Bangladesh Pharmaceutical Journal ***20****(2): 139-147, 2017*

Pharmacological and Biological Activities of Different Fractions Methanol Extracts of *Gardenia coronaria* Buch. -Ham. Leaves

Abdullah Al Faruq[1], Mohammed Ibrahim[1], M. Mohi Uddin Chowdhury[1], Mohiminul Adib[2], Mohammad R. Haque[2] and Mohammad A. Rashid[2]

[1]Department of Pharmacy, Southern University Bangladesh, Chittagong, Bangladesh
[2]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received: March 01, 2017; Accepted: February 28, 2017; Published (Web): July 31, 2017

Abstract

Ethanolic extract and its *n*-hexane and chloroform soluble fractions of the leaves of *Gardenia coronaria* Buch. Ham. were screened for their bio-activities. Preliminary phytochemicals screening of crude extract revealed the presence of glycosides, alkaloids, tannins, saponins, reducing sugars and flavonoids in different extracts. In antibacterial test, maximal zone of inhibition obtained against *B. cereus* (15.3 mm) and in antifungal test maximal zone found against *C. albicans* (12.7 mm) by the chloroform extract. The crude extract of the plant exhibited notable anti-inflammatory activity. The antioxidant activity was evaluated by DPPH free radical scavenging method where the scavenging activity was concentration dependent with IC_{50} values of 5.15µg/ml, 8.75µg/ml and 12.71µg/ml for ethanol, n-hexane and chloroform extract, respectively. In the castor oil-induced antidiarrheal assay, the crude extract of the plant significantly increased the latency and total count of defecation. Acetic acid-induced writhing reflex due to analgesia was inhibited by 31.18% (by ethanol), 23.67% (by *n*-hexane) and 24.73% (by chloroform) at 500 mg/kg body wt. in experimental mice. The extractives at doses from 31.25-500 µg/ml, produced inhibition of amylase activity in a dose dependent manner.

Key words: *Gardenia coronaria*, phytochemicals, antimicrobial, anti-inflammatory, antioxidant, analgesic, antidiarrheal, anti-amylase activity.

Introduction

Natural products are the valuable sources of structurally diverse compounds, which possess therapeutic potential for human diseases (De Smet, 1997). *Gardenia* is a genus of flowering plants in the coffee family Rubiaceae, native to the tropical and subtropical regions of Africa, Southern Asia, Australasia and Oceania (Chen and Taylor, 1761). *Gardenia coronaria* is an evergreen shrub and small tree growing to 1-15 meters tall.

In Bangladesh it is found in forests of Chittagong, Chittagong Hill Tracts, Cox's-Bazar, Sylhet and Moulvi Bazar districts. The plant is locally known as *Bela* (Sylhet region) and *Connari* or *Kannyari* (Chittagong region). The plant is traditionally used for ailments of many diseases like bronchitis, haemoptysis, haematemesis, melena, diarrheal diseases and skin disorders. The leaves of the plant consist of coronalolide, coronalolic acid, coronalolide methyl ester, ethyl coronalolate acetate triterpenes (secocycloartanes) and so forth (Govaerts, 2017).

Materials and Methods

Plant collection and identification: Fresh leaves were collected from the hill tracts of Sitakunda Eco Park, Chittagong, in the month of June and July, 2014 which is the flowering period of the plant. Taxonomical identification of this plant was made by the experts of Bangladesh Forest Research Institute

Correspondence to: Mohammad A. Rashid, Tel.: +88-02-9661900-73, Extn.- 8137; Fax: +88-02-9667222;
E-mail: rashidma@du.ac.bd

Herbarium (BFRIH), Chittagong. The herbarium sheet was prepared by following the standard procedure and specification suggested by the expert of the institute and preserved after identification.

