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Phytochemical profile and biological investigation of *Viburnum grandiflorum*

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ABSTRACT

The current research work, the methanolic stem extract of *V. grandiflorum* were investigated for phytochemical analysis, antibacterial, antioxidant, α-amylase inhibitory, and antiglycation activities. The phytochemical tests performed to represent the presence of a variety of phytochemicals. The biological investigation of the methanolic crude extract showed significant biological activities. The results obtained revealed that the maximum antibacterial inhibition pragmatic was 16.6 mm against *P. aureginosa* and minimum inhibition was 14.83mm against *B. cereus*. The maximum antioxidant activity was 2.42% found at concentration of 1mg/ml while the minimum activity recorded was 0.234% at concentration of 0.0075 mg/ml. The maximum α-Amylase inhibitory potential was 1.92 at concentration of 1mg/ml while minimum inhibition of 0.067 was observed at 0.0075mg/ml concentration. The maximum antiglycation activity of 2.88 was recorded at concentration of 1µg/ml while minimum antiglycation activity of 2.02 was founded at concentration of 0.0075µg/ml. The results obtained revealed the medicinal importance of the plant and will help the researchers to exploit the phytochemicals for antibacterial, antioxidant, α-amylase inhibitory and antiglycation activities.

Keywords: *Viburnum; phytochemicals; antibacterial; antioxidant; α-amylase; antiglycation.*

1. INTRODUCTION

The genus *Viburnum* possess a wide variety of important biological activities. It contains about 200 species, distributed in the temperate and subtropical regions of Asia, North America and Malaysia, only 21 of which have been studied phytochemically. The six species *V. opulus*, *V. tinus*, *V. cotinifolium* D. don,*V. mullaha* D. don, *V. foetns* Dene and *V. cylindricum* are found in the Northern Pakistan and in the State of Jammu and Kashmir [1, 2]. These species have use in the folk medicine system for their diuretic, antispasmodic and sedative properties mainly on uterine excitability [3-5]. The various classes of compounds such as iridoid glycosids, triterenoids, neovibsanin, flavon glucoside, triterpene, saponin, furcatin, norisoprenoids, phenolic compounds, lupane triterpenes and new vibsane diterpenes etc. has been reported from *Viburnum* genus[6-9].

Keeping in view the above-mentioned pharmacological importance of genus *Viburnum*, its species *V. grandiflorum* was selected to investigate its phytochemical profile, antibacterial, antioxidant, α-amylase inhibitory and antiglycation activities to further explore its hidden medicinal potential.

2. MATERIALS AND METHODS

2.1. Plant material.

V. grandiflorum stems were collected from Tehsil Trarkhal, District Sudhnothi. AJ&K, Pakistan. The plant was authenticated by Department of Botany, Abdul Wali Khan, University, Mardan, Pakistan. The air-dried plant stems were crushed in a grinder. The powdered plant material was then used for extraction.

2.2. Extraction.

The powder material was soaked in methanol at room temperature for three weeks. The methanolic extract of stem part of *V. grandiflorum* was filtered. The filtrate was concentrated through rotary evaporator at 45°C. The crude methanolic extract of *V. grandiflorum* stem (50gram) was obtained. The methanolic crude extract was further tested for phytochemical and biological potency.

2.3. Phytochemical analysis.

Qualitative phytochemical analysis of the crude extract of *V. grandiflorum* was carried out in order to detect the constituents as described by [10-12].

Alkaloids.

In this test 0.2g of methanolic extract was added with 2% H2SO4 and heated for two minutes, filtered followed by pouring of few drops of Dragendorffs reagent. Orange red precipitates specify the occurrence of alkaloids.

Tannins.

In order to detect the presence of tannins, a small quantity of methanolic extract was mixed with water and heated on water bath and filtered. Few drops of ferric chloride were added to the filtrate. The appearance of dark green solution indicates the presence of tannins.

Anthraquinones.

To determine the presence of anthraquinones, about 0.5 g of methanolic extract was boiled with 10 % HCl for a few minutes on water bath. The sample was then filtered and cooled. To the filtrate equal volume of $CHCl_3$ was mixed besides adding few drops of 10% ammonia. Appearance of rose-pink color authenticates the occurrence of anthraquinones.

Glycosides.

