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EVALUATION OF ANTIDIARRHOEAL, ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF *CINNAMOMUM TAMALA* LEAVES FROM BANGLADESH

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ABSTRACT

The crude ethanolic extract of the leaves of *Cinnamomum tamala* (family: Lauraceae) was evaluated for its possible phytochemical nature (group determination of plant constituent) and selected pharmacological activities (Antidiarrhoeal, Antimicrobial and Cytotoxic activity) growing in Bangladesh. Phytochemical analysis of the ethanolic extract of the leaves of *C. tamala* indicated the presence of reducing sugar, glycoside, tannins, steroid, amino acid & alkaloid types of compounds. The ethanolic extract of leaves of *C. tamala* on Castor oil induced diarrheal method in mice. The result showed that extract inhibited the mean number of defecation which were 24.49 % ($P<0.01$) and 40.82 % ($P<0.001$) at the doses of 250mg/kg and 500mg/kg respectively. The latent period for the extract treated group was ($p<0.01$ & $P<0.001$) increased as compared to control group. The extract of *C. tamala* (500 µg/disc) showed moderate anti-microbial activity against *Staphylococcus epidermidis*(10), *Vibrio cologet* (Zone of inhibition 11mm), *Streptococcus agalactiae* (9mm), *Shigella sonnei* (9 mm), *Streptococcus pyogenes* (9mm), *Staphylococcus saprophyticus* (11mm), *Staphylococcus aureus* (8 mm). The extract of leaves of *C. tamala* showed significant ($p<0.001$) toxicity to the brine shrimp nauplii. The concentrations of crude extract for 50% mortality (LC_{50}) and 90% mortality (LC_{90}) were 32 µg/mL and 74.29 µg/mL respectively. Therefore, the obtained results tend to suggest the antidiarrhoeal, antimicrobial and cytotoxic activities of *C. tamala* (Buch.-Ham.) leaves from Bangladesh and justify its use in folkloric remedies.

Keywords: *Cinnamomum tamala*, antidiarrhoeal, antimicrobial and cytotoxic activity

INTRODUCTION

Cinnamomum tamala (Buch.-Ham.) (family: Lauraceae) is a widely used medicinal plant by folklore medicinal practitioners in Bangladesh to treat diarrhea and various diseases. It is commonly named as tejpatta(Bengali,Hindi), Tejpat(Manipuri), Tamalapatram(Malayalam), Talisapatri, Talisha , Pat -taakulu(Telugu), Patraka(Kannada), Tezpat(Urdu). The leaves of *C. tamala* have been used extensively

in the cuisines of India (particularly in the Moghul cuisine of North India) and as spice in the food industry because of its special aroma [1], that is, clove-like-taste and pepper like odour. It also acts as an insect repellent. In Kashmir they are used as a substitute for paan (betel leaves). It is also used in industries as fragrance component in soaps, detergents, cosmetics and perfumes, and toothpastes. The leaves of this tree have medicinal properties and are used in treatment of numerous ailments [2, 3]. It is used as food, fodder, medicine, and timber in Uttarakhand Himalayan region [4].

Different extracts from leaves of *C. tamala* have shown anti-inflammatory [5], antioxidant [6], antiulcer [7], anticarcinogenic [8], antidiarrhoeal [9] effects, antidiabetic [10, 11] which is mainly contributed by Cinnamaldehyde (3-phenyl-2- propenal), a potential antidiabetic agent [12]. It is also used medicinally as a carminative, an anti flatulent, a diuretic, and in the treatment of cardiac disorders [13], analgesic in dental preparations [14–16] due to presence of eugenol (4-hydroxy-3-methoxy allylbenzene). Its bark is useful for the treatment of gonorrhoea [17]. Besides these, various pharmacological activities have been detected in natural products from *Cinnamomum* species. The essential oil from *C. tamala* exhibits antifungal [18, 19], antibacterial [20], anti-dermatophytic [21] antihypercholesterol-aemic, and antihyperglycaemic effects [22].

Since no literature is currently available to substantiate antidiarrhoeal, antimicrobial and cytotoxic activities from ethanolic extract of *C. tamala*, therefore the present study is a part of our on-going pharmacological screening of this selected Bangladeshi medicinal plant and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antidiarrhoeal, antimicrobial and cytotoxic activities that also confirm its use as antibiotic and other pathological conditions where antidiarrhoeal medications are implicated.

