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CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF *CORIANDRUM SATIVUM* LINN.

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ABSTRACT

The ethanolic extract of whole plant of *Coriandrum sativum* Linn., family: Umbelliferae, was assessed for its possible analgesic, antidiarrhoeal, antimicrobial and cytotoxic activity. Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, tannins, glycosides, and gums. In acetic acid induced writhing in mice, the ethanolic extract (250 and 500 mg/kg) exhibited significant ($p<0.001$ & $p<0.001$) inhibition of writhing reflex 62.12% and 72.73% respectively compared to standard diclofenac sodium. The extract showed a significant ($P<0.01$ and $P<0.001$) antidiarrhoeal activity against castor oil induce diarrhea in mice in which it decreased the frequency of defecation and increased the mean latent period at the dose of 250 mg/kg and 500 mg/kg body weight. The ethanolic extract showed moderate antibacterial activity against both gram-positive and gram-negative bacteria. In the brine shrimp lethality test, the extract showed cytotoxicity significantly with LC_{50} 40 µg/ml which was comparable to that of standard drug Chloramphenicol (LC_{50} 20 µg/ml). All the results tend to justify the traditional uses of the plant and require further investigation to identify the chemicals responsible for these effects.

INTRODUCTION: *Coriandrum sativum* Linn. (Family: Umbelliferae) is commonly known as "Dhania". It is a small strongly aromatic annual herb with odorous finely cut upper leaves, slender stems and small white or pink flowers producing little round fruits, cultivated as a spice plant throughout the country (Ghani, 2003).

Essential oil is the chief constituent of the fruits which is composed of about 70% of coriandrol (linalool), cymene, pinene, limonene, phellandrene, geraniol, and borneol, malic, oxalic and tannic acids. It also contains flavonoids, coumarins, phthalides and phenolic acids. Fruits also contains fixed oil, fatty matter, mucilage, tannin, malic acid, umbelliferone and scopoletin. Seeds contain quercetin-3-O-caffeyl and kaempferol-3-glucosides and beta sitosterol. The plant contains chlorogenic and caffeic acids, coumarins, quercetin and rutin (Ghani, 2003).

Infusion of the herb is a gentle remedy for flatulence, bloating and cramps. It settles spasm in the gut and counters nervous tension. Fruit extract is antimicrobial and the dried fruit is used as aromatic, refrigerant, stimulant, carminative, digestive, stomachic, diuretic, antibilious, aphrodisiac, antiseptic and tonic and chewed to remove foul breath. Fruits are also used as anti-inflammatory, anti-rheumatic, anti-scorbutic and anti-gripping agent. Various part of the plant is used in spleen complaints, sores, venereal sores and syphilis. Leaves are useful as carminative and antibilious agent. Coriander is also prescribed in rheumatism, neuralgia and bleeding piles (Ghani, 2003).

From the beginning of civilization plants have been used as remedies and still they play an important role in health care for about 80% of the world's population. The presence of diverse bioactive compounds like alkaloids, tannins, glycosides, and gums, etc. in plants has formed the therapeutic basis of herbal medication. Also plant can serve as a source of novel therapeutic agent for the treatment of disease still incurable. Thus emphasis is given on the biological screening of medicinal plants for further exploration of their active constituents.

MATERIALS AND METHODS:

Sample collection and extraction: The whole plant of *Coriandrum sativum* Linn. (Family: Umbelliferae) were collected from Jessore, Bangladesh in January, 2009 and identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB- 33875). A voucher specimen has been deposited in Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. The identified plant was dried under shade. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container.

About 500 mg of powder was extracted by maceration over 20 days with 1200 ml of 90% ethanol. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. The filtrate thus obtained was evaporated by using a rotary evaporator to get a viscous mass. The viscous mass was then vacuum dried to get a dried ethanolic extract (approx. yield value 10%). The extract thus obtained was used for experimental purposes.

Animals: Swiss-Albino mice of either sex (20-25 gm body weight) were collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animal were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University, Khulna under standard Laboratory conditions (relative

humidity 55-60%, room temperature 25±2^0 C and 12 hours light: dark cycle) for period of 14 days prior to performing the experiments.

Microorganisms: Both gram positive and gram negative bacterial strain were collected from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Drugs: The standard drugs Diclofenac sodium, Loperamide and Chloramphenicol were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Phytochemical tests: The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups (Evans, 1989). Then, the extract was used for pharmacological screening.

