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RESEARCH ARTICLE

Different Crude Extracts of *Cinnamomum tamala* with Antioxidant and Antibacterial Capabilities

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Highlights

- Methanolic and ethanolic extracts of young and mature leaves of *Cinnamomum tamala* were prepared.
- Antioxidant and antibacterial capabilities of the extracts were evaluated.
- The methanolic extract of young leaves is more potent in terms of antioxidant activity.
- All extracts showed potent antibacterial activity against gram-negative strain than gram-positive strain.

Abstract

In this present study, crude extracts of young and mature leaves of Cinnamomum tamala were evaluated for their antioxidant and antibacterial capabilities. The cold percolation method was carried out with polar solvents methanol and ethanol. The antioxidant activities of all the extracts were assessed by DPPH assay and antibacterial properties were performed against Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae by agar well diffusion method. All extracts were able to scavenge free radicals in which strong antioxidant activity was found in methanolic extract of young leaves, and its IC_{50} value was estimated as (67.19±14.96 µg/mL) at a concentration range of 31.25-500 µg/mL while IC_{50} value of standard ascorbic acid was found to be 33.53±0.97 µg/mL at the concentration range of 10-50 µg/mL. The ethanolic extract of leaves (ZOI = 19 mm) showed strong antibacterial activity while standard neomycin showed (ZOI = 23 mm) against Escherichia coli at a concentration of 50 µg/mL. These results may provide scientific evidence of the traditional uses of C. tamala. Isolation and characterization of pure active compounds should be done in the future.

Keywords*: C. tamala*, extracts, phytochemicals, antioxidant, antibacterial

Introduction

The physiographic and climatic variability have been reflected in a vibrant chemical diversity in Nepal [1]. The chemical variety of plants, animals, insects, and microorganisms has led to spectacular discoveries made over the last 150 years that have been credited to the indigenous peoples, who discovered and shared their knowledge of herbal and animal remedies between the communities and generations. Later, synthetic chemists used their skills to isolate, characterize, and reconstruct the molecular structures leading to the most fascinating bioactive natural products [2], [3]. For centuries, different medicinal plants are being used as a traditional medicine to heal some peculiar diseases in Nepal [4-6]. However, the knowledge of traditional healers will be extinct as peoples get more and more marginalized. Thus, scientifically exploitation of locally available medicinal plants is a blooming requirement for the therapeutic approach and the most effective formulation aspect of drugs with few or hardly adverse effects [7]. Among the various medicinal plants, the present study attempts to highlight *C. tamala* (Buch.-Ham.) T. Nees and Eberm (Lauraceae). This species is an evergreen tree naturally grown in Nepal between 450-2100 m in height [8], [9].

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The leaves of *C. tamala* have been used for flavouring food due to their aroma [9]. Besides these, it is also a traditional dye-yielding plant and natural food preservative for pineapple juice [10], [11]. Dried leaves and bark of *C. tamala* were prescribed for children for fever, anaemia, and body odour. Its seeds were crushed and mixed with honey or sugar for dysentery or cough. Additionally, parts of *C. tamala* have been used in the digestive system (acidity, lack of appetite, abdominal pain), respiratory disease (bronchitis, cold and cough), and circulatory system as reported [12]. Several previous studies reported *C. tamala* as an antioxidant, antidiabetic, anticancer and antiinflammatory [13-22].

Therefore, *in vitro* assessment of young and mature leaves of *C. tamala* for their antioxidant and antimicrobial capabilities is the main objective of this research.

Materials and Methods

Collection and authentication of plant materials

Fresh young and mature *C. tamala* leaves were collected separately from Machhegaun, Kathmandu, during July 2019. A herbarium specimen was authenticated with a voucher code (RM001) was deposited at the Central Department of Botany, Tribhuvan University. The collected young and mature leaves were shade dried, ground, and preserved in airtight bottles.

Extraction

The dried plant samples were extracted thrice with methanol and ethanol by cold percolation method for 72 hours in a conical flask with occasional shaking. The filtered extracts were then evaporated under vacuum at 40 ºC to dryness with the help of a rotary evaporator to obtain crude extract and left for dryness.

Antioxidant activity

In vitro antioxidant activity of respective crude extracts was evaluated by following the standard protocol [23]. An aliquot 100 μL of plant extract (31.25-500 μg/mL) was mixed with 100 μL of DPPH (0.1 mM) solution. The reaction mixture was then incubated for 15 min in the dark at room temperature, and absorbance was measured at 517 nm using a spectrophotometer (Synergy LX, BioTek, Instruments, Inc., USA). Ascorbic acid (10-50 μg/mL) was used as positive control and 50% dimethylsulphoxide (DMSO) only as a negative control. All the experiments of different concentrations of ascorbic acid/plant extract were carried out in triplicate. The inhibitory concentration (IC_{50}) value was then calculated by the software.

Statistical analysis

Gen 5 Microplate Data Collection and Analysis Software and Microsoft Excel were used for data analysis. The IC_{50} value was calculated using Graph Pad Prism version 8 software and the results were expressed as mean value ± standard error of the mean (SEM).

