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Antimicrobial activity and Cytotoxicity of Clerodane Diterpines from *Polyalthia longifolia* seed

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Two clerodane diterpines viz., Kolavenic acid (1) and 16-oxo-cleroda-3, 13(14) E-di ene-15-oic acid (2) were isolated from the petroleum ether (C_2H_5-O-C_2H_5) extract of the seed of *Polyalthia longifolia* and were screened against fourteen pathogenic bacteria for their antibacterial activities. The test materials exhibited strong activities against most of the test bacteria. The minimum inhibitory concentration (MIC) of the compound (1) and (2) was determined against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Shigella flexneriae* and *Shigella boydii* which were 64, 64, 32, 16 and 32μg ml^{-1} for compound (1) and 16, 32, 8, 4 and 16 μg ml^{-1} for compound (2), respectively. The cytotoxic activity of the compound (1) and (2) was determined by brine shrimp lethality bioassay. Both the compounds showed significant cytotoxic activities and LC_{50} values of Kolavenic acid (1) and 16-oxo-cleroda-3, 13(14)E-diene-15-oic acid (2) were 3.16 and 2.52μg ml^{-1}, respectively.

Key words: *Polyalthia longifolia*, clerodane diterpene, antimicrobial activity, cytotoxicity

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Introduction

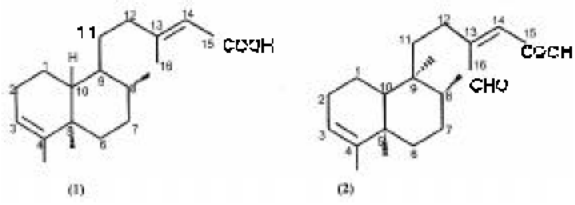
Polyalt hia longifolia (Family Annonaceae) is a tree, which is widely distributed in Bangladesh, Srilanka and throughout the hotter parts of India (Hooker and Clarke, 1875). In India, the seeds of this plant were used as febrifuge (Raghunathan and Mitra, 1982). The bark is also used as a febrifuge in the Balasore district of Orissa (Kirtikar, 1993). From the literature survey it is revealed that most of the plants of annonaceae family contain antitumor and anticancer principles (Chakrabarti and Mukherjee, 1968; Yamaguchi *et al*., 1964). The extract of stem bark and the alkaloids isolated from this extract were found to demonstrate a good antibacterial and antifungal activities (Hasan *et al*., 1988b). Its aqueous extract stimulates the isolated ileum and uterus, depresses heart, lowers the blood pressure and respiration in experimental animals (Achari and Lal, 1952). The crude extracts of the seeds of this plant also showed remarkable antibacterial activities (Sayeed *et al*., 1995). Two compounds, viz., Kolavenic acid (1) and 16-oxo-cleroda-3, 13(14) E-diene-15-oic acid (2) have been isolated and characterized from the petroleum ether (C_2H_5-O-C_2H_5) extract of *Polyalthia longifolia* seeds (Islam, 1995).

As a part of our continuing search for antimicrobial agents from the medicinal plants of Bangladesh, we have investigated the antimicrobial activity of the above two clerodane diterpines and report the result of such examination. In this paper we also report the cytotoxicity of the two clerodane diterpines.

Materials and Methods

Plant material: After identification of the plant *Polyalthia longifolia* by Department of Botany, University of Rajshahi, about 700gm of matured, healthy seeds were collected from the campus of Rajshahi University. The seeds were cleaned, washed with water and dried. Finally, the seeds were ground to course powder by a grinder.

Extraction and isolation of the compounds: The powder materials were extracted in a soxhlet apparatus with petroleum ether, (40-60)^{0}C (Morrison and Boyd, 1994). The petroleum ether (C_2H_5-O-C_2H_5) was then subjected to vacuum liquid chromatography followed by preparative thin layer chromatography (Egon and Stahl, 1969) to obtain two pure compounds which were identified as Kolavenic acid (1) and 16-oxo-cleroda-3,13 (14) E-diene-15-oic acid (2) by extensive spectroscopic [UV (Beckman DU-64); IR (Erkinelmer); EIMS; ^{1}H-NMR 500 MHZ; ^{13}C-NMR 50 MHZ] analysis [Islam, 1995].



