Full Length Research Paper

Evaluation of *Viburnum foetens* for anticancer and antibacterial potential and phytochemical analysis

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**Viburnum foetens** is an ethnobotanically important plant species traditionally used as purgative and also have sedative properties. Methanol extract from leaf explants was used to determine cytotoxic and antibacterial potential of this potent shrub. It was observed that cytotoxicity against MCF-7 cell lines gradually increases according to concentration of extract used. Further, the methanol crude extract was fractionated on polarity basis and each fraction was again tested for cytotoxic potential. Methanol fraction showed 83% inhibition that was highest from all other fractions. The crude methanol extract showed mild activity ranging from 10.30 to 12.00 mm zones of inhibition against bacterial strains tested by agar well diffusion method. Phytochemical analysis reveals that methanol fraction contain flavonoids, coumarins and tannins only, while crude extract include other phytochemicals along with these. It can be suggested that *V. foetens* contains some anticancerous compounds to be isolated and can be used as drug.

**Key words:** Anticancer, cytotoxic, MCF-7 cell line, MTT assay, phytochemical, *Viburnum foetens*.

**INTRODUCTION**

Plants have been used for medicinal purposes 60,000 years ago (Solecki and Shanidar, 1975). According to an analysis by World Health Organization, nearly 80% of the world population depends on herbal medicines for their health care problems (Farnsworth et al., 1985). The medicinal use of plants is actually due to the presence of active components that are effective for human body in many ways (Akinmoladun et al., 2007). Flavonoids, alkaloids, essential oils, tannins, terpenoids, saponins and phenolic compounds are some of the constituents responsible for bioactivity of plants (Edeoga et al., 2005; Tan et al., 2006). Synthesis of such compounds is one of the mechanisms responsible for the ability of plants to resist themselves from predators as well as to destroy pathogenic microorganisms and unwanted cells (Mans et al., 2000).

Among the medicinal plants, about one thousand plant species were found to have anticancer potential out of 250,000 plant species existing on earth (Mukherjee et al., 2001). Likewise, plant extracts exhibiting antimicrobial activity are a good source of antimicrobial compounds and hence antibiotic in nature (Cowann, 1999). Bioactivity guided isolation technique is the key to the discovery of some important anticancer agents like paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminata* (Kinghorn, 1994).

Genus *Viburnum* belonging to family Adoxaceae consists of about 200 species distributed from South America to South East Asia (Lobstein et al., 1999). Traditionally *Viburnum* species have been used in medicines due to their diuretic, antispasmodic and sedative effects (Cometa et al., 1998). Flavonoids, biflavonoids and coumarins are reported in different *Viburnum* species (Glasby, 1991; Plouvier, 1992). Hepatoprotective, antioxidant, antinoci-

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**Abbreviations:** DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
ceptive, anti inflammatory and gastroduodenal-protective activities have been studied in genus *Viburnum* (Kim et al., 2003; Mohamed et al., 2005; Yilmaz et al., 2007; Zayachkivska et al., 2006).

*Viburnum foetens* is a large deciduous shrub. It is widely distributed in Himalaya at an altitude between 1500 -3000 m from Swat eastward to Bhutan, South Tibet (Flora of Pakistan). Fruit of plant is edible and leaves have foetid aroma (Hedrick, 1972; Tanaka, 1976). Ethnobotanical survey shows that plant has been used traditionally as purgative and has sedative properties. Branches have been used by local people as tooth brush (miswaak) for cleaning teeth (Qureshi et al., 2009). Antidiabetic activity of the *V. foetens* has been studied (Hussain et al., 2003).

To the best of our knowledge, no other biological activity as well as phytochemical investigation of this plant has been done so far. The present investigation aims to do the preliminary phytochemical analysis and to evaluate potential of plant extract against breast cancer cell line as well as against gram negative and gram positive bacterial strains.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plant material was collected from northern areas of Pakistan (Ayubia NWFP Pakistan). Plant was identified by a Taxonomist, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

Drying and extraction

Plant material was thoroughly washed and dried under shade. Dried material was ground to fine powder. Cold maceration technique was used for extraction. Powdered plant material (1.5 kg) was dipped in methanol (200 ml) and kept at room temperature. After 7 days, the extract was filtered through Whatman filter paper No. 1 under vacuum. The residue was again dipped in methanol for seven days and filtered thereafter. The filtrate was combined and the methanol was evaporated under vacuum using rotary evaporator at 45°C. The dried extract was stored at 4°C until further analysis.

Fractionation

Four organic and one aqueous fraction were prepared. Fractionation of crude dry extract was carried out by suspending 250 g of extract in 100 ml water and then partitioned with different organic solvents (hexane, chloroform, ethyl acetate and methanol) in order of increasing polarity by using separating funnel (Chiu et al., 2006).

