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Short Communication

Vitality and Implication of Natural Products from Viburnum Grandiflorum: an Eco-Friendly Approach

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Abstract

In this study we screened *Viburnum grandiflorum* for bioactive secondary metabolites and biological activity. Secondary metabolites were detected by phytochemical tests, and biological activity was confirmed through antimicrobial and anti-oxidant assays. Phytochemical screening (alkaloidal, tannins, terpenoids, flavonoids, anthraquinones, and glycosides) was performed with methanol, and aqueous and ethyl acetate extracts. Antibacterial activity against four bacterial strains — *staphylococcus auries, Escherichia Coli, Bacillus subtillus,* and *salmonella typhi* – were measured. Methanolic extract showed maximum inhibitory activity with diameter of zone of inhibition (11.66 mm), followed by n-hexane extract (9.33 mm) and then ethyl acetate extract. Four different fungi (*Penicillium chrysogenum, Aspergillus flavus, Rhodotorula mucilaginosa,* and *Stachybotrys chartarum)* were also tested against plant stem extract using different solvents. Dimethyl sulfoxide extract showed a maximum zone of inhibition at 20 mg/ml. Anti-oxidant activity of stem extract of *Viburnum grandiflorum* was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Then we measured absorbance, and percentage activity at each concentration was found for three solvent extracts to get Ic50 values. These data support *Viburnum grandiflorum* as having enough potential to be used safely as an antimicrobial drug.

Keywords: natural products, phytochemical, secondary metabolites, antimicrobial, fungi, bioactivity

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Introduction

Caprifoliaceous is a small family of about 12 genera and 450 species [1]. In recent classifications on molecular phylogeny the genus *Viburnum* (Caprifoliaceae) was put in the Adoxaceae family [2]. It has spread from South America to Southeast Asia, and the majority are found in a particular place [3]. It is found mostly in the temperate regions of the northern hemisphere. Its 4 genera and 27 species are found in Pakistan [4]. Six species of the genus Viburnum (*V. opulus, V. cylindricum, V. grandiflorum, V. mullaha, V. cotinifolium, and V. tinus*) are commonly present in Pakistan [5]. *Viburnum grandiflorum* Wall ex D.C. has been used for treating wounds and malaria, and as a diuretic [6]. *Viburnum grandiflorum* is used locally to cure abdominal pain and as a purgative [7], antimalarial, and diuretic [8]. It is also used as a wound curative [9], and to treat upset stomachs [8, 10], whooping cough, respiratory diseases, toothaches, and typhoid [11], and as an anesthetic [12]. Uddin et al. [5] studied the conventional system of medicine and the role of *Viburnum grandiflorum* as an antipyretic in the treatment of typhoid and malaria. Uddin and his co-workers also performed scientific justification of the anti-inflammatory, anti-nociceptive, and antipyretic effects of *V. grandiflorum* with respect to its chemical composition. Viburnum species are also used for treating different diseases such as diarrhoea, rheumatoid arthritis, tumefaction, anti-diabetic, anti-oxidant and anti-bacterial [13-14]. The present study was undertaken to investigate the phytochemical composition and antioxidant and antimicrobial properties of the stem parts of *V. grandiflorum*.

Materials and Methods

Viburnum grandiflorum stem was collected locally from the Bagh Azad Kashmir District in Pakistan and dried in the shade. Dried stems were converted to powder by means of an electric grinder. Powder (10 g) was soaked in methanol in Erlenmeyer flasks for three days with continuous shaking. Extract was filtered and filtrate was evaporated to obtain dried extract that was stored at 4ºC. Phytochemical Analysis was performed following the methods reported in Tadesse et al. [15], and antibacterial assay was determined by the procedure described by Iqbal et al. [16].

Sabouraud dextrose agar (SDA) (65 g in 1,000 ml) was prepared for fungal growth (*Penicillium chrysogenum, Aspergillus flavus, Rhodotorula mucilaginosa,* and *Stachybotrys chartarum).* SDA was autoclaved at 121ºC for 15 minutes, cooled to 45-50ºC, and 20 ml of molten SDA medium was aseptically transferred into each sterilized petri plate. 150 µl fungal suspensions were spread uniformly over the agar in petri dishes using a sterile glass rod. Filter paper discs were prepared and soaked with different dilutions of plant extract, and one disc was soaked with water for negative control. Aseptically, the soaked discs were transferred to the inoculated plates with the help of sterile forceps. An antifungal disc and a water-soaked disc were placed in the plate for positive control and negative plate, respectively. The plates were incubated for 24 hours at 37ºC. After 24 hours fungal growth was observed in the petri plates and the diameters of zones of inhibition were measured. The antifungal activity was expressed as the mean of diameter of the inhibition.

