



Assessment of Bioactivity of *Cinnamomum tamala* (Buch.-Ham.)

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ABSTRACT

Bioactivity of medicinal plants is due to presence of various kinds of primary and secondary metabolites. Among studied phytochemicals polyphenol was in higher quantity (16.7 ± 0.7 g/100g). Antipathogenic efficacy of *C. tamala* has been tested against, *S. typhi* (MTCC 3216), *P. aeruginosa* (MTCC 7837), *S. aureus* (MTCC 3160), *P. mirabilis* (MTCC 1249) and *B. subtilis* (MTCC 736) by agar diffusion method and broth dilution method and all the strains were affected by methanolic and aqueous leaf extracts of *C. tamala*. The minimum inhibitory concentration (MIC) values in agar diffusion method were 1.25mg-5mg and 2.5mg-5mg for methanolic and aqueous leaf extracts respectively. Complete inhibition was observed in broth dilution method at 1.25mg/mL-9mg/mL and 1mg/mL-13mg/mL concentration of methanolic and aqueous extracts respectively. The leaf extracts did not show cytotoxic at 0.2mg/mL- 1mg/mL concentration of aqueous leaf extract but showed haemolysis at 1mg/mL concentration of methanolic leaf extract of *C. tamala*. *C. tamala* leaves showed high nutritional values (143.5 ± 0.5 Kcal/100g). The nutritional components like protein, fats, carbohydrates and edible fibres were 6 ± 0.5 , 8.5 ± 1.8 , 9.5 ± 0.5 and 30.5 ± 0.6 g/100g respectively. Therefore it can be concluded that leaf extract of *C. tamala* can be used safely against above mentioned human pathogens and for the preparation of new pharmaceutical drug.

Introduction

Infectious diseases are the foremost cause of premature death and kill about 50000 people every day in the world. In recent years, drug resistance pathogenic bacteria have been commonly reported from all over the world due to indiscriminate use of antibiotics and the situation alarming the development of potent antimicrobial agents (Robin et al., 1998).

S. aureus is a notorious suppurative (pus forming) pathogen, a major cause of hospital acquired (nosocomial) wound infection, urinary tract infection, and food poisoning. *B. subtilis* causes food poisoning and allergic reaction. *P. mirabilis* is known to cause urethritis, cystitis, pyelonephritis, prostatitis and pneumonia. *P. aeruginosa* is an opportunistic pathogen known to cause endocarditis, respiratory tract, central nervous system and ophthalmic infections in patients suffering cancer, cystic fibrosis and burns. *S. typhi* is the causative agents of two diseases – typhoid and acute gastroenteritis (Todar, 2012; Dandapat et al., 2013b).

Synthetic antibiotics are the most popular antimicrobial agents during their discovery. However, the emergence of multiple drug resistant pathogenic bacteria has a major cause of failure of the antibacterial drug (Mathias et al., 2000) and synthetic antioxidant are more effective but have side effects. That is why, it is very

essential to investigate newer drugs with less resistance as which are cheap and easily available.

Medicinal plants has been used as perennial source of traditional remedies for thousands of years and various modern drugs which are used in treatment of various diseases and disorders have been isolated from natural sources (Dandapat et al., 2013b). In fact, plants produce different bioactive molecules, which act as different types of medicines and play an important role in the maintenance of human health (Farombi, 2003). Nowadays awareness about the use and importance of medicinal plants have increased, because medicinal plants are easily available, less expensive and have no side effects (Cathrine and Nagaraj, 2011).

Cinnamomum tamala belonging to the family Lauraceae and has been used for various kind of ailments such as anorexia, dryness of mouth, bladder disorder, diarrhea, typhoid, urethritis etc (Rao, 2008).

The present study was undertaken to investigate the phytochemical screening, nutritional potentiality, cytotoxicity and antibacterial efficacy of methanolic and aqueous leaf *Cinnamomum tamala* extracts against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Bacillus subtilis*.

