

# Acetylcholinesterase activity of Cinnamon Zeylanicum extract

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## ABSTRACT

**Introduction:** Cinnamon zeylanicum (*C. zeylanicum*) is a tropical evergreen tree of Lauraceae family. It is one of the oldest culinary spices known. And because of containing analgesic, antiseptic, aphrodisiac and memory enhancing characteristics, cinnamon is traditionally used for treating many ailments, along with its culinary uses. Acetylcholinesterase (HGNC symbol ACHE), also known as AChE or acetylhydrolase, is the predominant cholinesterase in the body. **Materials and Method:** AChE inhibitory activities at different concentrations were taken (5, 10, 20, 40, 80, 160 and 320µg/ml) and were dissolved in a base-tris (0.05 M) buffer, following the spectrophotometric method. In this method, 200µl of acetylthiocholine iodide, 1000µl of DTNB (3mM), and 200µl of test extract solution at the different concentrations were mixed and incubated for 15 min at 30°C. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200µl of AChE solution was added to the initial mixture, to start the reaction and then the absorbance was determined. **Results and Discussion:** The inhibitory effect of different concentrations of cinnamon extract on the activity of acetylcholinesterase was examined. The plant extracts demonstrated the potent inhibition of the ACh. The IC<sub>50</sub> was found to be 53.25µg/ml. Maximum inhibition was found to be 87.09% at 320µg/ml. **Conclusion:** Because of possessing analgesic, antiseptic, aphrodisiac, astringent, carminative, haemostatic and insecticidal characteristics, cinnamon is considered as a traditional treatment for a lot of disease conditions. Cinnamon extract, on performing various phytochemical analysis, was found to break down the enzyme acetylcholinesterase, and is therefore considered an anti-acetylcholinesterase. This results in the assimilation of the acetylcholine by the brain.

**Keywords:** Acetylcholine, alzheimer's disease, inhibition, cinnamon zeylanicum, concentration.

## Introduction

Cinnamon belonging to the family Lauraceae, is a genus of trees found in continental Asia, eastern and southeastern Asia, Australia, the Pacific region, and a few species in Central and South America. In Malaysia, 21 species have been found in tropical rain forests where they grow at various altitudes from highland slopes to lowland forests and occur in both marshy places and on well-drained soils<sup>[1]</sup>. Cinnamon is reported to have several pharmacological activities including some antimicrobial<sup>[2]</sup> anti-fungal<sup>[3]</sup>, antioxidant<sup>[4]</sup> and anti-diabetic<sup>[5]</sup>. It has also been used as anti-inflammatory<sup>[6]</sup>, antitermitic<sup>[7]</sup>, nematocidal

<sup>[8]</sup> and anticancer agent<sup>[9]</sup> related properties worldwide. Cinnamon, the inner bark of a tropical evergreen tree has two main types, Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) and Chinese Cassia (*Cinnamomum aromaticum* Ness) and which when dried, rolls into a tubular form known as a quill. Cinnamon is either available as cinnamon sticks or as a ground powder. In our native Ayurvedic medicine, cinnamon is usually considered as a remedy for digestive, respiratory and gynaecological ailments<sup>[10]</sup>. Another important benefit of Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products<sup>[11]</sup>. Its bark contains procyanidins and catechins. Both procyanidin A-type and B-type linkages are included in the components of procyanidins. The antioxidant activities were observed in the procyanidins extracted from cinnamon and berries<sup>[12, 13]</sup>. Acetylcholinesterase (HGNC symbol ACHE), also called AChE or acetylhydrolase, is the predominant cholinesterase in the body. It is an enzyme that catalyses the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters. AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to end synaptic transmissions<sup>[14]</sup>.

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AChE activity is inhibited by many compounds. The number of known inhibitors is rather extensive. Two main types of inhibitors can be distinguished from a practical point of view: toxins and drugs [15].

Alzheimer's disease (AD) is a progressive and complex neurodegenerative disorder of the brain. It is suggested that deposition of amyloid- $\beta$ , accumulation of  $\tau$ -protein, release of inflammatory mediators and decreasing label of acetylcholine in the cholinergic system could be the possible reasons for enhancing neurodegenerative condition of the AD brain [16]. It is well known that patients with AD were deficient of acetylcholine, a neurotransmitter that navigates the memory process of central nervous system. This fact has led to develop the cholinergic hypothesis, which focuses on the impairment of cognitive function in AD related disorder [17]. Pathogenesis of Alzheimer's Disease is described as Microglia and astrocytes surrounding A $\beta$  neuritic plaques which secretes cytokine class of inflammatory mediators which gets increased in Inflammatory states, and it functions to regulate intensity and duration of immune response [18]. Level of acetylcholine in the brain decreases in the Alzheimer's disease, which is principally characterised by impaired memory and disturbed behaviour thus, Ache and BChE inhibitors are being developed for the symptomatic treatment of Alzheimer's disease [19].

Hence, considering the various medicinal benefits of cinnamon, an effort has been made to establish the scientific validity through screening of antioxidant, anticholinesterase, and antityrosinase inhibitory activities. The main objective of the study is to evaluate and access the acetyl cholinesterase activity of Cinnamon extract.

