

Short Communication

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

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Received: 18 July 2017

Accepted: 3 September 2017

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 subtilus*, 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

Introduction

Caprifoliaceae 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 4°C.

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 121°C for 15 minutes, cooled to 45-50°C, 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 37°C. 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:

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0\} \times 100\}$$

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	
<i>Staphylococcus aureus</i>	NZ	6	9.8	11.6	26	NZ	NZ	6.33	8.33	9.33	26	NZ	NZ	NZ	NZ	6.3	8	26	NZ
<i>Escherichia Coli</i>	NZ	NZ	7	8	26	NZ	5	7.33	8.33	9	26	NZ	NZ	NZ	NZ	7.33	8	26	NZ
<i>Bacillus Subtilus</i>	NZ	6	7.3	8.3	25	NZ	NZ	5	6.33	7.33	25	NZ	6	10.2	12	12.3	25	NZ	
<i>Salmonella entererous</i>	NZ	NZ	8	9	25	NZ	NZ	NZ	NZ	NZ	25	NZ	NZ	NZ	NZ	NZ	NZ	25	NZ

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

(*Rhodotorula mucilaginosa*), and 15.66 mm (*Stachybotrys Chartarum*). Methanolic 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-picryl-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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