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Antimicrobial and Free Radical Scavenging Activities of Some *Lamium* Species from Turkey

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Introduction

The genus *Lamium* L. (Lamiaceae) comprises almost 40 species spread throughout Europe, Asia, and Africa¹. Some *Lamium* species have been reported to possess application in traditional medicines worldwide for the treatment of trauma, fracture, putrescence, paralysis, leucorrhoea, hypertension, and some women afflictions, such as menorrhagia, uterine hemorrhage, vaginal, cervical inflammation etc.^{2,3}. There are evidences indicating various activities such as anti-inflammatory, antioxidant, free radical scavenging, and antiproliferative properties for *Lamium* plants⁴⁻⁶. In Turkey, thirty *Lamium* species are grown widely^{7,8}. *Lamium album*, *L. maculatum*, and *L. purpureum* have been reported to be used as tonics and for the treatment of constipation in Anatolia⁹. In western Anatolia (in particular in Manisa) whole plant of *L. album* and several other *Lamium* species are used to relieve pain in rheumatism and other arthritic ailments¹⁰. Phytochemical investigations in our laboratory on *L. garganicum* subsp. *laevigatum*¹¹ and *L. eriocephalum* subsp. *eriocephalum*¹² resulted in the isolation of some iridoid glycosides. Based on the traditional usage and previous research on *Lamium* species in Turkey and worldwide, the present activity screening study was performed on the extracts prepared with methanol, dichloromethane, *n*-butanol, and water from the aerial

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parts of *L. eriocephalum* Benth. subsp. *eriocephalum*, *L. garganicum* L. subsp. *laevigatum* Arcangeli, *L. garganicum* L. subsp. *pulchrum* R. Mill, and *L. purpureum* L. var. *purpureum* in order to evaluate their possible antimicrobial and free radical scavenging activities. Broth microdilution method recommended by National Committee for Clinical Laboratory Standards (NCCLS), was used to determine the antimicrobial activity^{13,14}. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used as radical for free radical scavenging activity.

Material and Methods

Plant Materials

Lamium eriocephalum Benth. subsp. *eriocephalum* and *L. garganicum* L. subsp. *pulchrum* R. Mill were collected from Aladağlar, Niğde at 2200 m in June 2002. *L. garganicum* L. subsp. *laevigatum* Arcangeli was collected from Uludağ, Bursa at 2300 m in July 2005. *L. purpureum* L. var. *purpureum* was collected from Sıhhiye Campus of Hacettepe University, Ankara at 850 m in March 2006. The plant specimens were autenticated by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Biology). Voucher specimens were deposited in the Herbarium of Hacettepe University Faculty of Pharmacy, Ankara, Turkey (HUEF 02045, 02046, 05004, and 06005, respectively).

Preparation of Extracts

Air-dried and powdered aerial parts of the plant material (25 g) was extracted with MeOH (250 mL) for 5 h at 40 °C under reflux and concentrated to dryness under reduced pressure. An aliquot of the concentrated crude methanolic extract was kept as the MeOH extract and the remaining extract was then suspended in H₂O (100 mL) and partitioned with CH₂Cl₂ (50 mL) and *n*-BuOH (50 mL), individually. CH₂Cl₂ and *n*-BuOH extracts, as well as the remaining H₂O phase were concentrated to dryness and lyophilized in *vacuo*. Yield extracts: *L. eriocephalum* subsp. *eriocephalum*-CH₂Cl₂ (LEEC) 4.76%; *L. eriocephalum* subsp. *eriocephalum*-*n*-BuOH (LEEB) 6.43%; *L. eriocephalum* subsp. *eriocephalum*-H₂O (LEEW) 9.45%; *L. eriocephalum* subsp. *eriocephalum*-MeOH (LEEM) 8.35%; *L. garganicum* subsp. *laevigatum*-CH₂Cl₂ (LGLC) 4.91%; *L. garganicum* subsp. *laevigatum*-*n*-BuOH (LGLB) 7.02%; *L. garganicum* subsp. *laevigatum*-H₂O (LGLW) 11.23%; *L. garganicum* subsp.

