

# Evaluation of antimicrobial, cytotoxic, thrombolytic, diuretic properties and total phenolic content of *Cinnamomum tamala*

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**Background:** The leaves of *Cinnamomum tamala* are aromatic, carminative, stimulant, diuretic, diaphoretic, lactagogue, and deobstruent. Other parts of the plant are also used as traditional remedies for various diseases. **Aim:** The aim of our study was to carry out the evaluation of the preliminary phytochemical and some pharmacological properties including cytotoxicity, antibacterial and antifungal sensitivity, total phenolic content, clot lysis and diuretic potential of the leaves of *C. tamala*. **Materials and Methods:** Phytochemical screening of ethanol extract was carried out by standard chromogenic reagents and total phenolic content was estimated in term of gallic acid equivalent by using Folin Ciocalteu's reagent. Brine shrimp lethality assay method was used to determine the degree of cytotoxicity. Antibacterial and antifungal sensitivity test was performed using disc diffusion method in agar medium and minimum inhibitory concentration was determined by micro dilution method. Clot lysis and diuretic potential were compared with streptokinase and furosemide, respectively. **Statistical Analysis:** The results were statistically interpreted using Student's "t" test. **Results:** The extract contained alkaloids, steroids, tannins and reducing sugars. Total phenolic content was found to be 276 gallic acid equivalent/100 g of dried plant material. In brine shrimp lethality assay, it showed moderate toxicity (LC<sub>50</sub> = 40 µg/ml and LC<sub>90</sub> = 160 µg/ml). The extract inhibited growth of tests bacteria and fungi with significant zones of inhibition. Highest activity was observed against the bacteria *Salmonella typhi* and the fungus *Aspergillus niger*. *In-vitro* clot lysis action of extract was comparable with streptokinase (48.22 ± 2.98%) and maximum effect (24.75 ± 1.27%) was found at a dose of 10 mg/ml. The extract showed diuretic activity which responded 29.16% and 37.50% diuretic activity in comparison with control (water) which indicates the plant mild diuretic activity. **Conclusion:** The results depicted that traditional uses of the plant are rational, however, advanced researches are needed to identify the active components as well as mechanism of action.

**Key words:** Antibacterial, antifungal, *Cinnamomum tamala*, cytotoxicity, diuretic, total phenolic content, thrombolytic

## INTRODUCTION

In Bangladesh, the spice plant *Cinnamomum tamala* Nees and Eberm (Synonym: *Cinnamomum albiflorum*) (Family- Lauraceae) is commonly known as 'Tejpata', which is also distributed in the Mediterranean region, West and Central Asia, South Asia, South East and East Asia, Africa, South East America, Australia, India, China and Myanmar. It grows throughout Bangladesh but cultivated more in southern region as spice as well as for medicinal value. This plant has

long been used as a traditional folk remedy for cold and cough, asthma, colic, blood dysentery, diarrhea, constipation, flatulence, indigestion, jaundice, hyperacidity, anorexia, dysmenorrhoea, leucorrhoea, postpartum haemorrhage, high fever, skin diseases, sore throat, sexual weakness and tuberculosis.<sup>[1]</sup> The leaves are carminative, stimulant, diuretic, diaphoretic, lactagogue, deobstruent and aromatic.<sup>[2]</sup> The bark is used to treat gonorrhoea. Leaves and bark are mixed with tea which is believed to cure coughs and colds and is a very popular stimulant and diuretic drink in Indian subcontinent.<sup>[3]</sup>

Previously, various researchers reported its phytochemical and pharmacological values by using standard experimental methods. For example, leaves of the plant are effective in diabetic rats<sup>[4,5]</sup> as well as has antioxidant,<sup>[6-10]</sup> hypoglycaemic,<sup>[11,12]</sup> anti-inflammatory and immunomodulation properties. The extract of the plant also has antidiarrhoeal<sup>[13]</sup> and gastroprotective<sup>[14]</sup>

