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Antimicrobial activity of endemic *Digitalis lamarckii* Ivan from Turkey

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Antimicrobial activity of the methanolic extracts of leaves and flowers of *D. lamarckii* Ivan, (Scophulariaceae), an endemic plant species of Turkey, was tested on ten bacterial and four yeast strains. Effective antibacterial activity was observed in four bacterial strains. Minimum inhibitory concentration (MIC) was calculated by use of liquid culture tests and in all the four effective bacterial strains, the MIC was found to be ≥199.5 mg/ml. The minimum bactericidal concentration (MBC) of *B. subtilis*, *S. aureus,* and *L. monocytogenes* was calculated to be ≥199.5 mg/ml, and MBC value for *Shigella* was calculated as ≥399 mg/ml.

Keywords: Antibacterial, Antimicrobial, *Digitalis lamarckii*

In Turkey, as well as in the entire world, many medicinal plants are found and are used in curing illnesses.

Turkey has a rich flora and is noted for its existing plant diversity. Anatolia is not only the origin and diversification centre of many genera and sections but also has ecological and phytogeographical diversity. As a consequence, many plant species are highly endemic in Anatolia[1,2].

Digitalis species is distributed in Europe, Western Asia and the Mediterranean region. Today, around 40 species belonging to different genera of Scophulariaceae family are known. The endemism rate of the species is around 50%[3]. The hazard category of *Digitalis lamarckii* species is stated to be of least concern and low risk[4].

The leaves of *Digitalis* species are used for medical treatment purposes as they have cardiac, diuretic, stimulant and tonic characteristics. *Digitalis*, which was first used by William Withering in 1785, was declared as cardiotonic in 1860 and in 1869, the glycoside called digitalin was isolated from the leaves of *Digitalis purpurea* by Natville. In the same year, Schiemiedeberg named this glycoside "digitoxin". These glycosides have hazardous effects for animals and humans but are used in cardiac drugs as they reduce pulse rate and slow down blood circulation[5,6] .

Digitalis is a general term for steroidal drugs prepared from the seeds and dried leaves of the genus *Digitalis*, which are used as a cardiac stimulant. More than a quarter of a century has elapsed since the first demonstration of the presence of digitalis-like compounds (DLC) in mammalian tissues at the cellular and molecular levels,. A large number of scientific reports that have appeared since then unequivocally support the notion that these compounds function as hormones in mammals; they are synthesized and released from the adrenal gland and by interacting with their receptor, Na^+, K^+, ATPase, they affect numerous cellular functions[7].

Many cardiologic and floristic studies have been conducted in *Digitalis* species[8,9]. However, there were no studies on antimicrobial property of this plant.

In this study the extract obtained from the leaves and flowers of *D. lamarckii* plant has been tested on some bacterial and yeast strains and its antimicrobial activity was evaluated.

Collection of plants — From the A4, Kastamonu: Azdavay; Karyatağı Mountain, Yanık Plateau and Iran-Turan, KG 1493 region, *D. lamarckii* plants were collected and dried in the shade. *D. lamarckii* was identified at Ankara University Faculty of Science Herbarium (ANK Herbarium), and its leaves and flowers were used in tests. The plant samples are being preserved at Ankara University Faculty of Science Herbarium (ANK Herbarium).

Preparation of extracts — For the determination of antimicrobial activity, 3 g of ground plant parts were soaked in 30 ml of methanol, and for the minimal inhibitory concentration (MIC) tests, 10 g of ground plants were extracted with 100 ml of methanol. It was heated until the mixture boiled and then cooled, kept overnight at room temperature. The extracts were filtered and dried. The dry weight of the remaining residue was calculated. The dried material was again

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re-dissolved in methanol and diluted with deionized water. After the prepared extract solution was sterilized at 121°C for 15 min, it was put in sterile tubes and centrifuged at 5000 rpm for 3 min. The supernatant of extract was used.