Materials and Methods

Collection of plant material

The fresh tender leaves were collected from Ranchi district, washed and disinfected by treating with $HgCl_2$ and washed again. The leaves were dried in shade under room temperature for six to seven days, powdered and sieved (Kumar et al., 2013a).

Extract preparation

50 g of the fine powder was subjected to extraction by soxhlet using methanol and distilled water separately for methanolic and aqueous extract. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at $45^\circ C$ for proper dehydration methanol free because methanol induce toxicity to living organisms. Percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies (Kumar et al., 2013h).

Phytochemical screening

Total phenol was determined by Folin-Ciocalteu reagent following Ramamorthy and Bano (2007). Tannins were quantified as standard published in the quality control methods for medicinal plant materials (WHO, 1998). Aluminium chloride colorimetric method was used with some modifications to determine flavonoids content following Lin and Tang (2007). Alkaloid was determined by the method used by Helrich (1990). Saponin content was determined following Obdona and Ochuko (2001). The details have been described elsewhere Kumar et al. (2013b).

Anti-Bacterial analysis

Antibacterial efficacy of methanolic leaf extract of *C. tamala* was carried out against *Salmonella typhi* (MTCC 3216), *Pseudomonas aeruginosa* (MTCC 7837), *Staphylococcus aureus* (MTCC 3160), *Proteus mirabilis* (MTCC 1429) and *Bacillus subtilis* (MTCC 736) by agar diffusion method comparing with standard antibiotic Gentamycin and by broth dilution method. The details have been described elsewhere Kumar et al. (2013e).

Cytotoxicity

Cytotoxicity of plant extract was determined by assessing the haemolytic activity of aqueous and methanolic leaf extract using goat blood RBCs. An erythrocyte suspension was prepared by adding 5% (by volume) of sodium citrate (36.5 g/L) to fresh blood and centrifuged at 1000 rpm for 5 min to separate the erythrocytes. 2% erythrocyte suspension was prepared by adding 49 mL phosphate buffer (pH 7.4) to 1 mL packed erythrocytes. Serial dilution of plant extracts were prepared using phosphate buffer. 1 ml of citrated blood was mixed with equal volume of diluted plant extracts and the volume was adjusted to 5 mL by phosphate buffer. The mixture was allowed to stand for 20 minutes at room temperature and O. D. was measured spectrophotometrically at 540 nm (WHO, 1998; Kumar et al., 2013h).

Nutritional potentiality

Crude fat, carbohydrate and protein were quantified following previously published standard tests (Watanble and Olsen, 1965; Jayarama, 2005), and nutritive values were calculated on the basis of formula following Nile and Khobragade (2009). The details have been described elsewhere Kumar et al. (2013a).

Results and Discussion

Phytochemical screening

The results of phytochemical analysis of the leaf samples of *Cinnamomum tamala* have been presented in fig – 1. The result revealed that polyphenols was highest (16.7 ± 0.7 g/100g) and flavonoids were lowest (1.0 ± 1.01 g/100g) among all the studied phytochemicals. Similarly Kumar et al. (2013d) reported 6.13 ± 0.13 g/100g tannin, 2.09 ± 0.17 g/100g saponin, 2.1 ± 0.21 g/100g flavonoids, 0.13 ± 0.1 g/100g poly phenols in *A. vasica*. Kumar et al. (2013f) also reported 1.38 ± 0.5 g/100g tannin, 4.5 ± 0.63 g/100g saponin, 0.65 ± 0.2 g/100g flavonoid, and 1.73 ± 0.4 g/100g polyphenol and 2.6 ± 0.5 g/100g alkaloid in *T. cordifolia*. Dandapat et al. (2013b) reported phytochemicals such as tannin, saponins, alkaloids, phenols and flavonoids possess direct or indirect correlation with therapeutic efficacy against various diseases.

