

Antimicrobial activity of some *Alnus* species

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Abstract. – OBJECTIVE: The increasing prevalence of resistant microorganisms forced scientists to find new antimicrobial substances from different sources like medicinal plants. The aim of this study was to determine the antimicrobial activities of leaf extracts of some *Alnus* sp. against some bacteria and a yeast.

MATERIALS AND METHODS: Aqueous and ethanolic leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens* were screened for their antimicrobial activities against *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. Broth dilution method was used to determine the antimicrobial activities of plant extracts.

RESULTS: Ethanolic extracts of tested species exhibited better antimicrobial activity than aqueous extracts. Ethanolic extracts of tested species possessed activity having MIC values of 0.125-0.250 mg/ml against the tested microorganisms. No antibacterial activity was observed against *B. subtilis*, *E. coli*, *P. aeruginosa* for all the aqueous extracts. Except these aqueous extracts, the others possessed activity having MIC value of 1.000 mg/ml against the tested microorganisms.

CONCLUSIONS: To our knowledge, this is the first investigation on the evaluation of antimicrobial activities on aqueous and ethanolic leaf extracts of these species. This study provides significant information about antimicrobial activities of leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens*. It is conceivable that one of the reasons for the usage of *Alnus glutinosa*, in treatment of wound healing in folk medicine, is because of its antimicrobial activity.

Key Words:

Antimicrobial activity, *Alnus* sp., *A. glutinosa*, *A. orientalis*.

Introduction

The genus *Alnus* Miller, belongs to Betulaceae family, consists of about 35 species and is distri-

buted in temperate regions of the northern hemisphere¹. There are six taxones of *Alnus* species growing wild in Turkey². The plants of the genus *Alnus* contain various types of plant secondary metabolites mainly diarylheptanoids, flavonoids, terpenoids, phenols, steroids and tannins³. *Alnus* species have been used as anti-perspirant⁴, for the treatment of rheumatism, uterus cancer⁵, hemorrhoids⁶, dental abscesses, nail inflammation⁴, various skin diseases such as chronic herpes, eczema, prurigo⁷ and also for wound healing⁶ in folk medicine.

The objective of this study was to evaluate the *in vitro* antimicrobial activities of leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens* against some bacteria and a yeast.

Materials and Methods

Plant Material

The plant materials used in this study, their localities, collection dates and voucher specimen numbers are presented in Table I. All voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Ankara University, Ankara, Turkey. *Alnus* species were identified by Prof. Dr. Hayri Duman.

Preparation of Extracts

5 g of each powdered dried plant material was extracted with water and 75% of ethanol in an ultrasonic bath for 1 h. The water extracts were filtered and then lyophilized. The ethanol extracts were filtered and then evaporated to dryness.

In vitro Antibacterial and Antifungal Activities of *Alnus* Species

Aqueous and ethanolic leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens* were screened for their potential *in vitro* antibacterial activities

Table I. Scientific names, studied organs, locations, harvest dates and voucher specimen numbers of the studied plants.

Scientific name	Studied organ	Location	Harvest date	Voucher specimen (AEF) No
<i>A. glutinosa</i> subsp. <i>glutinosa</i>	Leaves	Beylice Çukurhan Village, Bolu to Sakarya	May-11	25991
<i>A. orientalis</i> var. <i>orientalis</i>	Leaves	Köyceğiz, Sandras Mountain, Ağla	May-11	25990
<i>A. orientalis</i> var. <i>pubescens</i>	Leaves	Köyceğiz lakeside	May-11	25989

against *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and antifungal activities against *C. albicans* ATCC 10231. Prior to testing all extracts were filter-sterilized through a 0.45 µm membrane filter. Broth dilution assay was used for determination of the minimum inhibitory concentrations (MIC). The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). Serial two-fold dilutions ranging from 1.000 to 0.0625 mg/ml were prepared in medium. A set of tubes containing only inoculated broth were used as controls. After incubation for 18-24 h at 37±1°C for bacteria-48 h for fungi, the last tube with no microbial growth was recorded to represent MIC value (mg/ml)^{8,9}.