laevigatum-MeOH (LGLM) 9.51%; *L. garganicum* subsp. *pulchrum*-CH₂Cl₂ (LGPC) 3.98%; *L. garganicum* subsp. *pulchrum*-*n*-BuOH (LGPB) 5.86%; *L. garganicum* subsp. *pulchrum*-H₂O (LGPW) 8.51%; *L. garganicum* subsp. *pulchrum*-MeOH (LGPM) 7.48%; *L. purpureum* var. *purpureum*-CH₂Cl₂ (LPPC) 5.04%; *L. purpureum* var. *purpureum*-*n*-BuOH (LPPB) 6.21%; *L. purpureum* var. *purpureum*-H₂O (LPPW) 9.13%; *L. purpureum* var. *purpureum*-MeOH (LPPM) 7.87%.

Crude extracts were first subjected to phytochemical analysis by thin layer chromatography (TLC) and then applied to activity tests.

Chemicals

TLC Plates: Silica gel 60 F₂₅₄ 20x20 Merck; Organic Solvents: Carlo Erba; Ampicillin: Mustafa Nevzat; Fluconazole: Pfizer; DPPH (2,2-diphenyl-1-picrylhydrazil): Fluka; Ascorbic Acid: Aldrich

Thin Layer Chromatography

TLC analyses were carried out on pre-coated aluminium sheets (Merck). CH₃Cl-MeOH-H₂O (80:20:2; 61:32:7) and EtOAc-MeOH-H₂O (100:16.5:13.5) solvent systems were used for the development of the plates. Plates were examined by UV fluorescence and spraying 1% vanillin/H₂SO₄, followed by heating at 100 °C for 1-2 min.

Antimicrobial Activity

Test organisms: Plant extracts were tested against two standard Gram positive bacteria; *Staphylococcus aureus* ATCC 29213 (American Type Culture Collection) and *Enterococcus faecalis* ATCC 29212; two standard Gram negative bacteria; *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and three standard yeast-like fungi; *Candida albicans* ATCC 90028, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 22019.

Antimicrobial activity test: Broth microdilution method recommended by National Committee for Clinical Laboratory Standards (NCCLS), was used to determine the antimicrobial activity^{13,14}. Antibacterial activity test was performed in Mueller-Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA) and for antifungal test RPMI-1640 medium with L-Glutamin (ICN-Flow, Aurora, OH, USA), buffered with MOPS buffer (ICN-Flow,

Aurora, OH, USA) was used. The inoculum densities were approximately 5×10^5 cfu/mL and 0.5 - 2.5×10^3 cfu/mL for bacteria and fungi, respectively.

Each of plant extracts was dissolved in sterile distilled water. Final two fold concentrations were prepared in the wells of the microtiter plates, between 1024 - 0.25 $\mu\text{g/mL}$. Ampicillin and fluconazole were used as reference antibiotics for bacteria and fungi, respectively (64 - 0.0625 $\mu\text{g/mL}$). Microtiter plates were incubated at 35°C for 18 - 24 h for bacteria and for 48 h for yeast-like fungi. After the incubation period, minimum inhibitory concentration (MIC) values were defined as the lowest concentration of the extracts that inhibits the visible growth of microorganisms.

DPPH radical scavenging activity: Experiments were carried out according to a slightly modified procedure¹⁵. Briefly, 3 mL solutions of the proper extract dilution (25 , 50 , 100 , and 200 $\mu\text{g/mL}$) were added to a 1 mL solution of 1.5×10^{-5} M DPPH (2,2-diphenyl-1-picrylhydrazil) radical solution in methanol. The absorbance was measured at 517 nm after 30 min of incubation at room temperature. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. The percent of radical scavenging activity was calculated as the ratio of the absorption of the sample relative to the control DPPH solution by the following equation. % DPPH Radical Scavenging = $[\text{CA} - \text{EA}] / \text{CA} \times 100$

CA = Control absorbance

EA = Extract absorbance

The DPPH solution without extract was used as control solution. Ascorbic acid was used as reference compound.