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activity in rats. The stem bark has antibacterial potential.<sup>[15]</sup> It is also reported to have lipid lowering activity.<sup>[16]</sup> Leaf extract of the plant was reported to have antidiabetic and antioxidant activities in streptozotocin (stz) treated diabetic rats<sup>[17]</sup> and bark was reported to have anti-diabetic activity by using  $\alpha$ -amylase inhibition assay.<sup>[18]</sup>

As a part of our on-going pharmacological screening of selected Bangladeshi medicinal plants,<sup>[19-22]</sup> this report describes the presence of important phytochemical groups, total phenolic content as well as cytotoxicity, antimicrobial, thrombolytic and diuretic activities of *C. tamala* estimated by using standard *in vitro* methods and animal models for the first time.

## MATERIALS AND METHODS

### Collection and Identification of Plant

The fresh leaves of *C. tamala* were collected from Gollamari, Khulna, Bangladesh, during the month of April 2011 on the day time and were taxonomically identified by the experts of Bangladesh National Herbarium (Accession No.: DACB-31284).

### Animals

Swiss albino mice of either sex purchased from Animal branch of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and housed in polypropylene cages at a temperature of  $23 \pm 2^\circ\text{C}$  and relative humidity  $60 \pm 5$  with 12 hour each of dark and light cycles to adapt the laboratory environment. Standard pellets obtained from ICDDR, B were used as a basal diet during the experimental period. The investigation for cytotoxic property of the ethanol extract was done on *Artemia salina* (Brine shrimp). The Brine shrimp eggs of Artemia cysts (Brine shrimp eggs of Artemia cysts, Ocean Star International Inc. P.O. Box 643, Snowville, UT, U.S.A.) were purchased from the M/S. Jalil Hatchery and Nursing, Debhata, Satkhira. One spoon of cyst were hatched for 48 hours in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 L water. The cyst became nauplii. The experimental protocols were conducted in accordance with internationally accepted standard guidelines for care and use of laboratory animals.

### Bacterial and Fungal Strains

Ten Gram negative bacteria namely, *Escherichia coli*, *Pseudomonas aureus*, *Plesiomonas shigelloides*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, *Proteus vulgaris* and seven Gram positive bacteria such as *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Staphylococcus epidermis* were used for antibacterial activity test. For antifungal activity assay three fungal strains *Candida albicans*, *Aspergillus niger*,

*Sacharomyces cerevaceae* were used. The test strains were collected from the Microbiology Laboratory, ICDDR, B.

### Media

Nutrient agar (NA), Nutrient broth (NB), Potato dextrose agar (PDA) and Potato dextrose broth (PDB) media manufactured by HiMedia Laboratories Ltd., India were used for microbial studies.

### Growth and Maintenance of Test Microorganism for Antimicrobial Studies

The bacteria were maintained on NB medium at  $37^\circ\text{C}$  and the fungi were maintained on PDB medium at  $28^\circ\text{C}$ . Then the stock culture of each organism was sub-cultured onto appropriate selective media. Colonies of the pure organisms were cultured in 10 ml of broth medium in sterile test tubes and incubated at  $37^\circ\text{C}$  overnight. The cultures were adjusted to a suspension density equals to 0.5 McFarland turbidity standard, which has an approximate cell density of  $1.5 \times 10^6$  cfu/ml.<sup>[23]</sup>

### Preparation of Inoculums

The pre-cultured test bacteria were taken in a rotary shaker at  $37^\circ\text{C}$ , centrifuged at 10,000 rpm for 5 min, then the pellets were suspended in double distilled water and the cell density was standardized spectrophotometrically (A 610 nm) to obtain a final concentration of approximately  $1-2 \times 10^7$  cfu/ml. The inoculums of fungi were prepared from 5-10 days old culture grown on PDA medium. The Petri dishes were flooded with 8-10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A 595 nm) to obtain a final concentration of approximately 105 spores/ml.<sup>[23]</sup>

### Chemical and Drugs

Ethanol ( $\geq 99.5\%$ , Merck KGaA, Darmstadt, Germany) was used as solvent for maceration of the plant material. Sea salt (sodium chloride Crystal GR; Merck Ltd., Mumbai, India) was used as medium for hatching the eggs of shrimp. Folin Ciocalteu's reagent (Sigma-Aldrich Co. LLC, Missouri, United States) were employed for determination of total phenolic content. Streptokinase (Shanghai SIPI Pharmaceutical Co., Ltd., China) was used in thrombolysis activity test.

