

Short Communication

Antioxidant, Antimicrobial and Cytotoxic Activities of *Desmodium motorium* Merr.

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Abstract

The *n*-hexane, carbon tetrachloride, dichloromethane soluble fractions of methanol extract of the whole plant of *Desmodium motorium* were subjected to antioxidant activity by indicated method, inhibition of microbial growth by disc diffusion method and cytotoxicity by brine shrimp lethality bioassay. The methanol *i.e.*, crude extract showed highest antioxidant activity having IC₅₀ 9.5 µg/ml as compared to 9.0 µg/ml for standard agent BHT. The *n*-hexane, carbon tetrachloride and dichloromethane soluble fraction showed moderate inhibitory activity to microbial growth, while all the extractive exhibited strong cytotoxic property with the carbon tetrachloride soluble materials revealed the strongest cytotoxicity with LC₅₀ 1.54 µg/ml.

Keywords: *Desmodium motorium*; Leguminosae; Antioxidant activity; Brine shrimp lethality.

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1. Introduction

Medicinal plants are an important therapeutic aid for various diseases [1]. Traditional village doctors use the plants for making various medicines. Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various ailments. A large number of plants are constantly being screened for their possible medicinal value. For this purpose the use of plant extract in traditional medicine has been going on from ancient time. Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy [2]. In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the

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indiscriminate use of modern antibiotics [3]. Natural antioxidants protect the human body from free radicals, prevent oxidative stress and associated diseases. For these reasons they play a very important role in health care. Plants are a source of compounds with antioxidant activity such as phenolic acids, flavonoids, anthocyanins, tannins and carotenoids that may be used as pharmacologically active products [4]. Therefore, the demand for new and effective antimicrobial and antioxidant agents from natural sources are increasing day by day. *Desmodium motorium* Merr. (family- Leguminosae, Bengali name -Turuk chandal, English- Telegraph plant) is a small shrub with branches, which grows in different parts of Bangladesh. It is a self motorized and semaphore plant. In order to identify plant species having potential antioxidant, antimicrobial and other bio-activities, we herein, report the preliminary antioxidant, antimicrobial and cytotoxic activities of *D. motorium*.

2. Materials and Methods

2.1. Plant material

The plant *D. motorium* was collected from Auwsadhee gram, Natore and Bagerhat Districts in the month of November 2007. A voucher specimen for this collection has been deposited in Bangladesh National Herbarium, Dhaka (Accession no. 32896).

2.2. Extraction and fractionation

The powdered of whole plant (800 g) of *D. motorium* was soaked in 1.2 L methanol for 7 days with occasional shaking and stirring and filtered through a cotton plug followed by Whatman filter paper number-1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (5.0 g) of the concentrated methanol extract was fractioned by the modified Kupchan partitioning method [5, 6] into *n*-hexane (1.25 g), carbon tetrachloride (0.75 g), dichloromethane (0.80 g) and aqueous soluble materials (2.0 g).

2.3. Bioassays

The free radical scavenging activity of the plant extractives on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand- Williams [7, 8]. Here, 2.0 ml of a methanol solution of the samples at different concentration were mixed with 3.0 ml DPPH solution in methanol (20 µg/ml). The antioxidant potential was determined by a UV-Vis spectrophotometer from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that produced by the standard antioxidant agents of *tert*-butyl hydroxyl toluene (BHT) and ascorbic acid. Free radical scavenging activities of different partitionates of methanolic extract and standard tested by DPPH method. IC₅₀ value of the standard (BHT) obtained 10 µg/ml.

The antimicrobial activity of the extractives was determined by the disc diffusion method [9, 10]. The samples were dissolved separately in chloroform or methanol and applied to sterile filter paper discs (40 µg/disc) and kanamycin disc was used as standard (40 µg/disc). For cytotoxicity assay DMSO solutions of the plant extracts were applied against *Artemia salina* in a 1-day *in vivo* assay, the experimental details of which could be found elsewhere [11, 12]. For the experiment 4 mg of each of the Kupchan fractions was dissolved in DMSO and test solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. The median lethal concentration LC₅₀ of the test samples after 24 hours of exposure was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample of the concentration and vincristine sulphate (VS) was used as the standard.

