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## *in vitro* Antidiabetic and Antimicrobial Activity of Methanolic Bark Extract of *Cinnamomum obtusifolium* (Roxb.) Nees

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The present study was aimed to investigate *in vitro* antidiabetic and antimicrobial activity of methanolic bark extract of *Cinnamomum obtusifolium*. The  $\alpha$ -amylase and  $\alpha$ -glucosidase activities were measured in the presence of methanolic bark extract of *Cinnamomum obtusifolium* and acarbose, respectively. Antimicrobial activity was tested against six pathogenic bacterial strains and one fungal strain by agar well diffusion method. The results showed that methanolic bark extract of *Cinnamomum obtusifolium* had  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity with half-maximal inhibitory concentration values of 31.26 and 170.135  $\mu$ g/mL, respectively. The extract was found to exert low to moderate antimicrobial activity compared to a standard antimicrobial agent.

**Keywords:** *Cinnamomum obtusifolium*, Methanolic, Antidiabetic, Antimicrobial.

### INTRODUCTION

Today food plants [1], is not only considered a simple nutritional intake [2], but as a potential source of medicine [3,4]. Many wild edible plants, along with several cultivated plant species, can be defined as food-medicine [5] due to the presence of different classes of natural products [6]. Earlier studies have shown that the local Mediterranean foods are a good source of antioxidants [7], bioactive compounds [8] and have good estrogenic activity [9]. The consumption of indigenous fruits in Ohangwena and Oshikoto gives an important contribution to local communities' health and welfare [10]. According to WHO, herbal medicines serve the health needs of about 80 % of the world's population, especially for millions of people living in the rural areas of developing countries [11]. Humans have explored their environment for the use of a large number of medicinal plants which has resulted in the isolation and production of pure active compounds and later in the development of novel synthetic compounds based on natural products.

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [12]. According to WHO, it is estimated that 3 % of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3 %. Diabetes is a lifestyle disease characterized by lower physical activity and higher intake of high fat and

saturated carbohydrate containing diets. Hyperglycemia is a main symptom of type-2 diabetes mellitus and plays vital roles in most of the pathogenic features of the disease. This condition prevails when there is decreased insulin sensitivity or decreased insulin secretion from pancreatic  $\beta$ -cells, which can further inhibit insulin secretion from the pancreas and reduce insulin-mediated glucose uptake in peripheral tissues [13,14]. An important strategy to control hyperglycemia is through the inhibition of carbohydrate digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, which also play a vital role in preventing diabetic complications. The inhibitors of these enzymes delay the digestion of carbohydrates, therefore, reduce the rate of glucose absorption from the small intestinal tract, as well as reduce postprandial blood glucose level. Thus, inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is a key in the management and treatment of type-2 diabetes mellitus [13,15]. Currently, several conventional prescribed  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors are available in the market, which include acarbose, voglibose and miglitol. However, they have been shown to have some undesirable side effects such as flatulence, diarrhea and abdominal pain, which cause adverse reactions to patients [13]. This indicates the urgent need for the development of newer alternatives. Alternative medicines, predominantly herbal drugs are available for the treatment of diabetes. Common advantages of herbal drugs are effectiveness, safety and acceptability. The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase. The inhibition

of this enzyme delay carbohydrate digestion and protect overall carbohydrate digestion time, resulting in the reduction in glucose absorption rate and consequently dulling the postprandial plasma glucose rise. Several indigenous medicinal plants have a high potential in inhibiting  $\alpha$ -amylase enzyme activity [16].

Microbial resistance to antibiotics is a major threat to public health [17]. Worldwide, the increasing prevalence of multiresistant *S. aureus* strains has become a threat to public life [18]. Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants [19]. Therefore plant extracts [20] and essential oil are suitable alternatives now to treat resistant organisms [21]. Studies have shown that plants extracts possess significant antimicrobial properties [22]. In recent years various plants like *Cynodon dactylon* [23], *Hibiscus rosa sinensis* [24], *Hypericum perforatum* [25], *Cassia italic* [26], *Green tea* [27], *Helichrysum aureonitens* [28] are studied for their antimicrobial property.

