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An Antimicrobial Terpenoid from *Caesalpinia pulcherrima* Swartz.: Its Characterization, Antimicrobial and Cytotoxic Activities

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Abstract: A terpenoid, 3-oxo-(20S, 24S)-epoxydammarane-19,25-diacetate (CP-1) isolated from the chloroform extract of barks of *Caesalpinia pulcherrima* was characterized on the basis of spectral data coupled with chemical evidence. The compound showed significant antibacterial activity against a number of pathogenic bacteria and prominent antifungal activity against a few fungi. The minimum inhibitory concentration (MIC) values against *Bacillus cereus* and *Shigella dysenteriae* were 16 μg ml^{-1} and 32 μg ml^{-1} respectively. The compound was found cytotoxic against brine shrimp and LC_{50} value was 8.41 μg ml^{-1}. These findings suggested for further clinical trial.

Key words: *Caesalpinia pulcherrima,* terpenoid, antimicrobial and cytotoxic activities

INTRODUCTION

Bangladesh has a rich plant kingdom and some of which are good source of herbal medicines. The most important development of medicine in the present century is the introduction of chemotherapeutic agents such as sulfadrugs, antibiotics and also of other synthetic drugs. Inspite of these, interest (Hese *et al.,* 1966 and Dreyer, 1969) in the chemistry and pharmacology of medicinal plants has increased considerably in resent years. Morphine was isolated from opium by Sertuner (Burger, 1960) and ephedrine (Hese *et al.,* 1966) from *Ephedra vulgaris*. Many other chemical compounds have since then been isolated and their chemistry and pharmacological action were studied.

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 (Kirtikar and Basu, 1994). 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 aborticient, antiperiodic and cathartic as well as infusion of flowers is pectorial and febrifuge (Kirtikar and Basu, 1994). It is obvious that there are some sorts of pharmaceutically active principles in this plant, which may exhibit physiological functions after administration. The physiologically active principles of the plant are of great importance from the medicinal point of view. Therefore, isolation of physiologically active constituents from this plant was an interesting investigation to us.

In this paper, isolation, characterization and antimicrobial action as well as cytrotoxicity of a compound isolated from the chloroform extract of barks of *Caesalpinia pulcherrima* Stwartz have been reported.

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 plant barks (ca. 835 gm) 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. A pure, white solid compound, (CP-1), m.p. 134-137°C, was obtained.

Spectral characterization: IRν_{max}: 2918.1, 2850.6, 1728, 1380.9 and 1056.9 cm^{-1}, ^{1}H NMR: δ 4.21 (1H, *d*, *J*=11.7 Hz, H_a-19), 4.03 (1H, *d*, *J*= 11.7 Hz, H_b-19), 3.89 (1H, *dd*, *J*=9.0 Hz, H-24), 1.94 (3H, *s*, $OCOCH_3$), 1.93 (3H, *s*, $OCOCH_3$), 1.44 (3H, s, H-26), 1.42 (3H, *s*, H-27), 1.14 (3H, *s*, H-21), 7.21 (3H, *s*, H-29), 7.26 (3H, *s*, H-30) and 0.89 (3H, *s*, H-28). ^{13}C NMR: δ 34.20 (C-1), 33.80 (C-2), 216.70 (C-3), 45.80 (C-4), 52.31 (C-5), 19.60 (C-6), 34.40 (C-7), 40.00 (C-8), 51.70 (C-9), 38.61 (C-10), 23.10 (C-11), 25.80 (C-12), 43.01 (C-13), 49.80 (C-14), 31.70 (C-15), 27.01 (C-16), 49.40 (C-17), 16.50 (C-18), 64.50 (C-19), 86.70 (C-20), 27.40 (C-21), 34.60 (C-22), 26.71 (C-23), 84.81 (C-24), 82.80 (C-25), 22.00 (C-26), 22.50 (C-27), 29.40 (C-28), 19.30 (C-29), 15.41 (C-30), 171.20 ($OCOCH_3$), 170.50 ($OCOCH_3$), 20.90 ($OCOCH_3$), 21.80 ($OCOCH_3$).