Antibacterial activity

In vitro assessment of antibacterial activity of the methanolic and ethanolic extracts of young and mature leaves of *C. tamala* was performed using the agar well diffusion method as per guidelines of the Clinical and Laboratory Standards Institute (CLSI) [24]. Briefly, six wells (20 mm apart from one another) were bored in the bacteria cultured plates. The wells were loaded with 20 μL of the respective extracts (50 mg/mL) dissolved in DMSO. Neomycin is already marketed antibiotic used as positive control while 50% DMSO was used as a negative control. Petri plates were then incubated at 37 ºC for 24 hours and measured zone of inhibition (ZOI).

Results and Discussion

Antioxidant activity

Inhibitory concentration (IC_{50}) of standard ascorbic acid (10-50 μg/mL) and different extracts of *C. tamala* (31.25-500 μg/mL) were calculated and presented in **Table 1**.

Table 1: *IC_{50} values of antioxidant activity*

Sample	**IC_{50} (μg/mL)**
Ascorbic acid	33.53 ± 0.97
MEY	67.19 ± 14.96
MEM	75.70 ± 18.52
EEM	186.78 ± 16.87
EEY	388.19 ± 94.47

MEY: Methanolic extract of young leaves, MEM: Methanolic extract of mature leaves, EEM: Ethanolic extract of mature leaves, EEY: Ethanolic extract of young leaves.

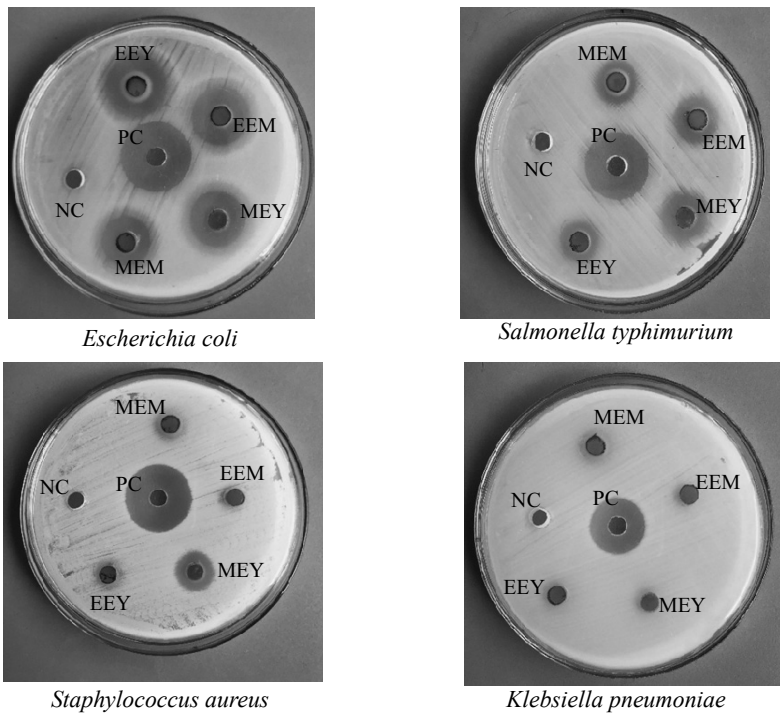
Antibacterial activity

The diameter of inhibition zone values of all extracts of leaves and young leaves of *C. tamala* was presented in **Table 2**.

Table 2: *Showing the diameter of zone of inhibition (mm) (Φ)*

Sample	Staphylococcus aureus	Escherichia coli	Salmonella typhimurium	Klebsiella pneumoniae
MEM	-	12	12	-
MEY	9	18	15	-
EEM	-	18	14	-
EEY	-	19	13	-
Neomycin*	24	23	22	14

-: No zone of inhibition, Concentration: 50 µg/mL in each well, Φ: Diameter of the well (6mm),*: positive control, Staphylococcus aureus: ATCC 25923, Escherichia coli: ATCC 25922, Salmonella typhimurium: ATCC 14028, Klebsiella pneumoniae: ATCC 700603



Escherichia coli *Salmonella typhimurium*

Staphylococcus aureus *Klebsiella pneumoniae*

Fig. 1*. Antibacterial activity of different extracts of Cinnamomum tamala on selected bacteria;*

positive control (PC), negative control (NC), MEY (Methanolic Extract of Young leaves), MEM (Methanolic Extract of Mature Leaves), EEY (Ethanolic Extract of Young leaves), EEM (Ethanolic Extract of Mature Leaves)

The result of antibacterial activity showed that all the extracts could inhibit *Escherichia coli* and *Salmonella typhimurium*. At the same time, *Staphylococcus aureus* showed the zone of inhibition only in MEY and no inhibition in *Klebsiella pneumoniae* by any of the extracts, as shown in table 2 and figure 1.