Test microorganisms — Fresh cultures of the microorganisms were grown in nutrient broth (acumedia). The density of microorganisms was adjusted to Mc Farland 0.5 standard. In the tests, a total of 14 microorganisms namely; *Enterococcus gallinarum* CDC-NJ-4, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* RSHI, *Escherichia coli* RSHI, *Shigella* RSHI, *Escherichia coli* ATCC 25922, *Streptococcus pyogene*s ATCC 19615, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC27853, *Saccharomyces cerevisiae* (Pakmaya), *Candida albicans* 845981, *C. albicans* 900628 and *C. crusei* ATCC 6258 were used.

Antibiotic discs — The discs amikacin (30 µg/ml; Eczacibasi), vancomycin (30 µg/ml; Mayne), penicillin (10 U/ml; I.E.Ulagay), gentamicin (10 µg/disc; I.E.Ulagay), rifampicin (5 µg/ml; Aventis), tetracycline (30 µg/ml; SIGMA), ampicillin (10 µg/ml; SELVA), chloramphenicol (30 µg/ml; SIGMA) and erythromycin (15 µg/ml; SIGMA) were used as positive control. In the MIC tests, gentamicin (Genta-120 mg; I.E.Ulagay) was used as the standard antibiotic. As negative control 1 ml of methanol, 5 ml of deionized water mixture was used.

Determination of antimicrobial activity — Fresh cultures of the microorganisms (100 µl) were inoculated on Muller Hinton Agar (Merck). Agar was allowed to dry for 15- 20 min in an incubator and on each plate, three drops of extract was added each of which was 20 µl. The plates were then incubated at 37ºC for 24 hr and the diameters of inhibition zones were measured and evaluated. The assays that were found to be effective were repeated three times. The positive and negative tests were performed under same conditions[10].

Determination of minimum inhibitory concentration (MIC) — On the sensitive bacterial strains, two-fold liquid dilution tests were made by using Mueller Hinton Broth (Merck). For each strain, two series of 10 tubes were used; while in the first series the plant extract was tested, in the second series standard antibiotic was tested. Whether bacterial growth occurred or not was determined by observing the turbidity of the cultures. The tube in which no growth occurred was evaluated as the minimum inhibitory concentration (MIC) and then the minimum effective dose of the extract was calculated[10].

Determination of the minimum bactericidal concentration (MBC) — The contents of the MIC tubes having no-growth were spread on Mueller Hinton Agar plates for colony counting. MBC was calculated by the determination of whether the activity of the extract was bacteriostatic or bactericidal according to the state of growth. If there was no growth, the extract was identified as bactericidal[10].

Many plants are commonly used as curative and as antiseptic for skin mucosa lesions and infections of other systems[11].

The leaves and flowers obtained from *D. lamarckii* were extracted together and their antimicrobial effect was tested on 10 bacterial and 4 yeast strains; and on 4 bacteria, effective inhibition zones were observed. It was noted that *D. lamarckii* extracts had no effect on the yeast strains tested (Table 1).

B. subtilis, a bacterium that was susceptible to the extract, exhibited an inhibition zone of 20 mm and it was found to be susceptible to all but one of the standard antibiotics that were tested. The plant extract exhibited an inhibition zone of 25 mm against *S. aureus*; and it was found to be susceptible to all but two of the antibiotics that were tested. *L. monocytogenes* exhibited an inhibition zone of 27 mm and it was found to be susceptible to five of the antibiotics while it was resistant against four. *Shigella* exhibited the smallest diameter of inhibition zone of 16 mm and showed one of the most effective results that the plant extract yielded. This particular *Shigella* strain was resistant against many antibiotics and susceptible to only two of the antibiotics. Hence it was a significant finding, as it was susceptible to *D. lamarckii* extract. When the susceptibility of bacterial strains to antibiotics and the plant extract were compared, it was observed that the plant extract had antibacterial activity no less than the antibiotics.