Results and Discussion

The results of antimicrobial activity screening are summarized in Table 1. Although all the extracts showed moderate antimicrobial activity, however, they possessed more activity against yeast like fungi than that of bacteria. Of the four extracts tested, the CH_2Cl_2 extracts of the title plants showed the highest antimicrobial activity for almost all microorganisms, thus indicating that CH_2Cl_2 fractions contain components with

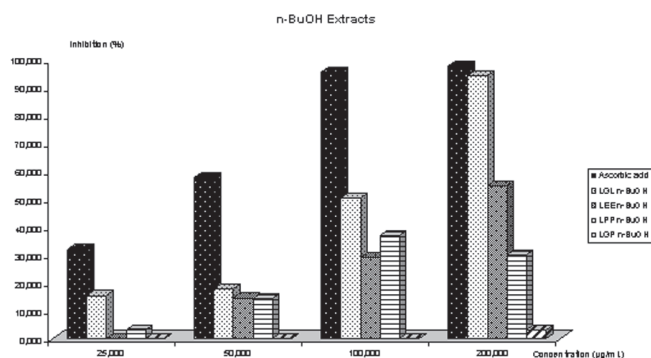
antimicrobial activity. Among them the CH_2Cl_2 extract of *L. eriocephalum* subsp. *eriocephalum* can be considered as the most active one against all tested bacteria and fungi. In addition, the CH_2Cl_2 extracts of *L. garganicum* subsp. *pulchrum* and *L. purpureum* var. *purpureum* as well as the MeOH extract of *L. garganicum* subsp. *pulchrum* possessed the same antifungal activity as the CH_2Cl_2 extract of *L. eriocephalum* subsp. *eriocephalum* on *C. albicans*.

TABLE I

Antimicrobial activity results of the extracts from *Lamium eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*

Test Materials	MIC ($\mu\text{g/ml}$)						
	Bacteria				Fungi		
	S. aureus	E. faecalis	E. coli	P. aeruginosa	C. albicans	C. krusei	C. parapsilosis
	ATCC 29213	ATCC 29212	ATCC 25922	ATCC 27853	ATCC 90028	ATCC 6258	ATCC 22019
LEEC	128	256	256	128	128	128	128
LEEB	1024	512	512	512	512	512	512
LEEW	>1024	>1024	>1024	>1024	1024	1024	1024
LEEM	>1024	1024	>1024	>1024	>1024	>1024	>1024
LGLC	256	512	512	512	256	256	512
LGLB	>1024	>1024	>1024	>1024	>1024	>1024	>1024
LGLW	>1024	>1024	>1024	>1024	>1024	>1024	>1024
LGLM	512	512	1024	1024	256	512	512
LGPC	512	512	512	512	128	256	256
LGPB	512	>512	512	512	256	512	512
LGPW	>512	>512	>512	>512	256	512	512
LGPM	256	512	512	512	128	256	256
LPPC	512	512	512	512	128	256	256
LPPB	512	512	512	512	256	256	512
LPPW	>512	>512	>512	>512	256	512	512
LPPM	512	512	512	512	256	512	512
Ampicillin	1	8	2	-	-	-	-
Fluconazole	-	-	-	-	1	64	8

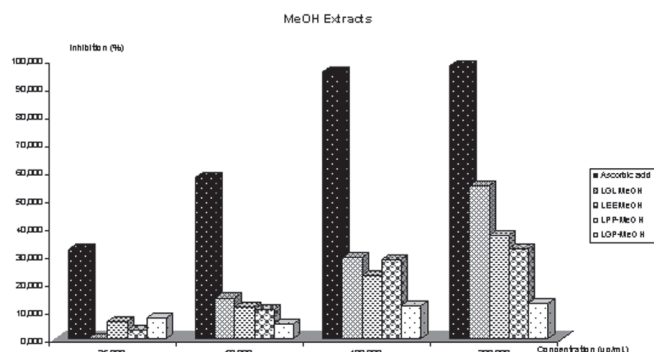
The DPPH radical scavenging activities of four *Lamium* species were found to be comparable to that of ascorbic acid (Fig. 1-4). The *n*-BuOH extracts of all species possessed the strongest activity, in all dose levels tested, whereas, the *n*-BuOH extract of *L. garganicum* subsp. *laevigatum* exhibited almost the same scavenging activity as ascorbic acid at 200 µg/ml dose level. Nevertheless, the MeOH and H₂O extracts showed lower activity, respectively, where the CH₂Cl₂ extracts of the title plants expressed weak activity. The IC₅₀ values of each extract were given in Table II.



