In vitro antioxidant and cytotoxic activity of ethanolic extract of *Cinnamomum tamala* (Tejpat) leaves

Salma Akter[1], Md. Ashraf Ali[*3], Ranjan Kumar Barman[2], Bytul Mokaddesur Rahman[2] and Mir Imam Ibne Wahed[2]

[1]Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh
[2]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh
[3]Department of Pharmacy, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh
*Email: ashrafali025@gmail.com

ABSTRACT

The present research was aimed to investigate in vitro antioxidant and cytotoxic effects of the ethanolic extract of the leaves of *Cinnamomum tamala* (Nees). The leaves of *Cinnamomum tamala* have shown good antioxidant and cytotoxic properties. Antioxidant activity of the extract was evaluated by using DPPH free radical scavenging assay and ascorbic acid used as a standard. The IC_{50} value of *Cinnamomum tamala* leaves was 13.55 µg/ml while the IC_{50} value of ascorbic acid was 5.35 µg/ml. Cytotoxic activity was evaluated by using brine shrimp lethality bioassay and vincristine sulphate used as a standard. In this assay, the LC_{50} value of the ethanolic extract of *Cinnamomum tamala* leaves was 17.82µg/ml whereas LC_{50} value of vincristine sulphate was 5.24µg/ml. Therefore, these results suggest that leaves extract of *Cinnamomum tamala* has antioxidant and cytotoxic activities.

Keywords: *Cinnamomum tamala (CT)*; DPPH radical; Brine shrimp; Antioxidant activity and Cytotoxic activity.

INTRODUCTION

Free radicals produced in the tissues of the body are capable of attacking the healthy cells causing them to lose their structure and function. Oxidative stress produced leads to the formation of altered physiological and pathological conditions. When the availability of antioxidants is limited this damage can become cumulative and debilitating [1]. Overall free radicals have been implicated in the pathogenesis of at least 50 diseases. Among them the prevalent disease are aging process, joint disorders, asthma, ischemia, inflammation, mongolism, neurodegeneration, parkinson's disease and dementia.

Cinnamomum tamala, also known as tejpata is a tree within the Lauraceae family which is native to Bangladesh, India, Nepal, Bhutan, and China. It can grow up to 20 m (66 ft) tall [2]. It has aromatic leaves which are used for culinary and medicinal purposes. The leaves, known as tejapatta or tejpatta in Hindi and in Nepali, tejpata in Bengali, are used extensively in the cuisines of Bangladesh, India, Nepal, and Bhutan. The bark is also sometimes used for cooking. The leaves of this plant have been reported on antidiarrhoeal [3], antidiabetic [4], antioxidant [4], hypoglycemic [5] and anti-inflammatory activities [5, 6]. *Cinnamomum tamala* also have cytotoxic activity against ehrlich ascites carcinoma (EAC) in mice [7]. Essential oil of this plant also has antibacterial [8] and antifungal properties [11]. The bark of this plant possesses antidiabetic properties [9]. The major constituents of the leaf essential oils of these species contain furanosesquiterpenoids as principal constituents. Furanogermenone (59.5%) was found to be the major compound in the leaf essential oil is β-caryophyllene (6.6%), sabinene (4.8%), germacrene D (4.6%) and curcumenol (2.3%). The leaf oil was characterized by a high content of sesquiterpenoids (96.8%), dominated mainly by furanosesquiterpenoids (79.3%) viz. furanodienone (46.6%), curzerenone (17.6%), furanodiene (1.8%) and curzerene (1.2%). Cinnamon leaf oil contains a variety of constituents including eugenol and cinnamaldehyde, which is a local mucous and dermal membrane irritant [10, 11, 12, 13, and 14]. So, the objective of present study was to evaluate the antioxidant and cytotoxic activity of ethanolic (95%) extract of *Cinnamomum tamala* leaves.

MATERIALS AND METHODS

Plant Materials

Fresh stem bark of the plant *Cinnamomum tamala* leaves was collected from local market of Dhaka city in March 2011 and the leaves authenticity was confirmed from the Bangladesh National Herbarium, Dhaka.

