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Anthelmintic, antimicrobial and antipyretic activity of various extracts of *Clerodendrum infortunatum* Linn. leaves

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SUMMARY

In the present study, various extracts of *Clerodendrum infortunatum* leaves have been studied for its anthelmintic, antimicrobial and antipyretic activities. In the anthelmintic and antipyretic study it was observed that the benzene extract was more potent than the other two extracts (ethanol and aqueous) even though all the three extracts were endowed with both the properties. The study reveals antimicrobial activity of the extracts against the tested strains of microorganisms between concentration ranges of 75 μg/ml and 350 μg/ml and shows effectiveness more against gram-positive bacteria than the gram-negative bacteria.

Key words: *Clerodendrum infortunatum* Linn.; Anthelmintic activity; Antimicrobial activity; Antipyretic activity

INTRODUCTION

The plant *Clerodendrum infortunatum* (Family-Verbenaceae) is a shrub, 9 - 24 m in height, gregarious, brachiate, blunty quadrangle, clothed with yellowish silk pubescence. The leaves of the plant are bitter in taste with a pungent flavor (Rastogi and Mahrotra, 2002; Anonymous, 1950).

The plant is used as tonic, aphrodisiac, antipyretic and anthelmintic. The leaves and roots are specifically used in tumors and skin diseases. It is very useful in cough, leucoderma, burning sensation and in diseases of blood (Kirtikar and Basu, 1957). The leaves extract of the plant mostly contains clerodin, sterol and flavone glucuronides viz. scutellarin and hispidulin-7-0-glucuronide with practically no free aglycone (Subramanian and Nair, 1973). Pharmacological work on the plant reported so far is limited, though, the genus *Clerodendrum* is reported to possess antioxidant as well as bronchodilator activity (Hazekamp *et al.,* 2001; Chae *et al.,* 2004). As the plant is having traditional uses in the treatment of helminthiasis, fever and in various skin diseases, it is thought worthwhile to investigate these activities of various extracts of the leaves of *Clerodendrum infortunatum* Linn. in a scientific manner.

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MATERIALS AND METHODS

Plant material

The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Shibpur, Howrah. After authentication, fresh leaves of the young and matured plants were collected in bulk from the rural belt of Salipur, Orissa, India during

early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder. A voucher specimen has been preserved in our Institute for future reference.

Preparation of the extract

The powdered plant material (100 gm) was extracted successively with benzene, ethanol and water in a soxhlet apparatus. The solvents were removed under reduced pressure, to get deep brown colour sticky residues of benzene extract (yield: 18% w/w with respect to the dried plant material), ethanol extract (yield: 14% w/w with respect to the dried plant material) and aqueous extracts (yield: 11% w/w with respect to the dried plant material) respectively. The masses were preserved in refrigerator for future use. The benzene extract, ethanol extract and aqueous extracts were referred to as BECI, EECI and AECI respectively.

Preliminary phytochemical studies

The three leave extracts of the plant were subjected to preliminary phytochemical studies using standard procedures (Harborne, 1984; Trease and Evans, 1989) to find out the nature of phytoconstituents present.

Drugs used

Piperazine citrate and albendazole were used as reference standards for anthelmintic study while Ciprofloxacin and clotrimazole were used as reference standards for the antibacterial and antifungal studies respectively and phenacetin was used as reference standard for antipyretic activity.

Microorganisms used

For the antimicrobial study, the microorganisms used include Staphylococcus aureus, Bacillus subtilis, Bacillus polymexia, Streptococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Escherichia coli,

Table 1. Preliminary phytochemical screening of different extracts of *C. infortunatum* leaves

Extracts	Phytoconstituents present
BECI	Lipids, Alkaloids, Steroids, Triterpenoids, cardiac glycosides, bitter principles
EECI	Cardiac glycosides, Flavonoids, Phenolic compounds
AECI	Carbohydrate, Proteins, Tannins.

Table 2. Anthelmintic activity of different extracts of *C. infortunatum* leaves

Group	Treatment	Concentration used (mg/ml)	Extracts suspended in Gum acacia	
			Time of paralysis (min)	Time of death (min)
I	Vehicle	-	-	-
II	Piperazine citrate	15	18.50 ± 0.31	-
III	Albendazole	10	34.66 ± 0.72	63.83 ± 0.79
IV	BECI	20	16.82 ± 0.61	34.96 ± 0.74
V	BECI	40	11.85 ± 0.54	29.88 ± 1.00
VI	BECI	60	8.89 ± 0.63	23.16 ± 5.29
IX	EECI	20	16.13 ± 0.9	34.84 ± 0.94
X	EECI	40	9.86 ± 0.52	29.85 ± 1.0
XI	EECI	60	4.86 ± 0.38	19.8 ± 1.46
XIV	AECI	20	64.78 ± 0.99	80.30 ± 5.39
XV	AECI	40	49.86 ± 3.36	69.84 ± 3.06
XVI	AECI	60	44.81 ± 3.06	59.80 ± 1.46

Results expressed as Mean ± S.E.M. from six observations.

