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Antimicrobial Properties and Chemical Composition of the Essential Oil of *Leucobryum glaucum* (Leucobryaceae)

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Abstract

In this study, a detailed study of the essential oil from *Leucobryum glaucum* (Hedw.) Angstr. was evaluated by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) methods as well as screened for antibacterial activities of the essential oil and solvent extracts (*n*-hexane and methanol). According to the results, a total of 47 compounds were detected, among which thujopsadiene (35.5%) and *β*-curcumene (25.4%) were the main components. In the second part of this study, the essential oil and solvent extracts were tested for its antimicrobial activity against 9 microorganisms with minimal-inhibitory-concentration (MIC) values in the range 61-4235 µg / mL.

Keywords: *Leucobryum glaucum*, Essential oil, Solvent extract, GC-FID/MS, Antimicrobial activity

Leucobryum glaucum (Leucobryaceae)'un Uçucu Yağının Kimyasal Bileşimi ve Antimikrobiyal Özellikleri

Öz

Bu çalışmada, *Leucobryum glaucum* (Hedw.) Angstr.'nin uçucu yağının detaylı çalışması gaz kromatografisi/ alev iyonlaştırma dedektörü (GC/FID) ve gaz kromatografisi/ kütle spektrometresi ile değerlendirildi hem de uçucu yağ ve çözücü ekstraktlarının (*n*-hekzan ve metanol) antimikrobiyal aktivitesi incelendi. Sonuçlara göre, thujopsadien (%35,5) ve *β*-curcumen (%25,4) başlıca bileşenler olmak üzere toplamda 47 bileşik belirlendi. Çalışmanın ikinci kısmında ise uçucu yağ ve çözücü ekstraktlarının antimiktobiyal aktivitesi 9 mikroorganizmaya karşı minimum-inhibisyon-konsantrasyonu (MİK) değerleri 61-4235 µg / mL aralığında test edildi.

Anahtar kelimeler: *Leucobryum glaucum*, Uçucu yağ, Çözücü ekstraktı, GC-FID/MS, Antimikrobiyal aktivite

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1. Introduction

Bryophytes, which are separated into three classes: Bryophyta (mosses), Marchantiophyta or Hepaticae (liverworts), and Anthocerotophyta (hornworts), consist of 25,000 species of mosses (Bryophyta) in the wide-spread in almost every part of our world (Saritas et al., 2001; Pannequin et al., 2017).

The species of *Leucobryum glaucum* (Hedw.) Angstr. related to the *Leucobryaceae* family (Bryophytes). The mosses of the genus *Leucobryum* is represented by 2 taxa, in Turkey. Many species from Bryophytes were used in folk medicine for various purposes in the world. In fact, besides its use as pharmacological activities, and for its antibacterial, antitumor, antiseptic, anticoagulant, insect antifeedant, nerve protecting, and cytotoxic qualities, providing at the same time (Boyom et al., 2003; Li and Zhao, 2009; Tosun et al., 2015). Additionally, previous studies have reported that the members of mosses have rich content including terpenoids, phenolics, glycosides, fatty acids, and the same rare aromatic compounds (Zeinsmeister and Mues, 1987; Zeinsmeister, et al., 1991; Jockovic et al., 2008; Sabovljević et al., 2009; Sabovljević et al., 2010). Recent phytochemical researches revealed that the dominant chemical essential oil components of mosses were aldehydes, terpenes, and aliphatic and aromatic compounds (Üçüncü et al., 2010; Cansu et al., 2013; Tosun et al., 2014). In contrast studies on the volatile composition and essential oil of mosses are still incompletely known (Tosun et al., 2015; Valarezo et al., 2018).

Our study represents the first and significant addition to solve the importance of the chemical composition of essential oil and antimicrobial activities of essential oil and solvent extracts in mosses in Turkey. Due to this, this paper aimed to identify the essential oil composition of *L. glaucum* and essential oil and solvent extracts (*n*-hexane and methanol) antimicrobial activities were investigated.

2. Material and Method

2.1. Sample collection

L. glaucum was collected from Ordu-Turkey (altitude: 1560-1700 m) in September 2013. The mosses were identifications immediately after collection. Voucher specimen diagnosed (Uyar and Çetin, 2004; Fedosov and Ignatova, 2009;) by Assoc. Prof. Dr. Nevzat BATAN and deposited in the Herbarium of the Department of Biology (KTUB: 1609), Faculty of Science, Karadeniz Technical University, Turkey. Plant material cleaned to remove any residual compost, dried under the shadow, and stored in an air-tight container until use and grounded before use.