In this phytochemical test, the methanolic extract was initially hydrolyzed with HCl followed by neutralization with NaOH solution. To the mixture few drops of Fehling solution A and B were mixed. Formation of red precipitates showed the existence of glycosides.

Reducing Sugars.

In this test methanolic extract was stirred with distilled water, filtered and boiled after the mixing of few drops of Fehling solution A and B. Formation of orange red precipitates confirms the presence of reducing sugars.

Saponins.

For the recognition of saponins, approximately 0.2 g of methanolic extract was mixed with 5ml of distilled water followed by heating to boil. Frothing (emergence of creamy small bubbles) exposed the occurrence of saponin.

Flavonoids.

The methanolic extract 0.2 g was dissolved in dilute NaOH and then to it, HCL was added. The appearance of a yellow solution that turns to colorless authenticates the existence of flavonoids.

***Phlobatanins*.**

The methanolic extract 0.5 g was dissolved in specified amount of distilled water and filtered. The filtrate was subjected to boil with 2% HCl solution. The appearance of red precipitate articulated the existence of phlobatanins.

Steroids.

In this test acetic anhydride (2 ml) was mixed to 0.5 g of the methanolic extract followed by adding 2 ml of H_2SO_4 .The color changed from violet to green or blue in some samples authenticate the presence of steroids.

Terpenoid.

To check the presence of terpenoids 0.2 g of methanolic extract was mixed one by one with a mixture of 2 ml of chloroform and conc. H_2SO_4 (3ml) to form a layer. The appearance of reddish-brown coloration at the interface was developed which specifies positive results for the existence of terpenoids.

2.4. Antibacterial activity.

In this biological evaluation, a total of five bacterial strains were chosen to be used. The bacterial strain used was classified as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella Pneumoniae, B.cereus* and *Staphylococcus aureus*. Through the method of agar well diffusion, in the presence of a cell suspension of roughly 1.5. 106 CFU/mL, arranged from Macfarland turbidity standard No. 0.5 [13], the anti-bacterial activity was tested. The shimadzu, UV-VIS spectrophotometer [14] was used to regulate the optical density to 0.1 at 600 nm, in order to standardize the suspension. On (8 mm thick) the Mueller Hinton agar (MHA) plate, holes with diameter of 6 mm were bored and filled with different concentration of the sample 50, 100, 150 and 200 µg/ml obtained by dissolution in DMSO with concentration 8 mg/1 ml and standard drug(s) were tested against different bacterial strains. For 24 hours, the plates after inoculation were incubated at temperature of 37°C. In order to determine the antibacterial activity, the zone of inhibition was calculated. The average diameter was measured by repeating the assay thrice. The standard antibiotic used in this bioassay for comparison was ciproflaxicine.

2.5. Antioxidant activity.

The antioxidant potential was determined according to the thiocyanate method [15]. The sample in 0.5ml methanol was combined with 5ml DMSO suspension (2.5mL, 0.02M, pH 7.0) and phosphate buffer (2 mL, 0. 2M, pH 7.0) in a test tube and located in dark at 37 OC to accelerate oxidation. The peroxide value was obtained by noting the absorbance at 750 nm with a spectrophotometer (Hitachi U-2000) after coloring with FeCl3 and thiocyanate at intervals during incubation.

2.6. α-amylase inhibitory assay.

Quantified amount of reducing sugar (maltose equivalent), which is liberated during the assay conditions was used for the estimation of α-amylase inhibition activity. The amount of maltose liberated during the reaction in units was used to represent the enzyme inhibition activity. A modified Dinitrosalicylic acid (DNS) procedure was used for the determination of maltose equivalent [16]. The experiment was initiated by pre-incubation of 1ml of the methanolic extract of *V. grandiflorum* for 30 min with α-amylase 1U/mL and then 1 ml (1% w/v) of starch solution was added. After that mixture was further incubated at 37°C for 10 min. At the end 1 mL of DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitro salicylic acid solution) was put in the mixture and then reaction was stopped. Meanwhile, the contents were heated in a boiling water bath for 5 min. Two more tests were performed, one blank test which was prepared without plant extract and the second which was prepared without enzyme amylase, being exchanged by equivalent quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, at pH 6.9 and 20oC). The calculated absorbance was 540 nm. With the help of standard graph, maltose equivalent was determined by the amount of reducing sugar which was eliminated from starch during the reaction. Acarbose acts as a positive control. To obtain the final concentration of 5mg/ml, 7mg/ml and 9mg/ml, the methanolic extract of *V. grandiflorum* was diluted in buffer. The anti-diabetic activity was calculated by the inhibition of α-amylase enzyme which was expressed as a percent inhibition and calculated by using the following equations.

