

## Evaluation of *in-vitro* Free Radical Scavenging Activity of *Cinnamomum zeylanicum* Bark Extract using Fenton Reaction

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### ABSTRACT

*Cinnamomum zeylanicum* is one of the species of cinnamon plant from the family of Lauraceae. It is not only the main ingredients of species but also exhibit many medicinal properties. Anti-microbial, anti-fungal, anti-inflammatory, anti-tyrosinase, anti-carcinogenic and anti-mutagenic are some of the activities shown by cinnamon. Cinnamon is used mainly as an aromatic condiment and flavouring additives in a wide variety of cuisines, sweet and savoury dishes, breakfast, cereals, snack foods, tea and traditional foods. The aroma and flavour of cinnamon derive from its essential oil and principal components, cinnamaldehyde as well as numerous other constituents including eugenol. In the present study the methanolic extract of bark of cinnamon was evaluated for antioxidant activity by the OH free radical scavenging activity using Fenton reaction. This study was conducted to investigate the effect of cinnamon bark extract using Fenton reaction. The dried bark of cinnamon was extracted with methanol using a Soxhlet extractor. The total phenolics content of bark as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. In this plant cinnamon bark extract, there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. The result suggests that the cinnamon extract can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analyzing the synergic association of extract with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts. In conclusions the present study indicated that cinnamon bark extract may be a potential source of natural antioxidants.

**Key words:** Antioxidant activity, Fenton reaction, Hydroxyl radicals, ascorbic acid, Cinnamon bark, TBARS.

### INTRODUCTION

*Cinnamomum zeylanicum* (Family Lauraceae), known as ‘Ceylon cinnamon’ or ‘true cinnamon’, grows as an ever-green tree native to Sri Lanka (earlier Ceylon), and India including other regions of tropical Indochina and Madagascar. This is one of the oldest traditional spice species used for culinary purposes in South Asian countries (Abdelgadir, 2020 and Abeysekera, et al., 2013). Additionally, according to toponymical and historical evidence, *C. zeylanicum* has been used for medicinal purposes since the establishment of Aryan settlements in the Anuradhapura kingdom (Patel, et al., 2020). Moreover, the indigenous species of Ceylon cinnamon has been used in the Ayurveda system of Sri Lanka

(Patel,2020 and Ranasinghe, et al., 2016). Ethnopharmacological studies show that *C. zeylanicum* has gained more importance in Ayurveda and folklore medicine as it can be used in concoctions and decoctions. The inner bark of *C. zeylanicum* is used for medicine preparation in flatulence control, indigestion and in flu-prevention in the Sri Lankan Ayurveda system. *C. zeylanicum* has also been found in various other folklore treatments against inflammation of eyes, dyspnoea, leucorrhoea, rheumatism, neuralgia, wounds, toothache and diabetes (Ranasinghe, 2016 and Kumar, et al., 2015).

The flavour of cinnamon is due to an aromatic essential oil that makes up 0.5 to 1% of its composition. This essential oil can be prepared by roughly pounding the bark, macerating it in sea water, and then quickly distilling the whole. It is of a golden-yellow colour, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent taste and scent come from cinnamaldehyde (about 90% of the essential oil from the bark) and, by reaction with oxygen as it ages, it darkens in colour and forms resinous compounds. (Yokomi, et al., 1 July 2009). *C. zeylanicum* and its compounds behaving as antioxidant, anti-inflammatory, antimicrobial, anticancer, anti-mutagenic, anti-tyrosinase and antidiabetic agents (Abdelgadir, 2020 and Abeysekera, et al., 2013). In fact, Ceylon cinnamon is considered one of the few plants in the world that have made it to the modern pharmacy in the form of pills, powders, oils and ointments.

A natural antioxidant is hereby the need of the hour. The methanol extract is said to have maximum anti-oxidant property as compared to the ethanolic and water extract (Mancini-Filho, et al., 1998). The antioxidant property is due to the eugenol component which inhibited peroxynitrite-induced nitration and lipid peroxidation in in vitro models (Chericoni, et al., 2005). The oil is said to form a phosphomolybdenum complex which is responsible for its antioxidant activity (Jayaprakasha, et al., 2003). This anti-oxidant effect has been recently extended to its application in liver disorders. The ethanolic extract has demonstrated to decrease the carbon tetrachloride induced lipid peroxidation resulting in a fallmarker of oxidative stress such as MDA (Moselhy, et al., 2009).

