Düzce Üniversitesi Bilim ve Teknoloji Dergisi

Araştırma Makalesi

Chemical Composition of *Lamium purpureum* L. and Determination of Anticancer Activity of Its Essential Oil on Melanoma

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ABSTRACT

The current study aimed to evaluate *Lamium purpureum* L. in terms of its chemical composition and the potential of its essential oil for anticancer activity. The profiling analysis of its chemical composition was carried out via GC-MS. For this purpose, the ethanol extract was subjected to GC-MS analysis to determine chemical compounds. To obtain essential oil, steam distillation of the whole plant (aerial, leaf, stem) was carried out in a Clevenger apparatus. The anticancer activitiy was determined via MTT assay. We observed that the maximum cell death was 14% at 50 µg/mL concentration. As a result of GC-MS analysis, palmitic acid, 7-tetradecenal, octadecadienoic acid, acetic acid, ethyl 9,12,15-octadecatrienoate, hexatriacontane, stearate and benzyl benzoate were identified as major components. We determined that the essential oil of *L. purpureum* L. had essential compounds which showed cytotoxic activities.

Keywords: *GC-MS analysis, Anticancer activity, MTT, Essential oil, Lamium purpureum* L.

Lamium purpureum L.'nin Kimyasal Bileşimi ve Melanomda Uçucu Yağının Antikanser Aktivitesinin Belirlenmesi

ÖZET

Bu çalışma *Lamium purpureum* L.'yi kimyasal bileşimi ve antikanser aktivitesi için uçucu yağ potansiyeli açısından değerlendirmeyi amaçlamıştır. Kimyasal bileşiminin profil analizi GC-MS ile yapılmıştır. Bu amaçla, etanol ekstresi kimyasal bileşiklerin belirlenmesi için GC-MS analizine tabi tutuldu. Esansiyel yağ elde etmek için, tüm bitkinin (hava, yaprak, gövde) buharla damıtılması bir Clevenger aparatında gerçekleştirildi. Antikanser aktivitesi MTT testi ile belirlendi. 50 µg/mL konsantrasyonunda maksimum hücre ölümünün% 14 olduğunu gözlemledik. GC-MS analizi sonucunda palmitik asit, 7-tetradekenal, oktadecadienoik asit, asetik asit,

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etil 9,12,15-oktadekatrienoat, heksatriakontan, stearat ve benzil benzoat ana bileşenler olarak tanımlandı. *L. purpureum* L.'ın uçucu yağının sitotoksik aktivite gösteren temel bileşiklere sahip olduğunu belirledik.

Anahtar Kelimeler: *GC-MS analizi, Antikanser aktivite, MTT, Esansiyel yağ, Lamium purpureum L.*

I. INTRODUCTION

Natural products have been employed in a therapuetic capacity since ancient times. Despite the important scientific and technological progress in the pharmaceutical industry, medicines from natural products still contribute greatly to the discovery of drugs today [1]. Recently, methadological studies have been initiated on the bioactive properties of phytochemicals with antimicrobial, anti-inflammatory, antimutagenic and antioxidant properties in the essential oils obtained from plants [2, 3]. *Lamium* species have attracted attention with their pharmacological properties and ethnobotany use worldwide and intensive phytochemical studies have been initiated on these species [4].

Turkey is considered as one of the important gene centers of the Lamiaceae family and in our country 48 genera and 782 taxa (603 species, 179 sub-species and varieties) are represented. Of them, 346 taxa (271 species, 75 subspecies and varieties) are endemic [5]. This family encompasses many species which are rich in essential oils and traditionally included in the Mediterranean cuisine [6]. Various members of the Lamiaceae family have yielded a number of biologically active essential oil isolates. Known for the presence of diterpenoids, this family includes many medical taxis used in the food and pharmaceutical industry and in traditional and modern medicine due to its antioxidant and antimicrobial essential oil content [7-9]. Therapeutic activities of *Lamium* species such as antimicrobial and antioxidant properties have been reportedly related to some biologically active substances such as volatile oils, iridoid, flavonoids, phenylpropanoids and benzoxazinoids [10]. Some biological features including the antimicrobial, anti-inflammatory, antinoseptic and antioxidant properties of different extracts of have been investigated. Although volatile oil analysis was performed, no bioactive properties were found. No study, however, has yet investigated the antiproliferative features of *Lamium purpureum* L.

