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ANTIBACTERIAL ACTIVITY OF *CORYLUS COLURNA* L. (BETULACEAE) AND *PRUNUS DIVARICATA* LEDEP. SUBSP. *DIVARICATA* (ROSACEAE) FROM USAK, TURKEY

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Abstract

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Hazelnut and plum are very popular fruits in the world being consumed in different form and presentations. These fruits are of economic importance, especially hazelnut. In this study, the antibacterial activity of Turkish tree hazel (*Corylus colurna*) and Chery plum (*Prunus divaricata* subsp. *divaricata*) were investigated. The leaves of plants were extracted successively with petroleum ether, dichloromethane, methanol and distilled water in a Soxhlet apparatus. The antibacterial effects of these extracts were tested by a disk diffusion method using gram positive bacteria (*Staphylococcus aureus* NRRL B-767, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* NRS-744, *Listeria monocytogenes* ATCC 7644) and gram negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* NRRL B-4420, *Klebsiella pneumonia ATCC 700603*). Extracts from both plants showed similar potencies against all tested microorganisms. The results show that the dichloromethane extract of *C. colurna* had the highest antimicrobial activity against *S. typhimurium*. In additionally, petroleum ether extract of *P. divaricata* subsp. *divaricata* had highest antimicrobial activity against *E. faecalis.* The present work demonstrates that *C. colurna* and *P. divaricata* subsp. *divaricata* might be a natural source of bioactive compounds that can be promoting human health and reducing disease risks.

Key words: antibacterial activity, Turkish tree hazel, *Corylus colurna,* chery plum, *Prunus divaricata* subsp. *divaricata*

Introduction

Nowadays, there is growing interest in discovering natural antimicrobial compounds that might be used as alternatives to the chemical preservatives used in food industry, and to antibiotics, thus decreasing their use, and lowering the probability of the occurrence of human resistance to these chemicals. Many studies have therefore focused on the antimicrobial agents and properties of plant-derived active principles, which have been used for some time in traditional medicine to overcome infections (Ramalhosa et al., 2011).

Hazel is the common name for the flowering plant genus *Corylus*, usually placed in the Betulaceae family, although some botanists consider it a separate family, Corylaceae. The genus *Corylus* comprises about 15 species, including the European *Corylus avellana* L., the common commercially grown hazelnut. It is a monoecious species, growing as shrubs or small trees, usually 2-5 m high (Contini et al., 2011). Nuts have been shown to contain substances such as tocopherols and polyphenols that significantly reduce the risk of coronary heart disease, some types of cancer, and several other diseases and physiological conditions and syndromes (Richardson, 1997; Yurttas et al., 2000). As far as we know, only two studies regarding determination of the hazelnut's antimicrobial potential have been carried out; one with hazelnut kernels (Oliveira et al., 2008), and one with leaves (Oliveira et al., 2007), and involving three cultivars: M. Bollwiller, Fertile de Coutard, and Daviana.

The Turkish tree hazel (*Corylus colurna* L.) is one of the wild species with in the genus *Corylus*. It grows in a tree form and can reach a height of 15–30 m with a trunk diameter up to 1 m (Ansin and Ozkan, 1997; Mitroviç et al., 1997, 2001). It

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occurs naturally in mixed temperate forests of Romania, the Balkans, northern Turkey, Transcaucasia, and northern Iran (Thompson et al., 1996). *C. colurna*, however, is not under cultivation as European hazelnut, in Serbia and Turkey, nuts of this species are collected and sold, eaten as a snack, or used by the confectionary industry in candies, chocolates, and sweets due to their small size (Mitroviç et al., 1997). Although Turkish tree hazel nuts are consumed and traded, there are limited reports of its nuts and their chemical composition, which are generally limited to protein and oil content. *C. colurna* used in this study is the first record for the Uşak region.

Species of the genus *Prunus* L. are distributed in the northern hemisphere. Most of the species occur in semiarid climats. Cultivated species of the genus are found under varying ecological conditions (Dönmez and Yıldırımlı, 2000). *P. divaricata* shows variations in its fruit colour and shape. Kovalev (1939) has divided the species into eleven ecotypes according to their morphological characters and ecological preference. There are different treatments on the *P. divaricata* and other names of this species, recently, the names *P. cerasifera* Ehrend. and *P. divaricata* (Ledeb.) Browicz (1996) proposes Schneider for the cultivated forms and wild forms of the species respectively. *P. divaricata* is native to the North America, Europe, Caucasia, Central Asia and Australia is a species of plum known by the common names cherry plum and myrobalan plum. Wild types are shrubs or small trees reaching 2-4 m, weakly thorny, young shoots and buds glabrous.

