol, 20 µL pure water, 25 µL of sodium borate buffer solution (0.1 M, pH 9) and 25 µL of type V soybean lipoxygenase solution in the buffer (pH 9, 20.000 U/mL). The mixture was pre-incubated at 25°C for 5 min. Then, 100 µL of 0.6 mM linoleic acid solution was added to solutions, mixed well and the change in absorbance at 234 nm was followed for 6 min. The results are expressed as IC_{50} value (mg/mL), the concentration of the sample that inhibits the activity of the enzyme by 50%. Each experiment was applied in triplicate.

In vitro Antidiabetic Activity Assay

The α-glucosidase inhibitor activity method suggested by Ramakrishna et al. (2017) was performed according to Sen et al. (2019) with some modifications. 10 µL of essential oils (5000–7.81 µg/mL) or standard acarbose (250–9.77 µg/mL), 40 µL of 0.1 M sodium phosphate buffer (pH 6.9), and 100 µL of a-glucosidase (obtained from *Saccharomyces cerevisiae*) were mixed in a well of the 96-well plate. After pre-incubation at 25°C for 10 min, 50 µL of 5 mM p-nitrophenyl-a-D-glucopyranoside (*p*NPG) to the solutions was added and re-incubated at 25°C for 5 min. The absorbance reading was taken before and after incubation at 405 nm using a microplate reader. The results are expressed as IC_{50} value (mg/mL), the concentration of the sample that inhibits the activity of the enzyme by 50%. Each experiment was applied in triplicate.

Evaluations for Cytotoxic Activity

To determine the cytotoxic effects, different concentrations (20, 100 and 250 µg/mL) of the essential oil of *H. bilgehan-bilgilii* were applied to A549 (human lung adenocarcinoma), MCF-7 (human breast carcinoma), L929 (mouse fibroblast), and CHO (Chinese hamster ovary) cell lines. These cell lines were obtained from ATCC and were cultured in DMEM-high glucose medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin, under incubation conditions of 37°C with 5% CO_2.

Prior to the experiment, 5000 cells per well were seeded in 96-well culture plates and allowed to adhere overnight. Essential oil prepared at a concentration of 2.5 mg/100 mL in ethanol were applied at different concentrations for 48 hours. Following exposure, the effect on cell viability was assessed using an MTT solution (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) prepared in PBS. The cells were incubated with the MTT reagent for 4 hours at 37°C in the dark to allow the formation of formazan crystals. The resulting formazan crystals were then dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. The effect of the treatment groups on cell viability was determined in comparison to the control group, and the IC_{50} values (concentration that inhibits 50% of cell viability) were calculated using GraphPad Prism version 9.5.1 (Ustuner et al., 2023).

Statistical Analysis

All tests were performed at least three times. The results were expressed as mean ± standard deviation (SD). The statistical significance of the data was analysed using Student's t-test (for anti-lipoxygenase and α-glucosidase inhibitory assays) or ANOVA with post hoc comparison by Tukey (for DPPH radical scavenging assay). $p < 0.05$ was considered statistically significant.

Results and Discussion

The essential oil yield of *H. bilgehan-bilgilii* was calculated as 0.06 (v/w). Forty-eight constituents were identified in HEO, which was equal to 97.3% of its total ingredients (Table 1).

It was observed that there were three dominant groups in the HEO: phloroglucinol derivatives (40.1%), sesquiterpenes (23.6%), and oxygenated sesquiterpenes (21.1%). 1-(2,4,5-trimethoxyphenyl) butan-1-one (27.7%) and 3-Methyl-1-(2,4,6-trihydroxyphenyl) butan-1-one (11.2%) were the main compounds of the essential oil (Figure 1 and Table 1). It is well known that secondary metabolite profiles of plants are affected by various parameters

Table 1. Essential oil composition of whole parts of *Hypericum bilgehan-bilgilii.*

RT[1]	RRI[2]	RRI[3]	Compounds	(%)	IM[4]
6.328	933	939	α-Pinene	2.0	RI, MS
7.616	977	980	β-Pinene	1.7	RI, MS
8.034	991	991	β-Myrcene	0.2	RI, MS
9.367	1028	1031	Limonene	0.9	RI, MS
11.656	1089	1088	*p*-Mentha-1,4(8)-diene	0.2	RI, MS
12.107	1101	1098	Linalool	0.7	RI, MS
12.288	1105	1101	Nonanal	0.2	RI, MS
12.676	1114	1117	Fenchol	0.1	RI, MS

Table 1. Continued.