Antibacterial screening: *In vitro* antibacterial activity of the isolated compound, CP-1, was studied against five gram-positive and nine gram-negative bacterial strains by the standard disc-diffusion method (Barry , 1980; Buer *et al.,* 1966; Berghe and Vlientinck, 1991). Nutrient agar was the bacteriological medium. Compound, CP-1, was dissolved in sufficient volume of methanol to get a concentration of 200 μg per 10 μl. Diameters of zones of inhibition produced by the isolated 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-1, was determined against one gram-positive (*Bacillus cereus*) and one gram-negative (*Shigella dysenteriae*) bacteria. The test was carried out by serial dilution technique (Reiner, 1982). 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. Compound, CP-1, was dissolved in sufficient volume of methanol to get a concentration of 200 μg per 10 μl. The *in vitro* antifungal activity of the compound was performed by disc diffusion method (Beur, 1966). Clotrimazole was used as a standard.

Cytotoxic evaluation: The cytotoxic effect of compound CP-1 was evaluated by LC_{50} of brine shrimp lethality test (Mayer *et al*., 1982 and Persoone, 1980). The compound was dissolved in dimethylsulphoxide (DMSO) separately 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 (Finney, 1947). The experiment was carried out quadruplicate and the mean LC_{50} value was recorded.

RESULTS AND DISCUSSION

The compound, CP-1, isolated from the chloroform extract of *Caesalpinia pulcherrima* was white powder, m.p. 134-137°C. IR spectrum showed a strong absorption band at 1728 cm^{-1} which indicated the presence of ester CO and ketonic CO groups. The spectrum also revealed a C-H streching vibration (in CH_3) at 2918.1 and 1380.9. ^{1}H NMR spectrum showed signals of nine methyl protons at δ 1.93 (3H, *s*, $OCOCH_3$), 1.94 (3H, *s*, $OCOCH_3$), 1.44 (3H, s, H-26), 1.42 (3H, *s*, H-27), 1.14 (3H, *s*, H-21), 7.21 (3H, *s*, H-29), 7.26 (3H, *s*, H-30), 0.89 (3H, *s*, H-28), two doublets at δ 4.21 (1H, *d*, *J*=11.7 Hz, H_a-19) and δ 4.03 (1H, *d*, *J*= 11.7 Hz, H_b-19) and a double doublet at δ 3.89 (1H, *dd*, *J*=9.0 Hz, H-24). ^{13}C NMR spectrum showed signals at δ 86.70 and δ 82.80 for C-20 and C-25 respectively. It displayed signals at δ 84.81 for oxygenated methyl carbon (C-2) and at δ 216.70 was discerned for carbonyl carbon (C-3). The presence of oxygenated carbon resonances at δ 86.7 (*s*) and δ 82.8 (*d*) suggested the presence of a 20,25-trisubstituted tetrahydrofuranyl system as ring E in Fig.1

Comparison of ^{13}CNMR resonances associated with the oxygenated carbons of the tetrahydrofuranyl unit (ring E) in compound, CP-1, with those of 3-oxo-(20S, 24S)-epoxydammarane-19,25-diacetate established the configuration of C-20 and C-24 as 20S and 24S in Fig. 1.

^{13}C NMR data of the compound, CP-1, closely correspond with the same carbon resonances of 3-oxo-(20S, 24S)-epoxydammarane-19,25-diacetate (Das, 1999). On the basis of physical and chemical characteristics as well as IR, ^{1}H NMR and ^{13}C NMR spectral data the compound, CP-1, was finally suggested as 3-oxo-(20S, 24S)-epoxydammarane-19,25-diacetate. This is the first report of isolation of the compound from this plant.

Results of antibacterial and antifungal activities are presented in Table 1 and 2. Compound, CP-1, showed significant antibacterial activity against all the fourteen bacteria tested. The concentration of the compound was taken 200 μg $disc^{-1}$. Zones of inhibition produced by the compound were in between 15 mm and 24 mm.

The compound, CP-1, showed antifungal activity against all the fungi tested except *Pigment yeast* and *Rhizopus oligasporum* and produced the zone of inhibition in between 7 mm and 10 mm (Table 2).

