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Preliminary Phytochemical Profile and Antibacterial Evaluation of *Viburnum grandiflorum* Wall

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Abstract: *Viburnum grandiflorum* Wall. ex DC., is a plant of Himalaya and Hazara region of Pakistan which was screened for bioactive secondary metabolite and screen for biological activity. Preliminary phytochemical profiling indicated the presence of different classes of secondary plant metabolites such as alkaloids, terpenoids, saponins, anthraquinone. These secondary metabolites vary in types in different parts of the plant, which also depends on the type of the solvent extraction. Ethyl acetate and methanolic fraction containing alkaloids and flavonoids while steroids, terpenoids, anthraquinones and saponins were present in *n*-hexane, chloroform, ethyl acetate and methanolic fraction respectively. *n*-Hexane, chloroform, ethyl acetate and methanol extracts of *V. grandiflorum* were most active, showing activity against three selected bacterial strains and thus showed highest inhibitory zone (20 mm) at tested concentration (22 mg/ml) which could be attributed to the presence these phytochemicals.

Key words: Phytochemical screening · Antimicrobial activity

INTRODUCTION

Viburnum, a genus of the plant family Adoxaceae (formerly Capripoliaceae), consist of more than 230 species. Mostly distributed in the temperate or subtropical zones from South America to South East Asia and the majority of them are endemic [1]. Six species of the genus *Viburnum* (*V. cotonifolium, V. tinus, V. cylindricum*, *V. opulus, V. mullaha* and *V. grandiflorum*) are present in Pakistan [2]. The genus *Viburnum* is well known in folk medicine for their Spasmolytic, sedative and anti-asthmatic properties [3]. *V. prunifolium* are specifically used for menstrual cramps, anti-abortive agent and for prevention of postpartum bleeding [4]. *Viburnum* species are also used in treatment of different diseases, such as diarrhea, rheumatoid arthritis and tumefaction [5]. Anti-diabetic, anti-oxidant and anti-bacterial, anti-cancer, cytotoxic, urine relaxant and antinociceptive activity, uterine excitability, molluscicidal and diuretic has been reported from *Viburnum* [6-14]. The triterpenoids, iridoids glycosides, flavones glucosides neovibsanin, triterpene saponins, furcatins, norisoprenoids, phenolic compounds, vibsane diterpenes and lupane triterpenes isolated from *Viburnum* genus [15-23]. Researchers also revealed that secondary metabolites are vary from plant to plant and even in different parts (leaf, stem, root) of the same plant species as well as at various growth stages i.e., vegetative and reproductive [24, 25].

MATERIALS AND METHODS

Plant Materials: *Viburnum grandiflorum* Wall ex DC., stem and roots were collected from Tandyani district Hazara, Khyber Pakhtunkhwa province of Pakistan in the month of July 2009. Plant was identified by an eminent taxonomist of Botany Department of Hazara University and a specimen voucher was preserved in the University Herbarium.

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Extraction: Shade dried plant materials (8.5 kg) were grinded to powder mechanically with heavy duty local grinder. Soaked with ethanol for three weeks and subjected to extraction until exhaustion of plant materials. The combined extract was concentrated under reduced pressure using rotary evaporator at low temperature as result, brownish syrupy crude residue was obtained which was suspended in distilled water and successively partitioned with distilled *n*-hexane, chloroform, ethyl acetate and methanol solvents.

Phytochemical Screening: Standard chemical tests were performed for qualitative analysis of different fractions (n- hexane, chloroform, ethyl acetate and methanol) of *V. grandiflorum* [26, 32]. The results were recorded in tabular form.

Micro Organisms Collection and Maintenance: The selected microorganisms (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis*) are used which were collected from stock culture of PCMD, HEJ research centre, University of Karachi, Karachi. The organisms were kept on agar in Muller-Hinton agar in refrigerator at 4°C prior to subculture.

Antibacterial Activity Test: Antibacterial activity was carried out on the modified agar well diffusion method as discussed earlier [26, 28, 29] to study antibacterial activity of *V. grandiflorum* seed fractions. The medium used was Muller-Hinton agar.

The cultures used in this study were prepared in triplicates. It was then incubated at a temperature of 37°C for 24 to 72 hours. The broth culture (0.6 ml) of the test organism was taken in a sterile petri dish to which 20 ml of sterile molten MHA was added. Well were bored in the medium and 0.2 ml of the extracts (*n*-hexane, chloroform, ethyl acetate, methanol) were potted.