2.2. Isolation of essential oil

The essential oil of *L. glaucum* was obtained from the air-dried above the ground part of the moss (85 g) by hydrodistillation in a Clevenger-type apparatus with a cooling bath (-15 °C) system (3 h) [yields: 0.05% (w/w)]. The obtained oils were dissolved in HPLC grade *n*-hexane (0.5 mL), dried over anhydrous sodium sulfate and stored at 4 °C in a sealed brown vial. Two µL of the essential oil was directly injected into the GC-FID/MS instrument.

2.3. Solvent (hexane and methanol) extracts

Air-dried grounded whole part of *L. glaucum* (10 g, each) extracted with *n*-hexane and methanol to give 0.035 g and 0.675 g extracts, respectively.

2.4. Gas Chromatography-Mass Spectrometry (GC-FID/MS)

GC analysis performed using a gas chromatography device (Shimadzu GC 2010 Plus, Kyoto, Japan) attached to a mass selective detector (Shimadzu QP2010 Ultra, Kyoto, Japan) according to the previously described method (Renda et al., 2016). The separation was carried out using a Restek Rxi-5MS capillary column (Bellefonte, PA, USA) 60 m length, 0.25 mm i.d. and a 0.25 µm phase thickness in split mode. The carrier gas was helium (99.99%) at a constant flow rate of 1 mL/min. Detection was implemented in electronic impact mode (EI); ionization voltage was fixed at 70 eV, scan mode (40-450 *m/z*) was used for mass acquisition.

Volatile compounds were compared to their retention index (RIs) (relative to C_6-C_{30} *n*-alkane standards) for identification (Adams, 2004). Mass spectral data were compared to those held in the FFNSC1.2 and W9N11 library of mass spectra (Bicchi et al., 2008; Kahriman et al., 2011; Özgenç et al., 2017). The sample was analyzed and the mean reported.

2.5. Antimicrobial activity

All tested microorganisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey). These were *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC 911), *Pseudomonas aeruginosa* (ATCC 43288), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (709 Roma), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 60193), *Candida tropicalis* (ATCC 13803) and

Saccharomyces cerevisiae (RSKK 251). The essential oil was dissolved in *n*-hexane (84.700 µg/mL) and solvent extracts were dissolved in dimethyl-sulphoxide (99.0%) (DMSO) to prepare extract solution within the range of 19.700-150.200 µg/mL. The screening results are shown in Table 2.

2.6. Agar dilution MIC assay

The antimicrobial effects of the essential oil, *n*-hexane, and methanol extracts were tested quantitatively in respective broth media by using the agar well diffusion method, and the minimal inhibition concentration (MIC) values (µg/mL) were examined and used in our previous work (Ahmad et al., 1998; Villanova, 1999; Tosun et al., 2014). The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The microdilution test plates were incubated for 18-24 h at 35 °C**.** Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35 °C (Wood et al., 2003). The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10,000 µg/mL), streptomycin (10.000 µg/mL), and fluconazole (2.000 µg/mL) were used as a standard antibacterial and antifungal drug, respectively. Dimethyl-sulfoxide (DMSO) with a dilution of 1:10 was used as solvent control.

3. Results and Discussion

3.1. Chemical composition

The essential oil *L. glaucum* (aerial parts) was obtained by hydrodistillation producing a yellow colored essential oil in the range of 0.05% (w/w). The combination of the essential oil of *L. glaucum* was identified by GC-FID and GC-MS and the components were determined by the association of their linear retention indices rates (against C_6-C_{30} *n*-alkanes on Restek Rxi-5MS capillary column) and mass spectra under identical experimental conditions (Table 1) (Üçüncü et al., 2010; Kahriman et al., 2011; Cansu et al., 2013; Tosun et al., 2015; Renda et al., 2016; Özgenç et al., 2017). The chemical components of the essential oil were grouped into nine classes, which were terpene/terpenoids (monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpenes), aliphatic hydrocarbons, aldehydes, ketones, alcohols, and other in Table 1. The chemical profile explained that essential oil contained 47 specific constituents, which accounted for 98.6% of the total amount. *L. glaucum* essential oil was included 1 monoterpene hydrocarbon (0.1%), 1 oxygenated monoterpene (0.1%), 15 sesquiterpene hydrocarbons (73.6%), and 9 oxygenated sesquiterpenes (19.5%). These chemical class variations of the mosses could be connected to the climatic conditions and environmental agents for example, ecospecies, location, season, soil properties, age of the plant, and extraction techniques (Tosun et al., 2015).