Preparation of Plant Extract

The collected stem barks were washed and sun dried under shadow for several days. The dried stem barks were powdered in an electrical grinder after overnight drying in an oven below 50°C. The powdered plant barks were extracted with 95% ethanol at room temperature. The bottle were kept at room temperature and allowed to

stand for several 7-10 days with occasional shaking and stirring. The extracts thus obtained were filtered through cotton and then through filter paper (Whatman Fitter Paper No. 1). The filtrate was defatted with petroleum ether for several times. Then, the defatted liquor was allowed to evaporate using rotary evaporator at temperature 40-45°C. Finally, a highly concentrated ethanol extract were obtained and kept in desiccators to dry to give a solid mass (1.5 %).

Drugs and Chemicals

DPPH (2, 2-diphenyl, 1-picrylhydrazyl) obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India. All chemicals and solvents were of reagent grade.

In vitro antioxidant activity of CT extract by DPPH free radical scavenging assay

The scavenging effects of samples for DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical were monitored according to the method of Brand-Williams, W. et al. [15]. Briefly, 0.004 gm weight of DPPH taken into a 100ml of a volumetric flask and then the volume is adjusted by methanol. Then the concentration of the solution is 0.004% of DPPH. The absorbance of this solution was taken at 517nm against methanol as a blank and recorded as a control solution standard. Accurately weight 0.025gm of ascorbic acid and dissolved it into 5ml of distilled water. The concentration of the solution is 5µg/µl of ascorbic acid. This solution is called stock solution. Take 0.025gm of plant extract and dissolved it into 5 ml of methanol. The concentration of the solution is 5µg/µl of plant extract. This solution is called stock solution. 200 µl of plant extract or standard of different concentration solution was taken in a test tube. 2ml of reagent solution was added in test tube. Incubate the test tube for 30 mins to complete the reaction. Then the absorbance of the solution was measured at 517nm against methanol as a blank by using UV spectrophotometer. A typical blank solution contained methanol. The mixture was mixed well and then left to stand in the dark for 30 min at room temperature, and its absorbance was read at 517 nm with a spectrophotometer against a blank. All measurements were done in triplicate.

The percentage (%) of scavenging of the DPPH free radical was measured by using the following equation:

$$\{(A_0-A_1)/ A_0\}\times 100$$

Where, A_0 = absorbance of the control

A_1 = absorbance of the extract/ standard

Then the percentage (%) of inhibition was plotted against concentration and from the graph IC_{50} was calculated.

In vitro cytotoxic activity of CT extract by Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds The cytotxic activity was determined according to the method of Meyer et al., 1982 and Zhao et al., 1992 [16, 17]. Briefly here, simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The dried cyst of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for 48 h day/dark cycles to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii at eight concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml. All doses were calculated by serial dilution technique. The eight test tubes were marked as 1, 2, 3, 4, 5, 6, 7 and 8 for each concentration of the extract. The sample (extract) was prepared by dissolving 4 mg of extract in 50 µl DMSO and volume adjusted to 5 ml with sea water (3.8% NaCl in water) to attain concentrations 800 µg/ml in the first test tube. Now 2.5 ml sample was transferred from first test tube to second test tube where volume adjusted to 5 ml with sea water and from this test tube 2.5 ml sample was transferred to third test tube and so on. In this case, all test tubes contained 2.5 ml sample with double concentrations of the test doses of the extract. Now, ten nauplii were transferred to each test tube and the final volume was made 5 ml by adding sea water. So, due to double dilution, our expected concentrations were then attained in the respective test tubes. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard vincristine sulphate was used as positive control.

Then the matured shrimps were applied to each of all experimental vials. After 24 h incubation, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The percentage (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \{N_t/N_0\}\times 100$$

Where, Nt = Number of killed nauplii after 24 h of incubation,

N_0 = Number of total nauplii transferred, that is 20.

Then the percentage (%) of mortality was plotted against log concentration and from the graph LC50 (Median lethal concentration) was determined.

RESULTS AND DISCUSSION

The outcome found for the antioxidant and cytotoxic activities of leaves of *Cinnamomum tamala* was discussed in detail in this chapter. It was shown that the sample has shown significant potential for antioxidant and cytotoxic activities.

