

Phytochemical Screenings and Bioactivities of *Duabanga grandiflora* (Roxb. ex DC.) Walp.

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(Received: October 5, 2018; Accepted: November 20, 2018; Published: January 17, 2019)

Abstract

The aim of this research was to find out the phytochemical constituents and to evaluate the antibacterial, antioxidant, antidiarrheal and antipyretic activities of different extracts of *Duabanga grandiflora*. Antibacterial study was carried out by the disk diffusion method where the *n*-hexane extract showed the inhibitory activity against *Staphylococcus aureus* (12.3 mm) and *Bacillus cereus* (11.3 mm) as compared to standard drug ciprofloxacin. A quantitative antioxidant assay was done by DPPH free radical scavenging activity, where at the dose of 100 µg/ml the different extracts of *D. grandiflora* leaves revealed the highest inhibition of 81.07, 82.48, and 80.33%, respectively in comparison to standard ascorbic acid (86.9%). The ethanol extract showed IC₅₀ value 1.91 µg/ml, which was found to be as potent as standard ascorbic acid (1.90 µg/ml). In antidiarrheal activity test by using castor oil induced diarrheal mice, the result showed different extracts (500 µg/ml) of plant possessed moderate antidiarrheal activity. It significantly increased the latency period and reduced the number fecal count in comparison to standard drug loperamide 3mg/kg body weight. In antipyretic activity test by using Brewer's yeast induced pyrexia in Swiss albino mice the different extracts of *D. grandiflora* demonstrated mild to moderate antipyretic activity as compared to standard paracetamol.

Key words *Duabanga grandiflora*, antimicrobial, antioxidant, antipyretic, antidiarrheal.

Introduction

Many therapeutic agents used to alleviate various diseases in human has been isolated and then developed from natural sources, especially of plant origin. Hence, plants will always remain one of the main focuses for the discovery and development of antibiotics, anti-cancer, antioxidant, antipyretic agents and other bio-molecules serving as a potential treatment (Gurib-Fakim, 2006).

The plant *Duabanga grandiflora* belongs to family Lythraceae, a moderate-sized family of angiosperms among which most genera are woody shrubs or small trees (Hooker, Fitch and Cathcart, 1855); the plant is a large evergreen plant (Bengali:

Duyabanga) and it is being used traditionally to treat skin diseases such as eczema or atopic dermatitis (Santiago *et al.*, 2015). *D. grandiflora* is instinctive to Nepal, Southeast Asia, Eastern India, Laos, Cambodia, Malaysia, Myanmar, Thailand, Vietnam and most importantly in the forest and hill tracks of Bangladesh (National Parks, 2018). Compounds such as, 5-formylfurfuryl esters, duabanganals A-D, together with sixteen known compounds, a known 5-formylfurfuryl ester, latifolinal, eight pentacyclic triterpenes, a benzofuran derivative, an ellagic acid derivative, vanillin, β-sitosterol, β-sitosterol glucoside, 3-hydroxy-4-methoxycinnamaldehyde, and 5-formylfurfurol were isolated from the stem

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bark of *D. grandiflora* where several of these metabolites were evaluated for cytotoxic activities against six cancer cell lines (Kaweetripob *et al.*, 2012). In another study, it has been found that the leaf extract of *D. grandiflora* actively affected several human skin cells such as skin whitening, anti-aging, and anti-inflammation (Tsukiyama *et al.*, 2010).

As parts of continuing studies with medicinal plants of Bangladesh, we investigated the antibacterial, antioxidant, antidiarrheal and antipyretic activities of *D. grandiflora* growing in Bangladesh, and here in, report the results of our preliminary investigation.

Materials and Methods

Collection and preparation of plant materials: Fresh leaves were collected from the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong, in the month of June and July of 2016 which is the period when the plant grows the most.