Looking at the consequences of the essential oil of *L. glaucum*, four of the most abundant components of this moss were thujopsadiene (31; 35.5%), *β*-curcumene (36; 25.4%), cedrol (43; 7.6%) and cis-isolongifolene (44; 5.0%). The chemical components of the essential oil of *L. glaucum* presented in this study were parallel and different following previous reports (Cansu et al., 2013; Sim-Sim et al., 2017; Valarezo, et al., 2018). For example, it was reported that essential oils of the mosses, was rich in monoterpene hydrocarbons (*α*- and *β*-pinene, camphene, *p*-cymene, myrecene, limonene, *α*-terpinene, and camphor) and aldehydes (n-heptanal, n-octanal, n-nonanal, 2(*E*),4(*E*)-decadienal, 2(*E*),4(*Z*)-decadienal, n-tetradecanal, benzaldehyde, and benzene acetaldehyde) with high percentages (Shaw and Goffinet, 2000; Adams, 2004; Özdemir et al., 2009; Tosun et al., 2014). Similarly, Üçüncü et al. presented of the essential oils of mosses *Tortula muralis* Hedw., *Homalothecium lutescens* (Hedw.) H. Rob., *Hypnum cupressiforme* Hedw. and *Pohlia nutans* (Hedw.) Lindb. from Turkey were rich in aliphatic and aromatic aldehydes: n-heptanal, n-nonanal, 2(E),4(E)-decadienal, benzaldehyde, phenylacetaldehyde, aliphatic alcohols: n-octanol, 1-octen-3-ol, and hydrocarbons: C12-C18, saturated, mono- and di- unsaturated (Üçüncü et al., 2010). In the essential oil of mosses species from Ecuador, ninety-four constituents were identified, the major components were epizonarene (8.7%) and *α*-selinene (6.7%) in the oil of *Breutelia tomentosa*, *β*-selinene (13.5%) and *α*-selinene (10.5%) in the oil of *Leptodontium viticulosoides*, selina-3,11-dien-6-*α*-ol (19.7%) and curcuphenol (10.6%) in the oil of *Macromitrium perreflexum*, epi-*α*-muurulol (15.1%) and *α*-cadinol (12.5%) in the oil of *Campylopus richardii*, *α*-cadinol (36.8%) and *α*-santalene (8.4%) in the oil of *Rhacocarpus purpurascens*, and phytol (21.7%) and valerenol (10.1%) in the oil of *Thuidium peruvianum* (Valarezo et al., 2018). Our results are in agreement which reported that aldehydes (3.8%), among which hexanal (1; 1.2%) were the important constituent in the essential oils of *L. glaucum*. In contrast to the previously reported,

our analysis of the essential oil of *L. glaucum* in Turkey found that sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the dominant class (Özdemir et al., 2009; Üçüncü et al., 2010; Cansu et al., 2013; Tosun et al., 2014; Tosun et al., 2015).

Table 1. Essential oil compounds identified from *L. glaucum*.

		A		
No	**Compounds**	**Area[b](%)**	**Exp.RI[a]**	**Ident.LRI**
1	Hexanal	1.2	817	802
2	Heptan-2-one	0.1	900	892
4	Heptanal	0.2	909	902
5	2(*E*)-Heptenal	0.1	962	959
6	1-Octene-3-ol	0.2	982	979
7	3-Octanone	0.1	990	984
8	2-Amylfuran	0.3	996	991
9	Octanal	0.2	1006	999
10	*α*-Terpinene	0.1	1010	1017
11	Benzene acetaldehyde	0.1	1050	1042
12	2(*E*)-Octenal	0.1	1062	1055
13	Octanol	0.2	1071	1068
14	Nonanal	1.2	1106	1101
15	2(*E*)-Nonenal	0.1	1163	1162
16	Decanal	0.1	1208	1202
17	2(*E*)-Decenal	0.1	1265	1264
18	2(*E*),4(*Z*)-Decadienal	0.1	1298	1293
19	Tridecane[c]	0.1	1300	1300
20	Undecanal	0.1	1310	1307
21	2(*E*),4(*E*)-Decadienal	0.2	1321	1317
22	Bicycloelemene	1.6	1348	1333
23	Eugenol	0.1	1361	1359
24	*β*-Bourbonene	0.2	1390	1388
25	Tetradecane[c]	0.5	1403	1400
26	Longifolene	0.2	1411	1408
27	(*E*)-Caryophyllene	0.4	1422	1419
28	*β*-Ylangene	3.7	1429	1421
29	*β*-Copaene	0.1	1436	1432
30	*γ*-Elemene	1.4	1440	1437
31	Thujopsadiene	35.5	1472	1468
32	*γ*-Gurjunene	1.0	1481	1477
33	*β*-Selinene	0.6	1493	1490
34	Valencene	1.6	1499	1496
35	(*E*)-*β*-Guaiene	1.6	1508	1503
36	*β*-Curcumene	25.4	1522	1516
37	Selina-3,7(11)-diene	0.1	1550	1547
38	Germacrene B	0.2	1563	1561
39	Ledol	0.3	1573	1569
40	Spathulenol	0.5	1579	1578
41	Caryophyllene oxide	3.8	1589	1583
42	Viridiflorol	0.5	1602	1593
43	Cedrol	7.6	1609	1601
44	cis-Isolongifolene	5.0	1617	1613
45	*β*-Eudesmol	1.4	1643	1651
46	Valerianol	0.1	1666	1658
47	Longifolol	0.3	1718	1715
Constituents				
Monoterpene hydrocarbon		0.1		
Oxygenated monoterpene		0.1		
Sesquiterpene hydrocarbons		73.6		

