

Volume 7 Issue 2 2021

ISSN 2454-3055

**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



Phytochemical Screening and Free Radical Scavenging Activity of *Cinnamomum tamala* Leaf Extract

Rani Raksha¹, Kumar Rajesh², Sharma Preeti¹, Hajam Younis Ahmad^{1*} and Rai Seema³

¹Department of Biosciences, Division Zoology, Career Point University, Hamirpur, Himachal Pradesh, India

²Department of Biosciences, Himachal Pradesh University, Shimla 175001, Himachal Pradesh, India

³Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur 495001, Chhattisgarh, India

*Corresponding Author

Received: 11th June, 2021; **Accepted:** 14th July, 2021; **Published online:** 19th July, 2021

<https://doi.org/10.33745/ijzi.2021.v07i02.008>

Abstract: Traditional plant-based medicines are still needed by the whole world for their primary healthcare benefits. The phytochemicals or plant extract could be used to treat different diseases and new formulation for the drug discovery in pharmaceuticals. *Cinnamomum tamala* is commonly called as Indian bay leaf or Tejpatta. The leaves and bark of *Cinnamomum tamala* are used to cure various diseases due to its various properties including astringent, stimulant and carminative. Hence, the objective of this study was to determine the comparative phytochemical screening and free radical scavenging activity of the leaf of *Cinnamomum tamala*. To achieve this, extract was prepared in three solvents (ethanol, ethanol, aqueous and chloroform). Phytochemical screening (qualitative and quantitative) was evaluated in all the three fractions to compare the solubility of various bioactive components. Phytochemical screening showed the presence of polyphenols, flavonoids, alkaloids, flavones and flavonols, tannins, carbohydrates, amino acids and proteins, saponins and glycosides in leaves of *Cinnamomum tamala*. Quantitative analysis showed that the total polyphenolic content, total flavonoids content, total alkaloids and total flavones and flavonols content in the hydroalcoholic extract was 48.1 mg GA (gallic acid)/g, 22.1 mg QE (Quercetin)/g, 59.9 mg/g and 1.75 mg RE (Rutin)/g, respectively. DPPH free radical assay revealed that *Cinnamomum tamala* hydroalcoholic leaf extract at a 100 µm/ml concentration showed 96.99± 0.99% inhibition activity. It can be concluded that most of the bioactive components are found soluble in hydroalcoholic solvent. *Cinnamomum tamala* hydroalcoholic leaf extract contains various bioactive and also exhibits significant free radical scavenging activity. Hence, it can be used as an alternative remedy for the treatment of various diseases.

Keywords: *Cinnamomum tamala*, Tejpatta, Medicinal plants, Bioactive components, Qualitative, Quantitative, DPPH, Scavenging activity

Citation: Rani Raksha, Kumar Rajesh, Sharma Preeti, Hajam Younis Ahmad and Rai Seema: Phytochemical screening and free radical scavenging activity of *Cinnamomum tamala* leaf extract. Intern. J. Zool. Invest. 7 (2): 376-386, 2021. <https://doi.org/10.33745/ijzi.2021.v07i02.008>

Introduction

Traditional plant-based medicines are still in need by the whole world for their primary healthcare benefits. This happens in many rural communities in Asia, Africa and Central and South America. In

these countries the use of medicinal plants and knowledge about their medicinal use are available and inexpensive. In other nations, most of the traditional plant-based medicines are being

included through rules and regulations into conventional health systems.

In India, the medicinal plants are now widely used by most of the people in different indigenous system of medicine like Siddha, Ayurveda, and Unani (Ravishankar and Shukla, 2007). About 4.5 million of plant species are found in India and among them only 250,000-500,000 plant species have been examined phytochemically for pharmaceutical or pharmacological activities (Singh and Kumar, 2017). The phytochemicals or plant extract could be used to treat different diseases and new formulation for the drug discovery in pharmaceuticals (Singh *et al.*, 2017). Medicinal plants play an important role in the continuation of livelihood improvement, especially women in an environmentally sustainable manner while maintaining the biodiversity of these natural products (Sharma *et al.*, 2017). World Health Organization has reported that about 80% of the world's population depends on traditional medicine for their preliminary healthcare needs. The presence of various active chemical substances in medicinal plants defines their medicinal value (Yadav *et al.*, 2017). Various kinds of primary and secondary metabolites are present in plants. Due to the presence of these bioactive compounds or secondary metabolites plants show various pharmacological activities such as anti-oxidative, antiallergic, antibiotic, hypoglycaemic and anti-carcinogenic. The body cells from any type of damage caused by free radicals are protected by these bioactive components (Krishnamachari and Nithyalakshmi, 2017). Therefore, there is a need to search for plants of medicinal value (Chavan, 2016).