Extraction and fractionation of plant materials: About 200 gm powder of the plant material was soaked in 800 ml of ethanol (99.8%) in a clean, sealed flat-bottomed amber colored glass container and kept for 20 days with occasional shaking. The whole mixtures were filtered by cotton followed by Whatmann filter paper and the solvent was evaporated under reduced pressure at room temperature. A gummy extract of brown color deposited in the bottom of container was collected and undergone hot extraction with 1000 ml of absolute ethanol in a Soxhlet Apparatus (Quickfit, England). The obtained extract was collected, filtered and evaporated to dryness *in vacuo* below 60°C temperature. The cold and hot ethanolic crude extract were combined and undergone solvent-solvent partitioning using the protocol design by Kupchan and modified by VanWagenen (VanWagenen *et al.*, 1993). The crude ethanol extract (15 gm) was dissolved in double distilled water (DDW) and then partitioned with n-hexane and subsequently with chloroform.

Phytochemicals screening: The bioactive components analyzed in the present study were alkaloids, steroids, tannins, saponins glycosides, reducing sugars, gums, amides and flavonoids. Following methods were applied to identify the bioactive compounds (Islam *et al*., 2012).

Bioactive compounds	**Methods of test**
Alkaloids	Mayer's test, Dragendorff's test; Wagner's test, Hager's test, Tannic acid test
Glycosides	Salkowski test, Libermann-burchared test
Steroids	Salkowski test, Libermann-burchared test
Tannins	Ferric chloride test, Potassium dichromate test, Keller-Killiani test
Flavonoids	Conc. HCl and alcoholic test
Saponins	Shake test (aq. solution)
Reducing sugars	Fehling's test, Benedict's test
Gums	Molisch's test
Amides	NaOH test

Screening for antimicrobial activities: The extracts of *Gardenia coronaria* were screened for antimicrobial sensitivity against important 11 human pathogenic bacteria and 7 fungi. Disc diffusion method was used for the preliminary screening of antimicrobial activities (Finer, 1983; Aboaba and Efuwape, 2001; Aboaba *et al.*, 2006). Standard discs were prepared by ciprofloxacin and fluconazole (30 µg/disc) for bacteria and fungi, respectively. Different fractionates were dissolved in respective solvents to prepare test samples of desired concentration (500 µg/disc). The antibacterial and antifungal sensitivities were determined by measuring and comparing the zone of inhibition (mm) of the extractives and standard drugs.

Anti-inflammatory activity: Heat induced protein denaturation method was used to perform this test. Egg albumin was taken as source of protein. In brief, 15 clean centrifuge tubes were taken. Three for standard, three for control and nine for each extract. The tubes were marked accordingly. 1 ml of 5% egg albumin solutions were kept into all treatment tubes, 2 ml of DDW was added to the control tubes. 2 ml acetyl salicylic acid (required concentrations) was mixed for positive control group on the other hand for test groups, 2 ml of test extracts were mixed as marked. The pH (5.6±0.2) of the all reaction mixtures

were adjusted by 1N HCl. All the reaction mixtures were heated at 60 °C for 5 minutes followed by cooling and filtering (Whatmann filter paper). The absorbance was measured spectrophotometrically at 660 nm. The test was repeated for three times for each extract (Ferrero *et al.*, 2007).

Antioxidant activity screening: Quantitative antioxidant activity was performed by DPPH free radical scavenging method (Sadhu *et al*. 2003) with minor modification. 0.1ml of ethanol, chloroform and n-hexane extracts, at various concentrations (20, 40, 60, 80 and 100 µg/ml), was added up to 3ml of 0.004% methanol solution of DPPH. All the reaction tubes were kept in dark for 30 minutes except negative control. After 30 minutes, absorbance of the resulting solution was measured against a blank at 517 nm. The percentage DPPH radical scavenging activities (% SCV) were calculated by comparing the results of the test with the control (not treated with extract) using following formula:

$$\% \text{ SCV} = \frac{\text{A0-A1}}{\text{A0}} \times 100$$

Where, SCV = Radical scavenging activity; A0 = Absorbance of the control; A1 = Absorbance of the test sample (extracts / positive control).