Antioxidant activity

The ethanolic extract of the plant was subjected to free radical scavenging activity by the method of Brand Williams W. *et al*., 1995 [15]. The antioxidant activity of the plant was measured by DPPH free radical scavenging assay and ascorbic acid was used as standard in this investigation. The IC50 value (concentration of sample require to scavenge 50% free radical or to prevent lipid peroxide by 50%) of all the extracts were calculated. The IC50 value of ethanolic extract of *Cinnamomum tamala* (leaves) was 13.55µg/ml and that of standard ascorbic acid was 5.35 µg/ml shown in **table 1** and **figure 1**. This result indicates significant antioxidant properties.

Table 1: IC_{50} Value of ethanolic extract of *Cinnamomum tamala* leaves and ascorbic acid

Sample	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
Ethanolic extract of *Cinnamomum tamala* leaves	500	87.61	13.55
	200	80.88	
	100	72.88	
	50	52.35	
	25	46.47	
	5	37.94	
	1	33.80	
Ascorbic acid	500	95.26	5.35
	200	89.96	
	100	78.59	
	50	70.23	
	25	51.47	
	5	47.58	
	1	43.76	

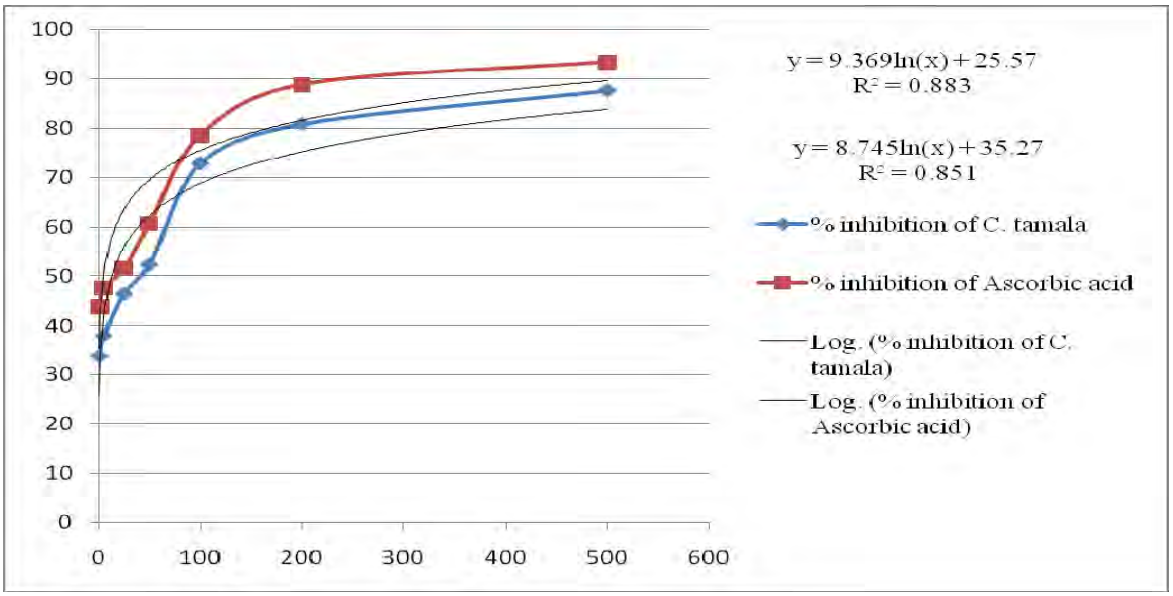

Figure 1: DPPH free radical scavenging assay of ethanolic extract of *Cinnamomum tamala* and ascorbic acid (% of inhibition vs concentration)

Cytotoxic activity through Brine Shrimp lethality bioassay

In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening and discovery of new biotic natural products. The ethanolic extract of samples was subjected to brine shrimp lethality bioassay and vincristine sulfate was used as standard in this investigation.

