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Phytochemical screening, antibacterial and anthelmintic activities of leaf and seed extracts of *Coix lacryma-jobi* L.

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ABSTRACT

Objective: To evaluate the possible phytochemical constituents, antibacterial and anthelmintic activity of *Coix lacryma-jobi* L. (Job's tears) using the chloroform leaves and seed extracts.

Methods: The test for antibacterial activity and minimum inhibitory concentration was conducted by the disc diffusion and two-fold dilution method, respectively. In anthelmintic activity test, using *Pheretima posthuma* model, vermifuge and vermucidal activity were determined by using the chloroform extract at various concentrations.

Results: The preliminary phytochemical screening of chloroform extracts of Job's tears leaves indicated the presence of alkaloid, carbohydrate, saponin, glycosides, flavonoids, phenols, tannins and steroids whereas the seeds extract contained glycosides, flavonoids, phenols and steroids, which revealed highest antimicrobial activity against *Bacillus cereus* and *Klebsiella pneumoniae*. The lowest minimum inhibitory concentration (12.5–50 mg/mL) was observed against all selected bacteria. On the other hand, it has been observed that chloroform leaves extracts showed shortest time of paralysis (P = 8.17 min) and death (D = 18.23 min) at 100 mg/mL concentration, in comparison with seed extracts (P = 36.83 min and D = 62.33 min) at 100 mg/mL concentration and albendazole (10 mg/mL) used as reference drug (P = 20.17 min and D = 43.67 min), which indicated the plant possessed mild anthelmintic activity.

Conclusions: The chloroform extracts (leaves and seeds) showed efficacy for both bacterial infections and parasitic diseases, which ensure the traditional uses of *Coix lacryma-jobi* L.

1. Introduction

Secondary metabolites or phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities as well as believed to be effective in combating or preventing disease due to their antioxidant effect[1]. Recently, World Health Organization suggested that approximately 80% of the rural people across the world confide on herbal remedies as homeopathic medicines for their primary health care because of their easy availability, efficacy and specially cost effectiveness compared to modern drugs[2]. There are more than 500 medicinal plants growing in our country, however, the

inventory is not complete and many plants with medicinal value are yet to be determined[3].

Prospective source of antimicrobial agents in different countries is plants as most of the developing countries use plant-derived products as medicines. Plants are rich in a variety of phytochemicals including tannins, saponins, alkaloids, and flavonoids[4,5] which have been found to have *in vitro* antimicrobial properties. Emergence of resistance to first-line antibiotics poses challenge in treatment of several human infections[6-8] and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy.

Infections caused by various species of parasitic worms (helminths) of the gastrointestinal tract are the most widespread of all chronic infections of humans in developing countries including Bangladesh and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis[9]. The current anthelmintic therapies act by incapacitating the parasite by paralysis, damaging the worm such that the immune system can eliminate it, or by altering its metabolic processes. Because the

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metabolic requirements of these parasites vary greatly from one species to another, drugs that are highly effective against one type of worm are ineffective against others and because of the prevalence of helminth infections, treatment of helminthiasis is of very great practical therapeutic importance as the synthetic drugs used in helminthiasis treatment have some potential side effects[10].

Coix lacryma-jobi L. (*C. lacryma-jobi*), commonly known as Job's tears, is a broad-leaved, grain-bearing tropical plant of the family Poaceae, which is considered a nutritious health food in Asian countries such as China, India, Bangladesh, Pakistan, Sri Lanka, Malaysia, Japan, the Philippines, Burma, and Thailand[11]. The plant parts can be used in ornamental purpose, rosaries and necklace. Its roots and grain are also used as traditional medicine and supplementary medical food in China. Job's tears are taken by people due to its beneficial effect for hay fever, high cholesterol, cancer, warts, obesity, arthritis, allergic disorder and respiratory tract infections. Many scholars have reported different biological activities of Job's tears, including antioxidant/free radical scavenging[12], anti-inflammatory[13,14], anti-tumor[12,15,16], hypolipidemic[17], hypocholesterolemic[18], anti-allergic[19,20], hypoglycemic[21], antiobesity[22], anti-mutagenic[23], anti-ulcer[24], prebiotic activity[25], hormonal modulation[26], osteoporosis preventing[27], abortifacient[28] and antimicrobial effect[29].