Determination of analgesic activity: The analgesic activity of the sample was studied using acetic acid induced writhing model in mice. Experimental animals were randomly selected and divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting of five (05) mice in each group. Control group received orally 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received orally diclofenac sodium at the dose of 25 mg/kg body weight.

Test group I and Test group II were treated with test sample orally at the dose of 250 and 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. After an interval of 5 minutes was given for absorption of acetic acid and number writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing (Whittle, 1964, Ahmed *et al.,* 2004).

Antidiarrhoeal activity test: Antidiarrhoeal activity of the ethanolic extract of the whole plant of *Coriandrum sativum* Linn. was tested using the model of castor oil induced diarrhea in mice (Chatterjee, 1993). All the mice were screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the experiment. The test animals were randomly chosen and divided into four groups having five (05) mice in each group.

Group I was kept as “control” and received 1% Tween-80 at the dose of 10 mg/kg body weight; Group II was “positive control” and received standard antimotility drug, Loperamide at the dose of 50 mg/kg body weight as oral suspension; and Group III Group IV were “test group” and treated with extract of *Coriandrum sativum* Linn. at the oral dose of 250 and 500 mg/kg body weight. Control vehicle and the extract were administered orally, 30 min. prior oral administration of castor oil at the dose of 0.5 ml.

Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in five hours study after the castor oil administration. Number of stools on any fluid material that stained the adsorbent paper were counted at each successive hour during the experiment (05 hrs). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for old ones.

Antibacterial activity: Antibacterial activity of the extract *Coriandrum sativum* Linn. was tested using the disc diffusion method (Bauer *et al.,* 1966; Ahmed *et al.*, 2003). Sample impregnated discs, standard antibiotic discs (Kanamycin) and negative

control discs were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then inverted and kept in refrigeration for about 2 h at 4°C to allow the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 24 h. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

Determination of Cytotoxic Activity: The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300ml). The flask were well aerated with the aid of an air pump, and kept in a water bath at 29-30^{o}C. A bright light was left on it. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% dimethyl sulfoxide (DMSO) to obtain a concentration of 5, 10, 20, 40, 80, 160 and 320 μg/ml. Each preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. For control, same procedure was followed except test samples. A series of same concentration as of sample was prepared for positive control, chloramphenicol. After making the test tube properly, 10 living shrimps were added to each of the test tubes with the help of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29^{0} C for 24 h in a water bath, after which each test tube was examined and the surviving brine shrimp counted and recorded. From this, the percentage of mortality was calculated at each concentration to determine the LC_{50} (Meyer *et al*., 1982).

Statistical analysis: Student's t-test was used to determine significant differences between the control group and test group.

RESULTS: In the preliminary phytochemical screening the extract showed the presence of alkaloids, tannins, glycosides, and gums (**Table 1**).

TABLE 1: RESULTS OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

Plant Extract	Alkaloids	Glycosides	Steroids	Gums	Tannins	Saponins	Flavonoids	Reducing sugar
Ethanolic extract of *Coriandrum sativum* Linn.	+	+	-	+	+	-	-	-

+ = Presence; - = Absence

Analgesic activity test: Analgesic activity of the ethanolic extract of *Coriandrum sativum* Linn. was tested by acetic acid induced writhing model in mice. The extract produced 62.12% (p<0.001) and 72.73% (p<0.001) acetic acid induced writhing inhibition in mice at the dose of 250 and 500 mg/kg body weight, which is comparable to diclofenac sodium 80.30% (p<0.001) at the dose of 25 mg/kg body weight (**Table 2**).

TABLE 2: EFFECT OF *CORIANDRUM SATIVUM* LINN. EXTRACT ON ACETIC ACID INDUCED WRITHING IN MICE

Animal Group	Treatment	Writhing Count (%Writhing)	%Writhing Inhibition
Control (n=5)	1% tween-80 solution in water	13.2 ± 0.59 (100)	0
Positive Control (n=5)	Diclofenac sodium (25mg/kg)	2.6 ± 0.51* (19.70)	80.30
Test group I (n=5)	Et. Extract (250mg/kg)	05 ± 0.45* (37.88)	62.12
Test group II (n=5)	Et. Extract (500mg/kg)	3.6 ± 0.52* (27.27)	72.73

Values are expressed as mean ± SEM, SEM=Standard error of Mean, n=No. of mice, Et. = Ethanolic, **P* < 0.001 vs. control