$$\% \text{ reaction} = (\text{maltose}) \text{ test} / (\text{maltose}) \text{ control} \times 100$$

$$\% \text{ inhibition} = 100\% \text{ reaction}$$

2.7. Antiglycation activity.

Reasonable amount of test sample almost 60 µl of the methanolic extract of *V. grandiflorum* was prepared by mixing with DMSO and the mixture of the sample (20 µl BSA + 20 µl of glucose anhydrous and test sample 20 µl). The glycated control contained 20 µl glucose, 20µl sodium phosphate buffer and 20 µl BSA, while the blank control contained 20 µl BSA and 40 µl sodium phosphate buffer. The test sample placed in incubator using 96 well plates for almost 7 days at 37°C and then removed from incubator and cooled out at room temperature. After incubation 60 µl of 100% TCA was placed into each well followed by centrifugation (15000 rpm) for 4 min at 4°C. After centrifugation and agitation performed at 14000 rpm for 4 min, the supernatant was eliminated which was contained on glucose, inhibitor, interfering substance and AGE-BSA pellet that was dissolved in PBS. In this bioassay of AGEs Spectrofluorimeter RF-1500

(Shimadzu, Japan) was used to observe the assessment of fluorescence spectrum (ex. 370 nm) and change in fluorescence intensity (ex. 370 to 440 nm). Rutin was used as standard in this activity. With the help spectrofluorometer, the intensity of fluorescence at 370 nm excitations and emission at 440 nm was compared with each other. Percentage inhibition was calculated using the following formula:

Inhibition (%) = 100 – [OD (test) / OD (blank)] x 100

3. RESULTS

3.1. Phytochemical analysis.

The results obtained from the phytochemical analysis of the stem part of *V. grandiflorum* revealed the presence of all compounds except the alkaloids and phlobatannins **Table 1**.

Table 1. Phytochemical screening of the methanolic extract of part stem of *V. grandiflorum.*

Phytochemicals	Methanolic Extract
Alkaloids	-
Tannins	+
Anthraquinones	+
Glycosides	+
Reducing sugars	+
Saponins	+
Flavonoids	+
Phlobatannins	-
Steroids	+
Terpenoids	+

3.2. Antibacterial activity.

For the determination of anti-bacterial activity, the methanolic extract of stem of *V. grandiflorum* was tested in resistance against five strains of bacteria, i.e. *E. coli*, *P. aureginosa*, *K. pneumoniae*, *B. cereas* and *S. aureus.* The results obtained are described as inhibition zone (mm) in **Table 2**. From the data given in the table it is clear that the methanolic extract of stem of *V. grandiflorum* showed inhibition against the tested microorganisms. The maximum inhibition observed was 16.6 mm against *P. aeruginosa* and minimum inhibition was 14.83mm against *B. cereus.*

Table 2. Antibacterial activity of the extract of stem part of *V. grandiflorum.*

Microorganism	Zone of Inhibition(mm)	
	Ciproflaxicine (Drug)	Methanolic Extract
Escherichia coli	15.6	15.6
Pseudomonas aeruginosa	17.16	16.6
Klebsiella Pneumoniae	16.16	15.6
B. cereus	15.33	14.83
Staphylococcus aureus	15.5	15.83

3.3. Antioxidant Activity.

The effects of methanolic extract of stem of *V. grandiflorum* on the per-oxidation of DMSO are shown in **Fig 1**. The oxidative impending of DMSO was subdued by the methanolic extract compared with the control assay. The maximum anti-oxidant action was observed at concentration of 1mg/ml which was expressed as 2.42% while the minimum anti-oxidant activity was observed at concentration of 0.0075 which was 0.234% inhibition of DMSO- peroxidation. This indicates the presence of polyphenols which are the most plentiful group of compounds in the methanolic extract of *V. grandiflorum* and seem to be meticulous for the antioxidant potential.

