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Antimicrobial and Cytotoxic Effects of a Glycoside from *Caesalpinia pulcherrima* Swartz

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Barks of *Caesalpinia pulcherrima* yielded a glycoside, Lathyrol-3-phenylacetate-5,15-diacetate (CP-2). The compound, CP-2, showed significant antibacterial and antifungal effects. The minimum inhibitory concentration (MIC) of CP-2 against *Bacillus cereus* and *Shigella dysenteriae* was determined and the values were 64 and 32 μg ml^{-1}, respectively. The cytotoxicity of CP-2 was determined by brine shrimp lethality bioassay and LC_{50} value was 5.39 μg ml^{-1}. The mortality rate by the compound with highest concentration suggests the drug to be used at highest doses and also suitable for further clinical trial.

Key words: *Caesalpinia pulcherrima,* glycoside, antimicrobial activity and cytotoxicity

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INTRODUCTION

Bangladesh has a rich plant kingdom and some of which are good sources of herbal medicines. The plants belonging to the family Leguminoceae have wide folkloric medicinal uses. *Caesalpinia pulcherrima* Swartz. vernacularly known as Radha chura in Bangladesh, belonging to the family Leguminoceae is widely distributed in Bangladesh and India[1]. Leaves, flowers, bark and seeds are largely used in Indian medicine (Watt). In Indo China, the plant is considered as a tonic, stimulant and emmenagogue. The bark is used as an abortifacient and an infusion of leaves is used as abortifacient, antiperiodic and cathartic as well as infusion of flowers is pectorial and febrifuge[1]. It is obvious that there are some sorts of pharmaceutically active principles in this plant, which may have physiological functions after administration. The physiologically active principles of the plant are of great importance from the medicinal point of view. Therefore, we have investigated further for the isolation of physiologically active principles from this plant.

We report here the isolation, characterization and antimicrobial effects as well as cytotoxicity of a glycoside isolated from the chloroform extract of barks of *Caesalpinia pulcherrima* Stwertz.

MATERIALS AND METHODS

Collection of the materials: Barks of *Caesalpinia pulcherrima* were collected from the rural area of the district of Gaibandha in Bangladesh after identification of the plant by Bangladesh National Herberium, Dhaka.

Extraction and isolation: The powdered bark materials (ca. 850 g) were extracted with chloroform at room temperature after washing the materials with petroleum ether to remove the fatty and waxy matters. The chloroform extract was concentrated by a vacuum rotary evaporator under reduced pressure and was subjected to column chromatography over silica gel followed by TLC and Preparative TLC. The compound, CP-2 was obtained in the pure state.

Spectral characteristics: White powder, m.p. 123-127°C; IRν_{max}:1740.2, 1728, 1647, 1278.7, 1263.2, 1238.1, 1128, 1010.9, 1057 cm^{-1}; ^{1}H-NMR: δ3.36 (1H, dd, J=14.8 Hz, H_α-1), 1.42 (1H, dd, J=14.11 Hz, H_β-1), 2.20 (1H, m, H-2), 5.58 (1H, t, J=3.5 Hz, H-3), 2.76 (1H, dd, J=10 Hz, H-4), 6.10 (1H, d, J=10 Hz, H-5), 2.20 (1H, m, H-7), 2.06 (1H, m, H-7), 2.02 (1H, m, H_α-8), 1.74 (1H, m, H_β-8), 1.15 (1H, m, H-9), 1.37 (1H, dd, J=11 Hz, H-11), 6.50 (1H,d, J=11 Hz, H-12), 0.71 (3H, d, J=6.5 Hz, H-16), 4.99 (1H, s, H-17), 4.72 (1H, s, H-17), 1.18 (3H, s, H-18), 1.15 (3H, s, H-19) and 1.69 (3H, s, H-20), 7.33-7.21 (2H, OPhAc), 3.63 (3H, d, J=15 Hz, OCO CH_3), 3.61 (3H, d, J=15 Hz, $OCOCH_3$). ^{13}C-NMR: δ 48.31 (t, C-1), 37.40 (d, C-2), 80.50 (d, C-3), 52.19 (d, C-4), 65.80 (d, C-5), 144.30 (s, C-6), 35.00 (t, C-7), 21.30 (t, C-8), 35.30 (d, C-9), 25.20 (d, C-10), 28.40 (d, C-11), 140.70 (d, C-12), 134.10 (s, C-13), 196.80 (s, C-14), 92.30 (s, C-15), 13.70 (q, C-16), 115.60 (t, C-17), 29.00 (q, C-18), 16.80 (q, C-19), 12.40 (q, C-20), 169.80 (s, PhAc), 135.30 (s, PhAc-2), 129.50 (d, PhAc), 128.50 (d, PhAc), 127.10 (d, PhAc), 41.50 (d, PhAc), 171.30 (s, Ac), 170.30 (s, Ac), 22.00 (q, Ac), 22.01 (d, Ac).