Analgesic activity study: Analgesic activity study was performed by acetic induced writhing method described by Koster and Turner with slight modification where necessary. Experimental animals (Swiss albino mice) were randomly selected and divided into four groups denoted as negative control, positive control, 250 & 500 mg/kg dose group for each extract consisting of 3 mice in each group. Each mouse was weighed properly and the dose of the test samples and control materials were adjusted accordingly. Test sample, negative control and diclofenac-Na were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Acetic acid solution (0.7%, 15 ml/kg) was administered intra-peritoneally. After an interval of five minutes, which was given for absorption of acetic acid, number of writhing was counted for 30 minutes (Koster *et al.*, 1959; Turner, 1971).

Screening for antidiarrheal activity: The study was conducted according to the method described by Shoba and Thomas (Shoba and Thomas, 2001). The animals were divided into control, positive control and two test groups containing three mice in each group. Control group received vehicle (1% Tween-80 in water) at a dose of 10 ml/kg body weight orally. The positive control group was given loperamide at the dose of 3 mg/kg orally whereas, test groups were given ethanol extract, *n*-hexane extract and chloroform extract of *G. coronaria* at the dose of 500 mg/kg and 250 mg/kg body weight orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhea was induced by oral administration of 0.4 ml castor oil to each mouse, 30 minutes after the above treatments. 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. A numerical score based on stool consistency was assigned (normal stool =1 and watery stool = 2).

Assessment of anti-amylase activity: Inhibition of α-amylase activity was measured using the starch-iodine method. 20 µl of α-amylase solution (0.050 mg/ml) was mixed with 1.3 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and 80 µl of ethanolic, chloroform and n-hexane extract of *G. coronaria*. After incubation at 37ºC for 20 min, 100 µl of the starch solution (0.1%) was added (except blank), and the mixture re-incubated for 20 min., after which 2 ml of 0.01% acidic iodine solution was added and the absorbance measured at 578 nm. Inhibition of amylase activity was compared with the reference standard, acarbose (Ngounou *et al.*, 2000).

Results and Discussion

Phytochemicals screening: Phytochemical screening revealed that the ethanol extract of *G. coronaria* contained glycosides, alkaloids, tannins, saponins and flavonoids. Chloroform extract contained glycosides, tannins and flavanoids and n-hexane extract showed the presence of glycosides,

alkaloids, tannins, saponins, flavonoids and reducing sugars (Table 1).

Screening for antimicrobial activities: The crude ethanol extract of *G. coronaria* showed maximum inhibition against *B. cereus* (15 mm) and *B. dermatitis* (11 mm). n-Hexane extract showed maximum zone of inhibition of 12.7 mm and 11.7 mm against *S. aureus* and *C. albicans*, respectively. Crude chloroform extract of *G. coronaria* significantly inhibited the growth of *B. cereus* (15.3 mm) and *C. albicans* (12.7 mm), which were considered as mild in comparison to reference standards (Tables 2 & 3).

Anti-inflammatory activity: In anti-inflammatory test, crude ethanolic extract of *G. coronaria* showed moderate activity (IC_{50} 4.24 µg/ml) compared to the reference standard, acetyl salicylic acid, ASA (IC_{50} 1.99 µg/ml). The n-hexane extract showed no significant activity (IC_{50} 10.08 µg/ml) and the chloroform extract showed inactivity (Table 4).

Antioxidant Activity: In the quantitative antioxidant test ethanol, n-hexane and chloroform extracts of *G. coronaria* produced significant inhibition of 53.92%, 30.79% and 20%, respectively as compared to reference standard, ascorbic acid (90.37%) shown in table 5 at the dose of 100 µg/ml. IC_{50} values for ethanol, n-hexane and chloroform extracts were 5.15, 8.75 and 12.71µg/ml, respectively whereas for ascorbic acid it was 0.47µg/ml (Table 6).

Table 1. Chemical groups for the plant extractives.

Chemical groups	Alkaloids	Glycosides	Steroids	Tannins	Flavonoids	Saponins	Reducing sugars	Gums	Amides
Ethanolic extract	+	+	-	+	+	+	-	-	-
Chloroform extract	-	+	-	+	+	-	-	-	-
n-Hexane extract	+	+	-	+	+	+	+	-	-

'+' = present, '-'= absent.