Kolavenic acid 16-oxo-cleroda-3, 13(14) E-diene-15-oic acid

Antibacterial screening: Fourteen pathogenic bacteria (four Gram positive and ten Gram negative) were selected for the test which were collected from the Department of Microbiology, University of Dhaka, Dhaka, Bangladesh. Nutrient agar was used as a bacteriological media. The isolated compounds (1) and (2) were dissolved separately in ethyl acetate ($CH_3COOC_2H_5$) to get a concentration of 100 and 200μg 10 μl^{-1}. Then *in vitro* antimicrobial activity of the compound (1) and (2) was carried out by the standard disc diffusion method (Berghe and Vlietuck, 1991) against selected organisms. The diameter of the zone of inhibition produced by the compound (1) and (2) were compared with those of a standard antibiotic (Kanamycin 30μg disc^{-1}).

Antifungal screening: Seven pathogenic fungi were selected for the test and collected from the Department of Botany, University of Rajshahi, Bangladesh. Potato Dextrose Agar (PDA) was used as a fungicidal media. The compound (1) and (2) were dissolved separately in sufficient volume of methanol (CH_3OH) to get a concentration of 200μg disc^{-1}. Then *in vitro* antifungal activities of the compound (1) and (2) were performed by disc diffusion method (Bauer *et al*., 1966). Clotrimazole (30μg disc^{-1}) was used as a standard disc.

Minimum inhibitory concentration (MIC): The MIC values of the compound (1) and (2) were determined against two Gram-positive (*Bacillus subtilis and Bacillus cereus*) and three Gram-negative (*Escherichia coli, Shigella flexneriae and Shigella boydii*) bacteria. The test were carried out by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Cytotoxic evaluation: The cytotoxic effect of compound (1) and (2) were evaluated by LC_{50} of brine shrimp lethality test (Mayer *et al*., 1982 and Persoone, 1980). The compound (1) and (2) were dissolved in dimethylsulphoxide (DMSO) separately and six graded doses 1, 2, 4, 8 and 16 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 hours and LC_{50} value was determined by Probit analysis (Gujrati, 1998 and Finney, 1947).

Results and Discussion

The antibacterial activities of compound (1) and (2) isolated from *Polyalthia longifolia* seed against fourteen pathogenic bacteria are presented in Table 1. The concentration of the compound (1) and (2) were taken 100 and 200μg disc^{-1}. All Gram positive and Gram negative bacteria showed remarkable sensitivity towards the compound (1) and (2) except *Salmonella typhi*A and *Klebsiella* species. *Bacillus subtilis, Bacillus cereus* and *Bacillus megaterium* (Gram positive) and *Escherichia col, Shigella flexneriae,* and *Shigella boydii* (Gram negative) bacteria were excellently sensitive to compound (1) and (2). The antifungal activities of the compound (1) and (2) are presented in Table 2. It is revealed that the compounds showed significant antifungal activity against seven pathogenic fungi.

Minimum inhibitory concentration (MIC) of compound (1) and (2) is presented in Table 3. Both the compounds showed significant activity against *Bacillus subtilis, Bacillus cereus, Escherichia coli, Shigella flexneriae* and *Shigella boydii*. As evident from the Table 4 the compounds, (1) and (2) showed promising cytotoxic activity when performed brine shrimp bioassay. After 24 hrs of observation, the number of survived nauplii was counted in both experimental and control. In control group the nauplii remained unchanged but in experimental groups, the percentage of mortality of brine shrimp nauplii was calculated for each concentration and the rate of mortality was found to be increased with increase in the concentration of samples (Table 4).