3. Results and Discussion

The antioxidant activity of various fractions of the methanolic extract was determined. The *tert* butyl-1-hydroxy toluene (BHT) was used as positive control. The IC₅₀ values for *n*-hexane (HX), carbon tetrachloride (CT), dichloromethane (DCM) and methanolic extract (ME) were found to be 14, 162, 19.5 and 9.5 µg/ml, respectively (Table 1).

Table 1. IC₅₀ values of test samples of *D. motorium*.

Sample	IC ₅₀ (µg/ml)
BHT	9.0
HX	14.0
CT	162
DCM	19.5
ME	9.5

In comparison with positive control (BHT) the antioxidant activity exhibited by the methanolic extract, *n*-hexane and dichloromethane soluble fractions were significant. This indicates the plant is a potential source of antioxidant. In the antimicrobial screening the extractives as *D. motorium* exhibited mild to moderate antimicrobial activity. The zone inhibition produced by the carbon tetrachloride, dichloromethane soluble fractions and methanolic extract was 9 to 17, 8 to 18 and to 16 mm, respectively (Table 2). The *n*-hexane soluble fraction did not inhibit the growth of microorganisms. Following a method (*Artemia salina* LEACH) [11, 12], the lethality *n*-hexane, carbon tetrachloride, dichloromethane soluble fractions of the methanolic extract to brine shrimp was determined. The LC₅₀ obtained from the best fit line slope were found to be 7.15, 1.54, 1.99 and 5.79 µg/ml for *n*-hexane, carbon tetrachloride, dichloromethane soluble fractions and methanolic extract, respectively (Table 3). In comparison with the positive control (vincristine sulphate LC₅₀ 0.46 µg/ml) the cytotoxicity exhibited by the carbon tetrachloride, dichloromethane soluble fractions and methanolic extract were significant.

Table 2. Antimicrobial activity of *D. motorium* extractives of 40 µg/disc.

Test microorganisms	Diameter of zone of inhibition (mm)			
	CT	DCM	ME	KAN ^a
Gram positive bacteria				
<i>Bacillus cereus</i>	11	8	14	35
<i>B. megaterium</i>	12	8	15	37
<i>B. subtilis</i>	11	8	9	34
<i>Staphylococcus aureus</i>	9	9	12	37
<i>Sarcina lutea</i>	14	9	9	37
Gram negative bacteria				
<i>Escherichia coli</i>	11	9	9	37
<i>Pseudomonas aeruginosa</i>	17	18	14	36
<i>Salmonella paratyphi</i>	16	8	8	35
<i>S. typhi</i>	9	8	9	33
<i>Shigella dysenteriae</i>	9	12	9	32
<i>S. boydii</i>	9	8	9	33
<i>Vibrio mimicus</i>	12	12	14	37
<i>V. parahemolipicus</i>	16	8	15	31
Fungi				
<i>Candida albicans</i>	9	8	16	33
<i>Aspergillus niger</i>	9	12	15	35
<i>Sacharomyces cerevacae</i>	9	9	9	34

^a Kanamycin

Table 3. LC₅₀ data of test samples of *D. motorium*.

Sample	LC ₅₀ (µg/ml)
VS	0.46
HX	7.15
CT	1.54
DCM	1.99
ME	5.79

The results of antioxidant, antimicrobial and cytotoxic activity screening support the folk uses of *D. motorium* in various diseases. Further work especially bioassay-guided fractionation is warranted in order to isolate and characterize the antioxidant and antimicrobial bio-active constituents responsible for the antioxidant and antimicrobial property.

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