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Sudipta Roy

Natural Product Chemistry Lab,
Department of Pharmacy,
Faculty of Science, University of
Rajshahi, Rajshahi, Bangladesh

Rafeza Khatun

Natural Product Chemistry Lab,
Department of Pharmacy,
Faculty of Science, University of
Rajshahi, Rajshahi, Bangladesh

Md Aziz Abdur Rahman

Natural Product Chemistry Lab,
Department of Pharmacy,
Faculty of Science, University of
Rajshahi, Rajshahi, Bangladesh

In vitro antimicrobial and cytotoxic activities of various methanolic fractions of *Viburnum foetidum* L. (Adoxaceae)

Sudipta Roy, Rafeza Khatun and Md Aziz Abdur Rahman

Abstract

The research was designed to determine the antimicrobial and cytotoxic activity of the wild edible species *Viburnum foetidum* (locally called motmoti, Family: Adoxaceae) found in hill tracts area of north-east Bangladesh and is used locally as a source of functional food. The dried course powder of the plant was extracted with MeOH (CME) and was partitioned to get petroleum ether (PETEF), Hexane (n-HF), chloroform (CHF), ethyl acetate (EAF) and aqueous fraction (AqF). The fractions were evaluated for antibacterial and cytotoxic potentials. All the extracts showed significant antimicrobial activity. The inhibition zone of CME was found to be 11-28 mm against bacteria and 18-20 mm against tested pathogenic fungi. In brine shrimp cytotoxicity assay PETEF, n-HF and CME showed significant lethality against shrimp nauplii with LC₅₀ of 25, 25 and 39.81 µg/ml, respectively when compared to standard vincristine sulphate (10.44 µg/ml). All these data suggested that the plant might be a good source for natural antimicrobial and cytotoxic remedy.

Keywords: *Viburnum foetidum*, Adoxaceae, Antimicrobial, Cytotoxic, Edible plant

Introduction

Viburnum, a genus of the plant family Adoxaceae (formerly Capripoliaceae), consists of more than 230 species, inhabitant to subtropical zones from south America to south east Asia and the majority of them are endemic [1]. *Viburnum* species are known to possess antiulcer, anticonvulsant and anthelmintic properties [2-3]. Plants from *Viburnum* are well known in folk medicine for their spasmolytic, sedative and anti-asthmatic properties [4-6]. *Viburnum foetidum*, a species of *Viburnum* found in mountain area of Bangladesh and is used in local medicine. The whole plant, fruits and leaves of *V. foetidum* L. are used in healing of various ailments such as cardiac discomfort, uterine disorders [7]. *V. prunifolium* are specifically used for menstrual cramps, anti-abortion agent and for prevention of postpartum bleeding [8-10]. A large number of research has been done on the various species of *Viburnum* but there is no complete report on phytochemical and pharmacological activity of *V. foetidum* L though the plant has been used for a long as a source of vitamin and functional foods for traditional peoples live in hill area [10]. To rationale the folkloric reputation, present investigations were carried out. In this paper we wish to report in vitro antimicrobial (disc diffusion assay) and cytotoxic activity (using brine shrimp lethality bioassay) of the different methanolic extracts of *V. foetidum*.

Materials and Methods**Collection of the plant materials**

Whole plant of *V. foetidum* was collected from hill area of Sylhet district, Bangladesh in September, 2014 and was identified by taxonomist Dr. AHM Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh. A voucher specimen is deposited in the department of Pharmacy, University of Rajshahi, Bangladesh.

Extraction and fractionation of the plant materials

Powdered plants (800 g) was soaked in methanol (4L) in an amber colour air tight container for 7 days with occasional shaking and stirring at room temperature. The solvent was then filtered through a cotton and was concentrated using rotary at low temperature (40-50°C) and pressure to yield 5.5% extract. Partitioning of the extract (CME) was performed using the modified method [8]. After successive partitioning five fractions were obtained and were labelled as petroleum ether (PETEF, 2.27 g), Hexane (n-HF, 1.1 g), chloroform (CHF, 2.7 g), ethyl acetate (EAF, 0.77) and aqueous fraction (AqF, 0.66 g).