In the present study, the organic soluble materials of the leaves and seed chloroform extracts of *C. lacryma-jobi* were evaluated for phytochemical screening, antimicrobial activity and minimum inhibitory concentrations (MICs) against some human pathogenic bacteria as well as anthelmintic activity against *Pheretima posthuma*.

2. Materials and methods

2.1. Collection, identification and processing of plant samples

The leaves of *C. lacryma-jobi* were collected from Dhaka, Bangladesh and then plant sample was submitted to the National Herbarium of Bangladesh, Mirpur-1, Dhaka for its identification and the voucher specimen was DACB-40674. Leaves were sundried for seven days in order to remove the moisture contents and then ground into coarse powder using high capacity grinding machine (Jaipan Designer Mixer Grinder, Jaipan, India) which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigations.

2.2. Extraction procedure

The powdered plant parts (30 g) were successively extracted in a Soxhlet extractor at elevated temperature using 500 mL of distilled chloroform (40–60 °C). After drying, all extracts were labeled and kept in refrigerator at 4 °C for future investigations.

2.3. Preliminary phytochemical screening

Chloroform extract was subjected to preliminary phytochemical

screening for determining nature of phytoconstituents by using standard protocols[30].

2.4. Antibacterial activity

The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method against Gram positive and Gram negative bacteria (Table 1) collected as pure cultures from the Department of Microbiology, Medinova Medical Services Limited, Bangladesh. Standard disc of Ciprofloxacin (5 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm[31].

2.5. Determination of MIC

The MIC of the extracted material was determined by two-fold dilution method. Subjected bacterial strains were grown in tryptone soya broth (HiMedia, India) until it reached to the exponential phase. *C. lacryma-jobi* extracts of different dilutions were prepared to give concentrations of 50, 25, 12.5, 6.25, 3.14, 1.56 and 0.78 mg/mL, respectively. After that, 0.5 mL extract of each concentration was added into separate test tubes containing 0.5 mL tryptone soya broth with bacterial suspension at a final concentration of 1 mL in each tube and incubated at 37 °C for 24 h. As negative control, 7% of 0.5 mL chloroform was added with 0.5 mL bacteria broth solution. After proper incubation, 100 µL of culture from each tube was transferred and spread over Mueller-Hinton agar (HiMedia, India) plate and incubated (Binder, Germany) at 37 °C overnight for bacterial count.

2.6. Experimental animal

For the experiment, earthworms were collected from moist soil at the Board of Intermediate and Secondary Education, Comilla and washed with normal saline to remove soil and fecal matter at the time of the experiment. The earthworms of 5–7 cm in length and 0.2–0.3 cm in width were used for the experimental protocol.

2.7. Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*[32] with minor modifications. In this experiment, *Pheretima posthuma* were used because of its anatomical and physiological similarity with intestinal roundworm parasites of human beings and they belonged to same group of Annelida. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Albendazole (10 mg/mL) was used as reference standard while saline water served as a control. Thirty-six earthworms were divided into six groups with equal size, each group containing six worms. 60 mL formulations containing two different concentrations of chloroform extract of *C. lacryma-jobi* leaves and seeds (50 and 100 mg/mL in distilled water) were prepared. All the test solution and standard solution were prepared

freshly before starting the experiments. The time for paralysis (in min) was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The time of death of the worms (in min) was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C).

2.8. Statistical analysis

In case of anthelmintic activity test, the experimental data were calculated as mean \pm SEM, evaluated by unpaired One-way ANOVA. Test values of $P < 0.01$ were considered statistical significant.

3. Results

3.1. Preliminary phytochemical screening

In primary phytochemical screening, leaf extract of *C. lacryma-jobi* was found to contain alkaloid, carbohydrate, saponin, glycosides, flavonoids, phenols, tannins and steroids whereas the seed extract contained glycosides, flavonoids, phenols and steroids.

3.2. Determination of antibacterial activity

Chloroform leaf extract of *C. lacryma-jobi* showed a wide range of antibacterial activity against *Staphylococcus epidermis* (*S. epidermis*), *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Vibrio cholerae* (*V. cholerae*) and *Klebsiella pneumoniae* (*K. pneumoniae*) at the concentration of 800 μ g/mL although *Escherichia coli* (*E. coli*) and *Shigella dysenteriae* (*S. dysenteriae*) showed resistance at the same concentration whereas the range of zone of inhibition was within 9–14 mm (Table 1). On the other hand, when chloroform seed extract of *C. lacryma-jobi* was subjected to antibacterial screening at 800 μ g/disc, it revealed antibacterial activity against *B. cereus*, *B. subtilis*, *P. aeruginosa*, *V. cholerae* and *K. pneumoniae* having the zone of inhibition ranging from 6 to 8 mm whereas the remaining tested microorganisms were found to be resistant at the same concentration (Table 1).