Cinnamomum tamala commonly called as Indian bay leaf or Tejpatta. It belongs to family Lauraceae which is native to India, Nepal, Bhutan, and China. This family contains about 55 genera and over 2000 species world-wide, mostly from warm or tropical regions. Tejpatta is an evergreen tree having aromatic oil in their leaves and barks. Because of its aroma, leaves are traditionally kept in

cloths or also chewed to disguise bad mouth odour. People use its dried leaves as an important ingredient in spices. Due to its various properties including astringent, stimulant and carminative; the leaves and bark of *Cinnamomum tamala* are used to cure various diseases and in earlier times it is used against rheumatism, colic, diarrhea, nausea and vomiting. It has been documented in ancient literature that dried leaves and bark of this plant were used in fever, anemia and body odour. Crushed seeds mixed with honey and sugar was given to children for dysentery or cough (Niyonzima and Vlientinck, 1993). There are several reports on the use of Tejpatta in gastrointestinal problems like acidity, lack of appetite, respiratory system related diseases like bronchitis, cold and cough and circulatory system. Essential oils of *Cinnamomum tamala* have various chemotypes such as eugenol type, cinnamaldehyde type or cinnamaldehydelinalool type (Baruah *et al.*, 2004; Rani *et al.*, 2017). Previous studies have revealed that Tejpatta contains various phytochemicals such as eugenol (Dighe *et al.*, 2005) and cinnamaldehyde (Mir *et al.*, 2004; Rani *et al.*, 2017). The flavonoids components were present in the leaf of *Cinnamomum* such as quercetin and kaempferol which are responsible for its antioxidant activity (Mir *et al.*, 2004; Rao and Gan, 2014). Previously, it has been reported that aromatic oil of leaves of Tejpatta have various chemical constituents and eugenol is the main component found in *C. tamala* (Mir *et al.*, 2004). Various studies have been done on the antimicrobial activity of aromatic oil and crude extracts of *Cinnamomum tamala* against the numerous pathogens (Mishra *et al.*, 2010; Mir *et al.*, 2012). Hence, the objective of this study was to determine the comparative phytochemical screening and free radical scavenging activity of the plant leaf of *Cinnamomum tamala* (Tejpatta).

Materials and Methods

Chemicals:

In this study, 1,1-Diphenyl-2-picryl hydrazyl (DPPH), trichloroacetic acid (TCA), L-ascorbic

acid, gallic acid, Folin-ciocalteu phenol reagent, distilled water, FeCl₂, and FeCl₃ hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), Acetic acid, aluminium chloride (AlCl₃) were of analytical grade.

Plant Material Collection and Identification:

Plant material (leaves) of *Cinnamomum tamala* Linn. were collected from Jogindernagar, Mandi (H.P.), India and the plant leaves were identified by a botanist from the Department of Botany, Career Point University (Hamirpur). The plant leaves were washed in potassium dichromate and then distilled water to make the plant dust free. Then leaves were allowed to dry under shade and dried leaves were crushed by using grinder to make fine powder. Finally the percentage yields were calculated of the dried extract.

Extract preparation:

The powder was processed for the preparation of extract in different solvents (70% ethanol, 70% chloroform and distilled water) to obtain the best fraction using Soxhlet extractor (Popular Traders) at ambient temperature. Standardized recent systematic method was used to make sure quality control of plant extract. The extract was dried at room temperature and was stored in a refrigerator at 4 C for further use.

Percentage yield determination:

The yield of extract of plant was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical (qualitative and quantitative) screening of extracts:

Following the standard methods, analysis of crude extract of *Cinnamomum tamala* leaves was done to determine the presence of a variety of bioactive components such as alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, and anthraquinones. The positive indicator observed for these tests was change in the colour or precipitate

formation. Further, examination of hydroalcoholic extract was also done for free radical scavenging activity through DPPH assay.

Detection of Alkaloids:

About 2 ml of hydrochloric acid (5%) was used to dissolve 15 mg of extract. After shaking, the extract was mixed with hydrochloric acid properly and then filtered and three separate aliquots were prepared. In each tube, drops of Mayer's reagent and Dragendorff reagent were added. Yellowish-white precipitate in Mayer's test, and red-orange precipitate in Dragendorff's test showed the presence of bioactive component.