The aim of this study was to analyze the chemical composition of the *Lamium purpureum* L. using GC-MS and to determine the cytotoxic and anticancer activity its essential oil.

II. MATERIALS AND METHODS

A. PLANT MATERIAL

The plant was collected from Golyaka (Duzce Province), Turkey, during the flowering stage in March, 2017- 2018. Collected plant materials were dried and ground in a blender.

B. GC-MS ANALYSIS AND ESSENTIAL OIL

The volatile oils were obtained by hydrodistillation of the *L. purpureum* L. plant material for 3 h by means of a Clevenger apparatus. Ether was then used to separate water and oil and anhydrous sodium sulphate was used to remove water following extraction, after which the essential oil was kept at 4 °C in a refrigerator.

The *L. purpureum* L. ethanol extract was analyzed via GC-MS (Agilent GC 6890N- Agilent MS5973) in accordance with the method defined previously by Canlı et al. [11], using a HP5-MS (30 m; 0.25 mm; 0.25 μm) capillary column with helium as carrier gas (1 mL/min) , the split ratio set at 10:1, the injector temperature at 350 °C, the pressure at 48.2 kPa, and the split flow at 9.9 mL/min. The MS scan was carried out at a transfer line temperature of 280 °C, an interface temperature of 280 °C, and an ion source temperature of 230 °C. For the identification of the components, the retention times were matched against the National Institute of Standards and Technology (NIST Mass Spectrometry DATA CENTER) data library and crosschecked with previously published data. Table 1 presents the results.

C. CELL LINES AND CULTURE CONDITIONS

The study utilized the melanoma cancer cell line B16F10. The protocol for standard cell culture was followed for maintenance of the cells. Culturing of the cells was performed using Dulbecco's-modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were cultered at 37 °C in 5% CO_2 in a humidified incubator.

D. CYTOTOXIC ACTIVITY

The MTT method, carried out as previously described with some modifications [12], was performed for the determination of cytotoxic activity of the *L. purpureum* L. essential oil. After a confluence of 70-80% was seen in the T75 flask, cells were cultured in 96-well plates. Equal volumes of 5×10^4 cells/100 μL were seeded in each well and incubated overnight. The stock solution consisted of 1 mg of plant extract dissolved in ethanol. Different dilutions were then prepared at 100 μg/mL, 50 μg/mL, 25 μg/mL, 12 μg/mL, 6 μg/mL, 3 μg/mL and 1,5 μg/mL, respectively, leaving one well for ethanol/DMEM, considered as the control.

III. Results And Discussion

Lamium plants have been used in medicine and folk remedies worldwide with many applications for their astringent, antispasmodic and anti-inflammatory properties as well as for use as a blood tonic. Moreover, they are used to treat various conditions such as fractures and trauma, strokes, hypertension, menorrhagia and uterine hemorrhage, prostate problems and skin and respiratory disorders [4,13].

This study represents the first report of the anticancer effect of *Lamium purpureum* L. essential oil. Table 1 and Figure 1 present the GC–MS analysis results for the ethanolic extract. In the extract of *L. purpureum* L., 49 compounds were identified, representing 99.67% of the total compound. The major compounds included palmitic acid (22.55%), 7-tetradecenal (20.65%), 9,12-octadecadienoic acid

(7.40%), octadecanoic acid (4.80%), (Cyclohex-2-enyl)acetic acid (4.48%), ethyl 9,12,15-octadecatrienoate (3.50%) and hexatriacontane (3.36%), with others at rates of less than 3%.