The aim of this study is to conduct an antibacterial screening study of the extracts obtained from the *C. colurna* and *P. divaricata* subsp. *divaricata*. This is the first study on the antibacterial effect of these species.

Materials and Methods

Plant materials

Naturally growing *P. divaricata* subsp. *divaricata* and *C. colurna* plants were collected at the flowering stage in March – June 2010 from Uşak regions of Turkey. A voucher specimens of the plants were collected and taxonomically identified by Dr. Mehtap Dönmez ŞAHİN at the Faculty of Education, the University of Uşak. These voucher specimens have been deposited at the Herbarium of the Department of Biology, University of Uşak, Turkey. The plant was identified immediately after collection and air-dried at room temperature for later analysis.

Preparation of the organic extracts

The air-dried and powdered leaves of *P. divaricata* subsp. *divaricata* and *C. colurna* were extracted successively with petroleum ether, dichloromethane, methanol and distilled water in a Soxhlet apparatus until the last portion of the extract became colourless. Solvents of all the extracts were removed under low vacuum by using rotary evaporation. Crude extracts were maintained at 4°C until used. Crude extracts were investigated for antimicrobial activity.

Antimicrobial Activity

Microorganisms and culture conditions

Escherichia coli ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 and *Klebsiella pneumonia ATCC 700603* were obtained from the American Type Culture Collection. *Staphylococcus aureus* NRRL B-767 and *Salmonella typhimurium* NRRL-B 4420 were obtained from Agricultural Research Service Culture Collection, USA. *Bacillus subtilis* NRS-744 were obtained from Collection of Nathan R. Smith, U.S. Department of Agriculture, Washington, D.C., USA.

The above mentioned bacteria were cultured in Nutrient Broth (NB) (Difco) at 37±0.1°C. Inocula were prepared by adjusting the turbidity of the medium to match the 0.5 Mcfarland Standard Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water were inoculated on NB, to check the viability of the preparation. The cultures of bacteria were maintained in Nutrient Agar (NA) (Difco) slants, in the dark at 4°C during the study.

Antimicrobial Assays

The antimicrobial activity was assayed by the disc diffusion method using bacterial cell suspensions whose concentration was equilibrated to a 0.5 McFarland standard (Collins, 1995; Murray et al., 1995). One hundred microliters of each bacterial suspension was spread on a Mueller-Hinton agar plate. 0.005 gr crude extracts were dissolved in 1 ml DMSO, under aseptic conditions empty sterilized paper discs (Whatman no.5, 6 mm dia) were impregnated with 50 µL of each extracts. The discs were allowed to dry and then placed on the inoculated agar. The plates were incubated at the appropriate temperature and time for microorganisms.

Paper disc moistened with aqueous DMSO was placed on the seeded petri plate as a vehicle control. A standard disc containing ciprofloxacin (25 µg/disc), chloramphenicol (30 µg/disc), eritromycin (15 µg/disc), penicillin (10µg/disc) and amikacin (10 µg/disc) were used as positive controls. At the end of incubation periods, diameters of no-growth zones around the discs were measured to the nearest 0.1 mm using vernier calipers. The experiment was performed in triplicate.

Results

Antimicrobial activity of different solvent extracts of *P. divaricata* subsp. *divaricata* and *C. colurna* has been evalu-

ated in vitro against four Gram-positive bacteria and three Gram- negative bacteria (Table 1) which are known to cause infections in humans.

All extracts used in this study showed antimicrobial effect against all gram positive and gram-negative bacteria with 7-11 mm inhibition zones. Dichloromethane extract of *P. divaricata* had an inhibitory effect against *S. aureus* with 7 mm and *E. coli* with 8 mm, respectively. In this study, petroleum ether extract of *P. divaricata* had shown the highest antimicrobial activity with 11 mm inhibition zone against gram-positive *E. faecalis*. In addition, this extract had antimicrobial effect to *S. aureus* and *L. monocytogenes* with 7 mm, and to *E. coli* and *S. typhimirium* with 8mm zone diameters. Gram-negative bacteria (*E. coli* and *K. pneumonia*) were found more sensitive than gram-positive bacteria against *P. divaricata* metanol extract. This extract had antimicrobial effect against *E. coli* with 9 mm inhibition zone, while had antimicrobial effect against *K. pneumoniae* with 8 mm inhibition zone.