RT[1]	RRI[2]	RRI[3]	Compounds	(%)	IM[4]
13.729	1139	1137	*trans*-Pinocarveol	0.7	RI, MS
14.734	1163	1164	Pinocarvone	0.3	RI, MS
14.868	1166	1169	Endo-borneol	0.4	RI, MS
15.947	1191	1189	α-Terpineol	0.6	RI, MS
16.182	1197	1191	Myrtenol	0.7	RI, MS
21.327	1317	1314	(*E*,*E*)-2,4- Decadienol	0.5	RI, MS
23.845	1376	1376	Copaene	0.3	RI, MS
24.587	1394	1387	(*E*)-β-Elemene	4.4	RI, MS
25.654	1420	1418	Caryophyllene	0.6	RI, MS
26.448	1440	1440	Aromadendrene	0.6	RI, MS
27.046	1454	1455	α-Humulene	0.2	RI, MS
27.333	1461	1462	Alloaromadendrene	0.5	RI, MS
28.012	1478	1477	γ-Muurolene	1.1	RI, MS
28.205	1483	1480	Germacrene D	3.3	RI, MS
28.405	1488	1485	β-Selinene	2.5	RI, MS
28.848	**1499**	**1495**	**Bicyclogermacrene**	6.1	RI, MS
29.198	1508	1504	Eremophilene	3.1	RI, MS
29.490	1515	1513	γ-Cadinene	0.2	RI, MS
29.860	1525	1524	δ-Cadinene	0.7	RI, MS
31.727	1573	1570	*cis*-3-Hexenyl-benzoate	0.2	RI, MS
32.044	**1581**	**1576**	**Spathulenol**	5.8	RI, MS
32.223	1586	1584	Globulol	0.9	RI, MS
32.587	1595	1595	Salvial 4(14)-en-1-one	0.3	RI, MS
33.287	1614	1607	Eudesm-6-en-4 α-ol	0.5	RI, MS
33.671	1624	1613	Rosifoliol	0.3	RI, MS
33.935	1631	1638	Hinesol	1.2	RI, MS
34.011	1633	1630	γ-Eudesmol	0.7	RI, MS
34.288	1641	1645	α-Muurolol	1.2	RI, MS
34.737	1653	1649	β-Eudesmol	3.3	RI, MS
34.873	1657	1652	α-Eudesmol	4.9	RI, MS
36.019	1688	1686	Germacra-4(15),5,10(14)-trien-1α-ol	1.0	RI, MS
37.272	1723	1714	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	0.6	RI, MS
38.750	1765	1763	Benzyl benzoate	0.9	RI, MS
39.062	1774	1751	1-(2,4,5-Trimethoxyphenyl)propan-2-one	1.2	RI, MS
41.537	1846	1845	Hexahydrofarnesyl acetone	0.4	RI, MS
42.666	**1880**	**1891**	**1-(2,4,5-Trimethoxyphenyl)butan-1-one**	27.7	RI, MS
45.011	**1952**		**3-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one**	11.2	MS
60.671	2500	2500	Pentacosane	0.2	RI, MS
65.956	2700	2700	Heptacosane	0.6	RI, MS
73.795	2901	2900	Nonacosane	1.4	RI, MS
			Oxygenated monoterpenes	3.7	
			Sesquiterpenes	23.6	
			Oxygenated sesquiterpenes	21.1	

Table 1. Continued.

RT[1]	RRI[2]	RRI[3]	Compounds	(%)	IM[4]
			Phloroglucinol derivatives	40.1	
			n-alkanes	2.2	
			Others	1.8	
			Total identified compounds	97.3	

[1]RT: Retention time.
[2]RRI: Experimental Relative retention indexes.
[3]RRI: Relative retention indexes from literature.
[4]IM: Identification method.