MATERIALS AND METHODS

Collection and identification of plant materials: *C. tamala* was collected from market of Khulna. The time of collection was December 2010. The leaves were fresh. The plant leaves were collected for identification. The Bangladesh National Herbarium Dhaka identified the plant (Herbarium Accession No-DACB-39290).

Preparation of ethanolic extract: The leaves of *C. tamala* were freed from any of the foreign materials. Then the leaves were air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 500g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1.4 liters of 80% ethanol. The container with its contents was sealed and kept for a period of 4 days accompanying occasional shaking and stirring. The ethanolic extract was filtered by Buchner funnel and the filtrate was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of extract (yield approx. 13.26%).

Drugs: Drugs employed in the study were: Loperamide (Square Pharmaceuticals Ltd., Bangladesh) and Kanamycin 30 µg/disc, Oxoid Ltd, UK.

Animal: Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20 -25 g, were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions (temperature: (24.0 ± 1.0°C), relative humidity: 55-65% and 12 h light/ dark cycle) and fed with rodent diet and water ad libitum. The ethics for use of experimental animals were followed carefully.

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent [23, 24,25].

Antidiarrhoeal activity: Antidiarrhoeal activity of the extract of *C. tamala* was tested using the model by castor oil induced diarrhoea in mice [26]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into four groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group-II received loperamide at 50 mg/kg; group-III and IV were 'test groups' and were treated with extract of *C. tamala*. at 250 and 500 mg/kg. Control vehicle and the extract were administered orally, 1/2 h prior to the oral administration of 0.5 ml castor oil. Individual animals

of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in four hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (4 hour). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones. Finally percent reduction of faecal output was calculated [27].

Testing for antimicrobial activity: The antimicrobial activity was investigated using disc diffusion assay [28,29]. Reference microorganisms from the stock were streaked onto nutrient agar plates and the inoculated plates were incubated overnight at 37°C. Using a sterile loop, small portion of the subculture was transferred into test tube containing nutrient broth and incubated (2-4 h) at 37°C until the growth reached log phase. Nutrient agar media seeded with standard inoculum suspension was poured in Petri-dishes and allowed to solidify. Measured amount of each test samples (Table-1) were dissolved in specific volume of solvent (chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri-dish under the laminar hood. Then discs were soaked with solutions of test samples and dried. Discs impregnated with extract, standard antibiotic disc (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and blank (solvent chloroform or methanol) discs were placed on the Petri-dishes with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Finally the inoculated plates were incubated at 37° C for 24 h and the zone of inhibition was measured in millimeters [28,30].

Assay for brine shrimp lethality: It was used for probable cytotoxic action [31]. The eggs of brine shrimp (Artemia salina Leach) were collected and hatched in a tank at a temperature around 37°C with constant oxygen supply.

Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). 4 mL of seawater was given to each of the vials. Specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 0.1, 0.5, 1, 10, 20, 40, 60, 80 and 100 µg/mL. In the control vials same volumes of DMSO (as in the sample vials) were taken. With a pasteur pipette, 10 living nauplii were put into each of the vials. After 24 h, the vials were observed and the number of nauplii that survived in each vial was counted. From this, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extract.

Statistical Analysis: For anti-diarrheal determination, data were presented as mean ± standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. p values < 0.05 were considered to be statistically significant (p indicates probability).

RESULTS

Chemical group test: Results of different chemical tests on the ethanolic extract of leaves of *C. tamala* showed the presence of reducing sugar, glycoside, tannins, steroid, amino acid & alkaloid (Table 1).

Antidiarrheal activity: Table 2 showed the effect of the ethanolic extract of leaves of *C. tamala* on Castor oil induced diarrheal method in mice. The result showed that extract inhibited the mean number of defecation which were 24.49 % ($P<0.01$) and 40.82 % ($P<0.001$) at the doses of 250mg/kg and 500mg/kg respectively. The latent period for the extract treated group was ($p<0.01$ & $P<0.001$) increased as compared to control group.

