ORIGINAL ARTICLE

In vitro study of antimicrobial activity of aqueous extracts of *Cinnamomum zeylanicum* bark against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Introduction: Various spices which we use in food are obtained from nature which show antioxidant, antimicrobial, anti-inflammatory properties etc. Various studies have been conducted in order to determine the effects of oils and extracts obtained from natural sources.The antimicrobial activities are also studied in the point from the extracts obtained from natural sources.The aim of this study was to investigate the antibacterial activity of aqueous extracts of bark of Cinnamon (Cinnamomum zeylanicum) against two food spoilage bacteria, Gram negative Escherichia coli and Gram positive Staphylococcus aureus.

Methods: The in vitro antibacterial activity was performed by disc diffusion method. Different concentrations of aqueous extracts were prepared by using distilled water.

Results: The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria. The maximum zones of inhibition at 100% concentrations were 24mm against Staphylococcus aureus and 25mm against Escherichia coli. A standard antibiotic Amikacin was also used to determine ZOI and it was compared with that result of aqueous extracts.

Conclusion: The results obtained in the present study suggest that the aqueous extracts of bark of Cinnamomum zeylanicum showed strong antibacterial activity against both test organisms.Thus cinnamon revealed a significant scope to develop a novel broad spectrum antibacterial herbal formulation.

Key words: Cinnamon, Cinnamomum zeylanicum, Antibacterial activity, Staphylococcus aureus, Escherichia. Coli, Disc diffusion, Aqueous extracts, Zone of inhibition.

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Introduction:

Medicinal plants are the source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Bangladesh is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity and the people of this country are very much habituated with the use of various types of herbs for their treatment purpose in many occasions.[1] The natural products are found to be more effective with least side effects as compared to commercial antibiotics, so that they are used as an alternate remedy for treatment of various infections.[2]

Cinnamon is a spice obtained from the inner bark of several trees from the genus *Cinnamomum* that is used in both

sweet and savoury foods. Cinnamon grows best in almost pure sand; it prefers a sheltered place, heavy moisture, and warm, unvarying temperature. The tree usually grows up to 30 feet high, has thick outer bark, and strong branches. The top sides of the leaves are shiny & emerald green in colour. The flowers are small in panicles; the fruit is an oval berry which is bluish when ripe. The cinnamon root bark smells like cinnamon and tastes like camphor, which it yields on distillation.[3]

Cinnamon has been used in food preparations and in traditional medicine by the Egyptians and the Chinese since ancient times.[4] In addition, this spice has been found to have strong antibacterial, antipyretic[5] and anti-inflammatory properties, which play an important role in tissue repair. The bark of cinnamon has been used as a spice and to make tea and also as an herbal remedy for the treatment of common colds, cardio-vascular diseases and chronic gastrointestinal and gynecological disorders in oriental herbal medicine.[6] Cinnamon has likewise been used for treating sore throats, cough, indigestion, abdominal cramps, intestinal spasms, nausea, flatulence and diarrohea. Moreover, it has been found that cinnamon slows down food spoilage and displays antifungal properties. It also clears up urinary tract infection, diabetes etc.[7] The present study was undertaken in order to evaluate the in-vitro antibacterial activity of cinnamon bark extract.

Methods:

Plant Material: The spice cinnamon barks (*Cinnamomum zeylanicum*) were purchased from local market of Charpara, Mymensingh, Bangladesh in February 2018. The spices were botanically identified.

Preparation of Extract: Fresh cinnamon barks were cleaned with deionized water and dried first in sunlight for two days and then in hot air oven at 40°C for 1 day. Finally the dried materials were pulverized into fine powdered substance by a grinder. After weighing with the electric balance 100 grams powder of cinnamon were taken in measuring conical flask and 200ml of distilled water was added. The flask was closed by foil paper and put on dark place for five days. The aqueous extract was then filtered by passing through Whatman No.1 filter paper and then concentrated under vacuum at 40°C by using a rotary evaporator. Thus the extract produced had 100 ml volume and 1g/ml or 100% concentration and it was used as Stock solution. This extract was stored in refrigerator at 4°C in small and sterile plastic bottles. Extract of the spice were further diluted to make different concentrations such as 80%,60%,40%, 20% and 10% by mixing with appropriate volumes of distilled water.