Determination of Steroids:

Liebermann's test:

A small quantity of *Cinnamomum tamala* leaf extract was added in a test-tube, in which 2 ml of acetic acid and chloroform were added. The whole reaction mixture was allowed to cool down and then few drops of concentrated sulphuric acid were added into it. The appearance of green colour showed that steroids are present.

Salkowski's test:

Double distilled water was used to dissolve a small quantity of *Cinnamomum tamala* leaf extract, in which 2.5 ml of concentrated sulphuric acid was added. The appearance of reddish brown color showed that steroids are present.

Detection of Phenols:

Phenol Test:

About 0.2 mg of *Cinnamomum tamala* leaf extract was added in a test tube, in which about 1 ml of 5% ferric chloride solution was added. Formation of intense colour indicated the presence of phenols.

Detection of flavonoids:

Shinoda test:

About 0.5 mg *Cinnamomum tamala* leaf extract was dissolved in isopropyl alcohol, in which 1 ml absolute alcohol and then 3 drops of concentrated

HCl were added to it. The appearance of red colour indicated the presence of aurones and chalcones. The appearance of orange, red or magenta colour showed the presence of flavonoids.

Sodium hydroxide test:

Three drops of 10% sodium hydroxide (NaOH) were added to 2 ml *Cinnamomum tamala* leaf extract dissolved in isopropyl alcohol. The reaction resulted the production of yellow-red, coffee-orange, purple-red color showed that xanthenes and/or flavones, flavonols, chalcones and anthocyanins were present.

Detection of Carbohydrates:

Molisch's Test:

A small quantity of *Cinnamomum tamala* leaf extract was added in a test-tube, in which small amount of Molisch's reagent was added and then shaken carefully. About 2 ml of concentrated sulphuric acid along the walls of test tube was added. Then it was allowed to stand for 2 min. The appearance of reddish violet ring showed the presence of carbohydrates.

Test for Flavones:

H₂SO₄ Test:

A small quantity of *Cinnamomum tamala* leaf extract was added in a test-tube, in which about 2 ml of concentrated sulphuric acid was added. The appearance of yellow colour showed the presence of flavones.

Detection of Tannins:

Ferric Chloride Test:

About 1 ml of 70% ethanol was used to dissolve 0.2 mg of *Cinnamomum tamala* leaf extract, in which 2 ml of double distilled water was added followed by the addition of 4-10 drops of FeCl₃ aqueous solution 10% w/v. The production of blue or green colour showed that the tannins are present.

Determination of Saponins by aqueous test:

About 2 ml of distilled water was added to the

Cinnamomum tamala leaf extract dissolved in isopropyl alcohol (20 mg/ml) in a test tube, and then the mixture was shaken vigorously. Froth is formed due to the vigorous shaking and the formation of foam layer indicated the presence of saponins.

Determination of glycosides:

Keller-Kilani test:

About 0.2 mg of *Cinnamomum tamala* leaf extract was added in a test-tube, in which 4 ml of glacial acetic acid and few drops of 2% ferric chloride were mixed and dissolved in it and then 1.5 ml of concentrated sulphuric acid was added. The appearance of brown ring showed that the glycosides are present.

Determination of Amino acids and Proteins:

Ninhydrin Test:

A small quantity of *Cinnamomum tamala* leaf extract was added in a test-tube, in which Ninhydrin reagent was added. The appearance of blue colour indicated the presence of amino acids and proteins.

Million's Test:

A small quantity of *Cinnamomum tamala* leaf extract was added in a test-tube, in which Ninhydrin reagent was added. White precipitates were turned into red upon heating and showed the presence of amino acids and proteins.

Quantitative assessment of phytochemicals:

Assessment of total polyphenolic content:

The total polyphenolic content was determined by Folin-Ciocalteu method (Sidduraju and Becker, 2003). Various concentrations of *Cinnamomum tamala* hydroalcoholic leaf extract were prepared in different test tubes. Then 50 µl of diluted Folin-Ciocalteu reagent (10%) and 2.5 ml of 20% sodium carbonate (Na₂CO₃) was added in it. The whole mixture was shaken properly and then incubated under dark condition for 40 min for colour formation. Following the incubation,

absorbance was taken at 765 nm. The results were expressed in mg/ GA/g of extract.

