

## ANTIOXIDANT PROPERTIES AND PHYTOCHEMICAL SCREENING OF COMMERCIAL CINNAMON BARK

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### ABSTRACT

Cinnamon (dalchini) commonly used as a spice or as a medicine is a bark of genus *Cinnamomum*. Phytochemical screening and antioxidant activity of a solvent extract of the commercial bark of Cinnamon have been studied for the presence of secondary metabolites and to find out its radical scavenging activity. The phytochemical screening and qualitative estimation of commercial Cinnamon revealed that these species are rich in alkaloids, flavonoids and saponins and were present in all the solvent extracts except for phenols which were absent in hexane extract while Oxalate was absent in all the extracts. The antioxidant activity was evaluated by DPPH radical scavenging method. The IC<sub>50</sub> value shows significant activity with 111.05 µg/ml of bark methanol extract and 57.97 µg/ml of standard ascorbic acid. The result provides the evidence that the bark of Cinnamon can be used as a potential source of antioxidants and help in combating free radical-mediated damage to the body.

**KEYWORDS:** Phytochemicals, Cinnamon, Antioxidant activity, DPPH.

### INTRODUCTION

*Cinnamomum* (family Lauraceae), a genus of evergreen aromatic trees and shrubs contains 250-350 species from all over the world.<sup>[19,37,51]</sup> It is distributed in tropical and subtropical regions of South East Asia, Australia, North America, Central America and South America.<sup>[27,24,1]</sup>

The term '*Cinnamomum*' is derived from the Greek word *Kinnamon* or *kinnamomum* which means sweet wood.<sup>[38]</sup> A number of species of the genus *Cinnamomum* are being cultivated commercially in different areas of the world for the production of leaf and bark. Several species of *Cinnamomum* such as *Cinnamomum zeylanicum* syn. *C. verum* (True Cinnamon, Ceylon Cinnamon, Sri Lanka Cinnamon), *C. cassia* (cassia or Chinese Cinnamon), *C. burmannii* (Indonesian Cinnamon), *C. loureiroi* (Saigon Cinnamon, or Vietnamese Cinnamon), *C. citriodorum* (Malabar Cinnamon) are commercially known as Cinnamon, as the bark of these species is often sold as commercial spice product "Cinnamon" in the global market.

Phytochemicals are the biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans more than those ascribed to micronutrients and macronutrients.<sup>[16]</sup> These are the secondary metabolites with valuable chemical constituents which are used in human therapy, veterinary, agriculture, scientific research and drug

development. Antioxidants are the substances that neutralize harmful free radicals generating in our body and works as a reducing agent. Natural antioxidants increase the antioxidant capacity of the plasma which helps in reducing the risk of certain diseases and recognized as potential modifiers of cancer.<sup>[34]</sup> The antioxidants act by preventing the attack of harmful free radicals on the basic components of the body like nucleic acid or lipids.<sup>[2]</sup>

Cinnamon has been used in medicine to treat diarrhea and other problems of the digestive system. It is high in antioxidant activity<sup>[29,41]</sup> and the essential oil of Cinnamon also has antimicrobial<sup>[25]</sup> antifungal<sup>[10]</sup> properties which aid in the preservation of certain foods. Being a popular spice, it imparts its beneficial activity in cosmetics, food, and pharmaceuticals<sup>[4]</sup> The medicinal value of natural products lies in several chemical substances which produce a definite physiological action on the human body.<sup>[3]</sup> Cinnamon has an ability to increase the flow of blood in the uterus and also helps in improving tissue regeneration.<sup>[38]</sup> Cinnamon has a powerful anti-diarrheal, anti-flatulent, stimulant and antiemetic activities<sup>[17]</sup> and also has anti-termitic,<sup>[7]</sup> larvicidal,<sup>[8]</sup> anti-diabetic,<sup>[22]</sup> nematicidal,<sup>[23]</sup> anti-inflammatory<sup>[49]</sup> and hepatoprotective activity.<sup>[31]</sup> Many of their benefits are primarily derived from the three active compounds cinnamaldehyde, cinnamyl acetate and

cinnamyl alcohol which are present naturally in the essential oil found in the bark of the Cinnamon tree.<sup>[28]</sup>

The present study focuses on preliminary Antioxidant properties and phytochemical screening of commercial Cinnamon bark using different extracts.