Antioxidant compounds present in foodstuffs play a vital role in human life, acting as health-protecting agents. In addition to this role, antioxidants are one of the key additives used in fats and oils. Even in the food processing industry, antioxidants have been used to delay or prevent food spoilage. Spices and medicinal plants have received rapid consideration as sources of beneficial antioxidants against various diseases (Suhaj 2006). Antioxidants have been considered the most important drivers in the progress and existence of human they respond to free radicals and damage in metabolic diseases and age-related syndromes of humans and other animals (Halliwell 2011).

## **MATERIALS AND METHODS**

### **Plant Materials**

*Cinnamomum zeylanicum* bark was collected from local market of Raipur (Chhattisgarh) India.

### **Chemicals and Reagents Samples**

The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

### Preparations of Extract

Dried powdered of *Cinnamomum zeylanicum* (10 g) were extracted by continuous mixing in 100 ml 50% methanol and distilled water, 24h at room temperature. After filtration, methanol was evaporated until only water remained through evaporation on water bath at 60-70°C temperature. The final extract was kept in petri plate and cover with silver foil.

### Fenton Reaction Assay to Assess OH-Radical Scavenging Activity

The OH- radical scavenging activity of *Cinnamomum zeylanicum* bark extract (10–100 µg/ml) was determined according to the Fenton reaction method of (Halliwell, et. al., 1987) in the presence of 1ml EDTA, FeCl<sub>3</sub>, H<sub>2</sub>O and ascorbic acid were prepared in degassed H<sub>2</sub>O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 IM EDTA, 1 mM H<sub>2</sub>O<sub>2</sub>, 100 IML- ascorbic acid, 100 IM FeCl<sub>3</sub>, H<sub>2</sub>O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38° C, 1hr, 0.5 ml 5% TBA and 0.5 ml 5% TCA were added to the reaction mixture which was then heated in a boiling water bath for 20 min. Once samples were cooled, the absorbances were read at 532 nm. The percent inhibition of hydroxyl radical was calculated as follows:

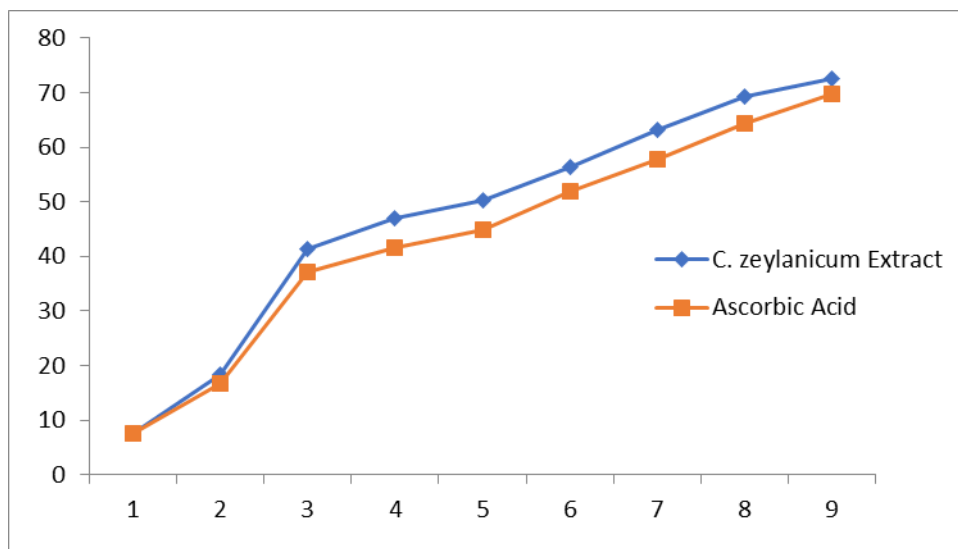
$$\% \text{ inhibition} = (\text{blank-ascorbic acid or drug})/\text{blank} \times 100$$

### RESULT

The result of the examined *Cinnamomum zeylanicum* extracts and control solution i.e.: ascorbic acid shows that they inhibited the production of OH<sup>+</sup> radicals. The percentage of free radical scavenging activity of the extract increases with an increase in concentration as shown in table 1. Extent of hydroxyl radical scavenging was determined by the increases in intensity of light pink colour which was determined at 532nm.