3.4. α-Amylase inhibitory activity.

In the current study methanolic extract *V. grandiflorum* was investigated to check its potential for the reticence of α-amylase enzyme. The methanolic extract fractions serial dilution of stem of the selected plant was tested for the inhibition of α-amylase activity **Fig 2**.

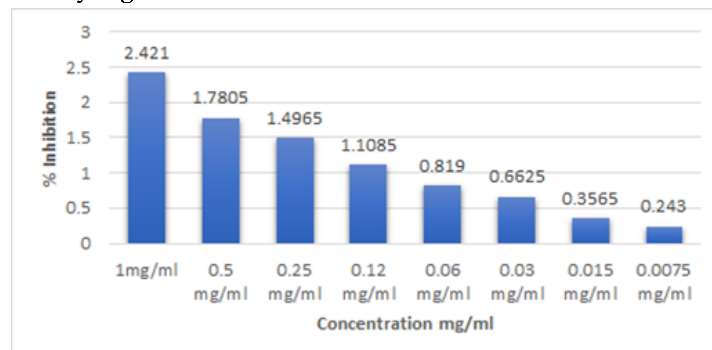

Figure 1. Antioxidant activity methanolic extract of stem part of *V. grandiflorum.*

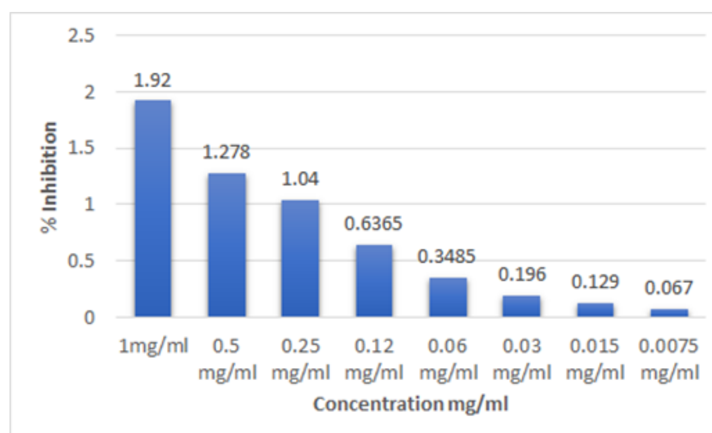

Figure 2. α-Amylase inhibitory potential of methanolic extracts from the stem part of *V. grandiflorum.*

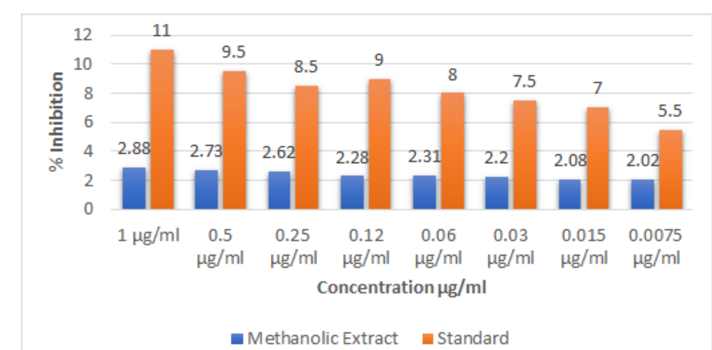

Figure 3. Antiglycation activity of methanolic extract of stem part of *V. grandiflorum.*

From the results, it is clear that maximum inhibition of 1.92 shown at a concentration of 1 mg/ml while minimum inhibition of 0.067 at 0.0075 mg/ml concentration. From the experimental data obtained it can be concluded that extract of *V. grandiflorum* has greater potential for the reduction of digestion-rate and carbohydrate's absorption and thereby play an important role in the efficient management of diabetes.

3.5. Antiglycation Activity.

The methanolic extract of *V. grandiflorum* was tested for anti-glycation activity based on serial dilution method with

concentration ranges from 1µg/ml to 0.0075 µg/ml. The results obtained are given in the **Fig 3**. From the results obtained it is clear that maximum anti-glycation activity observed was 2.88 at concentration of 1µg/ml while minimum anti-glycation activity was 2.02 at concentration of 0.0075 µg/ml.