## MATERIALS AND METHODS

### Collection of samples

The samples of Cinnamon bark (500 g) were procured from the spice market of Dehradun, Uttarakhand in the month of (July 2017) for phytochemical screening and evaluation of their antioxidant properties (Fig. 1).

### Preparation of extracts

The dried bark samples were powdered, weighed and stored in clean airtight containers at room temperature (25°C). 50 g of the powdered sample was extracted by Soxhlet extractor using 250 ml different solvents methanol, ethanol, acetone, chloroform and hexane. The extracts were concentrated using rotary evaporator to dryness and the extracts were stored at 4°C for further analysis.<sup>[42]</sup>

### Qualitative analysis of phytochemicals

Phytochemical screening was carried out on methanol, ethanol, acetone, chloroform, hexane and distilled water extracts of Cinnamon bark. The following tests were performed to detect various phytochemical constituents present in them:

#### Alkaloids (Wagner's Test)

To 2 ml of extract added few drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100mL distilled water). The formation of brownish-red precipitate, indicates the presence of alkaloids.<sup>[14]</sup>

#### Carbohydrates (Benedict Test)

To 1ml of extract 1ml of Benedict's reagent was added. The mixture is then heated for 2 minutes in a boiling water bath, the green appearance of the solution indicates the presence of reducing sugar.<sup>[15]</sup>

#### Flavonoids (Lead acetate Test)

The extract 2ml was treated with 2ml of 10% lead acetate solution appearance of yellowish-green color, indicates that flavonoid is present in the sample.<sup>[36]</sup>

#### Oxalate

The extract 2ml was treated with a few drops of glacial acetic acid. A greenish black coloration indicates the presence of oxalate.<sup>[30]</sup>

#### Phenols (Ferric Chloride Test)

The extract 0.5ml was added with few drops of neutral (0.5%) ferric chloride solution. Formation of dark green color signifies the presence of the phenolic compounds.<sup>[50]</sup>

#### Quinones

To 0.5ml of extract was treated with few drops of concentrated hydrochloric acid along the sides of the test tube. Formation of yellow precipitate or coloration indicates the presence of Quinones.<sup>[35]</sup>

#### Saponins (Foam Test)

To 5mL of distilled water was added to 2mg of the extracts and shaken for the formation of froth which confirms the presence of saponins.<sup>[40]</sup>

#### Tannins (Ferric Chloride Test)

The extract 1ml was added to 5ml of distilled water and kept for boiling in hot water bath. After cooling of the sample, 0.1% ferric chloride solution was added to it. The appearance of blue-black or brownish-green color confirms the availability of tannins.<sup>[44]</sup>

#### In-Vitro Antioxidant Activity

##### DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method

The free radical scavenging activity of the extracts was performed using established method.<sup>[5]</sup> DPPH solution was prepared in methanol at a concentration of 0.004 % and kept in dark. The working solution of extracts was prepared at different concentrations (500, 250, 125, 62.5, 31.25 µg/ml) by serial dilution from stock solution 1mg/ml. 2.7 ml of DPPH solution was added to in each test tubes and incubated in dark for 30 min. Ascorbic acid was used as a standard antioxidant. The absorbance was measured at 517nm against methanol as a blank by using UV spectrophotometer. DPPH solution without extract or standard served as a control. All the measurements were done in triplicates. The percentage (%) of scavenging of the DPPH free radical was calculated by using the following equation:

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

Where, A<sub>0</sub> = absorbance of the control

A<sub>1</sub> = absorbance of the extract/ standard

The IC<sub>50</sub> values were calculated from the regression equation, prepared from the graph plotted against concentration and percentage inhibition of the samples.