Table 1

Antibacterial activity by disc diffusion assay (mm).

Bacterial isolates	Zone of inhibition in diameter		
	Leaves extract	Seeds extract	Ciprofloxacin
Gram positive bacteria			
<i>S. epidermis</i>	12	-	35
<i>S. aureus</i>	10	-	30
<i>B. cereus</i>	14	7	34
<i>B. subtilis</i>	10	6	31
Gram negative bacteria			
<i>E. coli</i>	-	-	40
<i>P. aeruginosa</i>	12	6	31
<i>V. cholerae</i>	9	6	35
<i>S. dysenteriae</i>	-	-	40
<i>K. pneumoniae</i>	14	8	40

3.3. Determination of MIC

Inhibition of microorganism's growth at the lowest concentration

of plant extract is known as MIC. In MIC test, the leaves extracts of chloroform of *C. lacryma-jobi* was capable of inhibiting all types of Gram positive and Gram negative (*P. aeruginosa*, *K. pneumoniae*) bacteria at the concentration of 12.5–50 mg/mL except *E. coli*, *V. cholerae* and *S. dysenteriae* which were able to survive at 25 mg/mL concentration (Table 2). On the other hand, *B. cereus*, *B. subtilis*, *P. aeruginosa*, *V. cholerae* and *K. pneumoniae* were inhibited at the concentration 25–50 mg/mL of *C. lacryma-jobi* chloroform seeds extract, whereas, the MIC value of *S. epidermis*, *S. aureus*, *E. coli* and *S. dysenteriae* was 50 mg/mL (Table 3).

Table 2

MICs of the leaf extracts of *C. lacryma-jobi* against different bacteria.

Bacterial isolates	MIC of leaf extracts (mg/mL)						
	50	25	12.5	6.25	3.14	1.56	0.78
Gram positive bacteria							
<i>S. epidermis</i>	0	0	0	5	13	45	84
<i>S. aureus</i>	0	0	0	7	12	38	92
<i>B. cereus</i>	0	0	0	5	18	46	94
<i>B. subtilis</i>	0	0	0	4	14	25	64
Gram negative bacteria							
<i>E. coli</i>	0	5	25	64	102	162	218
<i>P. aeruginosa</i>	0	0	0	4	19	42	98
<i>V. cholerae</i>	0	2	5	7	25	67	112
<i>S. dysenteriae</i>	0	9	21	57	83	112	176
<i>K. pneumoniae</i>	0	0	0	2	5	14	44

Values are represented as the number of colony.

Table 3

MICs of the seed extracts of *C. lacryma-jobi* against different bacteria.

Bacterial isolates	MIC of seed extracts (mg/mL)						
	50	25	12.5	6.25	3.14	1.56	0.78
Gram positive bacteria							
<i>S. epidermis</i>	0	3	11	27	63	75	111
<i>S. aureus</i>	0	2	8	17	52	88	142
<i>B. cereus</i>	0	0	9	23	51	83	134
<i>B. subtilis</i>	0	0	4	17	61	87	134
Gram negative bacteria							
<i>E. coli</i>	0	9	31	59	83	133	198
<i>P. aeruginosa</i>	0	0	3	12	37	88	133
<i>V. cholerae</i>	0	0	3	11	44	93	157
<i>S. dysenteriae</i>	0	7	21	67	111	162	203
<i>K. pneumoniae</i>	0	0	2	6	19	43	102

Values are represented as the number of colony.

3.4. Anthelmintic activity

Time taken for paralysis and death of earthworms for both leaf and seed extracts and reference drug are given in Table 4. The chloroform extracts of the leaves of *C. lacryma-jobi* demonstrated paralysis and death of worms in a significant dose dependent manner as compared to seed extracts and albendazole especially at higher concentration of 100 mg/mL. Statistical variance of analysis on anthelmintic activity of *C. lacryma-jobi* leaf and seed extracts has been shown in Table 5.

Table 4

Anthelmintic activities of chloroform leaf and seed extracts of *C. lacryma-jobi*.