Flamini et al. analyzed the *Lamium purpureum* essential oil with GC-MS and characterized it with high germacren-D content [14]. In another study by Jones et al., α-pinene, β-pinene, 1-octen-3-ol, β-elemen and germacren-D were dominant in *L. purpureum* oil [15]. However, these components were not found in our study. Although Jones et al. found that palmitic acid had a low density (0.4% - 0.6% - 1.2%), our results showed that the main constituents of the chemical compounds of this plant included a high percentage of palmitic acid (22.55%). Another major component obtained in our study was 7-Tetradecenal. Tetradecenal is a long-chain fatty aldehyde derived from a hydride of a tetradecane [16]. According to the results of Flamini et al, tetradecane was found in *L. purpureum* oil. Tetradecane was also found in the *L. album* plant, which has been the most studied of the *Lamium* species [17].

Morteza-Semnani et al. used GC and GC-MS to analyze *Lamium album L.* (Lamiaceae) essential oil and detected forty-three components in its chemical composition. The most important of these were 6,10,14-trimethyl-2-pentadecanone (10.2 %) and 4-hydroxy-4-methyl-2-pentanone (9.1 %) [17]. In another study, the *Lamium album* collected from the Catalina region (Cluj-Napoca) was analyzed by GC-MS. It was shown to contain a high percentage of palmitic acid (256.6 µg/g) [18]. Essential oil production and its contents are not only dependent on the genetics of the plant. Endogenous factors, such as the developmental stages of the plant, as well as exogenous factors, such as the environment to which the plant is exposed, may alter the production and content of essential oils [19].

In this study, the essential oil of *L. purpureum* L. was evaluated for its anticancer and cytotoxic activity. The cytotoxic activity of the isolated essential oil was tested against the melanoma cancer B16F10 cell line using MTT assay. The results are shown in Figures 2 – 5 and graphically in Figure 6. The viability of the control group cells that were not dose administered was accepted as 100% for all time periods (Fig. 2) and the viability values of the other samples were calculated from the mean absorbance values. There was no significant change in cell viability compared to the control group as a result of *L. purpureum* L. treatment at a dose of 1.5 µg/mL. At the 3 µg/mL *L. purpureum* L. dose, the viability ratio again reached the control group level. A proliferative effect was observed in the application of 6 µg/mL *L. purpureum* L. and the viability of the cells was higher than the control. However, there was a decrease in cell viability, although not substantial, from the 12 µg/mL *L. purpureum* L. dose. The 50 µg/mL *L. purpureum* L. treatment of the cells showed the highest anti-proliferative effect, and a decrease in cell viability of approximately 14% was observed compared to the control group (Fig. 4). The cytotoxic activity of the oil showed partial effect on the cells.

A previous study in the literature reported that essential oils isolated from several members of the Lamiaceae family had exhibited an apoptotic impact. According to this study, the researchers had demonstrated the cytotoxicity activity of the *Lamium* species and suggested that the cytotoxic effect was due to various chemical constituents, including palmitic acid. Their study showed that palmitic acid exerted antitumor activities and indicated that palmitic acid might be a leading compound in anticancer drugs [20,21].

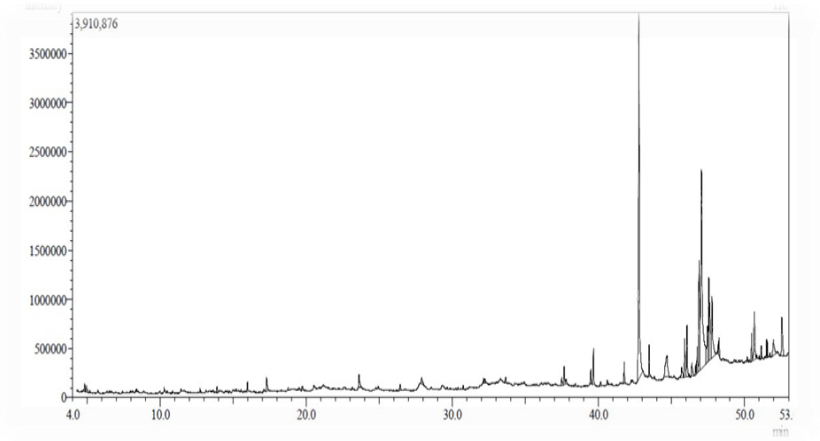

Figure 1. *GC-MS analysis of Lamium purpureum* L. *ethanol extract*

Table 1. *Chemical composition of L. purpureum* L.