Main stock content concentration of the plant extract used in MIC tests conducted by use of liquid dilution method was calculated as 399 mg/ml. The dose at which the plant extract was effective at a minimum level (MIC) was calculated. The minimum inhibitory concentration values of the incubation after 18, 24, 48 and 72 hr were all the same. At the 18th hr, no turbidity was observed in the tube that was diluted at a rate of 1/2 and no change occurred in the results as the incubation time increased. Even though the

Table 1—Comparison of antimicrobial activity of leaf and flower extract of *D. lamarckii* and nine standard antibiotics

Microorganisms	Plant	Diameter of inhibition zone (mm)								
		Standard antibiotics								
	Digitalis lamarckii (leaves & flowers)	Amikacin	Vancomycin	Penicillin	Gentamicin	Rifocin	Tetracycline	*Ampicilin*	Chloramphenicol	Erythnomycin
Enterococcus gallinarum CDC-NJ-4	-	-	12	-	15	13	-	-	-	11
Enterococcus faecalis ATCC 29212	-	16	12	-	16	14	-	-	-	11
Bacillus subtilis RSHI	20	24	19	22	25	23	12	-	13	24
Escherichia coli RSHI	-	18	-	-	18	-	-	-	-	-
Shigella RSHI	16	20	-	-	19	-	-	-	-	-
Escherichia coli ATCC 25922	-	16	-	-	17	-	-	-	-	-
Streptococcus pyogenes ATCC 19615	-	13	12	-	16	15	-	-	-	12
Staphylococcus aureus ATCC 29213	25	17	15	19	17	27	12	-	-	18
Listeria monocytogenes ATCC 7644	27	25	16	-	27	39	-	-	-	19
Pseudomonas aeruginosa ATCC27853	-	17	-	-	15	-	-	-	-	-
Saccharomyces cerevisiae (Pakmaya)	-	-	16	-	-	17	10	8	-	11
Candida albicans 845981	-	17	12	-	19	15	-	-	-	12
Candida crusei ATCC 6258	-	14	11	-	17	17	-	-	-	11
Candida albicans 900628	-	17	11	-	16	15	-	-	-	11

-, resistant

bacterial strains were different, the minimum inhibitory concentration values were the same. In other words, the MIC value for all the four bacterial strains was calculated as ≥ 199.5 mg/ml. These results were quite interesting. Excluding *Shigella*, the susceptibilities of the bacterial strains to gentamicin were the same as their susceptibilities to the extract (Table 2). Besides, no difference can be observed as regards to the effect of the extract depending on time. No turbidity was observed in the same MIC tube after 18, 24, 48 and 72 hr. The Gram negative bacterium *Shigella* and Gram positive organism *S. aureus, L. monocytogenes* and *B. subtilis* were observed to have the same minimum inhibitory concentrations. The determination of the MIC tubes is performed visually by looking at the turbidity in the tubes. Considering the possibility of a visual mistake, MIC results and MBC results were compared.

With the inoculations made on the agar plates from MIC tubes, MBC values were assessed and bacterial growth was not observed when inoculated on agar plates. When the MBC values were studied, such values of *B. subtilis*, *S. aureus* and *L. monocytogenes* were found to be ≥199.5 mg/ml in parallel to MIC values; and with respect to *Shigella*, the MIC value was found to be ≥199.5 mg/ml and the MBC value was found to be ≥399 mg/ml (Table 2). No bacterial colony was observed on the plates in the MBC tests. This result indicates that *D. lamarckii* plant extract had bactericidal effects.

Increase in number of resistant bacterial types and strains against classical chemotherapeutics and antibiotics (including Penicillin) reflect the necessity to search for newer antimicrobics. Discovery of plants with potentiable antimicrobial property has opened up a new horizon to search for newer antimicrobics in this field. Plants that have antibacterial activity inhibit bacteria through mechanisms that are different from the antibiotics that are currently in use. For this reason, they are able to take the resistant bacteria under control[12,13]. Scientists have identified many medicinal plants and the effects of many of these herbal drugs have been scientifically proven[14,15].