Sample	Concentration (mg/mL)	Time taken for paralysis in min	Time taken for death in min
Control	-	-	-
Standard	10	20.17 \pm 0.54	43.67 \pm 1.12
<i>C. lacryma-jobi</i> leaf extracts	50	18.33 \pm 0.80	37.67 \pm 1.15
<i>C. lacryma-jobi</i> seed extracts	100	8.17 \pm 0.79	18.33 \pm 1.31
<i>C. lacryma-jobi</i> leaf extracts	50	44.17 \pm 0.79	83.67 \pm 0.99
<i>C. lacryma-jobi</i> seed extracts	100	36.83 \pm 0.60	62.33 \pm 0.88

Table 5

Statistical variance of analysis on anthelmintic activity of chloroform leaf and seed extracts of *C. lacryma-jobi*.

Sample			SS	df	MS	F	P
<i>C. lacryma-jobi</i> leaf extracts	Paralysis time	Between	1366.78	2	683.39	151.86	0.000
		Within	67.50	15	4.50		
		Total	1434.28	17			
	Death time	Between	4010.78	2	2005.39	279.82	0.000
		Within	107.50	15	7.17		
		Total	4118.28	17			
<i>C. lacryma-jobi</i> seed extracts	Paralysis time	Between	1815.11	2	907.56	353.59	0.000
		Within	38.50	15	2.57		
		Total	1853.61	17			
	Death time	Between	4930.78	2	2465.39	453.75	0.000
		Within	81.50	15	5.43		
		Total	5012.28	17			

SS: Sum of squares; df: Degree of freedom; MS: Mean square; F: Fisher value; P: Probability value. Each value represents mean \pm SEM ($n = 6$). Data are found to be significant by testing through One-way ANOVA with replication at 1% level of significance.

4. Discussion

Activity of plant extract against both Gram positive and Gram negative bacteria is an indication of the broad spectrum of activity and thus can be used as sources of antibiotic substances for drug development that can be used in the control of these bacterial infections[33]. Extract of *C. lacryma-jobi* leaves showed a wide range of antimicrobial activity against Gram positive and Gram negative bacteria compared to the seeds extract. In agreement with this finding, Devi *et al.*[34] reported that antibacterial activity against *K. pneumoniae* and *P. aeruginosa* showed maximum efficiency in the chloroform extract followed by petroleum ether extract, aqueous extract, hexane extract and methanol extract whereas antibacterial activity tested against *E. coli* did not show any activity which is similar to the present study. In another report, antimicrobial activity against *E. coli* was found to be much more prominent in terms of ethanol extract[35].

It was found that the chloroform extracts (leaves) exhibited antibacterial activity against *S. epidermis*, *S. aureus*, *B. cereus*, *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* with MIC of 12.5 mg/mL. The extract was found to be bacteriostatic. On the other hand, the chloroform extracts (seeds) exhibited antibacterial activity with MIC of 25 mg/mL and 50 mg/mL against the selected bacteria. The phytochemical screening results indicated the presence of alkaloids, carbohydrates, saponins, glycosides, flavonoids, phenols, tannins and steroids as the main constituents that might be responsible for antibacterial activity of the test extracts. Less antibacterial activity of seed extracts may be due to the presence of less bioactive components or the composition of samples used and the extraction process (water or solvents).

The result of the present study indicates that all the leaf and seed extracts of *C. lacryma-jobi* tested for the anthelmintic activity showed significant effect compared to the albendazole, which is a broad spectrum anthelmintic drug, acting by inhibiting microtubules polymerization after binding to the colchicine sensitive site of β -tubulin. It causes decrease in microtubules in the intestinal cells, absorptive function and uptake of glucose by the adult and larval

forms of the parasites, thereby, depleting glycogen storage. Paralysis and death of susceptible gastrointestinal parasites occur slowly due to insufficient energy for the production of adenosine triphosphate and their clearance from the gastrointestinal tract may not be complete until several days after treatment[10]. Anthelmintic effects of plants are normally ascribed to secondary metabolites such as essential oils[36], flavonoids, alkaloids, terpenoids[37] or polyphenols such as proanthocyanidins[38], also known as condensed tannins. Presence of such secondary metabolites in the present study might be responsible for such kind of effect. Moreover, direct anthelmintic effects of purified condensed tannins have been confirmed in *in vitro* assays against, amongst others, *Haemonchus contortus*[39], *Ostertagia ostertagi*[40] and *Ascaris suum*[41] as tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death[10,42].

Conflict of interest statement

We declare that we have no conflict of interest.

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