Table 2. Zone of inhibition by the treatment groups against pathogenic bacteria.

	Zone of inhibition (MZI±SD) mm			
	Ethanolic extract of *G. coronaria*	n-Hexane extract of *G. coronaria*	Chloroform extract of *G. coronaria*	Reference standard (Ciprofloxacin)
Bacteria				
Gram positive species				
B. cereus	15.0 ± 1	9.0 ± 2.0	15.3 ± 1.2	16.0 ± 1.0
B. megateriuum	11.0 ± 1	9.7 ± 1.5	12.5 ± 1.8	14.7 ± 1.5
B. subtilis	12.0 ± 1	7.3 ± 1.5	13.0 ± 1.0	16.0 ± 1.0
S. aureus	13.0 ± 0	12.7 ± 0.6	13.3 ± 0.6	16.7 ± 1.5
Gram negative species				
E. coli	8.7 ± 1.2	9.3 ± 2.3	8.3 ± 0.6	15.5 ± 0.50
Sh. Dysenteriae	8.7 ± 1.0	8.7 ± 1.2	6.5 ± 0.5	14.7 ± 1.0
Sh. Sonnei	10.0 ± 1.0	NI	9.3 ± 0.58	13.8 ± 0.3
Sal. Paratyphi	11.0 ± 1.0	NI	NI	12.5 ± 1.5
Sal. Typhi	7.7.0 ± 0.6	NI	NI	13.0 ± 0.50
P. aeruginosa	12.0 ± 1.0	12.0 ± 1	10.0 ± 1	11.3 ± 1.0
V. cholerae	NI	NI	NI	13.8 ± 0.3

[a]$p<0.05$, [b]$p<0.001$; MZI: Mean zone of inhibition (mm); zone of inhibitions under 7 mm were considered as less active and were discarded. NI=No inhibition.

Table 3. Zone of inhibition by the treatment groups against pathogenic fungi.

Fungi	Zone of inhibition (MZI±SD) mm			
	Ethanol extract of *G. coronaria*	n-hexane extract of *G. coronaria*	Chloroform extract of *G. coronaria*	Reference standard (Fluconazole)
A. niger	9 ± 0.2^a	10.3 ± 0.2^b	10 ± 0.3^a	14.3 ± 0.3^a
B. dermatitidis	11 ± 0.5^a	11 ± 0.3^b	11.7 ± 0.2^b	12.3 ± 0.3^a
C. albicans	10 ± 0.42^b	11.7 ± 0.2^a	12.7 ± 0.5^a	15 ± 0.5^b
P. ovale	9 ± 0.23^a	10 ± 0.5^a	7.7 ± 0.2^b	13.3 ± 0.3^a
Tricho. sp.	NI	NI	NI	11 ± 0.4^a
Micro. sp.	NI	NI	NI	15.3 ± 0.5^a
C. neoformans	NI	NI	NI	14.3 ± 0.3^b

$^a p<0.05$, $^b p<0.001$; MZI: Mean zone of inhibition (mm); zone of inhibitions under 7 mm were considered as less active and were discarded. NI=No inhibition. (n=3).

Table 4. *In vitro* anti-inflammatory test.

Test groups	Dose	SD	SEM	% IPD
Control (DDW)	---	0.00	0.00	00.00
Standard (ASA)	500 µg/ml	0.0038	0.0027	69.4^a
	250 µg/ml	0.0070	0.0050	49.75^b
	125 µg/ml	0.0020	0.0014	31.37^b
Ethanolic extract of *G. coronaria*	500 µg/ml	0.0055	0.0039	41.26^a
	250 µg/ml	0.0038	0.0027	38.81^b
	125 µg/ml	0.0031	0.0022	29.08^a
n-Hexane extract of *G. coronaria*	500 µg/ml	0.0010	0.0007	24.75^a
	250 µg/ml	0.0010	0.0007	21.57^a
	125 µg/ml	0.0010	0.0007	17.65^b
Chloroform extract of *G. coronaria*	500 µg/ml	0.0010	0.0007	13.24^a
	250 µg/ml	0.0010	0.0007	12.25^b
	125 µg/ml	0.0010	0.0007	9.80^a

SD = Standard deviation, SEM = Standard error of mean, % IPD= % Inhibition of protein denaturation $^a p<0.0001$, $^b p<0.05$.