Also it has been seen that there is a constant need of alternative and efficient compounds for food preservation which aimed at partial or total replacement of antimicrobial chemical additives. Gould [29] has emphasized the possible use of spices and their derivatives as alternatives for inclusion in a new perspective of food conservation called "natural antimicrobial system", which relies on the synergistic effect of antimicrobial compounds from animal, plant and/or microbial origin in order to create an unfavourable environment for microbial survival in foods [30]. The microbiological quality of fresh-cut fruits and vegetables is particularly critical given their exposure during the cutting process, which can cause contamination by bacteria, fungi and yeast [31]. One effective way of limiting microbial growth is increasing the acidity of a particular food by adding an acidic substance acids attack cell walls, cell membranes, metabolic enzymes, protein synthesis systems and the genetic material of microorganisms [32]. The use of natural antimicrobials such as essential oils [33], organic acids [34], plant extracts could be a good alternative to ensure food safety.

The genus cinnamon had shown antibacterial and antifungal activity. Cinnamon is a well-accepted flavours in the North America. They are incorporated in many foods such as ice cream, beverages, biscuits, chocolate and other products [35]. Their antimicrobial property has been known since antiquity and currently many studies have confirmed their effect against different bacteria, yeasts and moulds. Cinnamon is reported to inhibit aflatoxin [36]. Cinnamic acid inhibit microbial growth in fresh-cut melon and kiwifruit at 4 and 8 °C [37]. *Cinnamomum* oil eliminate microorganisms that can be a hazard for the consumer in unpasteurized fruit juices [38]. *Cinnamon cassia* is reported to have antimicrobial activity against bacterial strains [39].

*Cinnamomum obtusifolium* (Roxb.) Nees is a medium to large-sized evergreen tree with aromatic leaves, stem bark and panicle, distributed in the central and outer parts of eastern Himalayas up to an altitude of 2,100 m and also in Andaman Islands. In Assam the plant is well distributed in the Jorhat, Sibsagar, Golaghat, Nowgong and Kamrup districts. It also grows in the Khasi, Garo and Jaintia districts of Meghalaya and

in a few places of Nagaland in northeast India. *C. obtusifolium* is known locally by different names such as Pati-Hunda, Nagaldalchini, Seerang-elsing, Sami-jong and Tejpat-manbi, among the different ethnic groups [40]. It was also observed that the bark, which was sold at the local markets, was used traditionally in the region as a spice in curry [41]. The bark and its infusions have local medicinal use for the treatment of bone fracture [42], stomach disorder [43], skin diseases [44], diarrhea [45], liver problem [46], urinary trouble, gall stones [47] and as a mouth freshener.

The potential antimicrobial properties of *Cinnamomum obtusifolium* bark extract have yet not been studied. The chief significance of the above study is to test the antimicrobial activity of cinnamon extract as alternatives to chemical preservatives so as to minimize their side-effects and simultaneously improving the shelf-life of the food products and for formulation of new broad spectrum antimicrobial agent.

## EXPERIMENTAL

**Collection of plant material and preparation of methanolic extract of bark of plant:** The bark of *Cinnamomum obtusifolium* was collected from Titabor, Jorhat, Assam, India during the month of October 2015. The plant was identified and authenticated by Dr. A.A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong.

Barks were cut into pieces, washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried bark pieces were then pulverized in a mechanical grinder to coarse powder and then stored in airtight containers free from moisture. 250 g of powdered crude drug of barks were extracted by soxhlet extraction (continuous hot extraction) with 1000 mL of methanol for 18 h after pretreatment with petroleum ether. The solvent was recovered at 50 °C by distillation under reduced pressure and the extract was concentrated to obtain an orange brown semisolid mass.

**Phytochemical screening:** Methanolic bark extract of *Cinnamomum obtusifolium* was tested for the presence of various phytoconstituents namely, alkaloids, amino acids, carbohydrates, fats and fixed oils, flavonoids, glycosides, saponins, gums, lignin, proteins, steroids, triterpenoids, tannins and phenolic compounds.