For hot extraction, about 180 g of the powdered leaf was treated with absolute ethanol in a Soxhlet apparatus (Quickfit, England). After evaporation of the solvent, a gummy concentrate was obtained which was designated as the hot ethanolic crude extract (5 g). Solvent-solvent partitioning was done using the protocol design by Kupchan and modified by Van-Wagenen *et al.* in 1993 (Moon, 2018). The crude ethanol extract (5 g) was dissolved in 10% aqueous methanol and fractionated in a separatory funnel with *n*-hexane and chloroform. The obtained fractions were evaporated in a rotary evaporator rendering a concentrate of *n*-hexane, chloroform and aqueous soluble fractions, which were properly labeled and stored at 4°C until use.

Antibacterial activity: The extracts of *D. grandiflora* were screened for antimicrobial sensitivity against 6 human pathogenic bacteria by the disc diffusion method (Brown and Kothari, 1975). All the microbial species were collected from the Microbiology Lab of Department of Pharmacy, Southern University Bangladesh, Chittagong.

Standard discs were prepared by ciprofloxacin and the antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of mm (Aboaba and Efuwape, 2001).

Antioxidant activity: The radical scavenging potential of the test samples was determined by evaluating the antioxidant activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and standard ascorbic acid was used as positive control (Brand-Williams *et al.*, 1995). The half maximal inhibitory concentration (IC₅₀) was determined graphically in comparison to standard ascorbic acid (Blois, 1958).

Antidiarrheal activity: The study was conducted as per the method where the Swiss albino mice were all screened initially where they were divided into control, standard and three test groups containing three mice in each group, e.g. control group receiving vehicle (1% Tween-80) at a dose of 10 ml/kg body weight orally, the standard group receiving loperamide at the dose of 3 mg/kg body weight orally and test groups receiving the ethanol, *n*-hexane and chloroform extract of *D. grandiflora* leaves at the dose of 500 mg/kg body weight orally (Shoba and Thomas, 2001). During the observation period (4 hrs), the total latency periods (first diarrheal stool after the administration of castor oil) and the number of diarrheic feces excreted by the animals were recorded.

Antipyretic activity: The antipyretic activities of extract were evaluated using Brewer's yeast induced pyrexia in the Swiss albino mice where the animals were divided into control, standard and one test group (Adams, 1960). Before yeast injection, rectal temperature of mice was recorded and at the 24th hrs after yeast injection, the distilled water at dose of 10 ml/kg body weight was administered orally to the control groups of animals and paracetamol at dose of 150 mg/kg was administered orally to standard group of animals and finally the different extracts of leaves of *D. grandiflora* were administered orally at a dose of 500 mg/kg of body weight.

Results and Discussion

The preliminary phytochemical screenings revealed that the ethanol extract of *D. grandiflora* contained alkaloids, steroids, tannins, saponins, and reducing sugars, whereas the chloroform soluble material contained steroids, saponins, reducing sugars and gums. On the other hand, the *n*-hexane soluble material contained alkaloids, saponins, and steroids. Glycosides and amides were not found in any test samples.

The result obtained from the antibacterial sensitivity of the extracts of *D. grandiflora* was compared with the standard drugs viz. ciprofloxacin testing. During the anti-bacterial screening, the *n*-hexane soluble material of ethanol extract showed inhibitory activity against all tested pathogenic bacteria. Among them, it displayed the highest inhibition against *S. aureus* (12.3 mm) and *B. cereus* (11.3 mm). The ethanol extract demonstrated weak inhibition against *L. casei* (8 mm) and *B. cereus* (7.8 mm) only. On the other hand, the chloroform-soluble fraction did not show any inhibition against the pathogenic bacteria (Table 1). However, the

antibacterial activity of the extractives was considered less significant when compared to that of the effects of the reference standard, ciprofloxacin.

During free radical scavenging assays at the dose 100 µg/ml, ethanol extract and its *n*-hexane, and chloroform-soluble fractions produced the significant inhibition of 81.07, 82.48, and 80.33%, respectively when compared with standard ascorbic acid (86.9%). The IC₅₀ for ethanol extracts and its chloroform and *n*-hexane soluble material were 1.91, 2.43 and 2.15 µg/ml, respectively whereas that for ascorbic acid the value was 1.90 µg/ml (Table 2 and 3). Therefore, the ethanol extract showed potent antioxidant activity to the standard ascorbic acid.