The dichloromethane extract in *C. colurna* extracts was found the highest antimicrobial effect against tested bacteria. This extract was showed antimicrobial effect against *E. faecalis* with 9 mm inhibition zone. In addition, other gram-positive bacteria were measured as the effect of 8 mm inhibition zones. The highest inhibition zone obtained from study against gram-negative bacteria belongs to dichloromethane extract of *C. colurna* with 11 mm. This inhibition zone was measured against *S. typhimirium*. Distilled water extract of *C. colurna* had effect only *E. faecalis* with 9 mm inhibition zone.

In this study, the highest antimicrobial activity against gram-positive bacteria showed petroleum ether extract of *P. divaricata* subsp. *divaricata*, while the highest antimicrobial activity against gram-negative bacteria have showed dichloromethane extract of *C. colurna*.

Discussion

No studies on antimicrobial activities of extracts of *P. divaricata* subsp. *divaricata* and *C. colurna* have been found in the literature concerned, although extracts belonging to the three cultivars (M. Bollwiller, Fertile de Coutard and Daviana) of the genus *Corylus* had an antimicrobial activity was reported in two studies (Oliveira et al., 2008; Oliveira et al., 2007). In both studies, the hazelnut kernels and leaves showed different antimicrobial activities. Oliveira et al. (2007) reported that the hazelleaf extracts presented high antimicrobial activity to gram positive bacteria (MIC 0.1 mg/ml for *B. cereus* and *S. aureus* and 1 mg/ml for *B. subtilis*). In the same study, a very low effect against gram negative bacteria and the fungi have been reported. Oliveira et al. (2008) in

Table 1
Antimicrobial activity of extracts of *P. divaricata* subsp. *divaricata* and *C. colurna* against test microorganisms.

Plant extracts	*P. divaricata* subsp. *divaricata*				*C. colurna*				Antibiotics					Control
	Petroleum ether	Dichloro-methane	Methanol	Distilled water	Petroleum ether	Dichloro-methane	Methanol	Distilled water	CIPS	C	E	P	AM	DMSO
Microorganisms	Inhibition zones, mm													
Gram-positive bacteria														
Staphylococcus aureus	7	7	(-)	(-)	(-)	8	(-)	(-)	32	28	24	9	19	(-)
Bacillus subtilis	(-)	(-)	7	(-)	(-)	8	(-)	(-)	36	32	26	20	20	(-)
Listeria monocytogenes	7	(-)	(-)	(-)	(-)	8	(-)	(-)	34	39	(-)	(-)	(-)	(-)
Enterococcus faecalis	11	(-)	8	(-)	8	9	(-)	9	32	30	18	(-)	11	(-)
Gram-negative bacteria														
Escherichia coli	8	(-)	9	8	7	0	8	(-)	34	30	(-)	(-)	(-)	(-)
Klebsiella pneumoniae	(-)	(-)	8	(-)	(-)	(-)	8	(-)	39	36	(-)	(-)	(-)	(-)
Salmonella typhimirium	8	(-)	(-)	(-)	7	11	(-)	(-)	39	35	9	10	10	(-)

DMSO: Dimethyl sulfoxide, CIP:Ciprofloxacin, C: Chloramphenicol, P: Penicillin, E: Eritromycin, AM: Amikacin, (-): no inhibition

other study with aqueous extracts of hazelnut kernels reported high antimicrobial activity was only found against gram positive bacteria; namely, *B.cereus, B.subtilis* and *S. aureus*. On the contrary, gram-negative test bacteria and fungi were found to be resistant to tested extracts at all the assayed concentrations. Our results showed that extracts of *C. colurna* effective against gram-negative bacteria. Especially dichloromethane extract was found effective against *S. typhimirium*. For this reason, these results are not compatible with our results. Orhan et al. (2011) investigated the antimicrobial activity of *C. avellana* oil and their results showed that *E. coli* was inhibited by 4 µg/ml concentration; *S. aureus and E. faecalis* by 8 µg/ml concentration of *C. avellana* oil. In another study (Amarowicz et al., 2008) reported antimicrobial activity from tannins. According to this study, 125-500 µg/ml tannin concentrations obtained from *C.avellana* measured as inhibition concentration for tested bacteria. In this study, to *E. coli* and *L. monocytogenes* 125 µg/ml, to *S. aureus* 250 µg/ml and to *S. typhimirium* 500 µg/ml were reported as the MIC value. These results may be important to show the different antimicrobial activities of different species of *Corylus*.