Table 5. DPPH inhibitory action of crude extracts and reference standard.

Concentration (µg/ml)	% inhibition by ethanol extract	% inhibition by n-hexane extract	% inhibition by chloroform extract	% inhibition by ascorbic acid
100	53.92	30.79	20.0	90.38
80	33.57	19.46	15.85	85.0
60	17.49	10.91	10.36	81.63
40	11.29	6.90	6.22	65.63
20	5.19	6.59	4.97	51.25

Table 6. IC_{50} of treatment groups.

Treatment groups	IC_{50} (µg/ml)
Ascorbic acid	0.47
Ethanolic extract	5.15
n-Hexane extract	8.75
Chloroform extract	12.71

Table 7. Effect of crude extracts on acetic acid induced writhing in mice.

Clinical groups	No. of mice	BW (gm)	WC	MW	% Writhing	% WI	No. of Writhing	SD	SEM	t- test (p values)
Negative control (1% Tween-80)	1	18	65	62.0	100	0	286 ± 7.495	14.99	7.495	-
	2	19	62							
	3	22	59							
Positive control (Diclofenac-Na, 25 mg/kg)	1	19	10	10.3	16.66	83.33	83 ± 0.408	0.577	0.408	89.47 (<0.05)
	2	22	11							
	3	24	10							
EEGC (250 mg/kg)	1	18	50	44.0	70.97	29.03	198 ± 4.425	8.85	4.425	2.281 (<0.05)
	2	20	43							
	3	20	39							
EEGC (500 mg/kg)	1	21	42	42.7	68.82	31.18	169 ± 6.219	12.438	6.219	3.098 (<0.05)
	2	21	45							
	3	22	41							
NEGC (250 mg/kg)	1	21	51	53.0	85.48	14.52	188 ± 4.575	9.15	4.575	3.57 (<0.05)
	2	20	55							
	3	23	53							
NEGC (500 mg/kg)	1	19	49	47.3	76.34	23.66	160 ± 5.85	11.705	5.853	3.06 (<0.05)
	2	18	46							
	3	25	47							
CEGC (250 mg/kg)	1	19	56	50.67	85.48	14.52	253.35 ± 3.34	4.73	3.34	4.83 (<0.01)
	2	18	49							
	3	25	47							
CEGC (500 mg/kg)	1	19	41	46.7	75.27	24.73	233.5 ± 3.63	5.13	3.63	3.76 (<0.05)
	2	18	48							
	3	25	51							

* BW = Body weight. WC = Writhing count, MW = Mean writhing, WI = Writhing inhibition, No. of writhing = (MW×5 ± SEM), SD = Standard deviation, SEM = Standard error of mean, EEGC = Ethanolic extract of *G. coronaria*, NEGC = n-Hexane extract of *G. coronaria*, CEGC = Chloroform extract of *G. coronaria*

Analgesic activity: The ethanol extract of *G. coronaria* exhibited inhibition of writhing reflex by 29.03% (P<0.05) and 31.18% (P<0.05), at the dose of 250 & 500 mg/kg body weight, respectively while the n-hexane extract inhibited writhing reflex by 14.52% and 23.67% (p< 0.05) at the dose of 250 & 500 mg/kg body wt. respectively (Table 7). Chloroform fraction of the plant showed similar inhibition of 14.52% and 24.73% at the same dose. The reference

standard, diclofenac-Na was used at the dose of 25 mg/kg body weight.

Antidiarrheal activity: The different extracts of the *G. coronaria* at the doses of 250 mg/kg and 500 mg/kg body weight of experimental animal, reduced the total number of stool count to a considerable extent as well as increased the latency period in comparison to the control groups of castor oil induced diarrheal mice. The n-hexane extract of *G. coronaria* significantly increases latency (75.67 minute) which was close to the reference standard, loperamide (85 minute) as shown in table 8. The chloroform extract also showed significant inhibition of diarrhea.