Table 3. MIC (μg/ml) of different extracts of *C. infortunatum* leaves

Microorganisms	Minimum Inhibitory Concentration (μg/ml)		
	BECI	EECI	AECI
Gram-positive bacteria			
Staphylococcus aureus ATCC 25923	150	175	200
Bacillus subtilis UC 564	125	150	175
Bacillus polymexia 474	75	100	100
Streptococcus faecalis ATCC 29212	100	125	200
Gram-negative bacteria			
Pseudomonas aeruginosa 25619	200	225	300
Salmonella typhi 57	175	200	250
Vibrio cholerae 824	200	250	275
Shigella dysenteriae ATCC C_3	175	200	225
Escherichia coli NCTC 8196	150	175	200
Fungi			
Penicillum notatum ATCC 11625	225	250	350
Aspergillus niger AB 41	175	200	250
Candida albicans ATCC 18804	150	200	225

Table 4. Zone of Inhibition (mm) of different extracts of *C. infortunatum* leaves

Microorganisms	Zone of Inhibition (mm)[a]									Standards[b]
	BECI (mg/ml)			EECI (mg/ml)			AECI (mg/ml)			
	2	5	10	2	5	10	2	5	10	
Gram-positive bacteria										
Staphylococcus aureus ATCC 25923	9.7	12.3	17.7	9.3	10.3	16.3	8.0	10.7	16.0	27.3
Bacillus subtilis UC 564	8.7	13.7	18.3	8.0	11.7	17.0	7.3	11.3	16.3	25.0
Bacillus polymexia 474	7.7	12.7	19.3	7.7	10.0	18.3	7.7	12.3	15.7	22.3
Streptococcus faecalis ATCC 29212	7.3	13.3	18.7	8.7	11.3	17.3	6.3	10.7	15.3	26.7
Gram-negative bacteria										
Pseudomonas aeruginosa 25619	7.3	14.3	16.7	6.7	12.7	15.7	6.7	12.3	13.7	24.3
Salmonella typhi 57	7.7	12.7	17.3	6.3	12.3	16.3	7.0	10.3	13.7	23.3
Vibrio cholerae 824	7.3	13.7	16.7	7.3	13.3	16.3	6.3	12.3	15.7	22.3
Shigella dysenteriae ATCC C_3	7.7	14.3	17.3	7.7	12.0	16.7	7.3	12.3	16.3	25.3
Escherichia coli NCTC 8196	8.7	12.3	18.3	6.7	13.3	16.7	7.7	10.3	16.3	21.0
Fungi										
Penicillum notatum ATCC 11625	8.3	11.7	15.7	7.7	11.3	15.0	7.3	8.7	14.3	20.3
Aspergillus niger AB 41	7.3	13.3	17.3	7.3	13.7	16.7	7.3	11.3	15.3	23.7
Candida albicans ATCC 18804	8.3	12.7	17.7	7.7	13.3	16.7	6.3	13.7	16.3	28.3

[a]Values are mean of three readings; [b]Standards: Antibacterial studies- Ciprofloxacin 5 μg/ml; Antifungal studies- Clotrimazole 25 μg/ml

Penicillum notatum, Aspergillus niger and Candida albicans respectively. Suitable strains of these microorganisms were procured from the microbiology laboratory of our institute.

Anthelmintic activity

The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with

Table 5. Antipyretic activity of different extracts of *Clerodendrum infortunatum* leaves on yeast-induced pyrexia in rabbits

Treatment	Dose (mg/kg)	Body temperature					
		0 h	5 h	6 h	7 h	8 h	9 h
Control		37.45 ± 0.08	39.19 ± 0.03	39.29 ± 0.01	39.38 ± 0.01	39.44 ± 0.01	39.57 ± 0.02
BECI	125	37.51 ± 0.03	39.21 ± 0.03	39.13 ± 0.02**	38.93 ± 0.03***	38.81 ± 0.03***	38.75 ± 0.03***
	250	37.38 ± 0.08	39.20 ± 0.03	39.01 ± 0.02***	38.58 ± 0.04***	38.37 ± 0.04***	38.20 ± 0.04***
EECI	125	37.43 ± 0.09	39.39 ± 0.09	39.33 ± 0.01	39.22 ± 0.09	39.18 ± 0.09	39.12 ± 0.08*
	250	37.39 ± 0.07	39.30 ± 0.05	39.21 ± 0.05	39.03 ± 0.05*	38.93 ± 0.05***	38.82 ± 0.05***
AECI	125	37.42 ± 0.08	39.35 ± 0.08	39.32 ± 0.07	39.27 ± 0.08	39.24 ± 0.08	39.20 ± 0.08
	250	37.40 ± 0.06	39.39 ± 0.07	39.31 ± 0.07	39.21 ± 0.07	39.14 ± 0.07	39.06 ± 0.07**
Phenacetin	100	37.37 ± 0.07	39.36 ± 0.08	37.77 ± 0.04***	37.67 ± 0.04***	37.47 ± 0.04***	37.37 ± 0.05***