Assessment of alkaloids:

About 22 g of *Cinnamomum tamala* hydroalcoholic leaf extract was dissolved in 66 ml of distilled water, in which 200 ml of 20% of acetic acid was added and then incubated for 4 h. After incubation period reaction mixture was filtered and then ammonium hydroxide was added drop by drop till the complete formation of precipitates takes place. The solution was allowed to settle down and the precipitate was collected and then weighed. The percentage of total alkaloid was calculated as:

$$\text{Percentage of total alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of flavonoids content:

The content of flavonoids was determined by using the method of Zhishen *et al.* (1999). About 22 g of *Cinnamomum tamala* hydroalcoholic leaf extract was dissolved in 66 ml of distilled water. Then 0.75 μl of 5% sodium nitrate solution was added and then incubated for 10 min. Following the incubation, 150 μl of 10% AlCl_3 was added and was again incubated for 5 min. After that about 2 ml of 4% NaOH solution was added and final volume was made up to 5 ml by adding distilled water. The reaction mixture was shaken and then again incubated for 20 min at room temperature. The appearance of pink colour showed that flavonoids are present. Absorbance was recorded at 510 nm.

Determination of flavones and flavonol content:

The AlCl_3 method of Cvek *et al.* (2007) was used to determine the total flavones and flavonol content. About 22 mg of *Cinnamomum tamala* hydroalcoholic leaf extract was dissolved in 66 ml of 70% ethanol, in which 0.2 ml of AlCl_3 , and then 2.8 ml of glacial acetic acid was added to make the final volume up to 5 ml. The whole reaction mixture was incubated for 30 min at room

temperature. Absorbance was recorded at 415 nm. The results were expressed in mg/g of extract.

Antioxidant Activity: DPPH free radical scavenging assay:

The method of Blois (1958) was used to estimate the free radical scavenging activity. 2, 2-diphenyl-1-picryl-hydrezy (DPPH) was used to determine free radical scavenging activity. About 0.2 mmol/l solution of 2, 2-diphenyl-1-picryl-hydrezy was prepared in methanol, and different concentrations of *C. tamala* hydroalcoholic leaf extract (50-250 $\mu\text{g/ml}$) were prepared in separate tubes, and 500 μl of DPPH solution was added to each tube. In all the tubes reaction mixture was shaken and then allowed to stand as such for 30 min at room temperature. Control solution (Group-I) was prepared in the same way without the addition of *Cinnamomum tamala* hydroalcoholic leaf extract and methanol was used for baseline correction. The absorbance was determined at 517 nm by using spectrophotometer. The decrease in absorbance revealed that there was increase in free radical scavenging activity. Vitamin C (ascorbic acid) was used as standard to compare the results. The potential of 2, 2-diphenyl-1-picryl-hydrezy free radical scavenging activity was calculated as:

$$\text{DPPH scavenging activity (\% inhibition)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of the control; A_1 is the absorbance of the sample extract.

Results

Determination of Percentage yield:

Results showed a significant difference in the percentage yield. Among solvents tested hydroalcoholic extract showed higher per cent yield (39.5%), followed by aqueous (19.6%), and chloroform (6.8%) (Table 1).

Phytochemical (qualitative and quantitative) screening of extract:

The screening of phytoconstituents in the hydroalcoholic extract of *Cinnamomum tamala*

leaves showed the presence of alkaloids, flavonoids, steroids, polyphenols, flavones and flavonols, tannins, saponins, glycosides, carbohydrates, proteins and amino acids (Table 2).

Table 1: Per cent yield was determined by using different solvents (Ethanol, Aqueous and Chloroform)

| S. No. | Solvent used | Per cent Yield |
|--------|----------------|----------------|
| 1. | Hydroalcoholic | 39.5% |
| 2. | Aqueous | 19.6% |
| 3. | Chloroform | 6.8% |

On the basis of per cent yield and qualitative analysis, it was found that most of the bioactive components are soluble in 70% alcohol. Therefore, hydroalcoholic extract was further screened for the quantitative assessment of various bioactive components.

Quantitative assessment of phytochemicals:

Antioxidant Activity (DPPH free radical scavenging assay):

DPPH free radical scavenging activity of *Cinnamomum tamala* leaf extract was observed as shown in Figure 1 and Table 3. The hydroalcoholic leaf extract of *Cinnamomum tamala* at different concentrations ranged from 1 to 100 $\mu\text{g/ml}$ was estimated with vitamin C (ascorbic acid) as standard. Figure 1 illustrates the % inhibition as a function of vitamin C concentration. The results revealed DPPH radical scavenging activity of *Cinnamomum tamala* hydroalcoholic leaf extract at various concentrations as shown in Table 3 and Figure 1. In *Cinnamomum tamala* hydroalcoholic leaf extract at 100 $\mu\text{m/ml}$ concentration, the % inhibition activity was 96.99 ± 0.99 .