The minimum inhibitory concentration (MIC) of the compound, CP-1, against *Bacillus cereus* and *Shigella dysenteriae* was determined and the values were 16 μg ml^{-1} and 32 μg ml^{-1} respectively (Table 3).

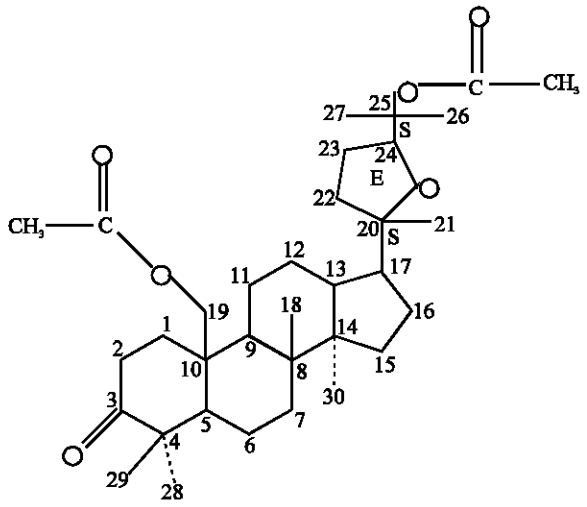

Fig. 1: Structure of compound, CP-1

Table 1: Antibacterial activity of compound, CP-1

	Diameter of the zone of inhibition (mm)	
Test organisms	A	B
Gram-positive		
1. *Bacillus cereus*	24	28
2. *Bacillus subtilis*	18	24
3. *Bacillus megaterium*	15	22
4. *Staphylococcus aureus*	16	26
5. *Streptococcus β-haemolyticus*	17	25
Gram-negative		
1. *Shigella dysenteriae*	22	27
2. *Escherichia coli*	21	26
3. *Shigella sonnei*	19	23
4. *Shigella shiga*	18	25
5. *Shigella boydii*	17	22
6. *Shigella flexneriae*	20	26
7. *Pseudomonas aeruginosa*	16	24
8. *Salmonella typhy*-A	17	26
9. *Salmonella typhy*-B	15	23

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

Table 2: Antifungal activity of compound, CP-1

	Diameter of the zone of inhibition (mm)	
Test organisms	A	C
1. *Aspergillus fumigatus*	10	22
2. *Candida albicans*	9	24
3. *Saccharomyces cerevaceae*	7	26
4. *Hensinela colifornica*	7	25
5. *Pigment yeast*	-	21
6. *Rhizopus aurizae*	8	23
7. *Rhizopus oligasporum*	-	22

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

Table 3: The minimum inhibitory concentration (MIC) of the compound, CP-1, against test organisms

	The minimum inhibitory concentration (MIC) in μg ml^{-1}	
Sample	*Bacillus cereus*	*Shigella dysenteriaei*
CP-1	16	32

The cytotoxicity of the compound was bioassayed against brine shrimp nauplii and the results were shown in Table 4. The 50% mortality concentration (LC_{50}) of the compound, CP-1, was 8.42 μg ml^{-1} and 95% confidence limits were 3.95-17.91. A regression equation of the compound Y=3.67+1.44X and χ^2 value 0.81 are observed from the probit analysis which were compared with the results reported for Kolavenic acid and Clerodane diterpine (Islam *et al.*, 2001); Isoflavone (M. Shah Alam Bhuyan *et al.*, 2003); Triterpenoid (Rahman *et al.*, 2002) and galic acid (Saker *et al.*, 1998).

In conclusion, the present study reports here the characterization, antibacterial and antifungal activities and cytotoxicity of the compound isolated from *Caesalpinia pulcherrima* Stwartz. This compound may be used as a versatile compound to the development of a potential antimicrobial and cytotoxicological agent.