4. CONCLUSIONS

In the current study, the medicinal plant *V. grandiflorum* was investigated for phytochemical profile, antibacterial, antioxidant, α-amylase and antiglycation activity. The biological investigation of the methanolic crude extract of the selected plant showed significant activity. The results obtained exhibit that this plant is very important from the medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents having antibacterial, antioxidant, α-amylase inhibitory and antiglycation activities.

5. REFERENCES

1. Nasir, E.; Ali, S.I.; Stewert, R.R. *Flora of West Pakistan*. Fakhri Printing Press, Karachi: Pakistan, 1972; pp. 695-696.
2. Parker, R.N. *A Forest Flora for the Punjab with Hazara and Dehli.* Ed. 3rd, Govt. Printing Press, West Pakistan 1956; pp. 265-275.
3. Nicholson, J.A.; Darby, T.D.; Jarboe, C.H. Viopudial, a Hypotensive and Smooth Muscle Antispasmodic from Viburnum opulus. *Proceedings of the Society for Experimental Biology and Medicine* **1972,** *140*, 457, https://doi.org/10.3181/00379727-140-36479.
4. Stanic, G.; Petricic, J. *J. Farm. Glass* **1979**, *35,* 231.
5. Tomassini L, Cometa M F, Foddai S, et al. Iridoid glucosides from Viburnum tinus[J]. Phytochemistry, 1995, 38(2): 423-425. https://doi.org/10.1016/0031-9422(94)00618-4.
6. Fukuyama, Y.; Minoshima, Y.; Kishimoto, Y.; Chen, S.I.; Takahashi, H.; Esumi, T. Iridoid Glucosides, and p-Coumaroyl Iridoids from Viburnum luzonicum and their cytotoxicity. *J. Nat. Prod.* **2004**, *67*, 1833-1838, https://doi.org/10.1021/np040138l.
7. Calis, I.; Yuruker, A.; Rugger, H.; Wright, D.A.; Sticher, O. Lantanoside, a monocyclic C. *Phytochem.* **1995**, *38*, 163-165, https://doi.org/10.1016/0031-9422(94)00585-H.
8. Parveen, M.; Khan, S.M.; Shafiullah, M.I. Luteolin 3'-xylosyl (1--> 2) glucoside from Viburnum grandifolium. *Phytochem.* **1998**, *49*, 2535-2538, https://doi.org/10.1016/S0031-9422(98)00184-8.
9. Kuba, M.; Minami, H.; Hayashi, E.; Kodama, M.; Kawazu, K.; Fukuyama, Y.Y. Neovibsanin C, a macrocyclic peroxide-containing neovibsane-type diterpene from Viburnum awabuki. *Tetrahedron. Let.* **1999**, *40*, 6261-6265, https://doi.org/10.1016/S0040-4039(99)01199-5.
10. Sofora, A. *Medicinal plants and traditional medicine in Africa.* John Wiley and son Ltd. 1993.
11. Evans, W.C. *Pharmacology.* 11th Edtn. Brailliar Tiridel and Macmillian Publishers, London 2009.
12. Harborne, J. *Phytochemical methods, a guide to modern techniques of plant analysis*. Chapman. London 1973.
13. Jan, A.K.; Shah, M.R.; Anis, I.; Marwat, I.K. In vitro antifungal and antibacterial activities of extracts of Galium tricornutum subsp. longipedunculatum. *J. Enzyme. Inhibition. Med. Chem***.** **2009**, *24*, 192-196, https://doi.org/10.1080/14756360802051255.
14. .Nisar, M.; Tariq, S.A.; Marwat, I.K.;Shah, M.R.; Khan, I.A. Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on Indigofera gerardiana. *J. Enzyme. Inhibition. Med. Chem.* **2009**, *24*, 224-229, https://doi.org/10.1080/14756360802051313.
15. .Yen, G.C.; Chen, H.Y. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimut agenicity. *J. Agric. Food Chem.* **1995**, *43*, 27-32, https://doi.org/10.1021/jf00049a007.
16. Bhutkar, M.A.; Bhise, S.B. In vitro assay of alpha amylase inhibitory activity of some indigenous plants. *Int. J. Chem. Sci.* **2012**, *10*, 457-462, http://dx.doi.org/10.31031/MAPP.2018.01.000518.

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