Cell culture

Human breast adenocarcinoma MCF-7 cell line was donated by Portsmouth University Cell Culture Laboratory, UK. The cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with FBS (fetal bovine serum), penicillin (10000 units), streptomycin (10 mg/ml) and L-glutamine (200 mM) at 37°C under 5% CO₂ and relative humidity 95%.

Dilution of extracts

Crude extract and fractions were weighed as needed. Crude/ fractionated extracts were dissolved in dimethyl sulfoxide (DMSO) at concentration of 2 mg/ml separately. Required dilutions in µg/ml were made under sterile conditions by adding calculated amounts of DMEM.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Standard MTT assay was used for evaluation of cell viability (Son et al., 2003). In 96 well plates, MCF-7 cells were seeded at the concentration of 5000 cells/well in 100 µl medium (RPMI 1640). Cells were allowed to attach overnight and then various concentrations of the crude extracts and fractions were added to wells. After 24 h incubation, 10 μl of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. After 4 h incubation, 100 μl of DMSO solution was added to each well to solubilize the MTT crystals. The plates were incubated overnight at 37°C, 5% CO₂ and relative humidity 95%. The plates were read for optical density at 570 nm, using a plate reader.

Antibacterial activity

Crude dry extract with a concentration of 20 mg/ml was tested for antibacterial activity by using agar well diffusion method (Fatima et al., 2009). Cefotaxime (2 mg/ml) was used as positive control and pure DMSO was used as a negative control. Bacterial strains used were *Bacillus subtilis*, *Micrococcus leuteus*, *Salmonella setubal*, *Salmonella aureus* and *Pseudomonas pickettii*. Tests were performed in triplicates for each microorganism and results were presented as arithmetic average.

Phytochemical analysis of crude extract and fractions

Different chemical tests were conducted for preliminary phytochemical analysis.

Test for alkaloids

Mayer's reagent

Mercuric chloride (0.3555 g) was dissolved in 60 ml of water and 5 g of potassium iodide was dissolved in 20 ml of water. Two solutions were mixed and volume was made up to 1000 ml with distilled water.

Dragendorff’s reagent

1. Solution A: Basic bismuth nitrate (1.7 g) and 20 g of tartaric acid was dissolved in 80 ml of distilled water.
2. Solution B: Potassium iodide (16 g) was dissolved in 40 ml of distilled water

Solution A and B were mixed in ratio of 1:1. Plant extract (0.5 - 0.6 g) was mixed with about 8 ml of 1% HCl, warmed and filtered. 2 ml of filtrate were treated separately with Mayer’s reagent and Dragendorff’s reagent. Turbidity or precipitation was observed to indicate the presence of alkaloids.
Table 1. Anticancerous activity of V. foetens crude extract against MCF-7 cell line.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Average absorbance</th>
<th>Standard deviation</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.2006</td>
<td>0.002547</td>
<td>28.4</td>
</tr>
<tr>
<td>25</td>
<td>0.1505</td>
<td>0.00108</td>
<td>55.6</td>
</tr>
<tr>
<td>50</td>
<td>0.1026</td>
<td>0.003921</td>
<td>81.6</td>
</tr>
<tr>
<td>100</td>
<td>0.0998</td>
<td>0.01549</td>
<td>83.1</td>
</tr>
<tr>
<td>200</td>
<td>0.0806</td>
<td>0.002221</td>
<td>90.5</td>
</tr>
<tr>
<td>300</td>
<td>0.0779</td>
<td>0.001197</td>
<td>92</td>
</tr>
<tr>
<td>400</td>
<td>0.0736</td>
<td>0.002171</td>
<td>94</td>
</tr>
<tr>
<td>500</td>
<td>0.065</td>
<td>0.001414</td>
<td>98.5</td>
</tr>
</tbody>
</table>

Test for anthraquinones

Plant extract (1 g) was boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene. The benzene layer was removed and then 10% NH₄OH was added. Formation of pink, violet or red color in alkaline phase was observed for the presence of anthraquinones.

Test for coumarins

Moistened plant extract (0.5 g) was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then filter paper was removed and examined in UV light for yellow florescence to indicate the presence of coumarins.

Test for flavonoids

Prepared extract (0.5 g) was shaken with pet ether to remove the fatty materials. The defatted residue was dissolved in 20 ml of 80% of ethanol and filtered. The filtrate was used for the following test:

a) Filtrate (3 ml) was mixed with 4 ml of 1% AlCl₃ in MeOH in a test tube. Formation of yellow color was observed to indicate the presence of flavonoids, flavones and/or chalcones.

b) Filtrate (3 ml) was mixed with 4 ml of 1% KOH. A dark yellow color was observed to indicate the presence of flavonoids.

Test for saponins

Plant extract (0.5 g) was dissolved in boiling water in a test tube, allowed to cool and shaken to mix thoroughly. Froth appears indicated the presence of saponins.

Test for tannins

Plant extract (0.5 g) was boiled in 20 ml of distilled water in a test tube and then filtered. 0.1% FeCl₃ was added to filtrate. Appearance of brownish green or blue black coloration showed the presence of tannins.