Correspondence**Md Aziz Abdur Rahman**

Natural Product Chemistry Lab,
Department of Pharmacy,
Faculty of Science, University of
Rajshahi, Rajshahi, Bangladesh

Phytochemical screening of CME

The crude methanolic extracts of *V. foetidum* was tested for the presence of alkaloids, steroids, tannins, saponins and glycosides. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Test for alkaloids

Few mg (about 15 mg) of each extract was separately stirred with 1% HCl (6 mL) on a water bath for 5 min and filtered. These filtrates were divided into three equal parts.

- Dragendorff's test:** To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution, 1 mL) was added. An orange red precipitate shows the presence of alkaloids.
- Mayer's test:** To one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution, 1 mL) was added. Formation of cream coloured precipitate gives an indication of the presence of alkaloids.
- Wagner's test:** Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate. A brown coloured precipitate indicates the presence of alkaloids [11-12].

Tests for terpenoids

The crude extract (about 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H₂SO₄ (2 mL) along the side of the test tube, a reddish brown coloration of the interface indicates the presence of terpenoid [13].

Test for tannins

Extract (leaf and bark, 0.5 g each) was separately stirred with distilled water (10 mL) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins [14].

Test for Saponins

Each of plant extracts (0.5 g) was separately shaken with distilled water (10 mL) in a test tube. The formation of frothing, which persists on warming in a water bath for 5 min, shows the presence of saponins [14].

Tests for glycosides

- Anthraquinone glycoside (Borntrager's test):** To the extract solution (1 mL), 5% H₂SO₄ (1 mL) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer gives indication of anthraquinone glycosides [11].
- Cardiac glycoside (Keller-Killiani test):** Extract (0.5 g) was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed by H₂SO₄ (1 mL) along the

side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring [13].

Antimicrobial Assay

In the present study, the antimicrobial assay was carried out by the disc diffusion method [15] against two gram positive: *Bacillus cereus*, *Staphylococcus aureus* and three gram negative: *Agrobacterium species*, *Shigella dysenteriae*, *Shigella sonnei*, and *Escherichia coli* bacteria and three fungi; *Candida albicans*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The diameter of zone of inhibition expressed in millimetre (mm) is then measured to determine antimicrobial activity of the test agent [15-16].

Cytotoxic Assay

Brine shrimp lethality bioassay was done [17] for evaluation of cytotoxic activity using concentration of 10-250 µg/ml of each extract. Different concentrations of vincristine sulfate were taken as standard sample. 120 µl of DMSO was added to each of the three remarked glass vials containing 5 ml of simulated sea water and 20 shrimp nauplii to use as negative control group. The percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extracts.

Statistical analysis

Statistical comparisons were performed using Microsoft Excel, 2016. Mean values ± S.D. were calculated for the parameters where applicable.

Results

Phytochemical screening

Table 1: Phytochemical screening of CME of *V. foetidum*

Test	Result
Alkaloids Dragendorff's Test	+
Mayer's Test	+
Wagner's Test	+
Terpenoids	+
Tannins	+
Saponins	-
Glycosides Anthraquinone	-
Cardiac Glycoside Flavonoidal	+
	+

+ = presence, = absence

Antimicrobial assay

The methanolic and other extracts from *V. foetidum* showed significant antimicrobial activity (Table 2 and Table 3). Ethyl acetate fraction from *V. foetidum* exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 12 to 24 mm. The maximum zone of inhibition produced by EAF was found to be 24 mm against *Agrobacterium species*. EAF also showed significant antifungal activity with 18 mm zone of inhibition. The results of in-vitro microbial screening *V. foetidum* indicated that all the fractions have significant antimicrobial activity. These can be further studied to explore potent antimicrobial agents.

Table 2: Zone of inhibition of CME, PETEF, n-HF, CHF, EAF, and AqF extractives of *V. foetidum* against various bacteria

Organism	CME	PETEF	n-HF	CHF	EAF	AqF	Kanamycin
Gram-positive	(100µg/disc)						(30µg/disc)
<i>Bacillus cereus</i>	18	15	16	18	12	8	25
<i>Staphylococcus aureus</i>	20	15	21	19	16	8	25
Gram-negative							
<i>Agrobacterium species</i>	28	21	20	18	24	15	28
<i>Escherichia coli</i>	20	18	16	15	14	11	25
<i>Shigella dysenteriae</i>	11	16	14	16	16	15	25
<i>Shigella sonnei</i>	17	15	13	15	12	8	25

The CME of *V. foetidum* and its five fractions (PETEF, n-HF, CHF, EAF and AqF) were also tested for their antifungal activity at a concentration of 200µg/disc against three fungi.