Antibacterial screening: *In vitro* antibacterial activity of the isolated compound, CP-2 was studied against five gram-positive bacteria and nine gram-negative bacteria strains by the standard disc-diffusion method[2-4]. Nutrient agar was used as the bacteriological medium. The compound was dissolved in sufficient volume of methanol to get a concentration of 200 μg/10 μl. The diameters of zone of inhibition produced by the agent were compared with those produced by the standard antibiotic (Kanamycin, 30 μg $disc^{-1}$). The experiment was performed in duplicate to minimize the error.

Minimum inhibitory concentration (MIC): The MIC value of the compound CP-2 was determined against one gram-positive (*Bacillus cereus*) and one gram-negative (*Shigella dysenteriae*) bacteria. The test was carried out by serial dilution technique[5]. Nutrient agar and nutrient broth were used as bacteriological media.

Antifungal screening: Seven pathogenic fungi were used for the antifungal test. Potato Dextrose agar was used as fungicidal medium. The compound was dissolved in sufficient volume of methanol to get a concentration of 200 μg/10 μl. The *in vitro* antifungal activities of compound CP-2 was performed by disc diffusion method[3]. Clotrimazole was used as a standard.

Cytotoxic evaluation: The cytotoxic effect of the compound was evaluated by LC_{50} of brine shrimp lethality test[6,7]. The compound CP-2 was dissolved in dimethylsulphoxide (DMSO) seperately and five graded doses 5, 10, 20, 40 and 80 μg ml^{-1}, respectively were used for 5 ml sea water containing 10 brine shrimp nauplii in each group. The number of survivors was counted after 24 h and LC_{50} value was determined by Probit analysis[8]. The experiment was carried out quadruplicate and the mean LC_{50} value was measured.

RESULTS AND DISCUSSION

The compound, CP-2 was isolated from the chloroform extract of *Caesalpinia pulcherrima* and the structure was identified on the basis of its spectral data incorporated with chemical evidences. The compound CP-2 was a white powder, m.p. 123-127°C. The IR spectrum of the compound showed a strong absorption at 1740.20 cm^{-1} and 1728 cm^{-1} which indicated the presence of ester CO and ketonic CO groups. ^{1}H-NMR spectrum of compound CP-2 showed two signals at δ 4.99 (1H) and δ 4.73 (1H) for the two olefinic protons at C-17. A multiplet at δ 7.33-7.21 (2H) indicated two protons for $-COCH_2Ph$ group and two doublets at δ 3.63 (3H, J=15 Hz) and δ 3.61 (3H, J=15 Hz) for the two acetyl methyl protons. A pair of doublet at δ 3.36 (1H, dd, J = 14, 8 Hz, H_α-1) and another