Compounds	Composition%	R.Time
PYRROLIDINE-.ALPHA.,.ALPHA.,.ALPHA.',.ALPHA.'-D4	0.19	4.832
PYRROLIDINE-.ALPHA.,.ALPHA.,.ALPHA.',.ALPHA.'-D4	0.34	4.960
D-Limonene	0.14	12.725
Isoxazole, 3,5-dimethyl- (CAS)	0.19	13.906
Ethyl 1-pyrrolidineacetate	0.36	15.973
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.78	17.288
Dianhydromannitol	0.16	19.727
Guaiacol <4-vinyl->	0.84	23.615
2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	0.50	27.895
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (CAS)	0.15	30.738
Fumaric acid, 2,4-dimethylpent-3-yl ethyl ester	0.24	32.149
Megastigmatrienone	0.17	32.265
Megastigmatrienone	0.21	33.645
Tetradecanoic acid	0.44	37.486
Benzyl Benzoate	1.01	37.651
(-)-Loliolide	0.33	37.775
Neophytadiene	0.63	39.474
Phytone	1.39	39.653
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.21	40.142
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	0.20	40.604
Hexadecanoic acid, methyl ester	0.86	41.760
Palmitic acid	22.55	42.770
Hexadecanoic acid, ethyl ester (CAS)	1.28	43.466
Hexatriacontane	2.95	44.676
1-Nonadecanol	0.80	45.693
9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	1.79	45.906
9,12,15-Octadecatrienoic acid, methyl ester (CAS)	2.69	46.062
9-Octadecenoic acid (Z)-, methyl ester (CAS)	0.15	46.195
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	0.83	46.392
Methyl stearate	0.41	46.649

Methyl 5,6-octadecadienoate	1.02	46.764
9,12-Octadecadienoic acid (Z,Z)-	7.40	46.892
7-Tetradecenal, (Z)-	20.65	47.050
n-Propyl 9,12-octadecadienoate	2.88	47.470
Octadecanoic acid	4.80	47.551
Ethyl 9,12,15-octadecatrienoate	3.50	47.626
(Cyclohex-2-enyl)acetic acid	4.48	47.778
Octadecanoic acid, ethyl ester	0.50	48.200
17-Octadecen-14-yn-1-ol	0.78	48.252
3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.22	50.180
Eicosane	1.15	50.482
Hexatriacontane	3.36	50.669
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	0.14	50.996
Eicosanoic acid, methyl ester (CAS)	0.53	51.147
3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-enolide	0.81	51.502
Cyclohexane, 1,4-dimethyl-2-octadecyl-	0.63	51.550
4,8,12,16-Tetramethylheptadecan-4-olide	0.23	51.742
Eicosanoic acid (CAS)	1.35	51.971
Stearate <ethyl->	2.49	52.550
Total	99.67	

