

ANTIMICROBIAL ACTIVITY OF TURKISH *VIBURNUM* SPECIES

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Abstract

Antimicrobial activities of aqueous and ethanolic extracts of *Viburnum opulus* L., *V. orientale* Pallas, *V. tinus* L. and *V. lantana* L. against *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* RSKK 574 and *Candida albicans* ATCC 10231 were carried out. The disc diffusion and tube dilution techniques were used to determine the antimicrobial activities of plant extracts. The ethanolic extracts of tested species of *Viburnum* exhibited better antimicrobial activity than that of aqueous extracts.

The genus *Viburnum* L. (Caprifoliaceae) comprises more than 230 species distributed from South America to Southeast Asia, the majority of them being endemic (Lobstein *et al.*, 1999). The plant is represented by four species in the flora of Turkey; *Viburnum opulus* L., *V. lantana* L., *V. orientale* Pallas, and *V. tinus* L. (Davis *et al.* 1988).

Viburnum L. species have been reported to contain sesquiterpenoids, triterpenoids and sterols; phenolic compounds and their glycosides such as tannins, flavonoids and anthocyanins and iridoid glycosides in their stem, root and leaves, and investigated to possess uterine sedative, diuretic, cardiovascular stimulant, antimicrobial, anti-inflammatory, anti-nociceptive, antispasmodic, antiasthmatic and astringent activities (Prabhu *et al.* 2011). *Viburnum* L. represented with four deciduous shrub species in Turkey (Yılmaz *et al.* 2008).

In Central Anatolia, a traditional beverage named gilaburu has been prepared from the fruits of *V. opulus* whose fruits have a dark-red color and are edible. The fruits of *V. opulus* have been used as antidiabetic, and the bark of *V. lantana* has been used as rubefiant and analgesic in Turkish folk medicine (Baytop 1999). The preventive effects of *V. dilatatum* on oxidative damage were reported in rats subjected to stress (Iwai *et al.* 2001), and in streptozotocin-induced diabetic rats (Iwai *et al.* 2004). Antioxidant activity of *V. opulus* and an antihyperglycemic effect of *V. dilatatum* have also been previously noted in different systems (Andreeva *et al.* 2004, Iwai *et al.* 2006). The alcoholic extract of *V. erubescens* has been reported to show antiviral activity (Dhar *et al.* 1968). Some iridoid aldehydes isolated from *V. luzonicum* exhibited a moderate inhibitory activity against HeLa S3 cancer cells (Fukuyama *et al.* 2005).

The objective of this study was to evaluate the antimicrobial activities of aqueous and ethanolic extracts of *Viburnum opulus*, *V. orientale*, *V. tinus* and *V. lantana* against six bacteria and a yeast. To our knowledge, this is the first comprehensive investigation on the evaluation of antimicrobial activities of these four *Viburnum* species.

Viburnum opulus (AEF No 23696), *V. orientale* (AEF 25988), *V. tinus* (AEF 25891) and *V. lantana* (AEF No 23543) were collected in the flowering stages from Kayseri, Artvin, Aydın

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and Ankara (Turkey), respectively. The plants were identified by Prof. Dr Hayri Duman, Department of Biology, Faculty of Sciences, Gazi University, Voucher specimens have been deposited in the Herbarium of Faculty of Pharmacy, Ankara University.

Preparation of extracts: Stem, leaf and fruit of *V. orientale*, *V. tinus*, *V. opulus* and leaf of *V. lantana* were separated and dried. Plant materials were powdered and then 5 g of each were weighted for the extraction. Aqueous and ethanolic (75%) extracts of each sample were prepared by stirring on a magnetic stirrer for 8 h at room temperature. The extracts were concentrated under pressure and the residues containing water were dried by lyophilisation.

Test solutions for antimicrobial activity were prepared by dissolving 10 mg of dried extracts in their solvents (water or 75% ethanol) and poured into filling to 10 ml in volumetric flask resulting a concentration of 1000 µg/ml.

Staphylococcus aureus ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* RSKK 574 and *Candida albicans* ATCC 10231 were used in the present investigation.

In vitro antibacterial and antifungal activities of Viburnum species: The disc diffusion method (Rangkadilok *et al.* 2012) and tube dilution technique were used to determine the antimicrobial activities of extracts. Minimum inhibitory concentrations (MIC) were determined with the effective *Viburnum* sp. extracts (Karunai-Raj *et al.* 2012). The extracts were sterilized through a 0.45 µm membrane filter.

Disc-diffusion method: The inocula were suspended in sterile saline and diluted according to 0.5 McFarland standard and then spread on solid culture media plates. Empty paper discs (6 mm in diameter) were soaked with 20 µl of the extract (1000 µg/ml) and placed on the inoculated plates. These plates, after remaining 2 h at 4°C, were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. Ampicillin (10 µg), ciprofloxacin (5 µg) and fluconazole (25 µg) were used as standards.

Determination of minimum inhibitory concentration (MIC): Broth dilution assay was used for determination of the minimum inhibitory concentrations (MIC). The cultures were obtained in Mueller Hinton Broth (Difco) for all the bacteria after 18-24 h of incubation at 37 ± 1°C and for *C. albicans* in Saboraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1°C. Serial two-fold dilutions ranging from 500 to 31.25 µg/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 and 48-72 h at 25 ± 1°C for *C. albicans*, the last tube with no microbial growth was recorded to represent MIC (µg/ml).