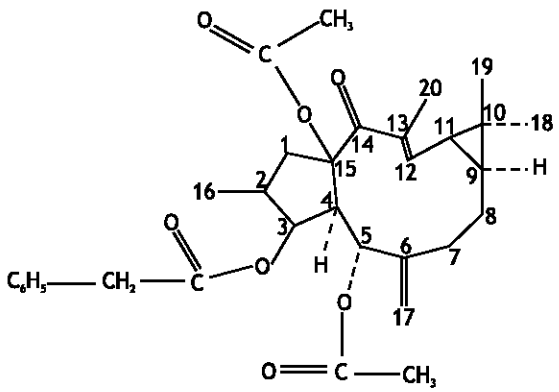

Fig. 1: Structure of CP-2

pair of a doublet at δ 1.42 (1H, dd, J=14, 11 Hz, H_β-1) were exhibited by the two protons at C-1. ^{13}C NMR spectrum of compound CP-2 showed signals at δ 144.30 (s) and at δ 115.60 (m) for the resonances of C-6 and C-17, respectively. Signals at δ 169.80 (s), 135.30 (s), 129.50 (d), 128.50 (d), 127.10 (d) and 41.50 (t) revealed the resonances for the corresponding $-COCH_2Ph$ carbons. Signals at δ 171.30 (s), 170.00 (s), 22.00 (q) and 22.01 (d) are exhibited for carbons of $-OCOCH_3$ groups. Carbon resonance in ^{13}C NMR spectrum of CP-2 were similar with those of Lathyrol-3-phenylacetate-5,15-diacetate[9]. Combination of IR, ^{1}H NMR and ^{13}C NMR coupled with physical and chemical evidences, the structure of CP-2 was finally characterized as lathyrol-3-phenylacetate-5,15-diacetate (Fig. 1). This is the first report of isolation of the compound from this plant.

Results of antibacterial and antifungal effects are presented in Table 1 and Table 2. Compound CP-2 showed significant antibacterial activity against all the bacteria tested. The concentration of the compound was taken 200 μg $disc^{-1}$. The zones of inhibition produced by compound were within the range from 16 to 26 mm.

Table 1: Antibacterial activity of compound, CP-2

	Diameter of the zone of inhibition (mm)	
Test organisms	A	B
Gram-positive		
Bacillus cereus	26	29
Bacillus subtilis	17	25
Bacillus megaterium	20	26
Staphylococcus aureus	19	27
Streptococcus β-haemolyticus	16	21
Gram-negative		
Shigella dysenteriae	24	28
Escherichia coli	19	25
Shigella sonnei	20	27
Shigella shiga	18	23
Shigella boydii	20	26
Shigella flexneriae	17	27
Pseudomonas aeruginosa	16	23
Salmonella typhy-A	17	23
Salmonella typhy-B	18	25

A= CP-2, 200 μg $disc^{-1}$ B= Standard Kanamycin, 30 μg $disc^{-1}$

Table 2: Antifungal activity of compound, CP-2

	Diameter of the zone of inhibition (mm)	
Test organisms	A	C
Aspergillus fumigatus	7	22
Candida albicans	-	24
Saccharomyces cerevaceae	9	26
Hensinela colifornica	8	25
Pigment yeast	-	21
Rhizopus aurizae	7	23
Rhizopus oligasporum	6	22

A=CP-2, 200 μg $disc^{-1}$ C= Standard Clotrimazole, 30 μg $disc^{-1}$
'-'= No sensitivity

Table 3: The minimum inhibitory concentration (MIC) of the compound, CP-2 against test organisms

	The minimum inhibitory concentration (MIC) in μg ml^{-1}	
Sample	*Bacillus cereus*	*Shigella dysenteriae*
CP-2	64	32

The compound, CP-2 showed antifungal activity against all the fungi tested except *Candida albicans* and *Pigment yeast* and produced the zone of inhibition in between 6 and 9 mm (Table 2).

The minimum inhibitory concentration (MIC) of the compound CP-2 against *Bacillus cereus* and *Shigella dysenteriae* was determined and the values were 64 and 32 μg ml^{-1}, respectively (Table 3).

The cytotoxicity of the compound was accomplished by brine shrimp lethality bioassay and the results were shown in Table 4. The 50% mortality concentration (LC_{50}) of the compound, CP-2 was 5.39 μg ml^{-1} and 95% confidence limits were 1.33-21.82. A regression equation of the compound Y=4.29+0.97X and χ^2 value 6.33 are observed from the probit analysis which were compared with galic acid[10] as a standard one.

Table 4: Cytotoxicity of the compound, CP-2 by brine shrimp lethality bioassay

Test sample	Concentration μg ml^{-1}	% Mortality	LC_{50}	95% Confidence limit	Regression equation	χ^2 value
	5	40				
	10	60				
Compound (CP-2)	20	70	5.39	1.33-21.82	Y=4.29+0.97X	6.33
	40	80				
	80	90				
Galic acid	-	-	4.53	3.33-6.15	Y=3.93+1.62X	1.25

In conclusion, the present study reports here the characterization, antimicrobial and antifungal effects and cytotoxicity of the compound CP-2 isolated from *Caesalpinia pulcherrima* Stwertz. This compound may be used as a potent compound to the development of a potential antimicrobial and cytotoxicological agent.

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