### *in vitro* Antidiabetic activity

**Inhibition of  $\alpha$ -amylase activity:** Starch solution (0.5 % w/v) was prepared by stirring potato starch (0.125 g) in 20 mM sodium phosphate buffer with 6.7 mM sodium chloride (pH 6.9; 25 mL) in a boiling water bath for 15 min. The  $\alpha$ -amylase solution was prepared by mixing 1 U/mL of  $\alpha$ -amylase in the same buffer. The colorimetric reagent was prepared by mixing equal volume of sodium potassium tartrate tetrahydrate solution and 96 mM 3,5-dinitro salicylic acid (DNS) solution. Starch solution (1000  $\mu$ L) was mixed with increasing concentration of an enzyme inhibitor such as methanolic bark extract of *Cinnamomum obtusifolium* and acarbose (20,40,60,80,100  $\mu$ g/mL) and to this 1000  $\mu$ L of  $\alpha$ -amylase solution was added and incubated at 25 °C for 3 min to react with the starch solution. A 1000  $\mu$ L of 96 m MDNS reagent was added to the above solution and the contents were heated for 15 min on a

boiling water bath. The final volume was made up with distilled water and the absorbance was measured at 540 nm using spectrophotometer [48]. The percentage inhibition and 50 % inhibitory concentration (IC<sub>50</sub>) value was calculated.

**Inhibition of  $\alpha$ -glucosidase activity:** The  $\alpha$ -glucosidase enzyme inhibition activity was determined by incubating 100  $\mu$ L of  $\alpha$ -glucosidase enzyme (1 U/mL) solution with 100  $\mu$ L of phosphate buffer (pH 7.0) which contains 100  $\mu$ L of enzyme inhibitor such as methanolic bark extract of *Cinnamomum obtusifolium* and acarbose (20, 40, 60, 80, 100  $\mu$ g/mL) at 37 °C for 60 min in maltose solution. To stop the  $\alpha$ -glucosidase action on maltose, the above reaction mixture was kept in boiling water for 2 min and cooled. To this, 2 mL of glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose by the action of  $\alpha$ -glucosidase enzyme [48]. The percentage inhibition and 50 % inhibitory concentration (IC<sub>50</sub>) value was calculated.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

### Antimicrobial study

**Microbial strains:** All the microbial strains were obtained from MTCC, Chandigarh, India. A total of six bacterial species were tested including *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 739), *Pseudomonas vulgaris* (MTCC 426), *Streptococcus pneumonia* MTCC 655, *Pseudomonas aeruginosa* (MTCC 1688) and fungal strain *Candida albicans* (MTCC 3017). The strains were maintained in the respective media as per the recommendations provided by the source.

**Preparation of the microbial culture media:** 3.7 % of Mueller Hinton agar and potato dextrose Agar media was mixed with hot distilled water and autoclaved at 15 lb pressure for 15 min. After autoclaving, it was allowed to cool and solidify. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 4 mm.

### Agar well-diffusion method

**Antimicrobial activity:** *in vitro* Antimicrobial activity was determined by using the agar well-diffusion method [49]. Bacterial and fungal suspension adjusted to 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL) was used to inoculate Mueller Hinton agar and potato dextrose agar, respectively using a sterile swab. Five wells were bored into each of the solidified agar plants using a cork borer and were treated with the standard drug, ciprofloxacin (10  $\mu$ g/mL) for bacteria and fluconazole (10  $\mu$ g/mL) for fungi. Three doses of the methanolic bark extract of *Cinnamomum obtusifolium* 10, 50 and 100

$\mu$ g/mL and one control of carboxy methyl cellulose. Each well was found to contain 75  $\mu$ L of the above samples. The plates were incubated at 37 °C for 24 h for bacteria and at 35 °C for 48 h for the fungi. The activity was determined by measuring the diameter of inhibition zones in millimeters. All the assessments were done in triplicates.

## RESULTS AND DISCUSSION

**Phytochemical screening:** Methanolic bark extract of *Cinnamomum obtusifolium* contains flavanoids, carbohydrates, glycosides, lignin, steroids, saponins, tannins and phenolic compounds. Qualitative determination of other constituents is underway which will provide the detailed amount of the various constituents present.