Standard antifungal Ketoconazole (50 µg/disc) discs were used for comparison. The results are shown in Table 3.

Table 3: Zone of inhibition of CME, PETEF, n-HF, CHF, EAF, and AqF extractives of *V. foetidum* against various fungi.

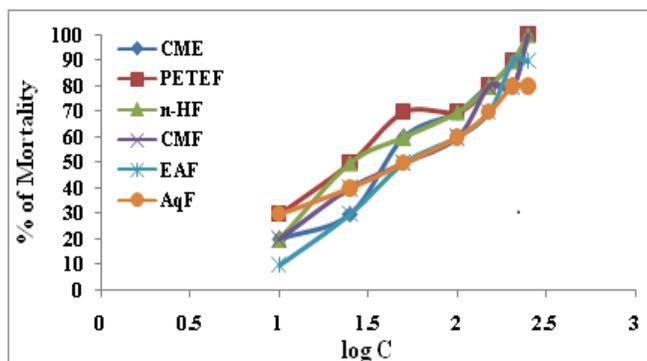
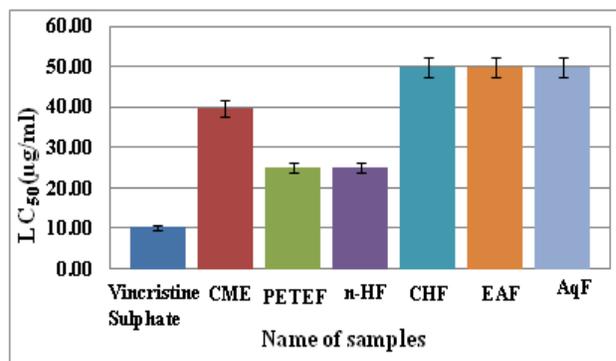
Organism	CME	PETEF	n-HF	CHF	EAF	AqF	Ketoconazole
	(200µg/disc)						(50µg/disc)
<i>Candida albicans</i>	18	15	20	20	15	-	15
<i>Aspergillus niger</i>	20	16	20	20	18	6	20
<i>Sacharomyces cerevicae</i>	12	10	15	18	16	6	20

Values are expressed as mean±SD (n=3). '-' Indicates no zone of inhibition

Cytotoxic assay

Cytotoxic activity was evaluated using brine shrimp lethality bioassay method [17]. The results are shown in Fig 1 and Fig 2. Vincristine Sulphate was used as positive control and DMSO (showed no mortality) as negative control. The PETEF and n-

HF fractions showed significant cytotoxicity with LC₅₀ around 25 µg/ml compared to standard with LC₅₀ 10.44 µg/ml. CME fraction showed a LC₅₀ value around 39.81 µg/ml. Activity of CHF, EAF and AqF were around 50 µg/ml.

**Fig 1:** Brine shrimp lethality bioassay of various fractions of *V. foetidum***Fig 2:** LC₅₀ values of various fractions of *V. foetidum* with Vincristine

Discussion

Phytochemical screening of *V. foetidum* showed the existence of terpenoids, alkaloids, tannins, and glycoside, Presence of these group of phyto-compounds can be correlated to the biological activities of the plant.

In the present study, almost all of the extracts of *V. foetidum* L (at different concentrations) exhibited low to moderate antimicrobial activity against various strains of gram-positive and gram-negative bacteria. The ability of the crude extracts of *V. foetidum* to inhibit the growth of bacteria is an indication of its antimicrobial potential which may be employed in the management of microbial infections. EAF showed the maximum potential antimicrobial activity indicating the presence of highest content of antimicrobial agent in the extract.

Various extracts of *V. foetidum* produced concentration dependent increment in percent mortality of brine shrimp nauplii. In brine shrimp cytotoxicity assay PETEF, n-HF and CME showed significant lethality against shrimp nauplii when compared to standard vincristine sulphate indicating the presence of cytotoxic principles in the extracts.

All these study suggest that *V. foetidum* can be used as antibacterial and cytotoxic agent in the development of new drugs. The observed effects rationale the traditional use of the plant. Further work is under progress to identify the bioactive principles and elucidate their mechanism of action of specific bioactivities.

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