For thin layer chromatography (TLC), a small spot of methanolic extract of *Viburnum grandiflorum* was applied to a plate about 1.5 centimeters from the bottom edge. Solvents used for TLC were ethyl acetate, methanol, n-hexane, dichloromethane, ethyl acetate + methanol, dichloromethane + n-hexane, and dichloromethane + methanol. The TLC plate was then placed in the chamber so that the spot(s) of the sample did not touch the surface of the eluent in the chamber, and the lid was closed. The plate was visualized using ultraviolet light and was sprayed by chemicals after elution.

For antioxidant activity, DPPH assay of the plant *Viburnum grandiflorum* was carried out as described in Bozin et al. [17]. The capability of scavenging the DPPH radical was calculated by using the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A1)/A_0) \times 100\}$$

Table 1. Antibiotic sensitivity activity of *Viburnum grandiflorum*-Guch stem's extract.

Microorganism	Methanol						Hexane						Ethyle acetate					
	Concentrations of extracts used (mg/ml) / Zones of inhibition (mm)																	
	5	10	15	20	A	W	5	10	15	20	A	W	5	10	15	20	A	W
Staphylococcus aureus	NZ	6	9.8	11.6	26	NZ	NZ	6.33	8.33	9.33	26	NZ	NZ	NZ	6.3	8	26	NZ
Escherichia Coli	NZ	NZ	7	8	26	NZ	5	7.33	8.33	9	26	NZ	NZ	NZ	7.33	8	26	NZ
Bacillus Subtilus	NZ	6	7.3	8.3	25	NZ	NZ	5	6.33	7.33	25	NZ	6	10.2	12	12.3	25	NZ
Salmonella entererous	NZ	NZ	8	9	25	NZ	NZ	NZ	NZ	NZ	25	NZ	NZ	NZ	NZ	NZ	25	NZ

NZ: no zone, A: standard antibiotic, W: Water

…where A_0 is the absorbance of the control reaction and A_1 is absorbance in the presence of all the extract samples and reference. All tests were performed in triplicate.

Results and Discussion

Phytochemical screening of *Viburnum grandiflorum* Guch stem's extract was performed for the recognition of secondary metabolites. Phytochemical screening was performed with methanolic, n-haxane, and ethyl acetate extract. The results revealed the presence of tannins and glycosides in all extracts, terpenoids, and saponins in methanolic and ethyl acetate extract and anthraquinones in ethyl acetate extract only. The results of the phytochemical screening are in line with the study conducted by Uddin et al. [5]. In their work they showed the presence of bioactive secondary metabolite constituents such as terpenoids, steroids, anthraquinones, flavonoids, and saponins [5].

The biological activity of *V. grandiflorum*-Guch stem's extract was confirmed through antibacterial assay. Table 1 shows the anti-bacterial activity of *Viburnum grandiflorum*-Guch stem's extract against Gram-positive bacteria (*Staph aureus and B. subtilis)* and Gram-negative bacteria (*E.coli and S. enterocous*) at different concentrations. Low concentrations of extract showed less anti-bacterial activity while in increasing concentrations anti-bacterial activity also increased. Samples with least concentration of extract showed no inhibitory activity. For *Staph aureus* the methanolic sample with the highest concentration (20 mg/ml) showed maximum inhibitory activity with diameter of zone of inhibition (DIZ) (11.66 mm). For *E. Coli,* n-hexane extract showed maximum inhibitory effect with highest DIZ (9 mm). Ethyl acetate extract showed maximum inhibition against *Bacillus subtillus* having DIZ (12.33 mm). Against *S. typhi*. only methanolic extract showed an inhibitory effect at higher concentrations with DIZ (9 mm). Uddin and his coworkers [5] checked antibacterial activity of *V. grandiflorum* extracts in (Methanol, n-hexane, ethyl acetate, and chloroform). At 22 mg/ml DIZ the results were 20, 15, and 14 for Methanol, n-hexane, and ethyl acetate solvents, respectively [5, 18]. In another study Ganjewala and Gupta showed the highest antibacterial activity with methanolic extract of *V. grandiflorum* at concentrations of 50 mg/ml and 100 mg/ml with DIZ from 13-21 mm [19].