### Extraction of Plant Material

The dried leaves (200 g) were ground into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China) and were extracted by ethanol (90%) at room temperature. The mixture was kept for 7 days and extract was filtered and air dried. The resulted semisolid mass was stored in air tight container in refrigerator at  $4^\circ\text{C}$ . The yield value was found to be 3.75% (w/w) and dried greenish black residue was designated as crude extract of *C. tamala* (ECT).<sup>[24]</sup>

### Phytochemical Studies

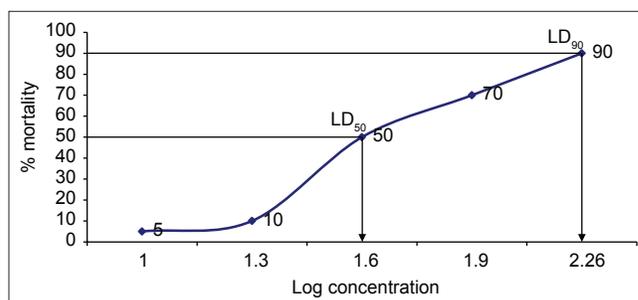
The extract was subjected for phytochemical study by using standard reagents for detection of different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins.<sup>[25]</sup> Ten percent (w/v) solution of the extract in ethanol was prepared for the tests.

### Determination of Total Phenolic Content

Dried plant material was weighed and 0.5 g of it was mixed with 50 ml of 80% aqueous ethanol. It was sonicated for 20 min. Two ml of the extract was centrifuged for 15 min at 14000 rpm. Total phenolic content was determined by folin ciocalteu's reagent.<sup>[26]</sup> In brief, gallic acid solution in ethanol was prepared to get concentrations of 20, 40, 60, 80, 100, 120 and 140 mg/ml. From each of the concentrations, as well as from the extract, 1 ml was transferred in 25 ml volumetric flasks containing 9 ml distilled water. Folin Ciocalteu's reagent (1 ml) was added to each volumetric flask and was mixed by shaking. After 5 min, 10 ml of 7% Na<sub>2</sub>CO<sub>3</sub> was added to it and the volume was adjusted to 25 ml by adding distilled water. Then kept for 25 min at room temperature, absorbance was measured at 750 nm against blank. Blank was prepared by following all the above steps except the addition of gallic acid. Standard curve was prepared using the absorbance of various gallic acid concentrations. Total phenolic content of the extract was determined from the standard curve and expressed as mg gallic acid equivalent (GAE)/100 g dried plant material.

### Brine Shrimp Lethality Bioassay

Cytotoxic potential of the plant extract was measured by using *in vitro* brine shrimp lethality assay.<sup>[27]</sup> At first, the shrimp eggs were hatched in simulated seawater (3.8% w/v sea salt in distilled water) at 25-30°C in front of a lamp. Eggs were left for 48 h to produce large number of larvae (nauplii). Then, test solutions were prepared in different concentrations 160, 80, 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 µg/ml by dissolving the extract in dimethyl sulfoxide (DMSO). For each sample four test tubes were used and in each test tube 10 shrimps were taken and the prepared extract solutions were applied in it. Finally, volume of liquid was adjusted by saline water. Ten shrimps were taken in a test tube (control) containing saline water and DMSO and were kept for observation under the same conditions with the test sample. The test tubes were kept for 24 h, and the surviving nauplii were counted. To calculate the lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>), percent mortality and log concentrations of the extract were plotted which produced an approximate linear correlation of concentration and mortality on graph. The 50% and 90% lethal concentrations (LC<sub>50</sub>) and LC<sub>90</sub>) were determined from the graph [Figure 1]. The results are summarised in Table 1.