Consequently, it may be concluded that *D. lamarckii* plant extract has antibacterial activity. The extract is found to be bactericidal in nature. Effective antimicrobial property obtained by the determination of the active compound from the plant can account for new resources to develop newer chemotherapeutics. Even at a trace level, the presence of antibacterial agents in the plant will allow development of new

Table 2 — MIC, MBC values of the *D. lamarckii* leaf and flowers extract (mg/ml) and level of sensitivity of gentamicin (µg/ml) on susceptible bacterial strains

Bacteria	Plant MIC (mg/ml)	Extract MBC (mg/ml)	Gentamicin sensitivity (µg/ml)
B. subtilis	≥ 199.5	≥ 199.5	≥ 1.875
Shigella	≥ 199.5	≥ 399	≥ 3.75
S. aureus	≥ 199.5	≥ 199.5	< 1.875
L. monocytogenes	≥ 199.5	≥ 199.5	< 1.875

biologically originated compounds as a result of obtaining that active agent from the plant through different methods and purification processes.

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References

1 Tan A, Türkiye'de bitkisel çeşitlilik ve bitki genetik kaynakları, *Anadolu J AARI MARA*, İzmir, 2 (1992) 50.

2 Dağcı EK, İzmirli M & Dığrak M, Kahramanmaraş ilinde yetişen bazı ağaç türlerinin antimikrobiyal aktivitelerinin araştırılması, *KSU Fen ve Mühendislik Dergisi*, 5 (2002) 38.

3 Davis PH, *Flora of Turkey and the East Aegean Islands* (University press, Edinburgh, U.K.) 1978, 825.

4 Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z & Adıgüzel, N *Red data book of Turkish plants* (Barışcan Ofset, Ankara) 2000, 246.

5 Timuralp B, Art Longa vita Brevis–*Digitalis purpurea*, *Anatolian J Cardiol,* 1 (2001) 4.

6 Wilkins MR, Kendall MJ & Wade OL, William Withering and digitalis, 1785 to 1985, *Br Med J,* 5 (1985) 7.

7 Nesher M, Shpolansky U, Rosen H & Lichtstein D, The digitalis-like steroid hormones–new mechanisms of action and biological significance, *Life Sci*, 80 (2007) 2093.

8 Simsek I, Aytekin F, Yesilada E, Yildirimli S, An ethnobotanical survey of the Beypazari, Ayas, and Gudul district towns of Ankara province (Turkey), *Economic Botany*, 58 (2004) 705.

9 Yousufuddin M, Cabrera Aguilera C, Oberlin A, Sagar S & Jahangir A, A potential molecular mechanism for digitalis cardiac toxicity in elderly, *J Mol Cell Cardiol,* 42 (2007) 114.

10 Bilgehan H, *Klinik Mikrobiyolojik Tanı* (Şafak Matbaacılık. 4. Baskı. Barış Yayınları Fakülteler Kitapevi, İzmir) 2004, 777.

11 Keleş O, Ak S, Bakırel T & Alpınar K, Türkiye'de yetişen bazı bitkilerin antibakteriyel etkisinin incelenmesi, *Turk J Vet Anim Sci*, 25 (2001) 559.

12 Eloff JN, Which extractant should be used for the screening and isolation of antimicrobial components from plants, *J Ethnopharmacol,* 60 (1998) 1.

13 Inuma M, Tosa H, Tanaka T, Kanamaru S, Asai F, Kobayashi Y, Miyauchi K & Shimamo R, Antibacterial activity of same *Garcinia* benzophenone derivatives against methicillin resistant *Staphyloccocus aureus, Biol Pharm Bull*, 19 (1996) 311.

14 Dülger B, Ceyhan M, Alitsaous M & Uğurlu E, *Artemisia absinthium* L. (Pelin)'un antimikrobiyal aktivitesi, *J Biol,* 23 (1999) 377.

15 Ertürk Ö & Demirbağ Z, *Scorzonare mollis* Bieb (Compositae) bitkisinin antimikrobiyal aktivitesi, *Ekoloji Çevre Dergisi*, 12 (2003) 27.