Streptomycin (2µgm) used as antibacterial standard. Inoculation was done for 1hr to make possible the diffusion of the antibacterial agent into the medium. The inoculation plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition of bacterial growth was measured in the plates in millimeter.

RESULTS AND DISCUSSION

The weight percentage yield of the crude fractions; *n*-hexane, chloroform, ethyl acetate and methanol of *V. Grandiflorum* stem and roots are presented in Table 1. The phytochemical screening of *n*-hexane, chloroform, ethyl acetate and methanol crude extract of *V. grandiflorum* stem is shown in Table 2. Chloroform and ethyl acetate fractions of the stem extract contain greater proportion of the component compounds while the ethyl acetate and methanol fractions of the roots extract have greater quantity of component compounds as shown in the Table 1.

The preliminary phytochemical screening results of *V. grandiflorum* stem extract showed the presence of bioactive secondary metabolite constituents such as terpenoids, steroids, anthraquinone, flavonoids, lignin's and saponins (Table 2).

Table 1: Extractive values of the Ariel part of *V. grandiflorum*

Parts	Hexane	Chloroform	Ethyl acetate	Methanol	Water fraction
Stem	24.5%	20.8%	13.3%	9.6%	31.5%
Roots	1.4%	11.5%	20%	23%	44.1%

Table 2: Phytochemical tests of *V. grandiflorum* stem fractions

Phytochemical constituents	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	-	-
Steroids	+	+	-	-
Terpenoids	-	+	+	+
Anthraquinones	-	+	+	-
Saponins	-	-	+	+
Reducing sugars	-	-	-	-
Glycoside	+	+	+	+
Phlobatanins	-	-	-	-
Flavonoids	-	-	-	-

Key words: (+) present, (-) absent

Table 3: Phytochemical tests of *V. grandiflorum* roots fractions

Phytochemical constituents	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	+	-
Steroids	+	+	-	-
Terpenoids	-	+	+	+
Anthraquinones	-	+	+	+
Tannins	-	+	+	+
Saponins	-	+	+	+
Reducing sugars	-	-	-	-
Glycoside	+	+	+	+
Phlobatanins	-	-	-	-
Flavonoids	-	-	+	+

Key words: (+) present, (-) absent

Table 4: Antimicrobial sensitivity activities of *V. grandiflorum* stem fractions

Microorganism	Gram	AL1	AL2	AL3	AL4	AL5
Staphylococcus aureus	+	15	14	14	20	28
Staphylococcus epidermidis	+	NA	10	12	NA	30
Escherichia coli	_	15	16	14	14	32
Klebsiella pneumonia	_	14	8	NA	20	28
Bacillus subtilis	+	12	13	20	14	26

Key words: AL1=*n*-Hexane, AL2= Chloroform, AL3=Ethyl acetate, AL4= Methanol, AL5= Streptomycin,
NA= not active

Table 5: Antimicrobial sensitivity activity of *V. grandiflorum* roots fractions

Microorganism	Gram	M1	M2	M3	M4	M5
Staphylococcus aureus	+	NA	12	14	10	28
Staphylococcus epidermidis	+	12	10	NA	12	30
Escherichia coli	_	12	12	16	13	32
Klebsiella pneumonia	_	NA	8	NA	8	28
Bacillus subtilis	+	14	12	NA	14	26

Key words: M1=*n*-Hexane, M2= Chloroform, M3=Ethyl acetate, M4= Methanol, M5= Streptomycin,
NA= not active

The phytochemical screening results (Table 3) showed that *V. grandiflorum* roots extract contain secondary metabolite like alkaloids, flavonoids, saponins, terpenoids, anthraquinone, glycosides and steroids. All these components possess high biological activity [6-14].

The biological activity of these fractions confirmed through antibacterial bioassay. The results revealed highest activity against certain bacterial strains like *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis* which clearly indicates that *V. grandiflorum* extracts/fractions possess chemical constituents having anti-bacterial properties. Present microbial study of *V. grandiflorum* extracts demonstrated that folk's medicine can be used as an effective and modern medicine to combat pathogenic microorganisms. More work will be needed to bioassay directed isolation to isolate the pure antimicrobial chemical constituents and standardize new antimicrobial drugs.

