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Antioxidant, Antimicrobial and Cytotoxic Activities of *Vitis trifolia* Linn.

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Abstract: The ethanol extract of the aerial parts of *Vitis trifolia* Linn. (Family-Vitaceae) was subjected to preliminary phytochemical, antioxidant, antimicrobial and cytotoxicity screenings. Primary phytochemical analysis indicated the presence of some major chemical groups in the plant extractive. The antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picryl-hydrazyl) and moderate free radical scavenging activity was found (IC_{50} 436.516 µg/ml). In case of antimicrobial screening, it showed moderate inhibition against bacterial growth. In addition, the extract also showed significant lethality to *Artemia salina* with a LC_{50} value of 2.80 µg/ml.

Key words: *Vitis trifolia* Linn., Vitaceae, antioxidant, antimicrobial, cytotoxicity

Introduction

Vitis trifolia Linn. (Family- Vitaceae, local name- Goali lata, syn.- *V. carnosa.* Wall.) is a glabrous climber grown throughout Bangladesh, Peninsular India, Himalaya, Sri Lanka, China, Indo-china and Malaya. The leaves of *V. trifolia* are ovulate or elliptic-obtuse, serrate. The flowers are green in corymbose paricles having four petals.[1, 2] The decoction of the whole plant is indigenously used as stimulant, stomachic and expectorant.[3] Previously epifriedelanol was isolated from *V. trifolia* which showed potent anti-tumor activity.[4] In the present study, antioxidant, antimicrobial and cytotoxic activities of *V. trifolia* are reported for the first time.

Experimental

***Plant*:** *V. trifolia* leaves were collected from Khulna in August 2007. It was identified in the National Herbarium of Bangladesh where a voucher specimen has been deposited (accession no DACB- 30227).

***Extraction and isolation*:** Fresh leaves of the plant were collected, dried and ground to a coarse powder. 1 kg of the powdered sample was extracted with ethanol for about 10 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator.

***Preliminary phytochemical analysis*:** The ethanol extract of leaves of *V. trifolia* was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test.[5]

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***Antioxidant activity*:** Free radical scavenging or antioxidant activity of the extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical in both qualitative and quantitative assay.

Qualitative assay: A suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted.[6]

Quantitative assay: Quantitative assay was performed on the basis of the method of Brand-Williams *et al.*[7] In the experiment, 2.0 mg of each of the extracts was dissolved in methanol. Solution of varying concentrations such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 2 ml of a methanol solution of the extract of each concentration was mixed with 4 ml of a DPPH-methanol solution (20 mg/L) and allowed to stand for 20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [\, 1 - (ABS_{sample} / ABS_{control})] \times 100$$

Then % inhibitions were plotted against respective concentrations used and from the graph the IC_{50} was calculated. Ascorbic acid, a potential antioxidant, was used as positive control.

***Antibacterial screening*:** Antibacterial activity of the crude extract was determined by disc diffusion method.[8, 9] The bacterial strains listed in Table-1 were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample was dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 400 µg/ disc. Discs were then carefully dried to evaporate the residual solvent. Standard kanamycin (30 µg/disc) was used as the positive control in the experiment.

***Cytotoxicity screening*:** For cytotoxicity screening, DMSO solutions of the plant extractives were applied against *Artemia salina* in a 1-day *in vivo* assay, the experimental details of which could be found elsewhere.[10, 11] For the experiment, 4 mg of each of the extracts was dissolved in DMSO. Solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg / ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 hours was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Result and Discussion

The preliminary phytochemical screening of the extract revealed the presence of alkaloids, reducing sugars, gums, steroids, saponins and flavonoids in the plant. However, in case of antioxidant screening, the color changes (yellow on purple

background) on the TLC plate were observed due to the bleaching of DPPH by some of the resolved bands. The quantitative study demonstrated moderate free radical scavenging activity with IC_{50} value 436.516 µg/ml.

Table 1: Antibacterial activity of leaves of *V. trifolia* ethanol extract

Bacteria	Zone of inhibition (mm)	
	Kanamycin (30 µg/disc)	Ethanol extract (500 µg/disc)
Gram Positive		
Staphylococcus aureus	44	11
S. epidermis	38	13
Gram Negative		
Enterobactor streptus	39	7
E. aerogenes	36	7
Escherichia coli	34	9
Shigella dysenteriae	32	13
S. flexneri	35	8
S. soniie	35	7
S. boydei	28	9

Besides, in the antimicrobial screening, the extract showed average zone of inhibition 07-13 mm (Table 1). Moderate inhibitory activity was noticed against the growth of *S. epidermidsi* and *S. dysenteriae* with the zones of inhibition 13 mm each.

During the brine shrimp lethality bioassay, the degree of lethality was found to be directly proportional to the concentration of the extract ranging from the lowest concentration (0.781 µg/ml) to the highest concentration (400µg/ml). The LC_{50} obtained from the best-fit line slope when the mortality of shrimp was plotted against the concentration of the sample were 0.200 and 2.80 µg/ml for positive control (vincristine sulphate) and crude methanol extract, respectively.

The results of the chemical and biological screenings of *V. trifolia* substantiate the folk uses of the plant in various diseases. Further, bioactivity guided isolation is required to isolate the active principles from the plant.

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