Antidiarrhoeal activity test: Antidiarrhoeal activity of the ethanolic extract of *Coriandrum sativum* Linn. was tested by castor oil induced diarrhea in mice. The extract caused an increase in latent period (1.05 and 1.36 h) *i.e.* delayed the onset of diarrheal episode at the dose of 250 and 500 mg/kg body weight respectively as compared to the standard antidiarrhoeal agent Loperamide where the mean latent period was 1.96 h (**Table 3a**). The extract also decreased the frequency of defecation at the dose of 250 and 500 mg/kg of body weight respectively where the mean number of stool at the 1st, 2nd, 3rd, 4th, and 5th hour of study were 1.8, 2.5, 2.2, 2.7, 2.7 and 2.3, 1.6, 2.3, 2.5, 1.8 respectively which was comparable to the standard drug Loperamide where the mean number of stool at the 1st, 2nd, 3rd, 4th, and 5th hour of study were 3.9, 1.5, 1.1, 1.3, and 0.7 respectively (**Table 3b**).

TABLE 3A: EFFECT OF ETHANOLIC EXTRACT OF *CORIANDRUM SATIVUM* LINN. ON CASTOR OIL INDUCED DIARRHEA IN MICE (LATENT PERIOD)

Animal Group/ Treatment	Dose (p.o)	Latent period (h)
Group I (Control) 1% Tween-80	10 ml/kg	0.62±0.05
Group II (Positive Control) Loperamide	50 mg/kg	1.96±0.09*
Group III (Test Group) Et. Extract	250 mg/kg	1.05±.11**
Group IV (Test Group) Et. Extract	500 mg/kg	1.36±0.08*

Values are expressed as mean ± SEM (n=5); *: *P*<0.001, ***P*<0.01 vs. control; p. o.: per oral

TABLE 3B: EFFECT OF ETHANOLIC EXTRACT OF *CORIANDRUM SATIVUM* LINN ON CASTOR OIL INDUCED DIARRHEA IN MICE (NUMBER OF STOOLS)

Animal Group/ Treatment	Dose (p.o)	Period of study (hr)	Total Number of Stool
Group I (Control) 1% Tween-80	10 ml/kg	1	4.9±0.80
		2	5.5±0.31
		3	2.6±0.16
		4	3.2±0.62
		5	3.0±0.25
Group II (Positive Control) Loperamide	50 mg/kg	1	3.9±0.25**
		2	1.5±0.52*
		3	1.1±0.12*
		4	1.3±0.32*
		5	0.7±0.22*
Group III (Test Group) Et. Extract	250 mg/kg	1	1.8±0.36**
		2	2.5±0.41**
		3	2.2±0.23
		4	2.7±0.15
		5	2.7±0.25
Group IV (Test Group) Et. Extract	500 mg/kg	1	2.3±0.27**
		2	1.6±0.21*
		3	2.3±0.24**
		4	2.5±0.21*
		5	1.8±0.23**

Values are expressed as mean ± SEM (n=5); *: *P*<0.001; **: *P*<0.01 vs. control, Et. = Ethanolic

Antibacterial activity test: Table 4 showed the results of antibacterial test. The antibacterial activity was assessed against a panel of 10 pathogenic bacterial strains (Both gram positive and gram negative) at the dose of 250 and 500 μg/disc, and the result were compared with the activity of the positive control, kanamycin (30 μg/disc). At 250 μg/disc, the extract showed activity against *Enterococcus faecalis* (5mm), *Streptococcus agalactiae* (7mm), *Shigella sonnei* (11mm) and *Streptococcus pyogenes* (12mm). At 500 μg/disc, the extract showed activity only against *Enterococcus faecalis* (6mm), *Streptococcus agalactiae* (10mm), *Shigella sonnei* (14mm) and *Streptococcus pyogenes* (17mm).

Cytotoxic activity test: Brine shrimp lethality bioassay indicates cytotoxicity of extract. The extract was found to show lethal activity against brine shrimp nauplii and LC_{50} was found at 40 μg/ml which was comparable to standard drug Chloramphenicol LC_{50} value was 20 μg/ml (**Table 5a and Table 5b**).

TABLE 4: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF THE *CORIANDRUM SATIVUM* LINN.