Oxygenated sesquiterpenes	19.5
Aliphatic hydrocarbons	0.6
Aldehydes	3.8
Ketones	0.2
Alcohols	0.4
Other	0.3
Total	98.6

[a]RI calculated from retention times relative to those of n-alkanes (C_6-C_{30}) on the same methyl silicone capillary column.

[b]Percentages obtained by FID peak-area normalization.

[c]Identified by authentic samples.

A: *Leucobryum glaucum*

3.2. Antimicrobial activity

The antimicrobial activities of the essential oil and solvent extracts (*n*-hexane and methanol) of *L. glaucum* were examined using minimal-inhibitory-concentration (MIC) values with different microorganisms (strains of bacteria, yeast, and fungi), which are listed in Table 2 (Barry et al., 1999; Woods et al., 2003; Tosun et al., 2015). All analyzed examples moderate to low antibacterial activity against all ten microorganisms with the MIC values varied from 61 µg/mL to 4235 µg/mL. Table 2 shows that, essential oil and solvent extracts (*n*-hexane and methanol) from *L. glaucum* no antimicrobial activities against Gram-negative bacteria (*Escherichia coli*, *Yersinia pseudotuberculosis*, and *Pseudomonas aeruginosa*). In general, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium smegmatis*, *Candida albicans*, and *Saccharomyces cerevisiae* (Gram-positive bacteria, acido-resistant mycobacterium, and yeast-like fungi) were selective microorganisms to the essential oil and solvent extracts (*n*-hexane and methanol) of *L. glaucum*. In addition, if compared to that of all studied samples, the hexane extract of *L. glaucum* exhibited good antibacterial activity (61-985 µg/mL). The highest bioactivity was detected against *Mycobacterium smegmatis* with MIC values (61-405 µg/mL).

Table 2. Screening for the antimicrobial activity of the essential oil and solvent extracts of *L. glaucum*.

Samples	Stock Sol. µg/mL	Microorganisms and minimal inhibition concentration (MIC, µg/mL)									
		Ec	**Yp**	**Pa**	**Ef**	**Li**	**Sa**	**Bc**	**Ms**	**Ca**	**Sc**
Essential oil	84.700	-	-	-	-	-	4235	2117	405	4235	4235
Methanol ext.	150.200	-	-	-	-	-	938	469	117	469	234
Hexane ext.	19.700	-	-	-	-	-	123	61	61	985	985
Amp.	10	10	18	>128	10	10	35	15			
Strep.	10								4		
Flu	5									<8	<8

Ec*:* *Escherichia coli* **(ATCC 25922), Yp:** *Yersinia pseudotuberculosis* **(ATCC 911), Pa:** *Pseudomonas aeruginosa* **(ATCC 27853)**, **Sa:** *Staphylococcus aureus* **(ATCC 25923)**, **Ef:** *Enterococcus faecalis* **(ATCC 29212)**, **Li:** *Listeria* monocytogenes **(ATCC 43251)**, **Bc:** *Bacillus* cereus **(709 Roma)**, **Ms:** *Mycobacterium smegmatis* **(ATCC607)**, **Ca:** *Candida albicans* **(ATCC 60193)**, **Sc:** *Saccharomyces cerevisiae* **(RSKK 251)**, **Amp.:** Ampicillin, Strep.: Streptomycin, **Flu.:** Fluconazole, —: no activity of test concentrations.

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