LEE: *Lamium eriocephalum* subsp. *eriocephalum*, LGL: *L. garganicum* subsp. *laevigatum*, LGP: *L. garganicum* subsp. *pulchrum*, LPP: *L. purpureum* var. *purpureum*. LPP: *L. purpureum* var. *purpureum*.

Figure 1

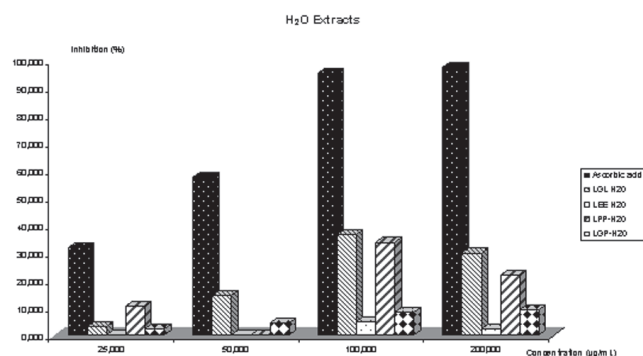
Comparison of DPPH radical scavenging activities of the *n*-BuOH extracts of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*.



LEE: *Lamium eriocephalum* subsp. *eriocephalum*, LGL: *L. garganicum* subsp. *laevigatum*, LGP: *L. garganicum* subsp. *pulchrum*, LPP: *L. purpureum* var. *purpureum*. LPP: *L. purpureum* var. *purpureum*.

Figure 2

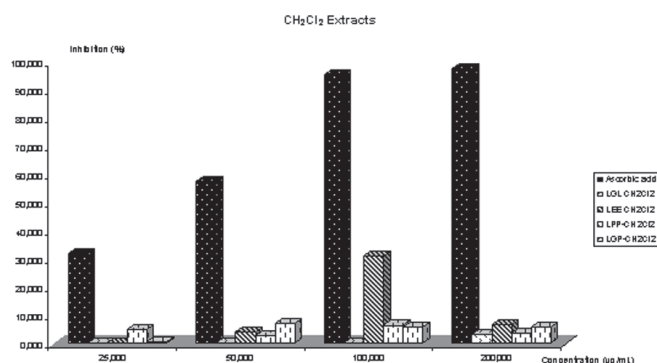
Comparison of DPPH radical scavenging activities of the MeOH extracts of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*.



LEE: *Lamium eriocephalum* subsp. *eriocephalum*, LGL: *L. garganicum* subsp. *laevigatum*, LGP: *L. garganicum* subsp. *pulchrum*, LPP: *L. purpureum* var. *purpureum*.

Figure 3

Comparison of DPPH radical scavenging activities of the H₂O extracts of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*.



LEE: *Lamium eriocephalum* subsp. *eriocephalum*, LGL: *L. garganicum* subsp. *laevigatum*, LGP: *L. garganicum* subsp. *pulchrum*, LPP: *L. purpureum* var. *purpureum*.

Figure 4

Comparison of DPPH radical scavenging activities of the CH₂Cl₂ extracts of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*.

The wide spectrum of therapeutic activities of *Lamium* species may be related to some biologically active substances such as, iridoids¹⁶⁻²⁶, flavonoids^{3,27}, phenylpropanoids^{3,27,28}, benzoxazinoids²⁴, and essential oil (29-31). There is only little information about antimicrobial activity of the examined species. Up to date, only the essential oil of *Lamium garganicum* subsp. *laevigatum* was reported to be active against some Gram positive and Gram negative bacteria²⁹. Essential oils of *Lamium* plants

TABLE II

IC₅₀ values of the extracts from *Lamium eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*

Extracts	IC ₅₀ (µg/ml)
LEEC	-
LEEB	-
LEEW	50.79
LEEM	88.73
LGLC	-
LGLB	99.19
LGLW	58.86
LGLM	96.19
LGPC	-
LGPB	96.82
LGPW	-
LGPM	-
LPTC	-
LPTB	75.87
LPTW	-
LPTM	64.54
Ascorbic Acid	42.82

are reported to be composed of mono- and sesquiterpenoids, as well as straight chain hydrocarbons with C₁₂ to C₃₁ carbon atoms and their esters²⁹⁻³¹. Concerning the preparation sequence of the studied extracts, it seems logical to speculate that the higher antimicrobial activity response of CH₂Cl₂ extracts of the title plant species among the others may be due to the presence of such active compounds in the extract.