Serial No.	Bacterial Strains	Type of Bacterial Strains	Diameter of Zone of Inhibition in mm			
			Blank	Kanamycin (μg/disc)	Extract (250μg/disc)	Extract (500μg/disc)
1	*Shigella flexneri*	Gram(-)	-	18	-	-
2	*Enterococcus faecalis*	Gram(+)	-	15	5	6
3	*Streptococcus agalactiae*	Gram(+)	-	20	7	10
4	*Shigella sonnei*	Gram(-)	-	27	12	14
5	*Shigella boydii*	Gram(-)	-	20	-	-
6	*Streptococcus pyogenes*	Gram(+)	-	26	11	17
7	*Shigella dysenteriae*	Gram(-)	-	20	-	-
8	*Pseudomonas aeruginosa*	Gram(-)	-	15	-	-
9	*Staphylococcus saprophyticus*	Gram(+)	-	10	-	-
10	*Escherichia coli*	Gram(-)	-	21	-	-

Gram (-):-Gram Negative Bacteria; Gram (+):-Gram Positive Bacteria; (-):- No inhibition

TABLE 5A: RESULT OF BRINE SHRIMP LETHALITY BIOASSAY OF ETHANOLIC EXTRACT OF *CORIANDRUM SATIVUM* LINN.

Conc. (μgm/ml)	Test-1	Test- 2	Avg. no of alive shrimp (sample)	Avg. no of alive shrimp (control)	% mortality	LC_{50}
5	10	10	10	10	0	40
10	9	9	9		10	
20	7	8	7.5		25	
40	5	5	5		50	
80	3	3	3		70	
160	2	0	1		90	
320	0	0	0		100	

TABLE 5B: RESULT OF BRINE SHRIMP LETHALITY BIOASSAY OF CHLORAMPHENICOL

Group	Conc. (µgm/ml)	S-1	S- 2	Avg. no of alive shrimp (sample)	Avg. no of alive shrimp (control)	% mortality	LC_{50}
Standard (Chloramphenicol)	5	9	9	9	10	10	20
	10	8	8	8		20	
	20	5	5	5		50	
	40	4	3	3.5		65	
	80	2	1	1.5		75	
	160	1	1	1		90	
	320	0	0	0		100	

DISCUSSION: To get preliminary idea about the active constituents present in the fruit extract different chemical tests were performed and found the presence of alkaloids, tannins, glycosides, and gums. Analgesic activity of the ethanolic extract of *Coriandrum sativum* Linn. was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul *et al.,* 2003). The writhing test is generally used for screening of antinociceptive effects (Koster *et al.*, 1959).

With respect to the writhing test, the research group of Deraedt *et al.* (1980) described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokanson, 1978). The ethanolic extract of *Coriandrum sativum* Linn showed significant inhibition on acetic acid-induced writhing response compared to reference drug diclofenac sodium in mice. Diclofenac sodium reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis (Small, 1989). Result of this test suggests that the extract might possess chemical constituent that have the capability to inhibit prostaglandin synthesis.

Antidiarrhoeal activity of the ethanolic extract of *Coriandrum sativum* Linn was tested by castor oil induced diarrhea in mice. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti-absorptive or anti secretory effect (Tripathi, 2001). The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface.

Most agreed view is that ricinoleate salts stimulate the intestinal epithelial cell's adenyl cyclase (Racusen *et al.,* 1979) or release prostaglandins, which results in an increase in the net secretion of water and electrolytes in the small intestine (Beubler *et al.,* 1979). The ethanolic extract of *Coriandrum sativum* Linn significantly and dose dependently inhibited and delayed the onset of diarrhea in mice. The maximum effect was found at 500 mg/kg of body weight. On the basis of this result it can be concluded that the ethanolic extract of *Coriandrum sativum* Linn might possess antidiarrhoeal activity. Antibacterial activity was tested by using disc diffusion method. The extract showed activity only against *Enterococcus faecalis*

(6 mm), *Streptococcus agalactiae* (10 mm), *Shigella sonnei* (14 mm) and *Streptococcus pyogenes* (17 mm) among 10 species of bacteria. On the basis of this result it can be concluded that the ethanolic extract of *Coriandrum sativum* Linn possesses moderate antibacterial activity. The results support the traditional use of this plant as a remedy of infectious diarrhea, dysentery, systemic shigellosis and gastrointestinal disturbances.

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the compound (Meyer *et al.*, 1982; McLaughlin *et al.*, 1988). The ethanolic extract of *Coriandrum sativum* Linn was found to show significant activity against the brine shrimp nauplii; LC_{50} was found 40 µg/ml and LC_{50} of Chloramphenicol was found 20µg/ml. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity.

CONCLUSION: According to above discussion *Coriandrum sativum* Linn contains important chemical constituents that confer upon it as a medicinal agent. It was revealed that the extract contains alkaloids, tannins, glycosides, and gums which have potential role in its Analgesic, Antidiarrhoeal, Antimicrobial, and Cytotoxic activity. This could provide a rationale for traditional uses of this plant and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

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