Antimicrobial activity: The ethanolic extract of the barks of *C. tamala* tested for anti-microbial activity against a number (16) of both gram positive and gram-negative bacteria. Standard antibiotic discs of Kanamycin were used for comparison purpose.

The table-3 showed that the ethanolic extract of the leaves of *C. tamala* (500 µg/disc) showed moderate anti-microbial activity against *Staphylococcus epidermidis*(10), *Vibrio cologet* (Zone of inhibition 11mm), *Streptococcus agalactiae* (9mm), *Shigella sonnei* (9 mm), *Streptococcus pyogenes* (9mm), *Staphylococcus saprophyticus* (11mm), *Staphylococcus aureus* (8 mm) .

Cytotoxic Activity: In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50} and LC_{90} were deduced (LC_{50} = 32 µg/ml; LC_{90} = 74.29 µg/ml) (Table 4).

DISCUSSION

Antidiarrhoeal activity of the ethanol extract of dried leaves of *C. tamala* was tested using the model of castor oil induced diarrhoea in mice [32]. Castor oil, which is used to induce diarrhoea in mice, 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 secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the 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 stimulates the intestinal epithelial cell's adenyl cyclase13 or release prostaglandin [33]. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of dried leaves of *C. tamala* might possess antidiarrhoeal activity. The plant is also reported to contain saponins There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic [34]. The cytotoxic activity of the ethanol extract of dried leaves of *C. tamala* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor. [35] The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *C. tamala* leaves possess significant antidiarrhoeal as well as antimicrobial activities. The potential of the extract of *C. tamala* as antidiarrhoeal, cytotoxicity and antimicrobial agents may be due to the presence of phytoconstituents like tannins, alkaloid, glycoside etc and might be responsible for its activity. However, extensive researches are necessary to search for active principles responsible for these activities.

Table 1: Results of different group tests of ethanolic extract of *C. tamala* leaves.

Phytoconstituents	**Ethanol extract of** *C. tamala*
Alkaloid	+
Reducing sugars	+
Tannins	+
Gums	-
Flavonoids	-
Saponin	-
Steroid	+
Amino Acid	+
Glycoside	+

+: Positive result; - : Negative result;

Table-2: Antidiarrheal activity of the *C. tamala* in castor oil induced diarrheal test method on mice.

Sample	Dose	Mean± SE		% inhibition
		Latent period	Defication	
Distilled water	2ml/mice, p.o.	1.52±0.21	9.8±0.66	--
Loperamide	50 mg/kg, p.o.	3.89±0.13**	4±0.32**	59.18
Et. Extract *C. tamala* (Leaves)	250 mg/kg, p.o.	2.81±0.31*	7.4±0.51*	24.49
	500 mg/kg, p.o.	3.29±0.10**	5.8±0.58**	40.82

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; * indicates P < 0.01; ** indicates P < 0.001, one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral.*

Table- 3 In vitro antibacterial activity of ethanol extract of *C. tamala*

Serial No	Bacterial Strains	Diameter of Zone of Inhibition in mm		
		Blank	Kanamycin (30 µg/disc)	Extract of *C. tamala* (500µg/disc)
	Gram Negetive (-) bacteria			
1	*Staphylococcus saprophyticus*	-	24	11
2	*Proteus spp.*	-	21	0
3	*Escherichia coli*	-	10	0
4	*Staphylococcus aureus*	-	21	0
5	*Shigella flexneri*	-	17	0
6	*Shigella sonnei*	-	14	10
7	*Shigella boydii*	-	21	12
8	*Vibrio cologet*	-	0	11
	Gram Positive (+) bacteria			
1	*Enterococcus faecalis*	-	24	0
2	*Streptococcus pyogenes*	-	0	12
3	*Staphylococcus epidermidis*	-	25	10
4	*Streptococcus agalactiae*	-	11	9

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

Table- 4: Result of Brine Shrimp lethality bioassay ethanolic extract of *C. tamala* leaves.

Test Sample	Conc. (µgm/ml)	Log conc.	Avg. no of alive shrimp (sample)	% mortality	LC_{50}	LC_{90}
Ethanolic extract	5	0.698	08	25	32	74.29
	10	1.000	07	35		
	20	1.301	06	35		
	40	1.602	04	60		
	80	1.903	01	95		
	160	2.204	0	100		

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