## RESULTS AND DISCUSSION

The cinnamon bark was tested for phytochemical screening and determination of their antioxidant activity. The extracts were prepared in solvents like methanol, ethanol, acetone, chloroform, hexane and distilled water which shows different activity in different tests. The antioxidant property of Cinnamon is a result of a biochemical process which these phytochemicals undergo. The result of phytochemical screening and antioxidant activity was reported below.

#### Solvent extraction percentage

The dried Cinnamon bark was extracted using different solvents. Extraction of components in different solvent is based upon the solubility of that component. The percentage yield of methanolic extract exhibited higher

percentage ( $11.36 \pm 0.69$ ) than other solvent followed by ethanol, acetone, chloroform and hexane with  $9.90 \pm 0.48$ ,  $2.60 \pm 0.14$ ,  $2.33 \pm 0.33$  and  $1.76 \pm 0.23$  respectively **Fig. 2**. This was supported by earlier report that methanol showed the highest extraction yield as compared to different types of solvents such as chloroform, hexane and ethanol.<sup>[18]</sup> Hence, polar solvent has ability to extract glycosidic and non-glycosidic phenolic compounds better than non polar solvent.

### Phytochemical screening

Secondary metabolites are the bioactive constituents of plant origin synthesized naturally in all parts of the plant such as bark, leaves, stem, root, flower, fruits, seeds, etc.<sup>[48]</sup> In the phytochemical screening, the presence of bioactive compounds such as saponins, quinones, tannins, carbohydrates, alkaloids, oxalate, phenols and flavonoids were done and tabulated in **Table 1**. The result shows that some of the phytochemicals such as alkaloids, flavonoids and saponins were present in all the mentioned extracts which were confirmed by the formation of brownish-red precipitate, yellowish-green color and froth formation respectively. Flavonoids in methanol and Phenols in both methanol and ethanol extract were strongly present. Carbohydrates, quinones and tannins were detected in methanol, ethanol and acetone extract while not detected in chloroform and hexane extract of Cinnamon bark. The oxalate compounds were not found in all the extracts cinnamon bark tested.

Different activities of Cinnamon depends upon the kind of phytochemicals it possesses. Phytochemicals are important constituents in our diet which have some beneficial effects on our health. The main purpose of performing the phytochemical test is to detect the presence of secondary metabolites such as phenols, saponin, alkaloids, carbohydrates, which have great medicinal importance. Saponins have antibiotic properties and help to prevent hypercholesterolemia<sup>[3]</sup> and alkaloids were used as analgesics.<sup>[47]</sup> Phenol and tannins are potent antioxidants.<sup>[12]</sup> Among the studies, the phytochemical components that were analyzed show their presence mostly in methanolic extract.<sup>[11,47,38]</sup> Oxalate was found absent in methanolic extract of cinnamon and a similar result was also reported by.<sup>[22,35]</sup> Majority of the compounds were found present in methanol, ethanol and acetone extracts of cinnamon bark but in chloroform and hexane extract minimum presence of these bioactive molecules were observed.

### Antioxidant activity

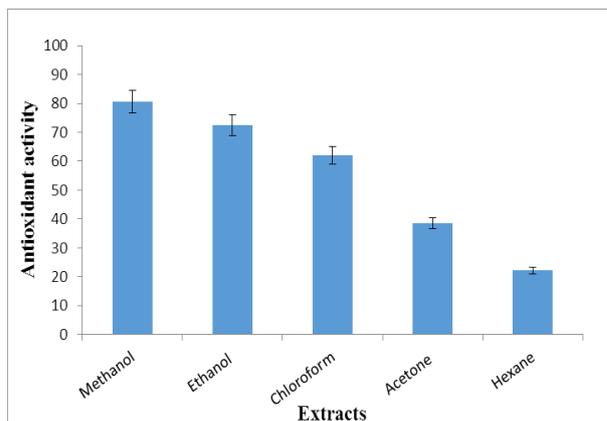
DPPH method is based on the reduction of DPPH which is a stable free radical.<sup>[13]</sup> It is based on different reaction mechanism that are available to determine the antioxidant activity of natural products. In this study, the antioxidant activity of different solvent extracts is shown in **Fig 3**. among all the extracts, the methanolic extract possessed highest antioxidant activity, followed by ethanol, chloroform, acetone and lastly by hexane with