Tested Bacterial strains: Two bacterial strains *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used in study. Pure cultures of these bacteria were obtained from the Department of Microbiology, Mymensingh Medical College, Mymensingh.

Maintenance of bacterial culture and inoculum preparation: Pure cultures were refreshed and maintained on nutrient agar slants and plates on regular basis. The cultures were streaked on sterile nutrient agar plates and kept in incubator for 24 hours at 37°C and stored at 4 °C. Bacterial cultures were refreshed after every 1 to 2 weeks to avoid contamination. Inoculum was prepared by growing the pure bacterial culture in nutrient broth over night at 37°C.

Antibacterial activity testing using disc diffusion method: Filter paper disc of 6mm diameter using Whatman no. 1 filter paper was prepared and sterilized. The test microorganisms were transferred from nutrient broth to sterile Mueller Hinton agar plates with the help of sterile cotton swabs. Using an ethanol dipped and flamed forceps the discs were aseptically placed over the Mueller Hinton agar plates seeded with the test microorganisms. Then with the help of micropipette 10 µl of 100%, 80%, 60%, 40%, 20% and 10% concentrations of aqueous extracts were transferred to different disc aseptically. Plates were incubated at 37°C for 24 hours. 10 µl of 95 % ethanol was added in sterile filter paper disc as negative control as because in this trace amount it has negligible antibacterial activity to suppress the growth of microorganisms in culture media. After 24 hours the results were recorded. The antibacterial activity results were expressed in terms of the diameter of zone of inhibition (ZOI) and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active[2].

Testing antimicrobial activity of a standard antibiotic: The test microorganisms i.e. *Staphylococcus aureus* and *Escherichia coli* were also tested for their activity against Amikacin (inj.500mg) by disc diffusion method.

Results:

In this research study bark of cinnamon (*Cinnamomum zeylanicum*) were found effective against the test bacterial strains. The maximum ZOI at 100% concentration was shown against *Escherichia coli* (25mm). But *Staphylococcus aureus* started showing definite activity from 20% conc. whereas *Escherichia coli* from 80% conc.The diameter of ZOI obtained against aqueous extract at 100% concentration by disk diffusion method was also compared to those obtained against a standard antibiotic Amikacin as shown in table-III. Aqueous extract produced a wider zone of inhibition as compared to Amikacin for

both *Staphylococcus aureus* (24mm) and *E.coli* (25mm). Amikacin was effective against both *Staphylococcus aureus* and *E.coli* forming zones of 17mm and 16mm, respectively.

Table I

Antibacterial activity of different concentrations of ACE measured in Zone of Inhibition by disk diffusion method.

Concentrations of ACE solutions in %	Zone of Inhibition (ZOI) in mm	
	Staphylococcus aureus	*Escherichia coli*
10	08	06
20	**15**	06
40	16	06
60	20	08
80	22	**18**
100	24	25
Control	06	06

Table-II

Antibacterial activity of Amikacin measured in Zone of Inhibition by disk diffusion method.

	Zone of Inhibition (ZOI) in mm	
	Staphylococcus aureus	*Escherichia coli*
Amikacin (500mg)	17	16

Table-III

Comparison of Antibacterial activity of ACE & Amikacin measured in Zone of Inhibition by disk diffusion method.

	Zone of Inhibition (ZOI) in mm	
	Staphylococcus aureus	*Escherichia coli*
ACE (100%)	24	25
Amikacin (500mg)	17	16