Total polyphenolic content:

The total polyphenolic content was quantified in the hydroalcoholic leaf extract of *Cinnamomum tamala*. The total polyphenolic content in the hydroalcoholic extract was estimated as 48.1 mg GA/g (Table 4).

Total flavonoids Content:

The hydroalcoholic extract of *Cinnamomum tamala* leaves was evaluated for total flavonoid content. The total flavonoid content in the hydroalcoholic extract of *Cinnamomum tamala* was 22.1 mg QE/g (Table 4).

Total alkaloids content:

The total alkaloid content was quantified in the hydroalcoholic leaf extract of *Cinnamomum tamala*. The total alkaloid content in the hydroalcoholic extract of *Cinnamomum tamala* was 59.9 mg/g (Table 4).

Total flavones and flavonols:

The hydroalcoholic extract of *Cinnamomum tamala* leaves was evaluated for total flavones and flavonols content. The total flavones and flavonols content in the hydroalcoholic extract of *C. tamala* was about 1.75 mg RE/g (Table 4).

Discussion

In the present study, various solvents (Ethanol, Aqueous, and Chloroform) were used for the preparation of *Cinnamomum tamala* leaf extract. Further, the *Cinnamomum tamala* leaf extract was used for the determination of per cent yield. The per cent yield is used to measure the efficiency of the solvent for the extraction of phytoconstituents. This study showed a significant difference in the percentage yield. Among solvents tested hydroalcoholic extract showed higher percentage yield (39.5%), followed by aqueous (19.6%), and chloroform (6.8%). Previous studies also reported that alcoholic extract of other plants has highest percent yield (Muhamad *et al.*, 2019). Therefore, hydroalcoholic extract was further processed for phytochemical (qualitative, quantitative and free radical scavenging activity) characterization.

Hydroalcoholic, aqueous, and chloroform extract of *C. tamala* leaf were also examined for the presence of different bioactive compounds. Results showed the presence of polyphenols, flavonoids, flavones and flavonols, alkaloids,

Table 2: Qualitative phytochemical screening of leaf extract of *Cinnamomum tamala* in different solvents

| S. No. | Phytochemicals | Test | Hydroalcoholic Extract | Chloroform Extract | Aqueous Extract |
|--------|--------------------------|---|------------------------|--------------------|-----------------|
| 1. | Alkaloids | a). Mayer's Test | ++ | + | - |
| | | b). Dragondroff's Test | + | - | + |
| 2. | Steroids | a). Salkowski's Test | + | - | +++ |
| | | b). Liebermann's Burchard's Test | + | + | - |
| 3. | Phenols | Phenol Test | +++ | - | + |
| 4. | Flavonoids | a). Shinoda Test | ++ | - | + |
| | | b). Sodium Hydroxide Test | ++ | - | + |
| 5. | Carbohydrates | Molisch Test | +++ | ++ | + |
| 6. | Flavones | H ₂ SO ₄ Test | +++ | + | + |
| 7. | Tannins | Ferric chloride Test | ++ | + | +++ |
| 8. | Saponins | Aqueous Test | ++ | + | ++ |
| 9. | Glycosides | a). H ₂ SO ₄ Test | ++ | - | + |
| | | b). Kellar Kilani Test | +++ | ++ | + |
| 10. | Proteins and Amino Acids | Millon's Test | ++ | - | + |

High concentration (+++); moderate concentration (++); low concentration (+); absence (-).

Table 3: Per cent inhibition activity for ascorbic acid and *C. tamala* leaf hydroalcoholic extract

| Concentration (µg/ml) | % inhibition by ascorbic acid ±SD | % inhibition by <i>C. tamala</i> extract±SD |
|-----------------------|-----------------------------------|---|
| 1 | 36.81 ± 0.91 | 30.01 ± 0.92 |
| 2 | 41.99 ± 0.98 | 36.78 ± 0.97 |
| 3 | 47.88 ± 0.95 | 38.88 ± 0.94 |
| 5 | 61.92 ± 0.88 | 48.99 ± 1.01 |
| 7 | 62.80 ± 0.96 | 56.89 ± 0.91 |
| 10 | 64.01 ± 0.94 | 56.94 ± 0.99 |
| 20 | 66.91 ± 1.99 | 60.98 ± 0.93 |
| 30 | 72.92 ± 0.89 | 63.91 ± 1.51 |
| 40 | 86.93 ± 0.99 | 78.99 ± 0.95 |
| 50 | 90.99 ± 0.87 | 81.02 ± 0.99 |
| 80 | 93.97 ± 0.83 | 85.04 ± 0.96 |
| 100 | 96.99 ± 0.99 | 90.88 ± 0.97 |