% radical scavenging activity  $80.63 \pm 0.86\%$ ,  $72.36 \pm 0.93\%$ ,  $62.06 \pm 0.44\%$ ,  $38.56 \pm 0.87\%$  and  $22.16 \pm 0.49\%$  respectively. Similar result were also demonstrated for methanol, chloroform and for ethanol having %inhibition approximately 85%, 68% and 69.76%.<sup>[32,51]</sup> The methanol extract at  $250\mu\text{g/ml}$  shows percentage inhibition 73.45%.<sup>[21]</sup> Similarly, acetone and hexane extract had shown the % inhibition of about 72.37% and 20%.<sup>[45]</sup>

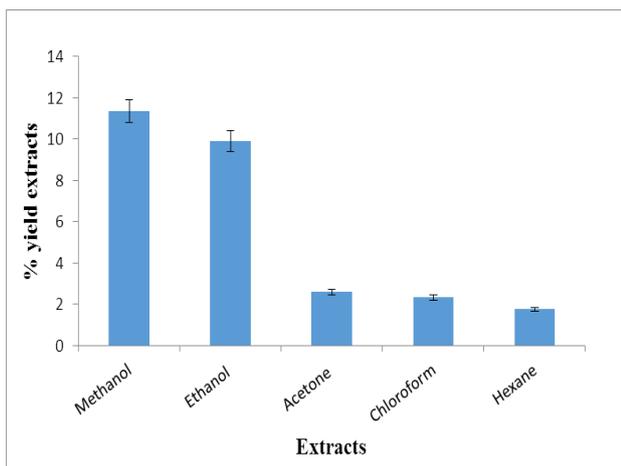
The IC<sub>50</sub> value (concentration of sample required to scavenge 50% free radicals) of methanolic extract of Cinnamon bark was evaluated and ascorbic acid was used as a standard in this investigation. The IC<sub>50</sub> value of Cinnamon bark extract is  $111.5\mu\text{g/ml}$  and that of standard ascorbic acid is  $57.97\mu\text{g/ml}$  which is presented in **Table 2 and Fig 4**. A similar result also has been reported in the methanolic extract of *Cinnamomum verum* with an IC<sub>50</sub> value of  $111.5 \pm 0.62\mu\text{g/ml}$  by DPPH assay.<sup>[47]</sup> Lower IC<sub>50</sub> values are associated with a higher radical scavenging activity.<sup>[9]</sup> Compared with the standard, methanol bark extract of Cinnamon has shown less antioxidant activity. At  $500\mu\text{g/ml}$  concentration, the highest radical scavenging activity of 81.89% was observed in the methanol extract of Cinnamon bark however at the same concentration the percentage inhibition of ascorbic acid was observed to be 90.40% which is greater than the extract. On dilution, the concentration of the radical scavenging activity decreases which proved that highest concentration revealed maximum activity. The DPPH solution becomes decolorized when mixed with sample extract due to their hydrogen donating ability. The change in color from deep violet to light yellow measured spectrometrically which showed that Cinnamon bark extract has potent antioxidant activity. The radical scavenging activities of the extracts also depend upon the type of solvent used and the scavenging activity of DPPH increases with increase in phenolic components such as phenolic acids, and phenolic di-terpenes. These phenolic components retain many hydroxyl groups which have efficient radical scavenging action and antioxidant power.<sup>[46]</sup> Although we have studied the extracts of Cinnamon bark, the essential oil of *Cinnamomum zeylanicum* bark shows the highest radical scavenging activity of (91.4%) in ethanol, among the 42 kinds of essential oil tested.<sup>[24]</sup> An alternate report revealed, that the IC<sub>50</sub> values of methanolic extract of *Cinnamomum cassia* indicate that extracts are more effective DPPH radical inhibitor ( $42.03\mu\text{g/L}$ ) than essential oil of Cinnamon bark  $147.23\mu\text{g/L}$ .<sup>[6]</sup>