**Inhibition of  $\alpha$ -amylase activity:** Methanolic bark extract of *Cinnamomum obtusifolium* produced 44.56 % inhibition of  $\alpha$ -amylase activity at 20  $\mu$ g/mL and 88.11 % at 100  $\mu$ g/mL concentrations, respectively and its IC<sub>50</sub> was found to be 31.26  $\mu$ g/mL. The standard drug acarbose exhibited 48.47 % inhibition of  $\alpha$ -amylase activity at 20  $\mu$ g/mL and 96.67 % at 100  $\mu$ g/mL concentrations, respectively and its IC<sub>50</sub> for acarbose was found to be 17.37  $\mu$ g/mL (Table-1).

**Inhibition of  $\alpha$ -glucosidase activity:** Methanolic bark extract of *Cinnamomum obtusifolium* exhibited 26.17 % inhibition of  $\alpha$ -glucosidase activity at 20  $\mu$ g/mL and 75.23 % at 100  $\mu$ g/mL concentrations, respectively and its IC<sub>50</sub> was found to be 170.135  $\mu$ g/mL. The standard drug acarbose produced 34.4 % inhibitory effect on  $\alpha$ -glucosidase activity at 20  $\mu$ g/mL and 80.2 % at 100  $\mu$ g/mL concentrations, respectively and its IC<sub>50</sub> was found to be 231.69 (Table-2).

**Antibacterial activity:** The extract showed significant antibacterial and antifungal activity against clinically isolated pathogenic microbial strains in comparison to standard ciprofloxacin and fluconazole. The observations were recorded (Tables 3 and 4). The different concentration of methanolic bark extract of *Cinnamomum obtusifolium* has been found to demonstrate appreciable antifungal activity against *Candida albicans* as compared to the standard drug fluconazole. It was highly effective against Gram-positive bacteria *Staphylococcus*, *Streptococcus* and Gram-negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa*. Among the bacteria selected, the *Bacillus subtilis* showed less response to the plant extracts compared to the other bacterial strains (Tables 1 and 2). This may be attributed to the increasing trend of these pathogens to resistance to the antimicrobial agents. The variation in the effectiveness of the extract against different microorganisms may be attributed to the phytochemical composition of the extract and membrane permeability of the

TABLE-1  
 $\alpha$ -AMYLASE INHIBITORY EFFECTS OF METHANOLIC BARK EXTRACT OF *Cinnamomum obtusifolium* AND ACARBOSE

Concentration ( $\mu$ g/mL)	Percentage inhibition (Mean $\pm$ SEM) of methanolic bark extract of <i>Cinnamomum obtusifolium</i>	IC <sub>50</sub> value ( $\mu$ g/mL)	Percentage inhibition (Mean $\pm$ SEM) of acarbose	IC <sub>50</sub> value ( $\mu$ g/mL) of acarbose
20	44.56 $\pm$ 0.315	31.26	48.47 $\pm$ 1.2250	17.37
40	57.06 $\pm$ 0.256		65.09 $\pm$ 1.1160	
60	60.66 $\pm$ 0.205		78.94 $\pm$ 0.8102	
80	75.23 $\pm$ 0.127		87.81 $\pm$ 0.5967	
100	88.11 $\pm$ 0.256		96.67 $\pm$ 0.3929	

The data represented as mean  $\pm$  SD (n = 3)

TABLE-2  
 $\alpha$ -GLUCOSIDASE INHIBITORY EFFECTS OF METHANOLIC BARK EXTRACT OF *Cinnamomum obtusifolium* AND ACARBOSE

Concentration ( $\mu\text{g/mL}$ )	Percentage inhibition (Mean $\pm$ SEM) of methanolic bark extract of <i>Cinnamomum obtusifolium</i>	IC <sub>50</sub> value ( $\mu\text{g/mL}$ )	Percentage inhibition (Mean $\pm$ SEM) of acarbose	IC <sub>50</sub> value ( $\mu\text{g/mL}$ ) of acarbose
20	26.17 $\pm$ 0.158		34.4 $\pm$ 0.4000	
40	37.00 $\pm$ 0.158		42.6 $\pm$ 0.4000	
60	44.56 $\pm$ 0.315	170.135	54.8 $\pm$ 0.4899	231.69
80	60.66 $\pm$ 0.205		75.8 $\pm$ 0.5831	
100	75.23 $\pm$ 0.127		80.2 $\pm$ 0.5831	

The data represented as mean  $\pm$  SD (n = 3)