Table 8. Effect of the different fractions of *G. coronaria* on the latent period of castor oil induced diarrheal episode in mice.

Treatment groups	No. of mice	Latency (min)	Mean latency (min)	SD of latency	SE of latency	no. of stool	mean of stool	SD of stool	SE of stool
(-) cont	1	40	47.67	7.51	5.31	15	15.67	2.08	1.47
	2	48				18			
	3	55				14			
(+) cont	1	90	85.00	6.24	4.42	4	5.33	1.53	1.08
	2	87				7			
	3	78				5			
EE-500	1	71	71.67	7.02	4.97	7	7.33	0.58	0.41
	2	65				7			
	3	79				8			
EE-250	1	65	65.67	4.04	2.86	8	9.33	1.15	0.82
	2	70				10			
	3	62				10			
NE-500	1	80	75.67	5.13	3.63	8	6.00	1.73	1.22
	2	70				5			
	3	77				5			
NE-250	1	64	69.67	4.93	3.49	12	10.33	1.53	1.08
	2	72				9			
	3	73				10			
CE-500	1	62	63.00	5.57	3.94	12	11.67	0.58	0.41
	2	69				11			
	3	58				12			
CE-250	1	50	52.00	4.36	3.08	13	13	2	1.41
	2	57				11			
	3	49				15			

(-) control = Negative control, (+) cont = Positive control, EE-500 = Ethanol extract at dose 500 mg/kg, EE-250 = Ethanol extract at dose 250 mg/kg, NE-500= n-Hexane extract at dose 500 mg/kg, NE-250 = n-Hexane extract at dose 500 mg/kg, CE-500 = Chloroform extract at dose 500 mg/kg, CE-250 = Chloroform extract at dose 250 mg/kg, SD = Standard deviation, SE = Standard error.

Anti-amylase activity: Consecutive doses of different extractives from 31.25-500µg/ml produced a dose graded inhibition of amylase activity (Table 10). Mild to moderate α-amylaese inhibitory activities were observed by different extractives of *G. coronaria* in comparison to the reference standard, acarbose.

Table 9. Anti-amylase activity of the standard and extract.

Treatment groups	Concentration (µg/ml)	% Inhibition of amylase activity
Acarbose	31.25	18.75
	62.50	27.9
	125	35.14
	250	47.06
	500	69.23
EEGC	31.25	11
	62.50	14.29
	125	20
	250	35.56
	500	43.75
NEGC	31.25	8.26
	62.50	17.69
	125	23
	250	35.29
	500	44.78
CEGC	31.25	2.6
	62.50	3.74
	125	7.07
	250	12.36
	500	18.6

EEGC, NEGC & CEGC = Ethanol extract, n-hexane extract and chloroform extract of *G. coronaria* respectively

Conclusion

Phytochemical screening suggested the presence of bioactive components from the fractional extractives of *G. coronaria*. Almost all Gram positive bacterial strains were significantly inhibited by the all three crude extractives in antibacterial study. However, among the fungi, *B. dermatitidis* & *C. albicans* were strongly inhibited. In case of *in-vitro* anti-inflammatory test, crude ethanolic extract of *G. coronaria* showed moderate activity compared to the standard acetyl salicylic acid. The ethanol fraction of the plant can be considered for further study as a potential source of anti-oxidant. Among the *in-vivo* tests on mice, the crude extracts exhibited considerable anti-diarrheal activity. Both ethanol and n-hexane extracts showed moderate anti-amylase activity.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgement

The authors are grateful to the Department of Pharmacy, Southern University Bangladesh for providing lab facilities. We would like to show our gratitude to the authority of Southern University Bangladesh for providing relevant facilities to perform this study. We also acknowledge the cordial helps of the taxonomists of Bangladesh Forest Research Institute (BFRI), and scientific officers of Bangladesh Council of Scientific & Industrial Research (BCSIR), Chittagong.

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