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Antibacterial Activity of Endemic *Lamium tenuiflorum*

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The organic solvents and aqueous extracts obtained from the leaves, rootstock and the combined formulation of endemic *Lamium tenuiflorum* Fisch. & Mey. (Lamiaceae) were tested for their antimicrobial activity against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169 by the well-in-agar method. All the organic solvent extracts exhibited a strong antibacterial effect against the bacterial cultures except for the aqueous extracts, which had no effect.

Key Words: Antibacterial activity, *Lamium tenuiflorum*, Turkey.

INTRODUCTION

The genus *Lamium* L. (Lamiaceae) comprises almost 40 species spread throughout Europe, Asia and Africa[1]. 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 antiinflammatory, antioxidant, free radical scavenging and antiproliferative properties for *Lamium* plants[4-6]. In Turkey, 30 *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[9].

Lamium tenuiflorum Fisch. & Mey. is endemic to Turkey[8]. During routine excursions, it is determined that this plant is used to treat cold, bronchitis and externally for boils and abscesses and urinary tract infections. Thus, the aim was to determine the antibacterial effects of some organic solvents and aqueous extracts obtained from this endemic plant against some bacteria.

EXPERIMENTAL

Aerial parts of plant were collected from Icel, Turkey in September, 2007. Voucher specimens of the plant were deposited in the Biology Department at Canakkale Onsekiz Mart University Canakkale, Turkey and identified by Mr. Ersin Karabacak from the same Department.

Preparation of extracts: The aerial parts of the plant were dried in an oven at 40 ºC (12 h) and powdered. Each dry powdered plant material (20 g) was soaked separately in distilled water and solvents (50 % concentration) (ethanol, acetone, methanol and chloroform) until complete saturation of the plant material. The extract was filtered using Whatmann filter paper no. 1. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extracts were stored in labeled sterile screw-capped bottles at -20 ºC. The extract (in the form of sticky black substances) amounting to around 2 g was dissolved in DMSO before testing. The combination of the plant extract (1:1 ratio) was used in this test[10].

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169 were used as test bacteria. Pure cultures were maintained on Mueller Hinton Agar (MHA) plates and Mueller Hinton Broth (MHB) in tubes.

Screening for antibacterial activities: The screening for antibacterial activity was done using a modified version of the well-in-agar method[11]. Each test bacterium (from MHB medium) was aseptically swabbed on the MHA medium to get uniform distribution of bacterium. Using a sterile cork borer, a well of 0.5 cm diameter was made in the inoculated medium and 0.2 mL of each extract (leaf and rootstock) was filled into the well and the plates were kept in room temperature for an hour to allow spread of extract in the medium. Later the agar plates were incubated for 35 ± 0.1 ºC for 24 h. The presence of zones of inhibition around the wells was observed and interpreted as an indication of antibacterial activity. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disk was applied, depending on the test bacteria for comparison.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts was quantitavely assessed by the presence or absence of inhibition zone and by measuring the diameter of the inhibition zone around the wells or disks. The antibacterial activity of the organic solvents and aqueous extracts of *Lamium tenuiflorum* are shown in Table-1. Besides, the inhibition zones formed by standard antibiotic disks are indicated in Table-2. As can clearly be seen from Tables 1 and 2,

all extracts except for aqueous have antibacterial effects against the bacterial cultures in various inhibition zones. Notably, the combination of plant extracts (both leaves and rootstock) contributes better activity against the tested bacteria than leaf and rootstock extracts. However, the aqueous extracts were inactive. *Staphylococcus aureus* is more susceptible to the combination ethanol extract, as compared to standard antibacterial antibiotics, except for OFX5 and TE30. In comparison to P10, Sam20 and VA30 standard, it was seen that *Listeria monocytogenes* is more susceptible to that extract. In addition, the acid fast bacterium *Mycobacterum smegmatis*, *Proteus vulgaris* and *Bacillus cereus* are more resistant to the extract in comparison to the standard antibiotics, except for CTX30, P10 and SAM20, respectively. Similarly, the other bacteria have been found to be resistant to the extract except for some standard antibiotics.