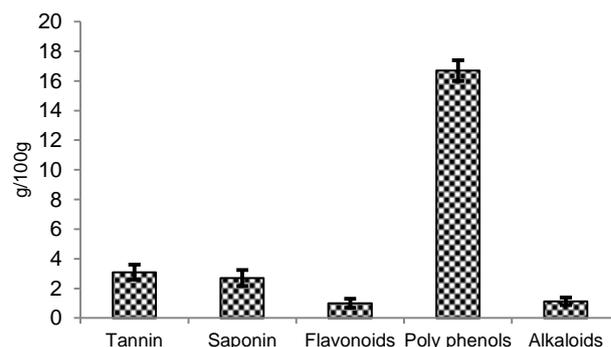


Figure 1 Proximate phytochemical composition of *Cinnamomum tamala* leaf ($M \pm SD$; $n = 3$).

Antibacterial efficacy

The antibacterial efficacy of methanolic extract of *C. tamala* leaf was quantitatively assessed on the basis of inhibition zone (ZOI) in mm and minimum inhibitory concentration (MIC) following the agar disk diffusion method and percent inhibition by broth dilution method. The test organisms were also inoculated with pure antibiotic-gentamycin to compare the efficacy of leaf extract for their microbial properties (Table – 2). In the present investigation the extract was found to be effective all the pathogens. The ZOI (Zone of inhibition) observed for the methanolic (M) and aqueous (A) extract using agar diffusion method is listed in table – 1.

The broth dilution method showed more pronounced antimicrobial activity through 100% inhibition of all the pathogens in the range of 1.25 – 10mg/mL concentration of methanolic leaf extract (Fig-2).

Table 1 The zone of inhibition and MIC (in mm) of methanolic leaf extract of *C.tamala*.

Concentration (mg/mL)	Zone of inhibition (ZOI in mm)									
	<i>S. typhi</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		<i>P. mirabilis</i>		<i>B. subtilis</i>	
	M	A	M	A	M	A	M	A	M	A
0.13	0	0	0	0	0	0	0	0	0	0
0.36	0	0	0	0	0	0	0	0	0	0
0.612	0	0	0	0	0	0	0	0	0	0
1.25	0	0	0	0	0	0	0	0	3	0
2.5	0	0	0	0	6	0	0	0	9	3
5	3	0	4	2	6	4	10	6	10	6
MIC(mg/mL)	5	0	5	5	2.5	5	5	5	1.25	2.5

M= Methanolic, A= Aqueous

Table 2 The zone of inhibition and MIC (in mm) of Gentamycin against the test organism.

Concentration (µg/mL)	<i>S. typhi</i>	<i>P.aeruginosa</i>	<i>S. aureus</i>	<i>P. mirabilis</i>	<i>S. subtilis</i>
25	2	0	13	9	8
50	13	0	18	13	10
100	16	1	21	18	15
200	21	3	25	21	19
400	25	8	27	25	22
800	27	14	34	27	25
MIC(µg/mL)	25	100	25	25	25

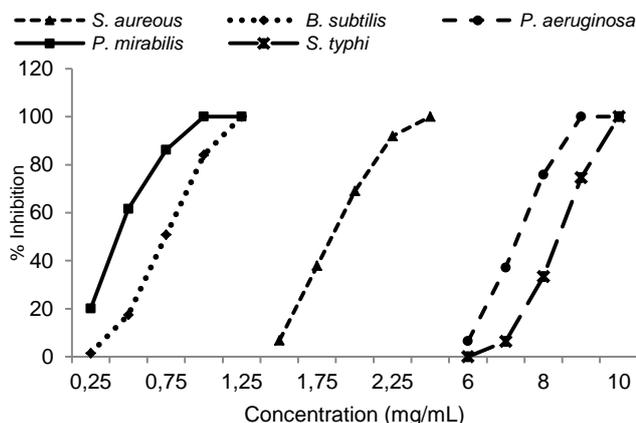


Figure 2 Inhibition (%) of methanolic leaf extract of *C. tamala* against the pathogens in broth dilution method.