**Figure 1:** Brine shrimp lethality bioassay of the ethanol leaves extract of *Cinnamomum tamala*

### Antibacterial and Antifungal Assay by Disc Diffusion Assay

Sterile blank discs (BBL, Cocksville, USA) were impregnated with test substances at the dose of 400 and 600 µg/disc. These discs along with positive standard disc (30 µg/disc) (Kanamycin, Oxoid Ltd., UK) and negative control discs were placed in petridishes containing the Mueller-Hinton agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates were then kept in an incubator (37°C) to allow the growth of the bacteria. The antibacterial and antifungal activities of the test samples were determined by measuring the diameter of the zone of inhibition in terms of millimeter.<sup>[25]</sup>

### Determination of Minimum Inhibitory Concentration

The MIC of the ethanol extract was determined by the broth dilution method.<sup>[28]</sup> In this method, extract solutions of increasing concentration were prepared using ethanol. Thirteen sterilised screw cap tubes each containing 3 ml of NB media were taken. Then, extract solution was added into the 10 test tubes to obtain different concentrations (200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,200 µg/ml, respectively), in which 10 µl (from log phase of growth; 5 × 10<sup>6</sup> cells/ml) of inoculum was taken. Additional three test tubes were also included as control for media (Cm; only media without inoculum, solvent and extract), control for inoculum (Ci; inoculum and medium without solvent and extract) and control for solvent (Cs; inoculum and solvent and media). The first control tube (Cm) was included to check the sterility of the media, the second (Ci) was to produce the growth and thus turbidity without any solvent extract and the third control tube (Cs) was to check whether the solvent itself caused any inhibition. Then, all the tubes were incubated over night at 37°C. After incubation, tubes having lowest concentrations of extract showing no turbidity considered as MIC to that particular solvent extract.

### Acute Toxicity Study

The acute toxicity (LD<sub>50</sub>) of the extract in mice was determined by oral route.<sup>[24]</sup> The study was divided into two phases. In the initial phase, the range of doses producing

**Table 1: Results of brine shrimp lethality bioassay of leaf extract of *C. tamala***

Test Sample	Conc. ( $\mu\text{g/mL}$ )	Log Conc.	No. of alive shrimp (Mean $\pm$ SEM)	% Mortality	LC <sub>50</sub> $\mu\text{g/mL}$	LC <sub>90</sub> $\mu\text{g/mL}$
ECT	10	1	9.5 $\pm$ 0.71	05	40	160
	20	1.3	9 $\pm$ 0	10		
	40	1.6	5 $\pm$ 0.71	50		
	80	1.9	3 $\pm$ 0	70		
	160	2.3	1 $\pm$ 0	90		

Values are expressed as mean $\pm$ SEM where  $n=3$ ; ECT – Ethanol extract of *C. tamala*

the toxic effects was established. Four groups of 3 mice each were selected. The first group received extract at a dose of 10 mg/kg body weight per oral (p.o.) while the second, third, and fourth groups received 100, 500, and 1,000 mg/kg b. wt. p.o., respectively. The animals were observed for signs of toxicity and death within 24 h. In the second phase, four groups of one mouse each were used. Specific doses were administered, which depended on the result of the first phase. The final LD<sub>50</sub> values were calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of consecutive doses for which 0% and 100% survival rates were recorded.