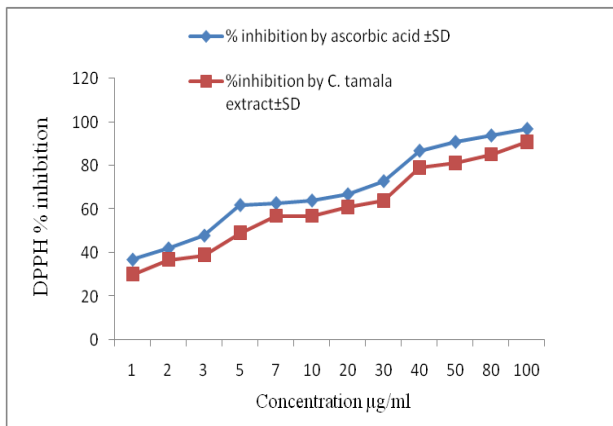


Fig. 1: Showing percentage inhibition by ascorbic acid and *Cinnamomum tamala*

Table 4: Quantitative phytochemical evaluation of *Cinnamomum tamala* hydroalcoholic leaf extract

| Phytochemicals (per gram of extract) | Concentration (mg/g) |
|--------------------------------------|----------------------|
| Polyphenols | 48.1 |
| Flavonoids | 22.1 |
| Flavones and flavonols | 1.75 |
| Alkaloids | 59.9 |

tannins, saponins and glycosides in *Cinnamomum tamala* leaf extract. However, hydroalcoholic fraction was found more suitable, because most of the bioactive components were found soluble in alcohol as alcohol group shows high polarity than most of the non-polar but lower polar than water. Previous studies also suggested that alcoholic extract contains more bioactive components as compared with other solvents extract (Roghini and Vijayalakshmi, 2018). The choice of the solvents depends on the polarity of various components and capability of the solvent to attracting all sorts of bioactive components like polar compounds and non-polar compounds (Mohanani *et al.*, 2018). Further, the *Cinnamomum tamala* hydroalcoholic leaf extract was analysed for quantitative analysis of free radical scavenging activity, the total polyphenolic content, total alkaloid content, total flavonoids content, total flavones and flavonols content.

The antioxidant property of *Cinnamomum tamala* hydroalcoholic leaf extract has been evaluated by DPPH free radical scavenging assay (Batool *et al.*, 2010; Upadhyaya and Kumar, 2010; Negi *et al.*, 2012; Mukhijal and Kalia, 2014; Karmakar *et al.*, 2015; Kanwal *et al.*, 2015). Previous studies reported that due to the presence of a number of phytochemicals such as polyphenols, flavonoids, and phenolic components, and mostly phenols in *Cinnamomum tamala* hydroalcoholic leaf extract, it might showed the radical scavenging activity. Polyphenols, flavonoids, and phenolic compounds, and most of the antioxidant activity of plants is because of the phenols (Phuyal *et al.*, 2020). The presence of natural antioxidants in various plants is responsible for preventing the damaging consequences of oxidative stress. For the determination of antioxidant ability of plants DPPH assay is the most suitable method among others (Mensor *et al.*, 2011).

The total polyphenolic content of the *Cinnamomum tamala* hydroalcoholic leaf extract was assessed. Finding of this study revealed that polyphenols are present in higher amount in the *Cinnamomum tamala* hydroalcoholic leaf extract. Previous studies reported that phenolic compounds show redox properties as they are essential plant components and having antioxidant activity (Soobrattee *et al.*, 2005). The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. It becomes clear from the present study that *Cinnamomum tamala* hydroalcoholic leaf extract is rich in polyphenols so that it might be used against oxidative stress or used to reduce free radicals.

The hydroalcoholic leaf extract of *Cinnamomum tamala* showed that alkaloids are present in higher amount. Alkaloids are the bioactive compounds of medicinal plants, which have broad biological activities. Alkaloids have extensive bioactivities and pharmacological activities (Wink, 2015), such as antimalarial (e.g. quinine), anticancer (e.g. homoharringtonine)

(Kittakoop *et al.*, 2014), antibacterial (e.g. chelerythrine) (Cushnie *et al.*, 2014), and antihyperglycemic activities (e.g. piperine) (Shi *et al.*, 2014). Alkaloids are extensively used in traditional pharmaceutical industries due to their medicinal properties. Alkaloids also possess psychotropic and stimulant activities. Other alkaloids possess psychotropic (e.g., psilocin) and stimulant activities (e.g., cocaine, caffeine, and nicotine) and have been used in many drugs (Shi *et al.*, 2014).