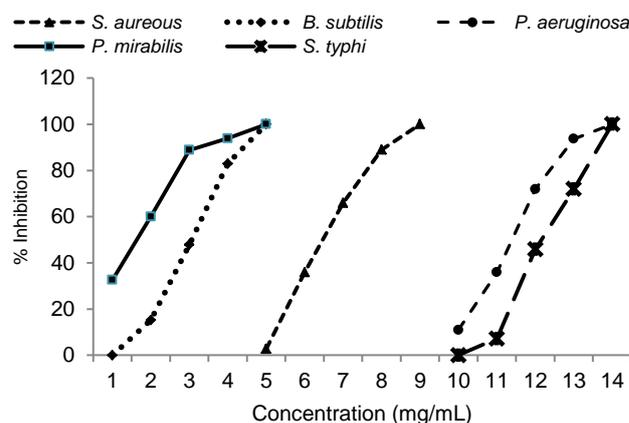


Figure 3 Inhibition (%) of aqueous leaf extract of *C. tamala* against the pathogens in broth dilution method.

However aqueous leaf extract also showed 100% inhibition against all the pathogens in higher concentration (1-13mg/mL) than methanolic extract presented in figure-3

Jeyasree and Dasarathan (2012) studied the antibacterial effect of *C. tamala* on *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. pneumoniae* and reported methanolic leaf extract was more effective than aqueous extract because polar and non polar components are effectively extracted in an organic solvent. *C.tamala* possess anti bacterial activity due to the presence of phenolic compounds such as cinnamic aldehyde, eugenol and cinnamic acid (Baratta et al., 1998). An important characteristic of leaf extract and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure and rendering them more permeable (Sikkema et al., 1994). Extensive leakages from bacterial cells or existence of critical molecules and ions will lead to death (Denyer and Hugo, 1991).

Cytotoxicity

Result of cytotoxicity performed by haemolytic activity of *C. tamala* and *A. marmelos* leaf extract has been presented in figure-4 and 5.

Aqueous leaf extracts of *C. tamala* did not show any changes in OD value at 0.2 mg/mL – 1 mg/mL concentration with increase in incubation time period and concentration but the methanolic leaf extract showed deflation at 1mg/ mL concentration after 15 minutes of incubation. Kumar et al. (2013g) reported 0.2 mg/mL - 0.4mg/mL leaf extract of *C. procera* safe but more concentrated leaf extract increase the hemolytic activity with time of incubation. The plant saponins are known to increase the membrane permeability and hence are considered as potential adjuvants and because due to same property, they cause hemolysis (Urbanska, et al., 2009; Noudeh et al., 2011). This hemolytic activity of a compound depends upon several factors, like the plasma membrane composition, saponin side chain, temperature and time of incubation (Noudeh et al., 2011). The present

results showed concentration -dependent increase in the hemolytic activity and a slight increase in the same with time of incubation. The hemolytic activity of few plant extracts have been reported (WHO, 1998; Urbanska et al., 2009; Chakraborty and Shah, 2011). By comparing the results, we may say that the haemolytic activity of *C. tamala* is not very pronounced and hence the use of leaf extract is safe.

Nutritional potentiality

The result of nutritional components of *C. tamala* leaves is very high represented in fig – 6 and calculated nutritional values were 143.5 ± 0.53 Kcal/100g of leaf sample. Among parameters carbohydrate content was 9.5 ± 0.5 g/100g and fat content was 6.0 ± 0.5 g/100g. The nutritive values of indigenous fruits and vegetables such as *Cucumis sativus*, *Pangiu medule*, *Brassica oleraceae*, *Spinacia oleraceae*, *Sinapis alba* have been reported as 15 Kcal, 227 Kcal, 22Kcal, 29Kcal, 34 Kcal respectively (Hoe and Siong, 1999). Comparing with the above values *C. tamala* can be used as fodder. Primary metabolites such as carbohydrates, proteins and fats etc, provide nutritional energy during illness (Bukhsh et al., 2007). Dietary fibre is an imperative constituent of a balance healthy diet (Trowel, 1976). Hoe and Sionga (1999) have quantified the crude fiber content of several medicinally important plants like *Magnifera grapfithii* (0.9%), *Solanum ferox*(1.9%), *Aleternanthera sessilis* (2.7%) and *Gnetum gnemon* (4.7%). *Cinnamomum tamala* can be considered a rich source of crude fibre having 30.5 ± 0.6 g/100g.