#### Determination of Diuretic Activity

Diuretic activity of the extract was investigated using the method as described by Lipschitz *et al.*<sup>[29]</sup> The test animals were randomly chosen and divided into five groups having 10 mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metallic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg b. wt., p.o. Group-2 and Group-3 were given urea solution at a dose of 500 mg/kg and Furosemide at a dose of 0.5 mg/kg b. wt., p.o. respectively. Group-4 and group-5, the test groups were treated with the extract at the doses of 200 and 400 mg/kg b. wt., p.o. respectively. From the graduated urine chamber of metallic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar.<sup>[30]</sup>

#### Determination of *In Vitro* Thrombolysis Activity

Phosphate buffered saline (PBS) (5 ml) was added to the commercially available lyophilised streptokinase vial (15, 00,000 I.U.) and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity. Experiments for clot lysis were carried following the method described by Chowdhury *et al.*<sup>[31]</sup> Firstly, 2 ml venous blood drawn from healthy volunteers was distributed in three different pre-weighted sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation,

serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighted to determine the clot weight. Then, 100  $\mu\text{l}$  of extract (10 mg/ml) was added to each microcentrifuge tube containing pre-weighted clot. As a positive control, 100  $\mu\text{l}$  of Streptokinase and as a negative non thrombolytic control, 100  $\mu\text{l}$  of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighted to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis expressed as percentage of clot lysis.

Clot weight was calculated as follows:

$$\text{Clot weight} = W_{CT} - W_T$$

Where,  $W_{CT}$  = Weight of clot containing tube and  $W_T$  = Weight of tube alone.

Percentage of clot lysis was estimated by using the following equation:

$$\% \text{ of clot lysis} = W_I - W_F$$

Where,  $W_I$  = Initial weight of clot and  $W_F$  = Final weight of clot.

#### Statistical Analysis

The experimental data were statistically evaluated by using Student's "t" test and values were expressed as mean  $\pm$  SEM, where the sample or positive control groups were compared with control group. Values with  $P < 0.05$  were regarded as significant.

## RESULTS AND DISCUSSION

#### Phytochemical Studies

In different chemical group tests it was revealed that ethanol extract of *C. tamala* leaves contained alkaloids, steroids, reducing sugars and significant amount of tannins [Table 2].

#### Determination of Total Phenolic Content

The total phenolic content of *C. tamala* leaves extract revealed to be 276 GAE/100 g of dried plant material.

**Table 2: Results of phytochemical group test of leaf extract of *C. tamala***

Test sample	Alkaloids	Steroids	Saponins	Tannins	Flavonoids	Reducing sugars	Gums
ECT	+	+	-	+	-	+	+

ECT – Ethanol extract of *C. tamala*; '+' indicates positive result and '-' indicates positive result

### Brine Shrimp Lethality Bioassay

In brine shrimp lethality bioassay [Table 1], the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. For the extract, the number of nauplii died and percent mortality was counted. From the plot of percent mortality versus log concentration on the graph paper [Figure 1], LC<sub>50</sub> and LC<sub>90</sub> were found to be 40 µg/mL and 60 µg/mL, while the both of 5-fluorouracil were 4.5 µg/mL and 6.5 µg/mL respectively. Control was used to see whether the solvent DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality.

### Antibacterial and Antifungal Activity

The antibacterial and antifungal properties of the extract was assessed by conventional disc diffusion method against 19 pathogenic bacterial and fungal strains, and the results were compared with the activity of the positive control, kanamycin (30 µg/disc) [Table 3]. The extract showed considerable antibacterial activities against most of the test organisms. The most prominent anti-bacterial profile was exhibited against *Salmonella typhi* with the zones of inhibition of 17 and 21 mm, in the concentration of 400 and 600 µg/disc respectively. The most noteworthy activity was observed against all three *Shigella* species tested (zones of inhibition 6 mm). Although all test microorganisms are pathogenic, the *Shigella* species are of particular importance, as they are one of the major causes of deadly diarrhoea and dysentery in Bangladesh.

Diarrhoea and dysentery, caused by *Shigella* infections are one of the major causes of morbidity and mortality particularly among children under 10 years of age, not only in Bangladesh, but also in many other poor and under-developed countries. Countries that are affected by recurrent flood, e.g., Bangladesh, are under specific threat from water and food borne *Shigella* infections. It is interesting to note that, the antibacterial activity of the leaves of *C. tamala* observed in this study is quite similar to that of the bark of this plant, previously reported elsewhere.<sup>[15]</sup> Thus, it is reasonable to assume that the leaves of *C. tamala* may possess same or similar chemical profile.