In antidiarrheal activity test, different extractives (ethanol, chloroform, *n*-hexane) of *D. grandiflora* leaves at 500 mg/kg body weight significantly increased latency period to 60.67, 100 and 91 minute, respectively in comparison to the standard drug loperamide (161 minute) and also reduced the total number of defecation (Table 4). Thus, the *n*-hexane and chloroform-soluble fractions showed moderate antidiarrheal activity.

Table 1. Antibacterial activity of *D. grandiflora* extractives and ciprofloxacin.

Test organisms	Zone of inhibition in diameter (mm)			
	Ethanol soluble material	<i>n</i> -hexane soluble material	Chloroform soluble material	Ciprofloxacin (standard)
<i>Staphylococcus aureus</i>	-	12.3	-	27.0
<i>Bacillus cereus</i>	7.83	11.3	-	29.3
<i>Escherichia coli</i>	-	8.00	-	26.3
<i>Salmonella paratyphi</i>	-	9.3	-	25.6
<i>Salmonella typhi</i>	-	8.8	-	24.3
<i>Lactobacillus casei</i>	8.0	8.8	-	25.3

Table 2. DPPH inhibitory action of different fractions of *D. grandiflora* and standard, ascorbic acid.

Concentration (µg/ml)	% Inhibition by ethanol extract	% Inhibition of <i>n</i> -hexane soluble fraction	% Inhibition of chloroform soluble fraction	% Inhibition by ascorbic acid (standard)
100	81.07	82.48	80.33	86.97
80	74.77	73.03	73.36	73.78
60	64.89	65.89	54.69	66.06
40	47.14	46.47	42.57	50.46
20	35.91	32.61	34.11	38.92

Table 3. IC₅₀ of different fractions of *D. grandiflora* and ascorbic acid.

Treatment groups	IC ₅₀ (µg/ml)
Ascorbic acid	1.90
Ethanol soluble material	1.91
Chloroform soluble material	2.43
<i>n</i> -hexane soluble material	2.15

Table 4. Antidiarrheal activity of *D. grandiflora*.

Test groups	% Defecation	% Inhibition of defecation	Total number of faeces (240 min)
Control(1% Tween-80)	100	0	31.8±1.08
Standard (loperamide)	40.6	59.4	12.9±1.08
Ethanol soluble material	72.6	27.4	23.1±2.48
Chloroform soluble material	44.3	55.7	14.1±0.41
<i>n</i> -hexane soluble material	44.3	55.7	14.1±0.81

Table 5. Antipyretic activity of *D. grandiflora*.

Treatment	Rectal temperature (°F)				
	0 hour	1 st hour	2 nd hour	3 rd hour	4 th hour
Control	100.37 ± 0.426	100.54 ± 0.460	100.47 ± 0.497	100.5 ± 0.495	100.37 ± 0.389
(distilled water)	100.27 ± 0.248	99.44 ± 0.268	98.47 ± 0.108	98.17 ± 0.108	98 ± 0.142
Standard (paracetamol)	100.37 ± 0.340	98.5 ± 0.050	97.3 ± 0.100	97.2 ± 0.087	98.1 ± 0.058
Ethanol extract	99.2 ± 0.389	97.7 ± 0.141	96.3 ± 0.0817	96.3 ± 0.227	97.4 ± 0.108
<i>n</i> -hexane soluble material	100.37 ± 0.426	97.7 ± 0.163	97.3 ± 0.163	96.8 ± 0.495	96.3 ± 0.108
Chloroform soluble material	99.8 ± 0.108	100.54 ± 0.460	100.47 ± 0.497	100.5 ± 0.495	98.1 ± 0.058

The test for antipyretic activity revealed that the ethanol extract reduced the pyresis in mice from 100.2°F to 98.1°F in (1st to 4th hrs) whereas the *n*-hexane and the chloroform soluble fractionate reduced the temperature from 99.2°F to 98.5°F and from 99.8°F to 98.5°F, respectively in 1st to 4th hour (Table 5). Therefore, it was evident that different extracts of *D. grandiflora* possess mild to moderate antipyretic activity.

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