**Table 1: Antioxidant activity of ascorbic acid and extract of *Cinnamomum zeylanicum***

S. No.	CONC. (µg/ml)	% Inhibition	
		<i>Cinnamomum zeylanicum</i> Extract (Mean ± SE)	Ascorbic Acid (Mean ± SE)
1.	10	7.52 ± 0.77	7.52 ± 0.46
2.	20	18.27 ± 0.56	16.8 ± 0.48
3.	30	41.28 ± 0.66	37.09 ± 0.84
4.	40	46.98 ± 0.69	41.50 ± 1.86
5.	50	50.21 ± 0.38	44.94 ± 1.13
6.	60	56.3 ± 0.56	51.82 ± 1.54
7.	70	63.11 ± 0.76	57.74 ± 1.17
8.	80	69.20 ± 0.46	64.40 ± 0.57
9.	90	72.69 ± 4.55	69.88 ± 1.19
10.	100	82.68 ± 0.75	79.73 ± 0.57



*Graph showing the Antioxidant activity of ascorbic acid and extract of Cinnamomum zeylanicum*



*Fig.1. Cinnamomum zeylanicum sample*



*Fig.2. Sample after crushed*



*Fig.3. Cinnamomum zeylanicum Extract*

## **DISCUSSION**

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesis hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety (Ahn,2017). *Cinnamomum zeylanicum* is one such medicinal plant. Its rich in minerals, vitamins such as vitamin C, vitamins A, vitamins D, vitamins E and the most vitamins B and amino acids essential for human health and growth. It contains high amount of potassium and rich in calcium. Manganese may help prevent osteoporosis, anemia and symptoms of pre-menstrual syndrome, cinnamon is naturally low in fat and sugar, and it can add flavor and zest to food without increasing its sugar, fat or calorie content.

The essential oils and some of the major compounds present in cinnamon, including (E)-cinnamaldehyde, eugenol, and linalool, were investigated in reference to peroxynitrite-induced nitration and lipid peroxidation. Eugenol and the essential oils were more effective than the other two compounds (Chericoni, et al., 2005). In a comparative study among 26 spices, cinnamon showed the highest antioxidant activity, indicating that it can be applied as an antioxidant used in foods (Shan, et al., 2005). Another study investigated the effectiveness of a mixture of spices on oxidative stress markers as well as the antioxidant activity in high fructose-fed insulin-resistant rats. The mixture, which consisted of 1g/100 g cinnamon bark, showed a significant antioxidant activity compared to the fructose alone group (Suganthi, et al., 2007). Volatile oils from *C. zeylanicum* showed significant biological activities (Jayaprakasha, et al., 2011).

The multiple benefits of *Cinnamomum zeylanicum* made it a true miracle of nature. Numerous studies have been conducted on different parts of *Cinnamomum zeylanicum*, but this plant has not yet developed as a drug by pharmaceutical industries. The present research reveals that the plant is used in treating various ailments. It elicits on all the aspects of the herb and throws the attention to set the mind of the researchers to carry out the work for developing its various formulations, which can ultimately be beneficial for the human beings as well as animals.

The results of the effects of the examined *Cinnamomum zeylanicum* extract as well as control solutions on OH- radical production. They show that all extract of *Cinnamomum zeylanicum* extract and control solutions as ascorbic acid inhibited the production of OH- radicals. The % of free radical scavenging activity of hydro-methanolic extract of *Cinnamomum zeylanicum* presented in Table 1 have reducing power, the free radical OH- scavenging activity of the extract increases with increasing the concentration.

## **CONCLUSION**

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the

bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc.

Antioxidant molecule inhibits the oxidation of other molecules from the body. Natural and artificial oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiol or ascorbic acid (vitamin C) terminate these chain reactions. The natural and synthetic antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as Cancer or coronary heart disease. The hypothesis that antioxidant supplements might promote health has not been confirmed experimentally (Abner, et al., 2011).

The result of the conducted experiments shows the *Cinnamomum zeylanicum* extract and ascorbic acid which is taken as control solution inhibited the production of OH<sup>+</sup> radical and that of percentage of free radical scavenging activity of the extract increases with the increase in concentration. Extent of hydroxyl radical scavenging was determined by the increases in intensity of light pink colour which was determined at 532nm. The antioxidant activity was compared with ascorbic acid which was taken as positive control. This study indicates that *Cinnamomum zeylanicum* shows good antioxidant activity.

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