Kumar et al. (2013a) quantify nutritional value of *Adhatoda vasicaleaves* was 106.00 Cal/ 100g. Nutritionally fibre is beneficial to human boy, since fibre aids absorption of trace elements in the gut and reduces absorption of cholesterol (Kelsay, 1981). Edible fibres are able to bind cancer-causing toxins in the colon, keep them away from the healthy colon cells and provide synergistic protection for colon cells from free radical damage to their DNA (Arvind et al., 2013). Carbohydrate constitutes a broad class of naturally occurring organic compounds that are essential for the maintenance of life (Ebun-Oluwa and Alade, 2007). Proteins contain amino acids utilized by the body cells to synthesize numerous proteins required for the body and also provide energy (Robinson, 2007). Due to moderate level of crude fat in the leaves, people suffering from overweight or obesity can consume in diet (Dandapat et al., 2013a).

Conclusion

The present study suggest *Cinnamomum tamala* leaf can be used as potent antibacterial medicine, which inhibits the growth of pathogenic bacteria, sources of many diseases, and the leaves can be used as fodder due to high nutritional value.

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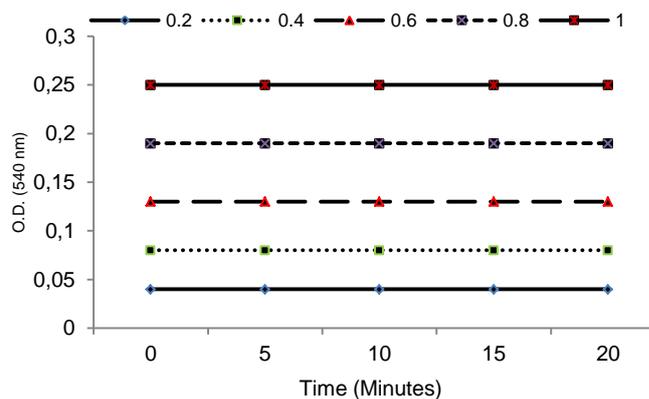


Figure 4 Haemolytic activity of aqueous leaf extract of *C. tamala*.

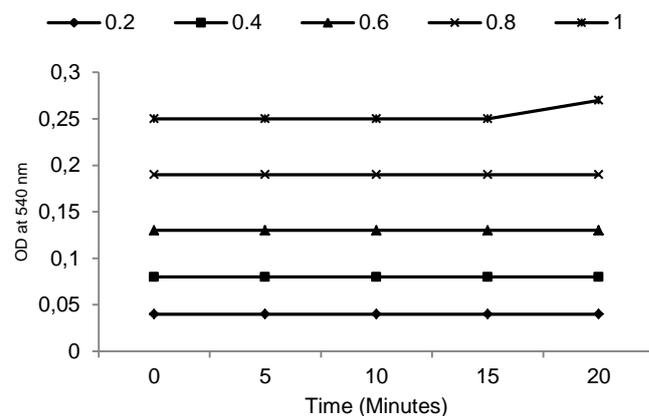


Figure 5 Haemolytic activity of methanolic leaf extract of *C. tamala*

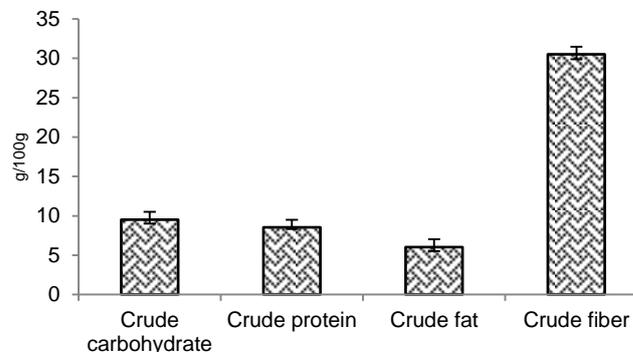


Figure 6 Nutritional composition of *C. tamala* leaf in g/100g (M \pm SD; n =3).

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