The presence of flavonoids was determined in large amount in the *Cinnamomum tamala* hydroalcoholic leaf extract. Previous studies also reported that flavonoids are present abundantly in various parts of plants (Ezeonu and Ejikeme, 2016; Al-Snafi, 2020). Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in various parts of plants both in free state and as glycosides. It is well known that flavonoids have antioxidant properties and prevents from the promotion and progression of tumor (Ezeonu and Ejikeme, 2016). It has also been reported in the previous studies that intake of flavonoids prevents from coronary heart diseases, also protects against platelet accumulation, microorganism, liver toxins, viruses, tumors, free radicals, and allergies (Ezeonu and Ejikeme, 2016). Apart from the antioxidant properties of flavonoid, other biological functions it possesses include protection against platelet aggregation, microorganisms, hepatotoxins, viruses, tumors, ulcers, free radicals, inflammation, and allergies (Ezeonu and Ejikeme, 2016).

Flavones and flavonols were also analysed in the *Cinnamomum tamala* hydroalcoholic leaf extract and it was found that these are present in high amount. Previous studies showed the presence of flavones and flavonols in various plants extract (Tungmunnithum *et al.*, 2018; Al-Snafi, 2020). Flavones and flavonoids are the phytoconstituents present in most of the plants

and are very important component of some human diets.

Conclusion

It is concluded that the hydroalcoholic leaf extract of *Cinnamomum tamala* showed high antioxidant activity and have various phytochemical properties. Extract of this plant is abundant in polyphenols, flavonoids, diterpenes, alkaloids, proteins, tannins, carbohydrates, saponins. This study also showed that biologically active phytochemicals are present in hydroalcoholic extract of *Cinnamomum tamala* leaves. Chloroform extract do not show the phytochemical activity. The medicinal properties of *Cinnamomum tamala* leaf extract may be due to the presence of active biochemicals and phytochemicals. The study showed that this plant is a source of significant natural antioxidant and may be beneficial in protection against oxidative stress.

Acknowledgements

Authors are grateful to CM-Startup Scheme, Govt. of Himachal Pradesh and Pioneer Incubator Career Point University, Hamirpur, Himachal Pradesh, India for providing necessary research facilities under startup project (Registration No. HPSTARTUP/2020/12/03) and also providing financial assistance to Raksha Rani.

References

- Al-Snafi AE. (2020) Phenolics and flavonoids contents of medicinal plants, as natural ingredients for many therapeutic purposes- A review. IOSR J Pharm. 10: 42-81.
- Ashfaq MH, Siddique A and Shahid S. (2021) Antioxidant activity of *Cinnamon zeylanicum*: (A review). Asian J Pharma Res. 11: 106-116.
- Baruah A and Nath SC. (2006) Leaf essential oils of *Cinnamomum glanduliferum* (Wall) Meissn and *Cinnamomum glaucescens* (Nees) Meissn. J Essent Oil Res. 18: 200-202.
- Batool F, Sabir SM, Rocha JBT, Shah AH, Saify ZS and Ahmed SD. (2010) Evaluation of antioxidant and free radical scavenging activities of fruit extract from *Zanthoxylum alatum*: a commonly used spice from Pakistan. Pak J Bot. 42: 4299-311.