RESULTS AND DISCUSSION

Plants have been used as traditional medicinal agents and serve as a base for modern medicines. Crude extract of V. foetens showed remarkable anticancerous activity against MCF-7 cell line at all concentrations in a dose dependent manner (Table 1). The average absorbance decreased by increasing the concentration of crude extract. Highest absorbance of 0.2006 was recorded when determining 28.4% inhibition at 10 µg/ml, while at the concentration of 500 µg/ml of crude extract, 0.065 absorbance was recorded with 98.5% inhibition. Cordell (1995) and Kusumoto et al. (1995) also suggested that crude extracts testing is beneficial for screening of bioactive components than isolation and testing.

On the basis of significant anticancerous activity of crude extract against breast cancer cell line MCF-7, the crude extract was fractionated and fractions at a concentration of 200 µg/ml were again tested against MCF-7 cell line. Highest percentage inhibition (83%) was measured by methanol fraction, while chloroform fraction showed 55.5% activity (Figure 1). Hexane fraction demonstrated 25.11% activity, while water fraction showed lowest activity (2%) against MCF-7 cell line. The maximum inhibition among fractions at a concentration of 200 µg/ml (83%) was less than crude extract inhibition at 200 µg/ml (90.5%). Same findings were reported by several investigations that crude extracts showed more activity than fractions or separated components (Pellati et al., 2006).

The crude extract of V. foetens was also tested against some bacterial strains to determine antibacterial potential of this potent shrub (Table 2). Maximum zone of inhibition was measured against Salmonella setuabal (12.0 ± 0.1), while mean zone of inhibition 11.2, 11.3 and 11.5 mm was found against Staphylococcus aureus, Bacillus subtilis and Micrococcus luteus, respectively. Minimum zone of inhibition 10.3 mm was measured against Pseudomonas pickettii. Although crude methanol extract of V. foetens proved strongly cytotoxic yet, it showed moderate antibacterial activity at a concentration of 20 mg/ml. These results are in agreement with findings of Hayet et al. (2007) that Salvia sclarea extract showed high cytotoxicity but moderate antimicrobial activity.

Preliminary phytochemical analysis showed presence of anthraquinones, saponins, tannins, flavonoids and coumarins in crude extract (Table 3). While methanol fraction that showed elevated anticancerous activity among all fractions was negative for presence of anthraquinones and saponins. So inhibition of crude extract might be from
any of these components. The same is reported by Meyer et al. (1982) that antiproliferative, antitumor, pesticidal and other activities in crude extracts might be due to active principles.

The role of phytochemicals in bioactivities is well established (Edeoga et al., 2005). Flavonoids are considered as potent agents against cancer, microbes and tumors (Farquar, 1996; Lopez-Lazaro, 2002). Tannins are also reported to have a strong inhibition of tumors. Likewise, coumarins and their derivatives are another class of cytotoxic compounds showing activity in plants (Cao et al., 1998). In view of strong cytotoxic potential of flavonoids, tannins and coumarins maximum inhibition of methanol fraction is strongly suggested to be due to these phytochemicals either synergistically as suggested by Choo et al. (2001) that activity of a fraction of plant extract might be due to synergistic effect of phytochemical constituents present in the extract sample or individually. Finally, the present assay-guided findings proved that methanol fraction had cytotoxic potential and can be proceeded for isolation studies in future as done in Viburnum awabuki in which bioactivity guided phytochemistry led to the isolation of four cytotoxic compounds from cytotoxic fractions (El-Gamal, 2008).

**Table 2.** Antibacterial activity of crude V. foetens extract.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. picketii</td>
<td>10.3 ± 1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>11.2 ± 0.5</td>
</tr>
<tr>
<td>S. satuball</td>
<td>12.0 ± 0.1</td>
</tr>
<tr>
<td>M. leutus</td>
<td>11.5 ± 0</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>11.3 ± 0</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>32 ± 0.2</td>
</tr>
</tbody>
</table>

**Figure 1.** Anticancerous potential of fractions of V. foetens methanol extract (200 µg/ml).

**Conclusions**

The present study further support the idea that ethanobotanically important plants can be promising source of anticancer agents and methanol fraction of V. foetens is a good candidate for isolation of anticancerous compounds. These results will form the basis for selection of this plant specie for further investigation of novel bioactive components and their application in pharmaceutical purposes.

**ACKNOWLEDGEMENTS**

We are thankful to Higher Education Commission, Pakistan for provision of grant for this research work and Portsmouth University, UK for Providing MCF-7 cell line.
Table 3. Phytochemical analysis of crude extract and fractions.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Crude extract</th>
<th>Hexane fraction</th>
<th>Chloroform fraction</th>
<th>Ethyl acetate fraction</th>
<th>Methanol fraction</th>
<th>Aqueous fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Coumarins</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

- = Absent, + = low, ++ = moderate, and +++ = high.

REFERENCES