Of the studied *Lamium* species, there is only one report on the methanolic extract of *L. album* and *L. purpureum* to exhibit free radical scavenging properties⁵. In this study DPPH scavenging activity of *L. album* was found to be higher than that of *L. purpureum*. However, in the same study some other methods such as phosphomolybdenum method and lipid peroxidation assay were also applied for free radical scavenging activity. In

the phosphomolybdenum assay at 90 °C the activity of *L. album* was found to be stronger than *L. purpureum*. But at 40 °C *L. purpureum* was noted to exhibit stronger activity. In lipid peroxidation assay the maximum inhibition of both *Lamium* species slightly exceed 70%. Antioxidant and free radical scavenging activities of the medicinal plants are mainly attributed to their phenolic contents like flavonoids and phenylpropanoids. Preliminary TLC analysis of different extracts of the titled *Lamium* plants have revealed the presence of flavonoids and phenylpropanoids in all extracts, whereas iridoids have been detected in methanol, *n*-butanol and aqueous extracts. Flavonoids^{15,32} and phenylpropanoids^{33,34} are reported to show antioxidant and free radical scavenging potentials. However, iridoids do not possess any antioxidant activity due to their non-phenolic nature^{33,34}. Thus, the higher radical scavenging potentials of the *n*-butanol extracts of four *Lamium* plants among different extracts tested might be related to the presence of active phenolic glycosides.

In conclusion, this study presents the antimicrobial and free radical scavenging activities of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*. The efficacy of each species in the studied activities differs depending on the chemical profile of the plants. However, further studies will require in order to clarify the bioactive principles responsible for these activities.

Acknowledgement

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Summary

Antimicrobial and Free Radical Scavenging Activities of Some *Lamium* Species from Turkey

In this study the MeOH, CH₂Cl₂, *n*-BuOH and H₂O extracts of *L. eriocephalum* subsp. *eriocephalum*, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum* were tested for their antimicrobial and free radical scavenging activities. Broth Microdilution method and spectrophotometric DPPH method were used to test antimicrobial and free radical scavenging activity, respectively. The CH₂Cl₂

extracts of all plants showed highest antimicrobial activity. Among the tested extracts the CH₂Cl₂ extract of *L. eriocephalum* subsp. *eriocephalum* is considered as the most active one against all tested bacteria and fungi. The *n*-BuOH extracts of all studied plants were found to be possess the highest free radical scavenging activity.

Key Words: Lamiaceae, *Lamium*, antimicrobial activity, free radical scavenging activity.

Özet

Türkiye’de Yetişmekte olan Bazı *Lamium* Türlerinin Antimikrobiyal ve Serbest Radikal Süpürücü Etkileri

Bu çalışmada, *L. garganicum* subsp. *laevigatum*, *L. garganicum* subsp. *pulchrum*, and *L. purpureum* var. *purpureum*’un MeOH, CH₂Cl₂, *n*-BuOH ve H₂O ekstraları antioksidan ve antimikrobiyal aktiviteleri yönünden test edilmiştir. Antimikrobiyal aktivite testi için sıvı mikrodilüsyon yöntemi, serbest radikal süpürücü etki tayini için spektrofotometrik DPPH yöntemi kullanılmıştır. Tüm bitkilerin CH₂Cl₂ ekstraları en yüksek antimikrobiyal aktiviteyi göstermiştir. Test edilen ekstralar arasında *L. garganicum* subsp. *laevigatum* bitkisinin CH₂Cl₂ ekstresi denenilen tüm bakteri ve mantarlara karşı en etkili ekstre olarak belirlenmiştir. Çalışılan bitkilerin *n*-BuOH ekstraları serbest radikal süpürücü etki yönünden en yüksek etkili ekstralar olarak bulunmuştur.

Anahtar Kelimeler: Lamiaceae, *Lamium*, antimikrobiyal aktivite, serbest radikal süpürücü aktivite.

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