The LC_{50} value of standard vincristine sulfate was 5.24 µg/ml and the LC_{50} value of ethanol extract of *Cinnamomum tamala* was 17.82 µg/ml which indicating good cytotoxicity properties of the plant extract shown in **table 2, 3 and figure 2.**

Table 2: LC_{50} value of ethanolic extract of *Cinnamomum tamala* leaves

Concentration (µg/ml)	Log of concentration	No. of nauplii added	No of nauplii alive	No. of nauplii dead	% of mortality	LC_{50} (µg/ml)
400	2.602	20	1	19	95	
200	2.301	20	2	18	90	
100	2.0	20	4	16	80	
50	1.698	20	6	14	70	17.82
25	1.397	20	10	10	50	
12.5	1.096	20	12	8	40	
6.25	0.795	20	13	7	35	
3.125	0.495	20	15	5	25	

Table 3: LC_{50} value of vincristine sulphate used as control

Concentration (µg/ml)	Log of concentration	No. of nauplii added	No of nauplii alive	No. of nauplii dead	%of mortality	LC_{50} (µg/ml)
400	2.602	20	0	20	100	
200	2.301	20	0	20	100	
100	2.0	20	0	20	100	5.24
50	1.698	20	2	18	90	
25	1.397	20	4	16	80	
12.5	1.096	20	8	12	70	
6.25	0.795	20	10	10	50	
3.125	0.495	20	14	6	30	

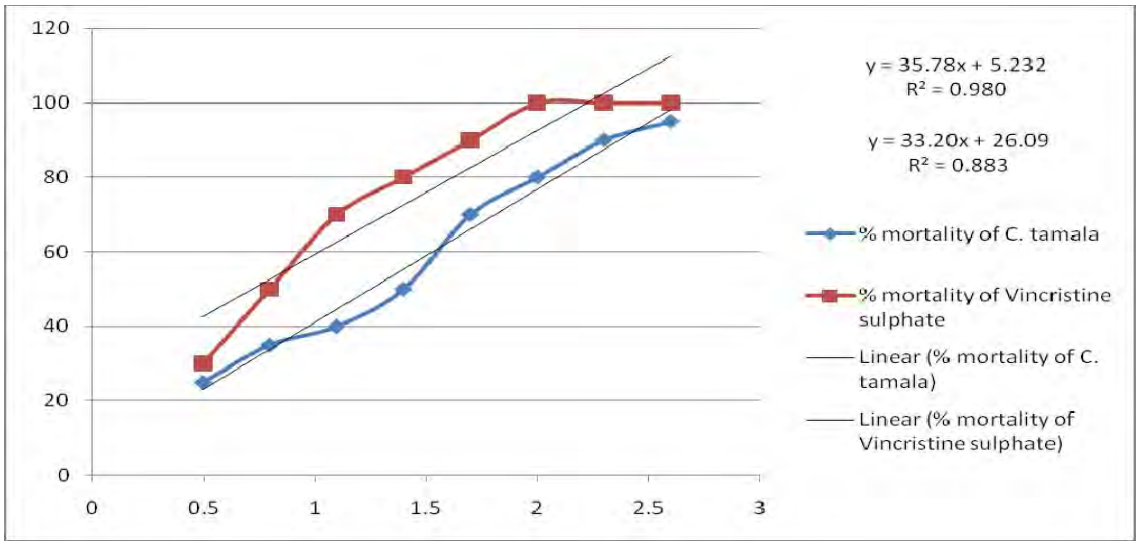

Figure 2: Brine Shrimp lethality bioassay (% of mortality vs log concentration)

Moreover, the leaves of this plant contain phenol, ascorbate, and carotenoids revealed that *Cinnamomum tamala* had high antioxidants [18]. Previously a report said that the presence of many phytochemical moieties such as phenolics, flavonoids, tannins, terpenoids, alkaloids and saponins in *C. tamala* leaf extract (19). Biological activities of plants may be due to the presence of these diverse groups of chemical compounds [20]. So, the

antioxidant and cytotoxic activity of *Cinnamomum tamala* leaf probable responsible due to the presence of polyphenolic compound, phenol, ascorbate, carotenoids, flavonoids, tannins, terpenoids, alkaloids and saponins.

CONCLUSION

The plant has been being used for a long time in herbal medicine without knowing the exact phytopharmacological properties that works against certain disease. The ethanolic extract of *Cinnamomum tamala* possesses antioxidant and cytotoxicity properties which were comparable to the well known standard ascorbic acid and vincristine sulphate respectively. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

Acknowledgements

The authors would like to express thanks, to Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh for providing lab facilities for this research work.