The 50% mortality (LC_{50}) of the compound (1) and (2) was 2.95 and 2.28μg ml^{-1} and 95 % confidence limits were 1.55-5.61 and 1.16-4.49, respectively. A regression

Table 1: Antibacterial activities of compound (1) and (2) isolated form *Polyalthia longifolia* seeds

	Diameter of zone of inhibition in mm after 24 hours of incubation				
	Compound (1)		Compound (2)		SK
Test organisms	100 μg disc^{-1}	200 μg disc^{-1}	100 μg disc^{-1}	200 μg disc^{-1}	30 μg disc^{-1}
Grams positive					
Bacillus cereus	29	34	30	35	24
Bacillus subtilis	30	35	25	32	26
Bacillus megaterium	27	32	26	32	28
Staphylococcus aureus	26	30	20	28	27
Gram negative					
Escherichia coli	24	30	31	36	27
Shigella flexneriae	36	45	30	35	29
Shigella shiga	26	37	27	33	22
Shigella dysenteriae	20	27	21	29	27
Shigella sonnei	34	40	9	12	30
Salmonella typhi-A	8	10	10	13	25
Salmonella typhi-B	14	19	9	12	28
Shigella boydii	32	39	20	29	25
Pseudomonas aeruginosa	14	20	8	11	23
Klebsiella species	9	12	10	15	19

Compound (1) = Kolavenic acid (1)
Compound (2) = 16-oxo-cleroda-3, 13 (14) E-diene-15-oic acid (2)
SK = Standard Kanamycin

Table 2: Antifungal activities of the compound (1) and (2) isolated form *Polyalthia longifolia* seeds

	Diameter of zone of inhibition in mm		
	Compound (1)	Compound (2)	SC
Test organisms	200 μg disc^{-1}	200 μg disc^{-1}	200 μg disc^{-1}
Aspergillus niger	16	17	27
Aspergilli fumigates	15	16	24
Hensinela californica	13	14	22
Pigment yeast	14	15	23
Phizopus arizae	16	15	26
Schizosporum species	17	13	25
Rhizopus arizae	14	11	21

Compound (1) = Kolavenic acid (1)
Compound (2) = 16-oxo-cleroda-3, 13 (14) E-diene-15-oic acid (2)
SC = Standard Clotrimazole

Table 3: The MIC values of the compound (1) and compound (2) against test organisms

	Minimum inhibitory concentration in μg ml^{-1}				
Samples	*Bacillus cereus*	*Bacillus subtilis*	*Escherichia coli*	*Shigella flexneriae*	*Shigella boydii*
Compound (1)	64	64	32	16	32
Compound (2)	16	32	8	4	16

Compound (1) = Kolavenic acid (1)
Compound (2) = 16-oxo-cleroda-3, 13 (14) E-diene-15-oic acid (2)

Table 4: Cytotoxicity of compound (1) and Compound (2) by brine shrimp lethality bioassay

Test sample	Concentration	%Mortality	LC50	95% Confidence limit	Regression Equation	χ^2 value
	1	20				
	2	40				
Compound (1)	4	50	2.95	1.55-5.61	Y = 4.35 + 1.38X	0.470
	8	70				
	16	90				
	1	30				
	2	50				
Compound (2)	4	60	2.28	1.1-4.49	Y = 4.49 + 1.42X	0.109
	8	80				
	16	90				
Gallic acid	-	-	4.53	3.33-6.15	Y = 3.93 + 1.62X	1.25

Compound (1) = Kolavenic acid (1) Compound (2) = 16-oxo-cleroda-3,13 (4) E-diene-15-oic acid (2)

equation of compound (1) and (2), $Y=4.53+1.38X$ and $Y=4.49+1.42X$ and χ^2 value 0.470 and 0.109, respectively are observed from the probit analyses which were compared with gallic acid (Saker *et al.*, 1998) as a standard one. An evaluation of cytotoxicity is also an important study for possible clinical use i.e., indicative of wide range of pharmaceutical activities of the drugs (Mayer *et al.*, 1982). In that sense, the mortality rate of the compounds with highest concentration suggest that the drug can be used at higher doses and also suitable for further clinical trial.

In conclusion, this study reports for the first time, the antibacterial, antifungal activity and cytotoxicity of the compound (1) and (2) isolated from the *Polyalthia longifolia* seed. However, further and specific studies are needed to better evaluate the potential effectiveness of the isolated the compounds from the *Polyalthia longifolia* seed as an antimicrobial agent.

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