The plant extracts (stem and roots) were subjected to antibacterial activity for exploring its bioactive chemical constituents. The wells-diffusion assay was used for the investigation of the antibacterial activity results of *V. grandiflorum* fractions against selected five microorganisms. The plant extract of *V. grandiflorum* showed significant reduction in bacterial growth in term of zone of inhibition, indicating that plant extract fractions exhibited significant antibacterial activity against selected microorganisms. The zone of inhibition was measured, recorded and presented in Tabulated form (Table 4 and 5). The different fractions of the plant showed significant activity against the selected bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis.* All fractions isolated from the stem extract showed significant activity against the selected bacteria, while roots extract fractions were found less active than stem extracts against these selected microorganisms. The chloroform fractions isolated from both stem and

roots extract exhibited inhibitory effect against all selected microorganisms with zone of inhibition ranging from 8 to 16 mm. The ethyl acetate and methanol fractions exhibited significant activity with highest zone of inhibition 20 mm. However, the ethyl acetate fraction of both roots and stem extract is non active against *Klebsiella pneumonia, Staphylococcus epidermidis* and *Bacillus subtilis* bacteria while methanol and *n*-hexane fractions showed no activity against *Staphylococcus epidermidis* and effective against microorganisms; *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis*. The recorded antibacterial bioassay data proves its importance as potential antimicrobial drug.

REFERENCES

1. Lobstein, A., G. Haan-Archipoff, J. Englert, J.G. Kuhry and R. Anton, 1999. Chemotaxonomical investigation in the genus Viburnum. Phytochemistry, 50: 1175-1180.
2. Ali, S.I., E. Nasir and R. Akhter, 1986. Flora of West Pakistan, pp: 5-11.
3. Jarboe, C.H., C.M. Schmidt, J.A. Nicholson and K.A. Zirvi, 1969. Uterine relaxant properties of Viburnum, Nature, 19: 837.
4. Bernal, H.Y. and J.E. Correa, 1990. Especies Vegetales Promisorias del Convenio Andres Bello. Editorial Guadalupe, Bogota DC, pp: 144.
5. Flora Yunnanica' Tomus, 1991. Institutum Botanicum kunmingense Academiae Edita, Science Press, Beijing,
6. Iwai, K., M.I. Kim, A. Onodera and H. Matsue, 2006. α-Glucosidase Inhibitory and Antihyperglycemic Effects of polyphenols in the fruit of *Viburnum* dilatatum Thunb. Journal of Agricultural Food Chemistry, 54: 4588-4592.
7. Chavolleau, A.S., A. Debal and E. Ucciani, 1992. Rev. Fr. Corps Garz, 39: 1-2.
8. Testsuo, I., Y. Sigetoshi, H. Tsunao, O. Tsutomu and K. Mujo, 1992. Gomojosides, labdane diterpenoids from *Viburnum* Suspensum Phytochemistry, 31: 1311-1315.
9. Fukuyama, Y., Y. Minoshima, Y. Kishimoto, I.S. Chen, H. Takahashi and T. Esumi, 2004. Iridoid Glucosides and *p*-Coumaroyl Iridoids from *Viburnum* l uzonicum and their cytotoxicity. Journal of Natural Products, 67: 1833-1838.
10. Fukuyama, Y., Y. Minoshima, Y. kishimoto, I.S. Chen, H. Takahoshi and T. Esumi, 2004. Iridoid glucosides and *p*-coumaroyl iridoids from *Viburnum luzonicum* and their cytotoxicity. J. Nat. Prod., 67: 1833-1838.
11. Duh, Y.C., A.H. Ali, E. Gamal and K.S. Wang, 2003. Vibsanin O, a novel diterpenoid from *Viburnum awabuki*. Tetrahedron Letter, 44: 9321-9322.
12. Calle, J., M. Toscano, R. Pinzon, J. Baquero and E. Bautista, 1999. Antinociceptive and uterine relaxant activities of *Viburnum toronis* alive (Caprifoliaceae), Journal of Ethnopharmacology, 66: 71-73.
13. Cometa, M.F., G. Nazzanti and L. Tomassini, 1998. Sedative and spasmolytic effects of *Viburnum tinus* L. and its major pure compounds, Phytotheraphy Research, 12: S89-S91.
14. Ibrahim, A.M., H.A. Shoeb, L.A. Rifahy, A.M. Saad, M.A. Mohamed and A.M. Abdou, 1994. Egypt. Medicinal Journal, 11: 7-20.
15. Stanic, G. and J. Petricic, 1979. Two Iridoids from *Viburnum lantana*, Journal of Farmacology Glossary, 35: 231.
16. Fukuyama, Y., H. Minami, H. Fujii and M. Tajima, 2002. Triterpenoids from Viburnum suspensum Phytochemistry, 60: 765-768.
17. Calis, I., A. Yuruker, H. Rugger, D.A. Wright, O. Sticher, 1995. Lantanoside, a monocyclic C_{10} Iridoid glucoside from Viburnum lantana, Phytochemistry, 38: 163-165.
18. Parveen, M., K. Sohrab and M. Ilyas, 1998. Luteolin 3'-xylosyl (1--> 2) glucoside from *Viburnum grandiflorum*. Phytochemistry, 49: 2535-2538.
19. Kuba, M., H. Minami, E. Hayashi, M. Kodama, K. Kawazu and Y.Y. Fukuyama, 1999. Neovibsanin C, a macrocyclic peroxide-containing neovibsan-type diterpenoid from *Viburnum awabuki*. Tetrahedron. Letter, 40: 6261-6265.
20. Machida, K. and M. Kikuchi, 1997. Viburnols: Six novel triterpenoids from Viburnum Dilatatum. Tetrahedron Letter, 38: 517-520.
21. Ahn, O.Y., M. Mizutani, H. Saino and K. Sakata, 2004. Furcatin hydrolase from *Viburnum furcatum* Blume is a novel disaccharide-specific Acuminosidase in glycosyl hydrolase Family 1*. Journal of Biological Chemistry, 279: 23405-23414.
22. Machida, K. and M. Kikuchi, 1996. Norisoprenoids from *Viburnum dilatatum*. Phytochemistry, 41: 1333-1336.
23. Zhu, X., X. Dong, Y. Wang, P. Ju and S. Luo, 2005. Phenolic compounds from *Viburnum cylindricum*. Helvetica Chimica Acta, 88: 339-342.
24. Kayani, S.A., A. Masood, A.K.K. Achakzai and S. Anbreen, 2007. Distribution of secondary metabolites in plants of Quetta-Balochistan. Pak. J. Bot., 39: 1173-1179.