R. Time: Retention Time

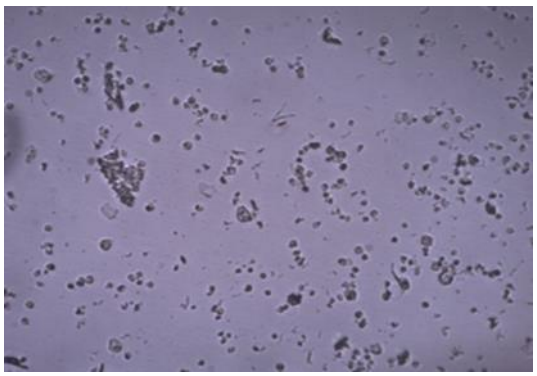

Figure 2. *L. purpureum* L. *control*

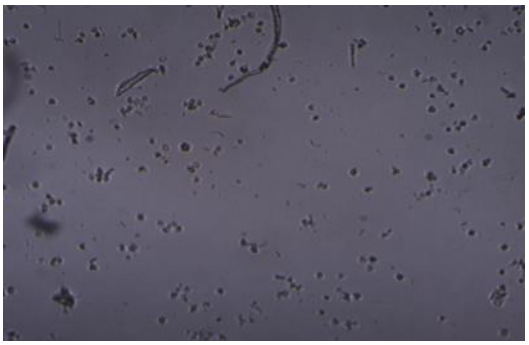

Figure 3. *L. purpureum* L. 25µg/mL

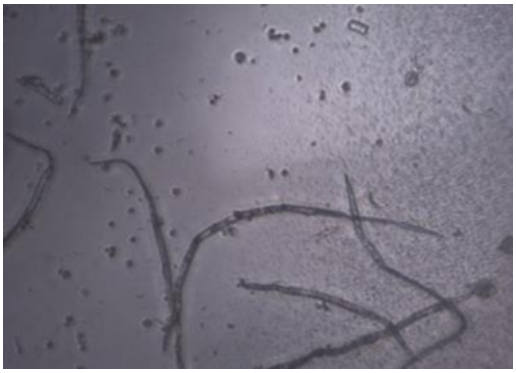

Figure 4. *L. purpureum* L. 50µg/mL

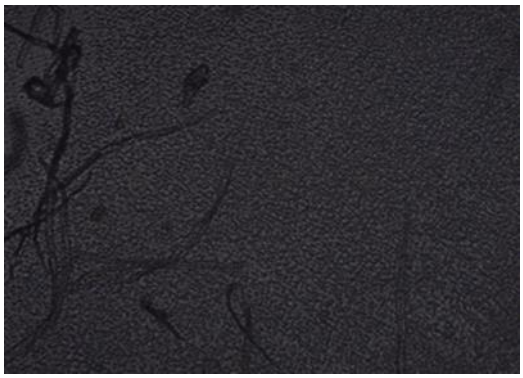

Figure 5. *L. purpureum* L. 100µg/mL

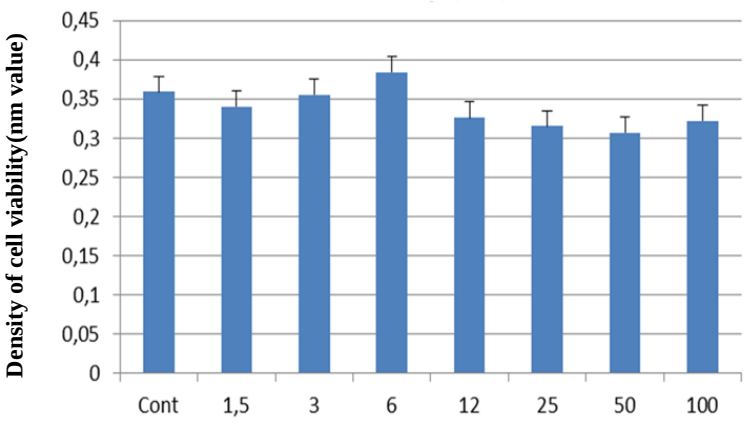

Figure 6. *MTT Assay* (*L. purpureum* L.)

IV. Conclusion

In this study, the chemical content of *Lamium purpureum* L., which is widely grown in Turkey, was analyzed. In addition, the cytotoxic effect was investigated and the anticancer effect of the essential oil obtained from this plant was first introduced into the literature. This study confirms information on the chemical composition of *L. purpureum* L. and contributes to a better understanding of its bioactive effects. Extensive studies are needed for the potential importance and therapeutic use of *L. purpureum* L. as an anticancer agent.

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