### Thrombolytic Activity

The percentage (%) clot lysis was statistically significant ( $P < 0.001$ ) when compared with control group. The plant extract showed moderate activity with  $22.33 \pm 1.23\%$ ,  $24.75 \pm 1.27\%$  and  $21.78 \pm 1.74\%$  clot lysis of samples from volunteer 1, 2 and 3, respectively; whereas,

**Table 3: Results of antimicrobial sensitivity test of leaf extract of *C. tamala***

Test organisms	Diameter of zone of inhibition in mm			
	Std	ECT400	ECT600	MIC(µg/ml)
Gram negative bacteria				
<i>Escherichia coli</i>	41	-	12	500
<i>Pseudomonas aureus</i>	23	-	-	-
<i>Plesiomonas shigelloides</i>	25	13	18	300
<i>Salmonella typhi</i>	41	17	21	250
<i>Salmonella paratyphi</i>	34	14	19	300
<i>Shigella dysenteriae</i>	35	-	8	500
<i>Shigella flexneri</i>	34	-	6	500
<i>Shigella boydii</i>	26	17	19	500
<i>Shigella sonnei</i>	34	-	6	500
<i>Proteus spp.</i>	41	15	17	300
Gram positive bacteria				
<i>Enterococcus faecalis</i>	43	-	14	500
<i>Staphylococcus saprophyticus</i>	25	-	-	-
<i>Staphylococcus aureus</i>	24	-	-	-
<i>Streptococcus pyogenes</i>	21	5	8	300
<i>Staphylococcus epidermis</i>	35	-	-	-
Fungi				
<i>Candida albicans</i>	34	5	9	300
<i>Aspergillus niger</i>	32	7	12	400
<i>Sacharomyces cerevaceae</i>	36	-	7	500

ECT400 – Ethanol extract of *C. tamala* at the dose of 400 µg/disc, ECT600 – Ethanol extract of *C. tamala* at the dose of 600 µg/disc, '-' indicates no zone of inhibition; MIC – Minimum inhibitory concentration

standard streptokinase produced  $48.22 \pm 2.98\%$  clot lysis [Table 4]. The effect of the extract on clot lysis activity is shown in [Figure 2].

### Diuretic Activity

The effect of the ethanol extract of the powdered leaves of *C. tamala* on the urination of mice was observed for 5 h which revealed that the extract has a mild diuretic effect in the test animals. This was comparable to that of standard drug Furosemide and diuretic agent urea [Table 5]. Electrolyte loss showed similar ratio ( $\text{Na}^+/\text{K}^+$  excretion ratio was 1.46 and 1.59 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.40).

## DISCUSSION

Based on the ethnobotanical uses of the plant in bacterial infections like skin diseases and dysentery, the crude extract was chosen for evaluation of antibacterial activity. The extract showed a wide spectrum of activity. The highest zone of inhibition (19 mm) was against *S. paratyphi*. While determining the MIC, pathogens against which *C. tamala*

leaf extract showed highest activity against *Salmonella typhi* (250 µg/mL). It is widely accepted that disc diffusion assay cannot be the sole method for screening antibacterial activity due to the inherent limitation of this technique. If the active compounds are non polar in nature, they may not diffuse in the polar media giving rise to a false negative result. This is also evident from the current experiment in which the extract showed no zone of inhibition against some of the strains of *Shigella* (500 µg/disc) in disc diffusion assay, but MICs were observed against the same organisms within 250-500 µg/mL. Since the extract showed activity against some bacterial strains in disc diffusion assay, it is possible that more than one compound of polar and non polar nature is associated with the antibacterial activity against different organisms included in the study.