Table 4: Cytotoxicity of the compound, CP-1, by brine shrimp lethality bioassay

Test sample	Concentration μg ml^{-1}	% Mortality	LC_{50} μg ml^{-1}	95% Confidence limit	Regression equation	χ^2 value
	5	40				
	10	60				
Compound , (CP-1)	20	70	8.42	3.95-17.91	Y=3.67+1.44X	0.812
	40	80				
	80	90				
Kolavenic acid	-	-	2.95	1.55-5.61	Y=4.35+1.38X	0.470
Clerodane diterpine	-	-	2.28	1.10-4.49	Y=4.49+1.42X	0.109
Isoflavone	-	-	24.92	11.91-52.11	Y=3.38+1.15X	0.201
Triterpenoid	-	-	15.39	8.87-26.68	Y=3.06+1.63X	0.215
Galic acid [Standard]	-	-	4.53	3.33-6.15	Y=3.93+1.62X	1.250

Clerodane diterpine=16-oxo-cleroda-3,13(14)E-diene-15-oic acid;
Isoflavone = 5-methoxy-4-hydroxy-2,2-dimethylpyrano(3,4,7,8) isoflavone;
Triterpenoid = Bet-20(29)-en-3-ol-28-oic acid;

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REFERENCES

Barry, A.L., 1980. Procedures for testing antimicrobial agents in agar media. In: Antibiotic in laboratory medicine. (V. Lorian Ed.) Williams and Wilkins Co. Baltimore, USA, pp: 1-23.

Berghe, D.A.V and A.J. Vlientinck, 1991. Screening methods for antibacterial and antiviral agents from higher plants. In: Assay for Bioactivity (Hostettmann K. Ed) Vol. 6 in the series, Methods in plant Biochemistry (Dey P. M. and J. B. Harborne, series Eds). Academic press, London, pp: 47.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standard disc method. Am. J. Clin. Pathol., 45: 493-496.

Burger, A., 1960. Medicinal chemistry. Inter Science publishers, Inc., New York, pp: 6-7.

Das, P.C., A. Patra, S. Mandal, B. Mallick, A. Das and A. Chatterjee, 1999. Cleogynol, a Novel Dammarane Triperpenoid from *Cleome gynandra*. J. Nat. Prod., 62: 616-618.

Dreyer, D.L., 1969. Tetrahydron Letters, Sept., 25: 4415.

Finney, D. J., 1947. Probit analysis: A statistical treatment of the Sigmoid response curve, Cambridge Univ. Press., London, pp: 333.

Hese, M. F., C.W.B. Gemenden, B.S. Joshi, W. I. Taylor and H., Schmid, 1966. Helventica Acta, 49:1173.

Islam A., A. Sayeed, G. Sadik, M. M. Rahman and G.R.M.A.M. Khan, 2001. Antimicrobial activity and Cytotoxycity of Clerodane Diterpines from *Polyalthia longifolia* seed, The Sci., 1: 320-323.

Kirtikar, K. R. and B. D. Basu, 1994. Indian medicinal plants, 2nd Ed., 3: 1730.

Mayer, B.N., N. R. Ferrigni, J.E. Putnam, L.B. Jacobson, D.E. Nichols and J.L. Mclaughlin, 1982. Brine Shrimp: A convenient bioassay for active plant constituents. Plant Medica, 45: 31-34.

Persoone, G., 1980. Proceeding of the international symposium on brine shrimp, *Artemia salina*, Vol. 1-3, University Press, Witteren, Belgium.

Rahman, A., A. Sayeed, A. Islam, D. Chowdhury, G. Sadik and G.R.M.A.M. Khan, 2002. Characterization and Biological Screening of a Triterpenoid from *Nymphoides cristatum*, O.J. Biol. Sci., 2: B46-48.

Reiner, R., 1982. Detection of antibiotic activity In: Antibiotic-An Introduction, Roche Schientific Service, Switzerland, pp: 21.

Saker, M.K., D. Ergil, A.U.Tamer and N. Sahin, 1998. Antimicrobial activity and cytotoxicity of *Abies nordmanniana* subsp. *Equi-trojani* extracts, Fitoterapia, 69: 457.

Sayeed, A., M.A. Sattar, M. Akteruzzaman, A. Islam, G. Sadik and M.S.A. Bhuiyan, 2003. A prenylated isoflavone from *Pouzolzia indica*: Its *in vitro* antimicrobial activity and cytotoxicity evaluation, Orient. J. Chem., 19: 35-40.