Antioxidant activity

The antioxidants/sample react with the stable free radical, i.e. 1, 1-diphenyl-2-picrylhydrazyl (deep violet colour) and transform it to 1, 1-diphenyl-2-picrylhydrazine with discolouration. The degree of discolouration indicates the free radical scavenging potentials of the sample/antioxidant, and it has been found that known antioxidant such as cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.) reduce and decolourize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability [25]. Among the four extracts, methanolic extracts of young leaves showed higher antioxidant activity having IC_{50} 67.19 ± 14.96 μg/mL. Ethanolic extract of young leaves showed the lowest antioxidant potency with IC_{50} 388.19 ± 94.47 μg/mL. The differences in antioxidant activity of different extracts from plants may be due to different phytochemicals in different extracts. Phenolics and flavonoids are the primary compounds responsible for exhibiting antioxidant activity [26].

It was previously reported that the IC_{50} value of standard ascorbic acid was 3.21 μg/mL, where the methanolic extract of *C. tamala* give the IC_{50} value of 6.0 μg/mL at the concentration range of 100 - 5 μg/mL [27]. The IC_{50} value of ethanolic extract of *C. tamala* leaves was 13.55 μg/mL, and that of standard ascorbic acid was 5.35 μg/mL at the concentration range of 500-1 μg/mL [28]. It was also revealed that the IC_{50} value of standard ascorbic acid was 22.78 mg/mL and that of methanolic extract of leaves of *C. tamala* was 157.58 mg/mL at the concentration range of 500-0.98 μg/mL [14].

Phenolic and flavonoids are responsible for reducing DPPH radical by their hydrogen donating ability, as reported [29], [30]. It was demonstrated that Kaempferol-7-O-rhamnoside was a well-known flavonoid glycoside, which has been reported to possess an excellent inhibitory effect on lipid peroxidation, DPPH free radical and superoxide radical in 70% ethanolic extracts of *Cinnamomum osmopholeum* twigs [31]. It was reported that *Cinnamomum osmopholeum* twigs extract in 70% acetone showed excellent antioxidant activities, and the active compounds are proanthocyanidin and condensed tannin [32]. The aqueous extract of leaves of *C. tamala* contains phenol (20.83 ±0.11), ascorbate (22.30±0.21) and carotenoids (0.82±0.04) mg/g dry wt., revealed that *C. tamala* had high antioxidants [33]. The findings mentioned above revealed variations in components of leaves and twigs extracts obtained using different solvents.

Polar sub-fraction was the most vital radical reducer compared with the non-polar one [14], [34]. In the pilot study, young and mature leaf extracts of *C. tamala* were prepared using solvents of different polarities as they differ in their phytochemical constitution. Although methanol and ethanol are both polar solvents, it might be assumed that cold maceration with ethanol did not successfully extract active constituents. Thus, the methanolic extract has less IC_{50} value as compared to less polar ethanolic extract. Besides these, the variation in antioxidant activity between young and mature leaves might be considered the different antioxidant constituents in them [13].

Antibacterial activity

In the pilot study, antibacterial activity against gram-negative strains (*Escherichia coli* and *Salmonella typhimurium*) are shown to be effective in both methanol and ethanol extracts of young and mature leaves as compared to gram-positive bacteria *Staphylococcus aureus*, as shown in table 2 and figure 1. Among four extracts, a better antibacterial activity was recorded in ethanolic extract of young leaves against *Escherichia coli and* methanolic extract of young leaves against *Salmonella typhimurium*, while only methanolic extract of young leaves showed inhibition against *Staphylococcus aureus*. However, there was no inhibition against *Klebsiella pneumoniae* by any extracts at the concentration of 50 μg/mL.

Previous studies reported that the different solvent extracts including n-hexane, dichloromethane, water, and isobutanol extract of leaves of *C. tamala* exhibited an extra degree of inhibition against selected gram-positive and gram-negative bacteria [35]. Many authors reported that the antimicrobial constituents of *Cinnamomum* extracts are cinnamaldehyde and eugenol [36], [37]. Cinnamaldehyde was identified in *Cinnamomum zeylanicum* that inhibits amino acid decarboxylase activity and has been

active against many pathogenic bacteria [38], [39]. Cinnamaldehyde interferes with biological processes involving electron transfer and reacts with nitrogen-containing components including, proteins and nucleic acids [40]. Gram-negative bacteria were slightly less susceptible to the action of cinnamaldehyde since they possess an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering [41], [42].

However, the results from this current study revealed that gram-negative bacteria were more susceptible because extracts of *C. tamala* might contain certain constituents, which enable the extract to overcome the barrier in gram-negative bacteria. Besides these, the variation of antibacterial activity between young and mature leaves might be considered to be the presence of different antibacterial constituents in them. Therefore, the bioactive components with antimicrobial properties should be analyzed for further study.

Conclusions

In this study, the different solvent extracts of *C. tamala* were screened for antioxidant and antibacterial activity. The antioxidant activity revealed that methanol extract inhibits more effectively as compared to ethanol extracts. Besides these, methanol and ethanol extract had a wide range of antibacterial activities against *Escherichia coli* and *Salmonella typhimurium* among four tested bacteria. This study further helps to pave the pathway to the scientific community regarding the pharmacological potentials of *C. tamala* and provides the rationale for further identification, isolation and characterization of its bioactive principles.

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