To get a better insight into the total phenolic content of the extract, Folin Ciocalteu's reagent was used. Total phenolic content of the extract was similar to some of the high phenol content fruits like strawberry and plum (244.1 and 303 mg GAE/100 g, respectively).<sup>[26]</sup> Antioxidant activity of plant extract is to some extent due to the presence of phenolic components like flavonoids, chalcones, polyhydroxy benzoic acid derivatives.<sup>[32]</sup> The antioxidant activities of the different parts of *C. tamala*<sup>[7,9-11]</sup> may be due to the presence of phenolic compounds. Advanced investigations are required to carry out to isolate the phenolic antioxidant components.

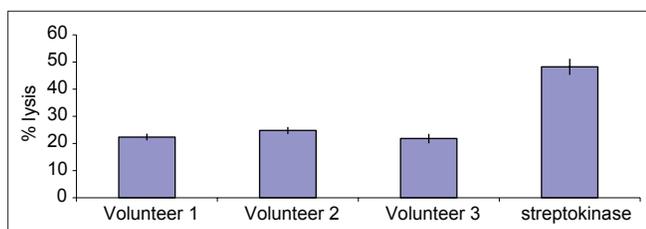


Figure 2: Clot lysis activity of *Cinnamomum tamala* leaf extract

Table 4: Results of thrombolytic activity of leaf extract of *C. tamala*

Sample no.	Percent of clot lysis (%)
Volunteer 1	22.33±1.23*
Volunteer 2	24.75±1.27*
Volunteer 3	21.78±1.74*
Streptokinase	48.22±2.98*

Values are expressed as mean±SEM where n=3; \*P<0.001

Table 5: Effect of leaf extract of *C. tamala* on urine excretion parameters in mice

Treatments	Volume of urine (ml)	Concentrations of ions (m.eq.l <sup>-1</sup> )			
		Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup> /K <sup>+</sup>
Control	2.87±0.2	72.54±1.19	47.89±1.21	76.78±1.12	1.51
Urea	3.72±0.03	107.98±1.28**	74.18±1.56*	82.63±1.71*	1.45
Furosemide	4.69±0.18	122.84±1.29*	87.39±1.77*	92.67±1.79*	1.40
ECT200	2.98±0.12	97.51±1.35*	66.52±1.22*	72.37±1.31**	1.46
ECT400	3.43±0.07	109.61±1.43**	68.92±1.58**	66.23±1.48*	1.59

ECT200 – Ethanol extract of *C. tamala* leaves at the doses of 200 mg/kg and ECT400 – Ethanol extract of *C. tamala* leaves at the doses of 400 mg/kg; Values are expressed as mean±SEM where n = 10; \*\*P<0.001 \*P<0.05.

Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity.<sup>[27]</sup> In the present study, the extract showed LC<sub>50</sub> at a low concentration indicating that the extract is quite potent. It is of interest to find whether the observed antibacterial activity and cytotoxic activity are due to the same compound (s) or not. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells.<sup>[33]</sup> It is necessary to test this extract against various cancer cell lines as well as normal cell lines to justify the potential to further investigate this plant for anticancer activity. The toxicity could also be due to compounds with a different pharmacological activity since pharmacologically active compounds often tend to be toxic in high doses.<sup>[34,35]</sup> Further investigation is required to find the responsible compound (s) for the cytotoxic activity observed for *C. tamala*.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilising Na<sup>+</sup> and Cl<sup>-</sup> from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na<sup>+</sup> and Cl<sup>-</sup> load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume.

These findings suggest that the traditional uses of the plant are relevant to its experimental results. Further studies are necessary to identify the specific compounds which are exactly producing these actions. However, this study will provide preliminary idea about the therapeutic importance of the plant and the compounds contained in it may appear as new or novel therapeutic agents.

## CONCLUSION

Present study is based on the report of preliminary biological screening of *C. tamala* leaf extract. The results are quite promising and demands further investigation. Advanced studies including Liquid chromatography–mass spectrometry (LC-MS) can be carried out to get a bigger picture of the chemical constituents present in the plant. Screening methods applying various cell lines or bacterial enzymes can be carried out to find the underlying mechanism for the observed biological activities. On the basis of the results from above studies, bioassay guided approach can be undertaken to isolate and identify the active component(s).

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