TABLE-1
ANTIBACTERIAL ACTIVITY OF LEAF AND ROOTSTOCK EXTRACTS OF *Lamium tenuiflorum*

Part used	Extract	Zone of inhibition (mm)*								
		1	2	3	4	5	6	7	8	9
Leaf	Aqueous	-	-	-	-	-	-	-		-
	Ethanol	13.2	16.4	13.6	12.2	10.0	12.0	11.2	16.2	15.8
	Chloroform	11.0	14.4	12.8	11.0	9.6	11.6	10.0	14.0	13.4
	Methanol	10.0	12.8	11.6	10.8	9.0	10.0	9.6	14.2	12.8
	Acetone	10.2	11.8	11.2	9.6	9.0	9.6	9.0	13.0	13.2
Rootstock	Aqueous	-	-	-	-	-	-	-	-	-
	Ethanol	12.0	13.8	12.8	11.4	9.6	11.2	10.8	14.2	14.0
	Chloroform	11.2	13.2	11.0	10.0	9.0	9.8	9.6	12.8	11.8
	Methanol	10.0	11.0	9.8	9.6	9.2	9.0	9.0	11.8	12.2
	Acetone	9.8	12.0	9.2	9.0	9.0	9.0	9.2	13.0	11.6
Leaf and rootstock (1:1 ratio)	Aqueous	-	-	-	-	-	-	-	-	-
	Ethanol	14.4	18.4	15.2	12.8	11.8	14.0	12.2	17.0	16.8
	Chloroform	11.2	16.6	14.0	11.6	10.6	13.2	11.8	16.8	16.2
	Methanol	10.6	15.0	13.8	10.0	9.8	11.8	11.2	15.0	15.2
	Acetone	11.8	14.4	14.6	10.8	10.2	9.6	9.6	13.2	14.0

*(-): no activity
1 = *Escherichia coli*, 2 = *Staphylococcus aureus,* 3 = *Klebsiella pneumoniae,*
4 = *Pseudomonas aeruginosa,* 5 = *Proteus vulgaris,* 6 = *Bacillus cereus,*
7 = *Mycobacterium smegmatis,* 8 = *Listeria monocytogenes,* 9 = *Micrococcus luteus*

Based on the results, it is possible to conclude that ethanol extract has stronger and broader spectrum of antibacterial activity as compared to the others. This information confirmed the evidence in previous study reported that ethanol is a better solvent for extraction of antimicrobial substance from medicinal plants than water and methanol[12,13].

TABLE-2
ANTIMICROBIAL ACTIVITIES OF SOME STANDARD ANTIBIOTICS

Microorganisms/antibiotics	Zone of Inhibition (mm)					
	P10	SAM20	CTX30	VA30	OFX5	TE30
Escherichia coli	18.2	12.2	10.4	22.0	30.8	28.2
Staphylococcus aureus	13.4	16.8	12.6	13.4	24.4	26.4
Klebsiella pneumoniae	18.2	14.4	13.4	22.4	28.2	30.6
Pseudomonas aeruginosa	8.6	10.8	54.2	10.8	44.0	34.8
Proteus vulgaris	10.2	16.2	18.4	20.0	28.6	26.2
Bacillus cereus	14.4	12.4	14.6	18.6	30.2	25.4
Mycobacterium smegmatis	15.8	21.0	11.8	20.0	32.2	24.6
Listeria monocytogenes	10.6	12.4	16.6	26.4	30.2	28.2
Micrococcus luteus	36.2	32.0	32.2	34.2	28.8	22.4

P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, CTX30 = Cefotaxime 30 µg, V30 = Vancomycin 30 µg, OFX 5 = Oflaxacin 5 µg, TE30 = Tetracyclin 30 µg.

Bacteria used in this study were chosen primarily on the basis of their importance as pathogens in humans. Methicillin resistant *Staphylococcus aureus* (MRSA) remains an important nosocomial pathogen. According to the latest report from the National Nosocomial Infection Surveillance System (NNIS), *ca.* 60 % of all *S. aureus* nosocomial infections in intensive care units (ICUs) were methicillin resistant in 2003, representing an 11 % increase in resistance compared to the preceding five year period[14]. Notably, *Staphylococcus aureus* is the most sensitive bacterium to the extracts in this study. So, the results of the present study indicate that the extracts have the potential to generate novel metabolites. The extracts demonstrating especially antibacterial activity against *Staphylococcus aureus* could result in the discovery of novel antibacterial agents, showing demonstrating broad spectrum activities, this may help to discover new antibiotics that could serve as selective agents against infectious diseases. In addition, the result supports the folkloric usage of the studied plant and suggests that the plant extract possess certain constituents with antifungal properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

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