25. Achakzai, A.K.K., P. Achakzai, A. Masood, S.A. Kayani and R.B. Tareen, 2009. Response of plant parts and age on the distribution of secondary metabolites of plants found in Quetta. Pak. J. Bot., 41: 2129-2135.
26. Shen, Y.C., C.L. Lin, S.C. Chien, A.T. Khalil, C.L. Ko and C.H. Wang, 2004. Vibsane diterpenoids from the leaves and flowers of *Viburnum odoratissimum*. J. Nat. Prod., 67: 74-77.
27. Shen, C.Y., S.V.C. Prakash, T.L. Wang, T.L. Chein and C.M. Hung, 2002. New vibsane diterpenes and lupane triterpenes from Viburnum odoratissimum. J. Nat. Prod., 65: 1052-1055.
28. Uddin, G., A. Rauf, M. Qaisar, T.U. Rehman, A. Latif and M. Ali, 2011. Preliminary phytochemical screening and antimicrobial activity of *Hedera helix* L. Middle-East Journal of Scientific Research, 8: 198-202.
29. Uddin, G., A. Rauf, B.S. Siddiqi and S.Q. Shah, 2011. Preliminary comparative phytochemical screening of *Diospyros lotus* Stewart, Middle-East Journal of Scientific Research, 10: 78-81.
30. Uddin, G., W. Ullah, A. Rauf, B.S. Siddiqui, T.U. Rehman, S. Azam and M. Qaisar, 2011. Phytochemical screening and antimicrobial activity of *Cornus macrophylla* Wall, ex Roxb. Middle-East Journal of Scientific Research, 9(4): 516-519.
31. Uddin, G., A. Rauf, M. Arfan, M. Ali, M. Qaisar, M. Saadiq and M. Atif, 2012. Preliminary phytochemical screening and antioxidant activity of *Bergenia caliata*. Middle-East Journal of Scientific Research, 11: 1140-1142.
32. Usman, R., A. Khan and A. Rauf, 2012. Preliminary comparative phytochemical screening and biological evaluation of *Desmodium elegans*, Middle-East Journal of Scientific Research, 11: 1591-1594.