Conclusion

In conclusion, antibacterial activity results of this study are the first record for *P. divaricata* subsp. *divaricata* and *C. colurna*. In addition, *C. colurna* is the first record for Usak region. Both plant species (*Corylus colurna* and *Prunus divaricata* subsp. *divaricata*) are edible and with industrial importance. As a result, in which continued searching for alternative antibiotics in these days, the antimicrobial activity of *Corylus colurna* and *Prunus divaricata* subsp. *divaricata* is very important for these fruits have a lot consumed.

References

Amarowicz, R., A. Troszynska, A. Kosinska, G. Lamparski, F. Shahidi, 2008. Relation between sensory astringency of extracts from selected tannin-rich foods and their antioxidant activity. *J. Food Lipids*, **15**: 28-41.

Ansin, R. and Z. C. Ozkan, 1997. Tohumlu Bitkiler (Spermathophyta): Odunsu Taksonlar, Karadeniz Teknik Universitesi, Orman Fakultesi Yayınları, Trabzon, Turkey, pp. 306–311.

Browicz, K., 1996. Chorology of Trees and Shrubs in South-West Asia and Adjacent Regions (Suppl), *Prunus* L. Poznan, 16-17.

Collins, C. H., 1995. In: P.M. Lyne and J.M. Grange (eds.), Microbiological Methods. Seventh ed. Butterworths, London, p. 493.

Contini, M., M. T. Frangipane and R. Massantini, 2011. Antioxidants in Hazelnuts (*Corylus avellana* **L.).** In: V.R. Preedy, R.R. Watson, V.B. Patel (Ed), Nuts & Seeds in Health and Disease Prevention (1st ed.). London, Burlington, San Diego: *Academic Press,* pp. **611-625.**

Dönmez, A. A. and Ş. Yildirimlı, 2000. Taxonomy of the Genus *Prunus* L. (Rosaceae) in Turkey. *Turk J. Bot*., **24**:187-202.

Kovalev, N. V., 1939. New species of Plums. Comptes (Doklady) de Akademie des Sciences de 1'URSS, **23** (3).

Mitroviç, M., D. Ogasanovic, N. Micic, Z. Tesovic and R. Miletic, 1997. Biodiversity of the Turkish Haazel (*Corylus colurna* L.) in Serbia. *Acta Hort*., **445**: 31.

Mitroviç, M., M. Stanisavljevic and D. Ogasanovic, 2001. Turkısh Tree Hazel Bıotypes in Serbia. *Acta Hort*., **556**: 191.

Murray, P. R, E. J. Baron and N. A. Pfaller, 1995. Manual of Clinical Microbiology, 6th ed. *ASM Press,* Washington, p. 1482.

Oliveira, I., A. Sousa, P. Valentao, P. B. Andrade, I. C. F. R. Ferreira, F. Ferreres, A. Bento, R. Seabra, L. Estevinho and J. A. Pereira, 2007. Hazel (*Corylus avellana* L.) leaves as source of antimicrobial and antioxidative compounds. *Food Chem*., **105**: 1018–1025.

Oliveira, I., A. Sousa, J. S. Morais, I. C. F. R. Ferreira, A. Bento, L. Estevinho, J. and A. Pereira, 2008. Chemical composition and antioxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L.) cultivars. *Food Chem. Toxicol.,* **46**: 1801-1807.

Orhan, I., B. Ozcelik and B. Sener, 2011. Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. *Turk J. Biol*., **35**: 251-258.

Ramalhosa, E., T. Delgado, L. Estevinho and J. Alberto Pereira, 2011. Hazelnut (*Corylus avellana* L.) cultivars and antimicrobial activity. In: V.R. Preedy, R.R. Watson, V.B. Patel (Ed), Nuts & Seeds in Health and Disease Prevention (1st ed.). London, Burlington, San Diego: *Academic Press*, pp. 627-636.

Richardson, D. G., 1997. The health benefits of eating hazelnuts: Implications for blood lipid profiles, coronary heart disease, and cancer risks. *Acta Hort*., **445**: 295–300.

Thompson, M. M., H. B. Lagerstedt and S. A. Mehlenbacher, 1996. Hazelnuts. In: J. Janick and J. N. Moore (eds) Fruit Breeding; Vol. 3 Nuts, *John Willey & Sons,* New York, pp. 125–184.

Yurttas, H. C., H. W. Schafer and J. J. Warthesen, 2000. Antioxidant activity of nontocopherol hazelnut (*Corylus* spp.) phenolics. *J. Food Sci*., **65**: 276-280.

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