REFERENCES

[1] Mark P: Antioxidants. Clin Nut Insights 1998, 96: 1-4.
[2] Xi-wen Li, Jie Li & Henk van der Werff. "Cinnamomum tamala". Flora of China. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA
[3] Rao, Chandana Venkateswara, Vijayakumar, M; Sairam, K; Kumar, V: Antidiarrhoeal activity of the standardised extract of Cinnamomum tamala in experimental rats. Journal of Natural Medicines 2008, 62 (4): 396–402.
[4] Usha Chakraborty and Hariswami Das: Antidiabetic and Antioxidant Activities of Cinnamomum tamala Leaf Extracts in Stz-Treated Diabetic Rats. Global Journal of Biotechnology & Biochemistry 2010, 5 (1): 12-18
[5] Kar A, Choudhary BK, Bandyopadhyay NG: Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol. 2003, 84(1):105-8.
[6] Chaurasia JK, Tripathi YB: Chemical characterization of various fractions of leaves of Cinnamomum tamala Linn toward their antioxidant, hypoglycemic, and anti-inflammatory property. Immunopharmacol Immunotoxicol 2011, 33(3): 466-72
[7] Manmeet S Saluja, B Sangameswaran and Ajay Sharma: Cytotoxic activity of Cinnamomum tamala linn against ehrlich ascites carcinoma (EAC) in mice. The Pharma Research 2010, 3; 232-242.
[8] Prabuseenivasan S, Jayakumar M, Ignacimuthu S: In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine 2006, 6: 39-45
[9] Roux GF, Perrier J, Forest E, Mouren GM, Puigserver A, and Santimone M: Screening of bark of Cinnamomum tamala (Lauraceae) by using aamylase inhibition assay for anti-diabetic activity. Biochim Biophys Acta 1998, 1388: 10-20
[10] Sood RP, Padha CD, Talwar IP, Jamwal RK, Chopra MM and Rao PR: Essntial oils from the leaves of Cinnamomum tamala Nees and Ebern. Growing in Himachal Pradesh, Indian Perfumer 1979, 23: 75-78
[11] Simic A, Sokovic MD, Ristic M, et al.:The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytother Res. 2004, 18: 713-770.
[12] Shah M, Panchal M: International Journal of Pharmaceutical Sciences Review and Research 2010, 5(3): 141-144.
[13] Ahmed, Aftab Choudhary, M. Iqbal; Farooq, Afgan; Demirci, Betül; Demirci, Fatih; Can Başer, K. Hüsnü: Essential oil constituents of the spice Cinnamomum tamala (Ham.) Nees & Eberm. Flavour and Fragrance Journal 2000, 15 (6): 388–390.
[14] [14] Dighe, V. V., Gursale, A. A.; Sane, R. T.; Menon, S.; Patel, P. H: Quantitative Determination of Eugenol from Cinnamomum tamala Nees and Eberm. Leaf Powder and Polyherbal Formulation Using Reverse Phase Liquid Chromatography. Chromatographia 2005, 61 (9 - 10): 443–446.
[15] Brand-Williams, W., Cuvelier, M. E., & Berset, C: Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technology 1995, 28: 25–30.
[16] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JE, Nichols DE, McLaughlin JL: Brine shrimp: A convenient general bioassay for active plants constituents. J. Med. Plant Res. 1982, 45: 31-34.
[17] Zhao GY, Hui JK, Rupprecht JLM, Wood KV: Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of Asimina triloba. J. Nat. Prod. 1992, 55(3): 347-356.
[18] Usha Chakraborty and Hariswami Das: Antidiabetic and Antioxidant Activities of Cinnamomum tamala Leaf Extracts in Stz-Treated Diabetic Rats. Global Journal of Biotechnology & Biochemistry 2010, 5(1): 12-18,
[19] Mishra, A.K., Singh, B.K. and Pandey, A.K.: In vitro antibacterial activity and phytochemical profiles of Cinnamomum tamala (Tejpat) leaf extracts and oil. Reviews in Infection 2010, 1: 134-139.
[20] Kishore DV, Rahman R. Int. J. Pharm. Sci. Res. 2012, 3(5): 1452-1456.