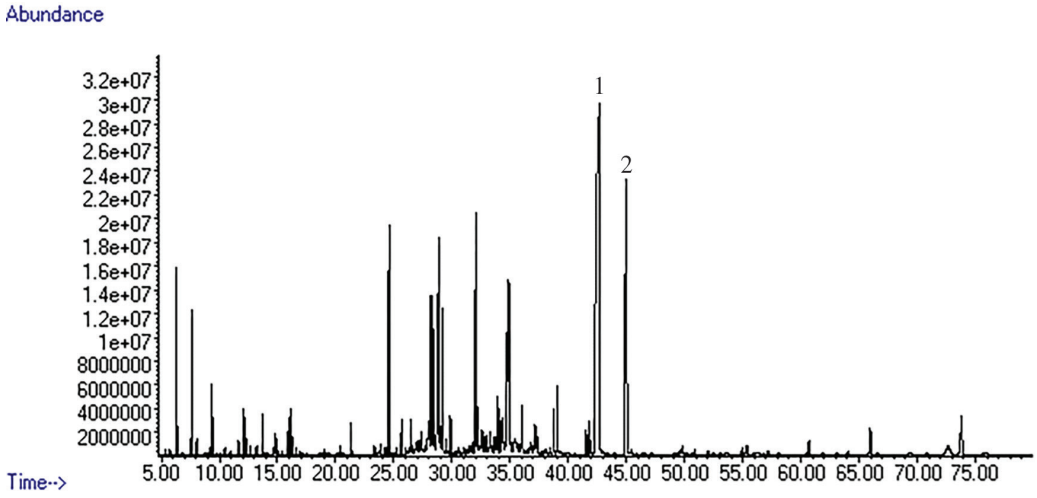

Figure 1. GC-MS chromatogram of essential oil of *H. bilgehan-bilgilii* whole part (1: 1-(2,4,5-Trimethoxyphenyl)butan-1-one; 2: 3-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one).

such as climate, soil condition, genetic factors, etc. (Barak et al., 2023a; Sen et al., 2019; Barak et al., 2024). Correspondingly, these variations were reported for *Hypericum* species. Previous studies showed that increasing the sum of sunlight is correlated with higher amounts of hypericin, hyperforin, and pseudohypericin contents of *H. perforatum* (Odabas et al., 2008). On the contrary, warm winters negatively affect secondary metabolites of *H. perforatum* (Khorshidi et al., 2020). Consistently, relative humidity and annual rainfall increase hypericin content while augmented sunshine hours positively affect hyperforin content (Riazi et al., 2015).

Even though it is the first study that investigates the chemical composition of HEO, several reports have revealed the essential oil ingredients of *Hypericum* species. These previous studies demonstrated that essential oils of *Hypericum* species from Türkiye are rich in monoterpenes, sesquiterpenes, *n*-alkanes, and fatty acid derivatives. α-pinene (12.3–88.3%) was determined as the main monoterpene compound in the essential oils of *H. empetrifolium* Willd., *H. hircinum* L., *H. kotschyanum* Boiss., *H. lydium* Boiss., *H. microcalycinum* var. *microcalycinum* Boiss. & Heldr, *H. origanifolium* var. *depilatum* (Freyn & Bornm.) N.Robson, *H. scabrum* L., *H. thymopsis* Boiss., *H. triquetrifolium* Turra., and *H. uniglandulosum* Hausskn. ex Bornm. (Babacan & Bagci, 2017; Boga et al., 2021; Eroglu et al., 2013; Kıyan et al., 2014; Serbetci et al., 2012; Tabanca et al., 2015; Yuce & Bagci, 2012). Spathulenol (12.9%), iso-longifolene (11.2%), germacrene D (30.2%), allo-aromodendrene (24.7%), caryophyllene oxide (18.3%), α-eudesmol (11.3%), *α*-selinene (19.6 or 18.7%), and *β*-selinene (15.0-37.1%) were identified as main sesquiterpenes in the essential oils of *H. capitatum* Choisy, *H. saturejifolium* Trevir, *H. empetrifolium*, *H. lydium*, *H. orientale* Boiss, *H. origanifolium* Willd., and *H. pruinatum* Boiss. & Balansa (Bertoli et al., 2015; Boga et al., 2016; Boga et al., 2021; Cirak & Bertoli, 2013; Kıyan et al., 2014). Nonacosane (11.1-42.7%), 1-hexanal (18.8%), 3-methylnonane (12.5%), undecane (19.2%) were major *n*-alkane components of essential oils of *H. kotschyanum* Boiss., *H. salsugineum* N. Robson & Hub.-Mor., *H. triquetrifolium*, and *H. uniglandulosum* Hausskn. ex Bornm. (Babacan & Bagci, 2017; Eroglu et al., 2013; Yuce & Bagci, 2012). Hexadecanoic acid (17.7 and 23.2%) was found in high amounts in the essential oils of *H. salsugineum* and *H. scabroides* (Eroglu et al., 2013).