- Chavan PA. (2016) Evaluation of antimicrobial activity of various medicinal plants extracts of Latur Zone against pathogens. *Int J Life Sci Sci Res.* 2: 612-618.
- Cushnie TT, Cushnie B and Lamb AJ. (2014) Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int J Antimicrob Agents* 44: 377-386.
- Cvek J, Medić-Šarić M, Jasprica I, Zubčić S, Vitali D, Mornar A, Vedrina-Dragojevic I and Tomić S. (2007) Optimisation of an extraction procedure and chemical characterisation of *Croatian propolis* tinctures. *Phytochem Anal: Int J Plant Chem Biochem Tech.* 18: 451-459.
- Dighe VV, Gursale AA, Sane RT, Menon S and Patel PH. (2005) Quantitative determination of eugenol from *Cinnamomum tamala* Nees and Eberm. Leaf powder and polyherbal formulation using reverse phase liquid chromatography. *Chroma.* 61: 443-446.
- Ezeonu CS and Ejikeme CM. (2016) Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New J Sci.* 2016: 5601327. doi.org/10.1155/2016/5601327.
- Kanwal R, Arshad M, Bibi Y, Asif S and Chaudhari SK. (2015) Evaluation of ethnopharmacological and antioxidant potential of *Zanthoxylum armatum* DC. *J Chem.* 2015: 25654. doi.org/10.1155/2015/925654.
- Karmakar I, Haldar S, Chakraborty M, Dewanjee S and Haldar PK. (2015) Antioxidant and cytotoxic activity of different extracts of *Zanthoxylum alatum*. *Free Radicals Antioxid.* 5: 21-28.
- Kittakoop P, Mahidol C and Ruchirawat S. (2014) Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Curr Top Med Chem.* 14: 239-252.
- Krishnamachari H and Nithyalakshmi V. (2017) Phytochemical analysis and antioxidant potential of *Cucumis melo* seeds. *Int J Life Sci Sci Res.* 3: 863-867.
- Mir SR, Ali M and Kapoor R. (2004) Chemical composition of essential oil of *Cinnamomum tamala* Nees et Eberm. leaves. *Flavour Fragr J.* 19: 112-114.
- Mishra AK, Singh BK and Pandey AK. (2010) In vitro-antibacterial activity and phytochemical profiles of *Cinnamomum tamala* (Tejpat) leaf extracts and oil. *Rev Infect.* 1: 134-139.
- Muhamad SHA, On S, Sanusi SN, Hashim AA and Zai MA. (2019) Antioxidant activity of Camphor leaves extract based on variation solvent. *J Phys Conf Ser.* 1349: 012102. doi:10.1088/1742-6596/1349/1/012102.
- Mukhijal M and Kalia AN. (2014) Antioxidant potential and total phenolic content of *Zanthoxylum alatum* stem bark. *J Appl Pharm.* 6: 388-397.
- Negi JS, Bisht VK, Bhandari AK, Bisht R and Kandari Negi S. (2012) Major constituents, antioxidant and antibacterial activities of *Zanthoxylum armatum* DC essential oil. *Iranian J Pharmacol Ther.* 11: 68-72.
- Niyonzima G, Scharpe S, Van Beeck L, Vlietinck AJ, Laekeman GM and Mets T. (1993) Hypoglycaemic activity of *Spathodea campanulata* stem bark decoction in mice. *Phytother Res.* 7: 64-67.
- Phuyal N, Jha PK, Raturi PP and Rajbhandary S. (2020) Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci World J.* 2020: 8780704. doi.org/10.1155/2020/8780704.
- Rani A, Pande C, Tewari G and Patni K. (2017) A review on aroma profile of *Cinnamomum* species in north and north east India. *World J Pharm Res.* 6: 200-221.
- Rao PV and Gan SH. (2014) Cinnamon: A multifaceted medicinal plant. *Evid Based Complement Alternat Med.* 2014: 642942. doi: 10.1155/2014/642942.
- Ravishankar B and Shukla VJ. (2007) Indian systems of medicine: a brief profile. *Afr J Tradit Complem.* 4: 319-337.
- Roghini R and Vijayalakshmi K. (2018) Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *Int J Pharm Sci Res.* 9: 4859-4864.
- Sharma A, Singh H and Kumar N. (2017) Studies on traditional knowledge of medicinal flora and its contribution to livelihood enhancement in the doon-valley, Uttarakhand (India). *Int J Life-Sci Sci Res.* 3: 951-960.
- Shi Q, Hui S, Zhang AH, Hong-Ying X, Guang-Li Y, Ying H and Xi-Jun W. (2014) Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese J Nat Med.* 12: 401-406.
- Siddhuraju P and Becker K. (2003) Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Food Chem.* 51: 2144-2155.
- Singh P, Singh R, Sati N, Ahluwalia V and Sati OP. (2017) Phytochemical and pharmacological significance of genus: *Impatiens*. *Int J Life Sci Scienti Res* 3: 868-881.
- Singh V and Kumar R. (2017) Study of phytochemical analysis and antioxidant activity of *Allium sativum* of

- Bundelkhand region. Int J Life Sci Scienti Res.3: 1451-1458.
- Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI and Bahorun T. (2005) Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res Fundam Mol Mech Mutagen. 579: 200-213.
- Tungmunnithum D, Thongboonyou A, Pholboon A and Yangsabai A. (2018) Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5: 93.
- Upadhyay NK, Kumar MY and Gupta A. (2010) Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaves. Food Chem Toxicol. 48: 3443-3448.
- Wink M. (2015) Modes of action of herbal medicines and plant secondary metabolites. Medicines 2: 251-286.
- Yadav R, Khare RK and Singhal A. (2017) Qualitative phytochemical screening of some selected medicinal plants of shivpuri district (MP). Int J Life Sci Scienti Res. 3: 844-847.
- Zhishen J, Mengcheng T and Jianming W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chem. 64: 555-559.