Values are expressed as mean ± S.E.M. n = 6 animals. *P < 0.05, **P < 0.01, ***P < 0.001 when compared with control

the intestinal roundworm parasites of human beings (Thorn *et al.,* 1977; Vidyarthi, 1977; Vigar, 1984). The method of Ghosh *et al.* (2005) was followed for anthelmintic screening. Sixteen groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released in to 50ml of desired formulation. Each group was treated with one of the following: vehicle (1% gum acacia in normal saline), piperazine citrate (15 mg/ml), albendazole (10 mg/ml) or extracts (20, 40 or 60 mg/ml). Observations were made for the time taken to paralyse and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour.

Antimicrobial Activity

Determination of minimum inhibitory concentration (MIC).

Minimum inhibitory concentration of the extract was determined by broth dilution method (Ghosh *et al*, 2006) at concentrations ranging from 75 µg/ml to 350 µg/ml in Dimethylsulphoxide against all the test microorganisms.

Determination of zone of inhibition

The zone of inhibition of the extracts was performed by agar disc diffusion method (Cruickshank, 1968) at concentrations of 2 mg/ml, 5 mg/ml and 10 mg/ml of the extracts dissolved in Dimethylsulphoxide. Ciprofloxacin (5 µg/ml) and Clotrimazole (25 µg/ml) were used as reference controls for the antibacterial and antifungal studies respectively. Solvent control (only Dimethylsulphoxide) was also maintained throughout the experiment.

Antipyretic activity

Antipyretic activity was measured by slightly modifying the method described by Bose *et al.* (2007). An aliquot of 3 ml/kg of 10% yeast suspension was subcutaneously injected into the rabbit back. After 5 h, anus temperature rising was observed and the animals showing at least 1 ^{o}C temperature increase were selected for experiments. Vehicle (1% Tween-80 solution, 3 ml/Kg), doses of extract (125 mg/kg and 250 mg/kg) and phenacetin (100 mg/kg) were administered orally and the temperature was measured at 6, 7, 8 and 9 h after induction of pyrexia (n = 6).

Statistical analysis

For anthelmintic study the values are mean ± S.D. for n = 6 worms. For zone of inhibition determination in antimicrobial study the values are the average of three readings. Values are mean ± S.E.M. for n = 6 animals for antipyretic activity. Statistical significance was determined by One Way Analysis of Variance (ANOVA) followed by Dunnet's *t*-test to compare group means. The levels of significance were fixed at P < 0.05, P < 0.01 and P < 0.001.

RESULTS

In the anthelmintic study it was observed that the BECI was more potent than the other two extracts (EECI and AECI) eventhough all the three extracts were endowed with anthelmintic property. The results of Minimum inhibitory concentration study revealed the antimicrobial activity of the extracts against the tested strains of microorganisms between concentration ranges of 75 μg/ml and 350 μg/ml. The zone of inhibition study revealed that the extracts possess antimicrobial activity in a concentration dependent manner against the test microorganisms and was comparable with the standard drugs. Further, from the antimicrobial study it was observed that the order of activity was BECI > EECI > AECI. The gram-positive bacteria were observed to be more susceptible than gram-negative bacteria. Among the tested strains of bacteria, the extracts were most effective against *B. polymexia* and least against *P. aerugenosa*, which is naturally resistant to antibacterial agents (Walker and Edward, 1999). Tested on yeast-induced pyrexia in rabbits, the different extracts significantly reversed hyperthermia similar to the standard drug used. The order of antipyretic activity was also found to be BECI > EECI > AECI.

DISCUSSION

The anthelmintic activity revealed concentration dependent nature of different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis or death of the worms. The observations of antimicrobial study are more likely to be due to the fact that gram-negative bacteria possess an outer lipid membrane that acts as a barrier to many environmental substances including antibiotics (Jigna *et al*., 2005). Therefore it may be a reason that the plant extracts are more active against gram-positive microorganisms than gram-negative microorganisms. The antipyretic study seems to support the view that *C. infortunatum* leave extracts have some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature (Bose *et al*., 2007). The present study reveals that the anthelmintic, antimicrobial and antipyretic activities decrease with increasing polarity of the extracts. Our results from the present study indicate the potential usefulness of *C. infortunatum* in the treatment of helminthiasis, various pathogenic diseases as well as in pyrexia. Further study regarding the isolation and characterisation of the active constituents responsible for such activities is currently under progress.

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