Fatty acid derivatives were not found in the essential oil of the present study. Additionally, there are quantitative differences in the main monoterpenes, sesquiterpenes, and *n*-alkanes. Even though there is a significant sesquiterpene amount in HEO, and the major ingredients were determined as phloroglucinol derivatives. More than 400 phloroglucinol derivatives have been identified in *Hypericum* crude extracts to date. Nevertheless,

these metabolites were not previously detected in the essential oils. To our knowledge, these compounds are considered as the main responsible principles for the therapeutic bioactivities of the genus (Bridi et al., 2018). The volatile oil composition of the current study displayed a different chemical profile from that of the volatile oils of other *Hypericum* species. This dissimilarity in the present study may be related to the plant's habitat conditions or genetic factors.

HEO showed a low DPPH free radical scavenging activity, having an IC_{50} value of 1.012 mg/mL compared to standards ($p < 0.05$, Table 2). Till date, no study is presented with the antioxidant capacity of *H. bilgehan-bilgilii* essential oil, however antioxidant activity studies were carried out on another species of *Hypericum*. In one of these studies, Kamila et al. (2018) indicated that essential oil obtained from the leaves and tender parts of *H. gaitii* Haines had an IC_{50} value of 105.12 µg/mL against DPPH radical. In another study, it was determined that the IC_{50} value of the essential oil of *H. perforatum* subsp. *veronense* aerial parts was 23.07 µg/mL against DPPH radical (Vuko et al., 2021).

DPPH radical scavenging activity of *H. bilgehan-bilgilii* essential oil in our present study was found to be lower than the results reported by other investigators. HEO presented a poor α-glucosidase inhibitory activity, having an IC_{50} value of 1.513 mg/mL compared with acarbose ($p < 0.05$, Table 2). There are no studies that have focused on the α-glucosidase inhibitory activity of not only the essential oil of *H. bilgehan-bilgilii* but also the essential oils of *Hypericum* species.

HEO exhibited a significant 5-lipoxygenase inhibitory activity with an IC_{50} value of 0.039 mg/mL when compared with indomethacin (0.022 mg/mL) used as the positive control ($p < 0.05$, Table 2). Similarly, no study in the literature analyzes the 5-lipoxygenase inhibitory activity of essential oils of any *Hypericum* species, including HEO. Hyperforin, a phloroglucinol derivative found in the *H. perforatum*, has been reported to have significant 5-LOX inhibitory activity (Albert et al., 2002). In another study, it was reported that different phloroglucinol derivative compounds (3-geranyl-1-[2′-methylpropanoyl] phloroglucinol and 3-geranyl-1-[2′-methylbutanoyl] phloroglucinol) found in *H. empetrifolium* have 5-LOX enzyme inhibitory activity (Crockett et al., 2008). Spathulenol, one of the major compounds of the essential oil of *H. bilgehan-bilgilii* has also been suggested to exhibit a good docking score against 5-LOX in an *in silico* studies (Fenanir et al., 2022). Therefore, phloroglucinol derivatives in particular, along with other compounds, may be responsible for the activity of the essential oil of the current study. Cytotoxic potential of HEO was likewise investigated in the current study. There are several studies in the literature that investigated the cytotoxic properties of different *Hypericum* species.