## Materials and Methodology

### Plant materials:

Cinnamon extracts used in the study were obtained from Green Chen herbal extracts and formulations, Bengaluru, India.

### Chemicals used:

Acetylthiocholine iodide, DTNB were obtained from sigma Aldrich.co.india. All other chemicals used in this study were up to analytical grade.

### In vitro acetylcholinesterase (ACh) inhibition assay

Cinnamon extract were examined for their AChE inhibitory activities at different concentrations (5, 10, 20, 40, 80, 160 and 320 $\mu$ g/ml) and were dissolved in a base-tris (0.05 M) buffer, following the spectrophotometric method developed by [20] as described by [21].

In this method, 200 $\mu$ l of acetylthiocholine iodide (15mM), 1000 $\mu$ l of DTNB (3mM), and 200 $\mu$ l of test extract solution at the different concentrations were mixed and incubated for 15 min at 30°C. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200 $\mu$ l of AChE (0.3U/ml) solution was added to the initial mixture, to start the reaction and then the absorbance was determined.

The control contained all the components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

**IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100** All treatments were performed in triplicate with two replicates.

### Estimation of IC<sub>50</sub> values:

The concentrations of the tested extracts that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC<sub>50</sub>) were determined by a linear regression analysis between the inhibition percentages against the extract concentrations by using the Graph pad prism.

## Results

Different concentrations of cinnamon extract were evaluated for the inhibitory effect on the activity of acetylcholinesterase. The plant extracts exhibited potent inhibition of the ACh. The IC<sub>50</sub> was found to be 53.25 $\mu$ g/ml. Maximum inhibition was found to be 87.09% at 320  $\mu$ g/ml.

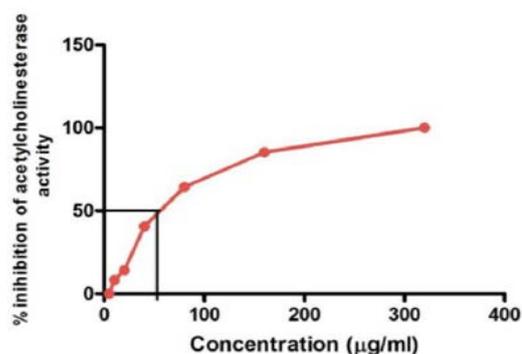


Figure 1. Graph showing percentage of acetyl cholinesterase activity

## Discussion

Cinnamon which is the inner bark of a tropical evergreen tree includes two main types, Ceylon cinnamon and Chinese Cassia which are dried and rolled into a tubular form known as a quill. Cinnamon can be in the form of a whole quill (cinnamon sticks) or as ground powder. Cinnamon was regarded as a treatment for respiratory, digestive, and gynaecological diseases in native Ayurvedic medicine [22]. Many potentially beneficial health effects of cinnamon such as anti-inflammatory and anti-microbial characteristics have been shown in studies done recently [2, 6, 18]. In this study, the acetyl cholinesterase inhibitory activity of cinnamon is examined. The results are clearly indicative of the fact that cinnamon is inhibitory to acetyl cholinesterase activity, and thus can be used as a potent suppressor of the central nervous system.

Another study indicated that the cinnamon oil of bay leaves has more affinity towards AchE than BChE and was found to be more active in comparison to the methanol extract. Plants therefore provide a useful resource of therapeutic lead components [23, 24].

Recent examination of several monoterpenoids compounds have revealed the potent anti-cholinesterase activity against the pest insects, among them fenchone, geraniol and linalool have shown the highest anti cholinesterase inhibitory activity [25].

A study has also shown that both AchE and BChE play critical role in maintaining chemical neurotransmitter (ACh) in the

region of the human brain. However, the ratio of AChE/BChE label in certain region of brain is altered in due course of Alzheimer's disease. These changes provide strong evidence of decline in label of acetylcholine in the region of dementia brain [26].

Several studies have proved that cinnamon is used as a part of Ayurvedic treatment for various diseases. Now, the fact that it has been confirmed about having the inhibitory effect of acetylcholinesterase activity, if it has the Ayurvedic action of the particular extract which was examined. The inhibitory level of cinnamon is maximum at the concentration of 320 micro grams per ml, and thus this has been incorporated into pharmacological formulations which may help the society in several ways. This will help the patients in particular diseases as well.

The present study can be further improved by incorporating more number of neurotransmitters and checking the effect of the same extract with those potent neurological transmitters.

## Conclusion

Cinnamon is considered as a folk remedy for many disease conditions because of including analgesic, antiseptic, astringent, carminative, haemostat and insecticidal properties. Cinnamon extract, on performing various phytochemical analysis was found to break down the enzyme acetylcholinesterase, and is therefore considered an anti-acetylcholinesterase. This results in the assimilation of the acetylcholine by the brain. The outcome of the study further explores that the cholinesterase inhibitory activity of cinnamon provides evidence of anti-cholinesterase agents for the treatment of Alzheimer's disease. Therefore, proving that Cinnamon truly is a potent inhibitor of AChE [27].

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