Table 2 shows the anti-fungal activity of *Viburnum grandiflorum*-Guch stem's extract against a fungus named *penicillium chrysogenum, Aspergillus flavus, Rhodotorula mucilaginosa*, and *Stachybotrys Chartarum.* Meconazole was used as anti-fungal for positive control, and water was placed as negative control. Only dimethyl sulf-oxide extract exhibited an inhibitory effect against these fungal species, while methanol, n-hexane, and ethyl acetate extract showed no inhibition. Dimethyl sulf-oxide extract exhibited maximum inhibitory effect against this fungus having DIZ 12.66 mm (*penicillium chrysogenum*), 11.66 (*Aspergillus flavus*), 12 mm

Table 2. Antifungal sensitivity activity of *Viburnum grandiflorum*-Guch stem's extract.

Microorganism	Concentrations of extracts used (mg/ml) / Zones of inhibition (mm)																					
	Methanol					Hexane					Ethyle acetate					DMSO						
	5	10	15	20	A	W	5	10	15	20	A	W	5	10	15	20	A	W	5	10	15	20	A	W
Penicillium chrysogenum	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	6	10.3	12	12.6	20	NZ
Aspergillus flavus	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	NZ	9.3	11.6	NZ	20	NZ
Rhodotorula mucilaginosa	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	6	8.6	10.6	12	20	NZ
Stachybotrys chartarum	NZ	NZ	10	11.3	20	NZ	NZ	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ	NZ	20	NZ	5.6	9.6	12.6	15.6	25	NZ

NZ: no zone, A: standard antibiotic, W: Water

(*Rhodotorula mucilaginosa*), and 15.66 mm (*Stachybotrys Chartarum*). Methaolic extract also showed an inhibitory effect on *Stachybotrys Chartarum* with diameter of zone of inhibition (11.33 mm).

The results are also in line with Alam et al. [13], who checked the antifungal activity of *V. grandiflorum* stem's ethanolic extracts in methanol, n-hexane, ethyl acetate, and chloroform against *Aspergillus flavus,* and no activity was found for these solvents. In another study they also found antifungal activity of *V grandiflorum* ethanolic extracts of its oil in DMSO against *Aspergillus flavus* [20]. TLC was also performed by using different solvent systems. Ethyl acetate, ethanol, n-hexane, dichloromethane, and ethyl acetate were used at 100% concentrations each or 50% concentrations. The total number of components detected from the stem's extract is: 7, 3, 1, 5, 6, 4, and 6, respectively. Maximum components were detected by the ethyl acetate solvent system.

The free radical scavenging activity of extracts of *V grandiflorum*-Guch stems was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), and absorbance was measured at 517 nm. Among the tested extracts the aqueous extract displayed the most potent antioxidant activity (58%) at 400 mg/ml concentration, followed by ethyl acetate (58%) at 600 mg/ml and methanol (33%) at 250 mg/ml. The lower concentration of extracts showed lower activity in different solvents, e.g., the lowest activity (14.10%) was shown in ethyl acetate extract followed by methanolic extract (24.24%) and aqueous extract (25.84%) at 10 mg/ml concentrations. The higher concentration of the extracts showed higher percent activity, which may be due to using stem extract. The antioxidant potential of Viburnum grandiflorum showed better scavenging activity (IC50 = 255 µg/ml) in aqueous extract followed by ethyl acetate extract (IC50 = 322 µg/ml) and methanolic extract (IC50 = 742 µg/ml).

Medicinal plants are still widely used for conservation of biodiversity, traditional culture, drug development, and health care. Natural products obtained from different plant species possess a variety of biologically active compounds and are subsequently being tested for pharmacological activities. An ethnobotanical study of Viburnum Grandiflorum has been attempted to highlight its medicinal significance. It has been tested and shown to have antibacterial, antifungal, antimicrobial, antiseptic, anti-inflammatory, antioxidant, anti-malarial, and anti-rheumatic activities [22-29].

Conclusions

All phytochemicals were found to be present in all extracts. Methanol, n-hexane, and ethyl acetate extract showed no inhibition for the tested three fungi except *Stachybotrys chartarum*. Dimethyl sulfoxide extracts showed maximum inhibition followed by methanolic extract for *Stachybotrys chartarum*. Through TLC on stem extract, maximum components were detected by the ethyl acetate solvent system. Antioxidant activity of stem extract of *Viburnum grandiflorum* was evaluated by 1, 1-diphenyl-2-picry l-hydrazyl (DPPH). This plant was selected due to the presence of different compounds of medicinal value and to determine the antimicrobial and antioxidant capacities of these compounds.

We have concluded that the aerial parts (stem) of *V. grandiflorum* can be safely used as antibacterial and antifungal drugs, and the recorded data of the study proves its importance as a potential antimicrobial drug.

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