Table 1 represents the *in vitro* antimicrobial activities of aqueous and ethanolic extracts of *Viburnum* sp. against six bacteria and *C. albicans* by disc diffusion method. Ethanolic extracts prepared from *Viburnum* sp. showed antimicrobial activity against all the tested microbes. It was observed that the ethanolic extracts of leaf and stem of *V. opulus*, fruit of *V. orientale*, leaf, fruit and stem of *V. tinus* showed activity against *S. aureus* and MRSA (*S. aureus* ATCC 43300); fruit of *V. orientale* showed activity against *B. subtilis*; leaf and fruit of *V. opulus*, fruit and stem of *V. orientale*, fruit of *V. tinus*, leaf of *V. lantana* showed activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae*; leaf of *V. orientale* and *V. tinus* showed activity against *E. coli*, *K. pneumoniae*, stem of *V. orientale* showed activity against *C. albicans*. No antimicrobial activity was observed against *S. aureus*, MRSA, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans* for the aqueous extracts but aqueous extracts of leaf of *V. opulus*, *V. orientale*, *V. tinus*, *V. lantana* showed weak antibacterial activity against *K. pneumoniae*. These extracts possessed activity having MIC values of 125-250 µg/ml against the tested microorganisms (Table 2). The activity of the extracts of *Viburnum* sp.

Table 1. Antimicrobial activity of *Viburnum* species against tested microorganisms using disc diffusion method.

Plant extracts	Inhibition zone (mm) owing to different microorganisms						
	<i>S. aureus</i>	MRSA	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
<i>V. opulus</i>	S ^a	E ^d	-	-	-	-	-
		W ^e	-	-	-	-	-
	F ^b	E	-	-	9	10	8
<i>V. orientale</i>		W	-	-	-	-	-
	L ^c	E	10	-	8	8	8
		W	-	-	-	-	7
	S	E	-	-	9	8	8
		W	-	-	-	-	-
<i>V. tinus</i>	F	E	10	8	8	9	10
		W	-	-	-	-	-
	L	E	-	-	8	-	8
		W	-	-	-	-	7
	S	E	8	-	-	-	-
<i>V. lantana</i>	F	E	-	-	-	-	-
		W	-	-	-	-	-
	L	E	8	-	9	8	8
		W	-	-	-	-	-
	S	E	9	-	9	-	8
Antibiotics		W	-	-	-	-	8
		E	-	-	-	-	-
	F	E	-	-	-	-	-
		W	-	-	-	-	-
	L	E	-	-	-	-	-
Not tested							-
	W	-	-	-	-	-	-
Ampicillin		E	-	-	8	8	8
Ciprofloxacin		W	-	-	-	-	7
Fluconazole		E	34	11	20	22	32
	W	-	-	22	20	22	22
	E	-	-	-	-	-	-
	W	-	-	-	-	-	28

^aS = Stem, ^bF = Flower, ^cL = Leaf, ^dE = Ethanol, ^eW = Water, "-" = represent s no activity.

Table 2. MIC values ($\mu\text{g/ml}$) of *Viburnum* species against tested microorganisms

Plant extracts		Inhibition zone (mm) owing to different microorganisms						
		<i>S. aureus</i>	MRSA	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
<i>V. opulus</i>	S ^a	125	125	-	-	-	-	-
	E ^d	-	-	-	-	-	-	-
	W ^e	-	-	-	125	125	125	-
F ^b	E	-	-	-	125	125	125	-
	W	-	-	-	-	-	-	-
	W	125	125	-	125	125	125	-
L ^c	E	-	-	-	125	125	125	-
	W	-	-	-	-	-	-	-
	W	-	-	-	125	125	125	125
<i>V. orientale</i>	S	-	-	-	-	-	-	-
	E	-	-	-	125	125	125	-
	W	-	-	-	-	-	-	-
F	E	125	125	125	125	125	125	-
	W	-	-	-	-	-	-	-
	W	-	-	-	125	125	125	-
L	E	-	-	-	125	125	125	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
<i>V. tinus</i>	S	125	125	-	-	-	-	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
F	E	125	125	-	250	250	125	-
	W	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
L	E	125	125	-	125	125	125	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
<i>V. lantana</i>	S	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
F	E	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-
L	E	-	-	-	125	125	125	-
	E	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-

^aS = Stem, ^bF = Flower, ^cL = Leaf, ^dE = Ethanol, ^eW = Water, "-" = represent s no activity.

was less than that of the standards. The ethanolic extracts of four tested *Viburnum* species exhibited better antimicrobial activity compared to aqueous extracts.

A preliminary organic analysis on the alcoholic stem extracts showed the presence of phenolic compounds such as flavonoids, tannins, anthocyanins, phenolic acids and their derivatives (glycosides) as their principal constituents, which may be attributed to a mild and pronounced antibacterial activities of the extracts (Prabhu *et al.* 2011).

Yilmaz *et al.* (2008) investigated the antimicrobial activities of essential oils (in hexane) of *V. opulus*, *V. lantana* and *V. orientale* against the bacteria *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 10145, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. cereus* 709 Roma and the fungus *C. tropicalis* ATCC 13803 and observed no activity against all the test microorganisms for *V. opulus* and *V. lantana* however the essential oil of the *V. orientale* showed weak antibacterial activity against Gram-positive bacteria. In another study Ucar-Turker *et al.* (2012) evaluated the antibacterial activity of fruit extracts of *V. lantana* and they indicated that the hot ethanolic extract of *V. lantana* showed better antibacterial activity than the cold ethanolic extract against *S. aureus*, *S. epidermidis* and *S. pyogenes*.

From this findings it could be concluded that, the ethanolic extracts of *Viburnum* sp. exhibited antimicrobial activity against the tested microorganisms.

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