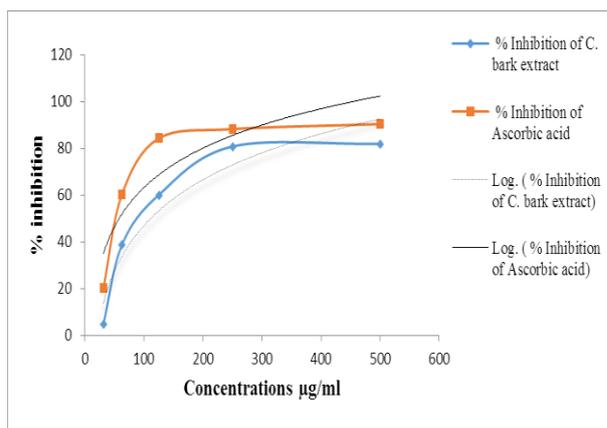
**Fig 1: Cinnamon bark.**



**Fig 3: Extracts Antioxidant activity of extracts of Cinnamon bark.**



**Fig 2: Percentage yield of extracts from different solvent of Cinnamon bark.**



**Fig 4: DPPH free radical scavenging activity of Cinnamon bark methanol extract and ascorbic acid (% inhibition vs. concentration).**

**Table 1 : Phytochemical screening of secondary metabolites of Plant Extracts of Cinnamon bark.**

Phyto-constituents	Methanol extract	Ethanol extract	Acetone extract	Chloroform extract	Hexane extract
Alkaloids	++	++	+	+	+
Carbohydrates	+	+	+	-	-
Flavonoids	++	+	+	+	+
Oxalate	-	-	-	-	-
Phenols	++	++	+	+	-
Quinones	+	+	+	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	-	-

-- = negative test, + = weak positive test, ++ = strongly positive test.

**Table 2: The IC<sub>50</sub> Value of methanol extracts of Cinnamon bark and Ascorbic acid.**

Sample	Concentration (µg/ml)	% Inhibition	IC <sub>50</sub> (µg/ml)
Methanolic extract of Cinnamon bark	500	81.89	111.05
	250	80.82	
	125	60.05	
	62.5	38.58	
	31.25	4.66	
Ascorbic acid	500	90.40	57.97
	250	88.24	
	125	84.23	
	62.5	60.18	
	31.25	20.08	

**CONCLUSION**

From this study, it is concluded that all the experiment which was performed on the commercial bark of Cinnamon have confirmed the presence of phytochemicals tested by different methods. The availability of saponins, quinones, tannins, carbohydrates, phenols, alkaloids, oxalate, phenols and flavonoids have proved that the bark has possessed potent medicinal activity which is beneficial for health. Saponins and Alkaloids were found in abundance in all the extracts whereas flavonoids and oxalate were found missing to them. The methanolic extract of Cinnamon bark reveals good antioxidant activity in reaction with DPPH (2,2, Diphenyl-1-picryl hydrazine) with an IC<sub>50</sub> value of 111.5µg/ml. The antioxidant activity more or less depends upon the type of solvent used basically methanol is regarded as a good solvent for extraction and thus shows the effective action. Therefore the bark of Cinnamon which is used as a spice has some remarkable effects on our health and considered to be a good source of natural antioxidants. Further research is required to investigate the bioactive molecules of Cinnamon.

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