Results

The MIC results of the tested extracts are shown in Table II. Ethanolic extracts of tested species exhibited better antimicrobial activity than aqueous extracts. Ethanolic extracts of tested species possessed activity having MIC values

of 0.125-0.250 mg/ml against the tested microorganisms. No antibacterial activity was observed against *B. subtilis*, *E. coli*, *P. aeruginosa* for all the aqueous extracts. The MIC values of aqueous extracts were 1.000 mg/ml against both *S. aureus* strains and *C. albicans*.

Discussion

A. glutinosa (L.) Gaertn., commonly known as “black alder” or “european alder”, is naturally widespread across all of Europe, from mid-Scandinavia to the Mediterranean countries, including northern Morocco and Algeria¹⁰. In Turkey, the leaves of *A. glutinosa* have been used as a folk remedy to treat wounds and cuts, and one glass of the infusion of leaves is drunk three times per day to treat rheumatism¹¹.

In the previous studies, antioxidant^{12,13}, hepatoprotective and anti-inflammatory³ activities of *A. glutinosa* have been reported. Middleton et al¹² also demonstrated the antibacterial activity of the seed methanolic extract of *A. glutinosa* against eight bacterial species (*Citrobacter freundii*, *E. coli*, *Klebsiella aerogenes*, *Lactobacillus plan-*

Table II. MIC values (mg/ml) of *Alnus* species against tested microorganisms.

Extracts		Microorganisms					
		<i>S. aureus</i> ATCC 25923	<i>S. aureus</i> ATCC 43300	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
<i>A. orientalis</i> var. <i>pubescens</i>	E	0.250	0.250	0.125	0.250	0.250	0.125
	W	1.0	1.0	-	-	-	1.0
<i>A. orientalis</i> var. <i>orientalis</i>	E	0.250	0.250	0.250	0.250	0.125	0.125
	W	1.0	1.0	-	-	-	1.0
<i>A. glutinosa</i> subsp. <i>glutinosa</i>	E	0.250	0.250	0.125	0.250	0.125	0.125
	W	1.0	1.0	-	-	-	1.0

E: ethanol, W: water, ‘-’ = represents no activity.

tarum, *P. aeruginosa*, *S. aureus*, *E. coli*) and the most potent activity was seen against *E. coli* with an MIC value of 1.25×10^{-1} mg/ml. In 2014, Dahiya et al¹⁴ indicated that methanolic leaf extracts of *A. glutinosa* possessed antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. In 1995, Saxena et al¹⁵ demonstrated that the bark methanolic extract of *A. rubra* has antibacterial effect against Gram positive and Gram negative bacteria. Diarylheptanoid oregonin and its glycoside were identified as the two constituents responsible for this activity.

In a study that the antioxidant, cytotoxic, and antimicrobial activities of leaves, bark, and cone extracts of *A. incana* (L.) Moench ssp. *incana* and *A. viridis* (Chaix) DC ssp. *viridis* were evaluated, all the extracts were found to possess antimicrobial activity against 15 microorganisms. The most active extracts were found as the extracts of cones of *A. incana* and *A. viridis* with MIC values ranging from 0.117 to 0.129 mg/ml¹⁶.

In a study, the ethanolic extract of the barks of *A. pendula* and the fractions obtained from it were evaluated for their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). By using the broth microdilution method, the extract found to possess antibacterial activity with MIC values ranging from 0.125 to 0.250 mg/ml against different strains of *S. aureus* and 16 clinical isolates. The isolated compounds from the extract, oregonin and hirsutanone were also evaluated for their antibacterial activities and they were found to be active against MRSA strains with MIC values ranging from 0.03125 to 0.250 mg/ml¹⁷.

In this study ethanolic leaf extracts of *Alnus* species exhibited better antimicrobial activity than aqueous extracts. The MIC values of ethanolic extracts were 0.125-0.250 mg/ml against the tested microorganisms. No antibacterial activity was observed against *B. subtilis*, *E. coli*, *P. aeruginosa* for aqueous extracts. The MIC values of aqueous extracts were 1.000 mg/ml against both *S. aureus* strains and *C. albicans*.

Conclusions

This study provides significant information about antimicrobial activities of leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens*. It is conceivable that one of the reason for the usage of

Alnus glutinosa, in treatment of wound healing in folk medicine, is because of its antimicrobial activity. To our knowledge, this is the first investigation on the evaluation of antimicrobial activities on aqueous and ethanolic leaf extracts of these species.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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