IC_{50} values of three different species of *Hypericum* were reported in a study conducted by Akdeniz et al. (2023). Results showed that *H. linarioides* Bosse had 26.39 µg/mL IC_{50} value while *H. lydium* Boiss. had 26.89, which indicates a significant difference. In addition, Vuko et al. (2021), investigated proliferation inhibition potentials of hydrosol obtained from *H. perforatum* subsp. *veronense* against three different cancer cell lines. Results demonstrated that the volatile content of hydrosol showed significant inhibitory potential against HeLa, HCT116, and U2OS cell lines. As literature stated, significant variations might be seen in cytotoxic properties of essential oils of *Hypericum* species. In this study, three different concentrations of HEO were investigated against four different cell lines (A549, L929, MCF-7, and CHO). Results showed that HEO exhibited notably low cytotoxicity, as none of the IC_{50} values fell within the tested concentration range (up to 250 µg/mL) (Table 3). This observation is further supported by the fact that all cell viability remained relatively unaffected, even at the highest concentration tested. These values are considerably higher than those reported for other *Hypericum* species with known cytotoxic or antiproliferative effects, such as *H. linarioides* and *H. lydium* (Akdeniz et al., 2023) or *H. perforatum* hydrosol (Vuko et al., 2021).

Taken together, the current findings indicate that HEO does not exhibit strong anticancer activity under the tested conditions. Rather, its low cytotoxicity profile may support its potential use in formulations where minimal toxicity is required, such as in dermatological or supportive therapeutic applications.

Table 2. Biological activity of *Hypericum bilgehan-bilgilii* essential oil.

HEO*/standards	**Antioxidant activity**	**Anti-inflammatory activity**	**Antidiabetic activity**
	DPPH radical scavenging activity	**Anti-lipoxygenase activity**	**α-glucosidae inhibitory activity**
	IC_{50} (mg/mL)		
HEO	1.012 ± 0.059[d]	0.039 ± 0.000[b]	1.513 ± 0.016[b]
Ascorbic acid	0.018 ± 0.000[a]		
Trolox	0.015 ± 0.000[a]		
Butylated hydroxyanisole	0.057 ± 0.000[b]		
Butylated hydroxytoluene	0.214 ± 0.015[c]		
Indomethacin		0.022 ± 0.000[a]	
Acarbose			0.192 ± 0.001[a]

*HEO shows essential oil of whole parts of *H. bilgehan-bilgilii*. **Each value in the table is represented as mean ± SD (n = 3). Different letter superscripts in the same column indicate significant differences ($p < 0.05$). ***Different letters in the same row indicate significance ($p < 0.05$).

Table 3. IC_{50} values (µg/mL) of HEO on different cell lines.

L929 (mouse fibroblast)	CHO (Chinese hamster ovary)	A549 (human adenocarcinoma)	MCF-7 (human breast carcinoma)
6112	374.9	367.9	720

Conclusion

The present study determined the chemical composition and biological activities of the essential oil of *H. bilgehan-bilgilii* as well as phloroglucinol derivatives were identified in the essential oil of *Hypericum* species for the first time. In the present study, 1-(2,4,5-trimethoxyphenyl)butan-1-one and 3-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one were determined as the main compounds of HEO and this is the first report for this compounds in *Hypericum* essential oils, to our knowledge. In addition, it was displayed in this study that HEO is rich in sesquiterpenoid compounds which is parallel with literature. Results showed that phytochemical analysis of the HEO may be beneficial for enhanced perspective on its biochemical systematics and HEO might be a valuable source for medical purposes as a source of anti-inflammatory agents. HEO did not display antioxidant, cytotoxic, and antidiabetic activities.

Ethics

Ethics Committee Approval: The manuscript does not include any studies on human or animal topics. Ethics committee approval is not required.

Data Sharing Statement: Data can be shared upon reasonable request to authors.

Footnotes

Author Contributions: Conceptualization: H.S., Design/methodology: H.S., and H.T., Execution/investigation: H.S., T.H.B., A.Ş., and S.K.E., Resources/materials: H.T., B.T.Ü., and C.İ., Data acquisition: H.S., T.H.B., A.Ş., and S.K.E., Data analysis/interpretation: H.S., and S.K.E Writing – original draft: H.S., and T.H.B. Writing – review & editing/ critical revision: A.Ş., S.K.E., H.T., B.T.Ü., and C.İ.

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