TABLE-3  
 ANTIBACTERIAL ACTIVITY OF THE METHANOLIC BARK EXTRACT OF *Cinnamomum obtusifolium* USING AGAR WELL DIFFUSION METHOD

Microorganisms	Zone of inhibition (mm)			Ciprofloxacin (10 $\mu\text{g/mL}$ )
	Methanolic bark extract of <i>Cinnamomum obtusifolium</i> ( $\mu\text{g/mL}$ )			
	10	50	100	
<i>Staphylococcus aureus</i> MTCC 96	12.3 $\pm$ 0.25**	15.4 $\pm$ 0.50**	23.7 $\pm$ 0.43	22.8 $\pm$ 0.80
<i>Streptococcus pneumonia</i> MTCC 655	14.2 $\pm$ 0.20	22.8 $\pm$ 0.80**	30 $\pm$ 1.155**	15.8 $\pm$ 0.60
<i>Bacillus subtilis</i> MTCC 441	3.7 $\pm$ 0.20**	6.7 $\pm$ 0.20**	11.3 $\pm$ 0.33**	23 $\pm$ 0.31
<i>Escherichia coli</i> MTCC 739	6.5 $\pm$ 0.31**	8.7 $\pm$ 0.20**	13.3 $\pm$ 0.12**	17.8 $\pm$ 0.12
<i>Pseudomonas aeruginosa</i> MTCC 1688	16.5 $\pm$ 0.61**	21.2 $\pm$ 0.48	24.3 $\pm$ 0.12**	21.8 $\pm$ 0.73
<i>Pseudomonas vulgaris</i> MTCC 426	14.6 $\pm$ 0.24**	18.9 $\pm$ 0.24**	25.2 $\pm$ 0.48	25.5 $\pm$ 0.31

Values are mean  $\pm$  SEM (n = 5), statistical significance: \*P < 0.05, \*\*P < 0.01, compared with standard drug ciprofloxacin

TABLE-4  
 ANTIFUNGAL ACTIVITY OF THE METHANOLIC BARK EXTRACT OF *Cinnamomum obtusifolium* USING AGAR WELL DIFFUSION METHOD

Microorganisms	Zone of inhibition (mm)			Ciprofloxacin (10 $\mu\text{g/mL}$ )
	Methanolic bark extract of <i>Cinnamomum obtusifolium</i> ( $\mu\text{g/mL}$ )			
	10	50	100	
<i>Candida albicans</i> MTCC 301	17.2 $\pm$ 0.20**	27 $\pm$ 0.94**	31.6 $\pm$ 0.40**	21.1 $\pm$ 0.24

Values are mean  $\pm$  SEM (n = 5), statistical significance: \*P < 0.05, \*\*P < 0.01, compared with standard drug flconazole

microbes for the chemicals and their metabolism. The antimicrobial activity of methanolic bark extract of *Cinnamomum obtusifolium* was found to be higher in case of the higher dose which proved that the antimicrobial activity of methanolic bark extract of *Cinnamomum obtusifolium* should increase in a dose dependent manner.

**Statistical analysis:** All result was expressed as the mean  $\pm$  standard error of mean (SEM) using one-way Analysis of variance (ANOVA) followed by Dunnett's tests. The software used was Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA, USA). The results are analyzed for statistical significance and significance was expressed by P value, as mention in the Tables. P<0.01 was considered as statistically significant.

## Conclusion

Based on this study, it can be concluded that methanolic bark extract of *Cinnamomum obtusifolium* possesses effective  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects. The results displayed by the methanolic bark extract of *Cinnamomum obtusifolium* are interesting enough to stimulate the isolation of pure bioactive compounds and further *in vivo* study using an animal model of diabetes. The present study also provided the potential antimicrobial properties of cinnamon bark. From the experiment it is found that the methanolic bark extract of *Cinnamomum obtusifolium* has shown significant antibacterial

activity against five strains of bacterium, namely, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa* and *Pseudomonas vulgaris* and *Escherichia coli* in a dose dependent manner. The least susceptible strain was found to be *Bacillus subtilis*. The antifungal activity against *Candida albicans* as compared to the standard drug fluconazole is appreciable. This fact can support their use to control microbial growth during food processing.

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