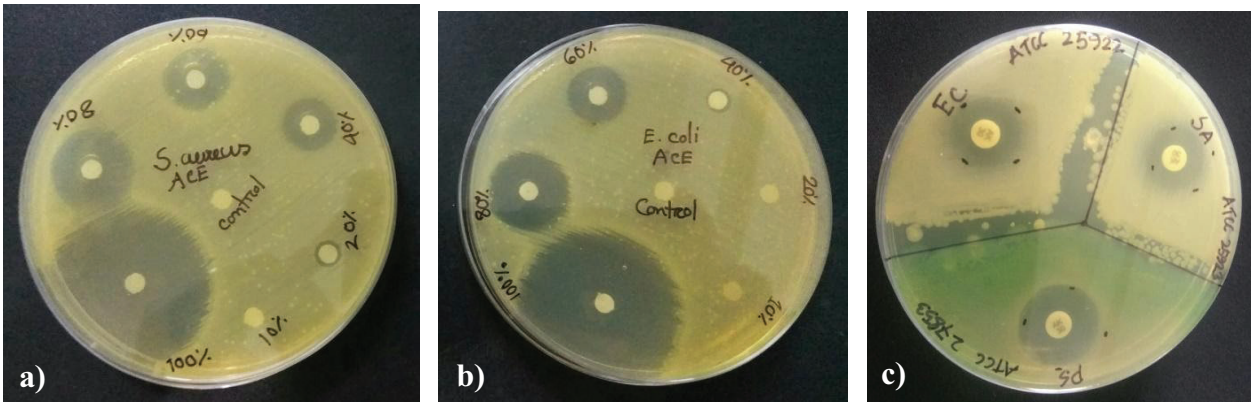

Fig.-1: *Testing antibacterial activity of aqueous cinnamon extract against a) Staphylococcus aureus, b) Escherichia coli and c) testing antibacterial activity of Amikacin against above organisms.*

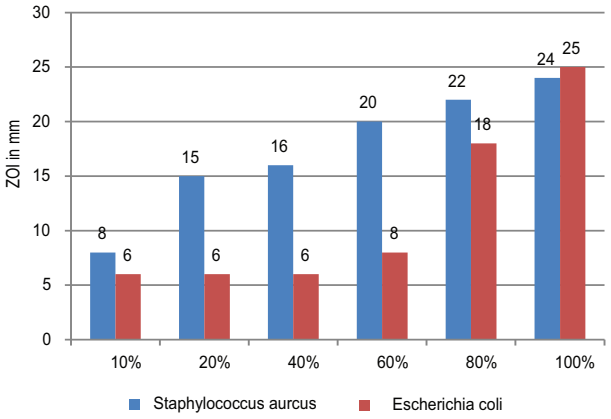

Fig.-2: *Multiple bar diagram showing ZOI at different concentration of ACE againstStaphylococcus aureus Escherichia coli*

Discussion:

Aqueous extract of cinnamon bark were found active against both *Staphylococcus aureus* and *E. coli*. The extract showed relatively better zones of inhibition against *Staphylococcus aureus* than *E.coli* and when compared with Amikacin it produced larger zone than Amikacin.

Aqueous extract produced zone of inhibition of 08mm,15mm,16mm,20mm,22mm and 24mm against *Staphylococcus aureus* and 06mm,06mm, 06mm, 08mm, 18mm and 25mm against *E. coli* at 10%,20%,40%,60%,80% and 100% conc. respectively (table-I). Amikacin produced zone of inhibition of 17mm against *Staphylococcus aureus* and 16mm against *E. coli*. The findings agree with the work of Zainab A. Al-dhaher (2008).[11] In that study the antibacterial activity of aqueous extract of cinnamon bark

and clove were investigated against *Staphylococcus aureus* by Agar diffusion technique. The ZOI of aqueous extract of cinnamon were 17mm, 15.5mm, 13mm, 8mm and 0mm at 70%, 60%, 40%, 20% and 10% concentration. In this study they were 22mm, 20mm, 16mm, 15mm and 8mm at 80%, 60%, 40%, 20% and 10% conc. respectively. There is quite similarity with this study except at 20% conc. He also stated that Fan et. al (2001), Yuste et.al (2006) also found similar antibacterial activity in their studies.

Sana Mukhtar and Ifra Ghori in 2012 investigated the antibacterial activity of aqueous and ethanolic cinnamon bark extract against *E.coli* ATCC 25922 by disc diffusion method.[2] This study showed that both aqueous and ethanolic extracts were active against gram negative bacteria *E.coli* but ethanolic extracts comparatively showed better results which were similar with this study. ZOI were (9.3 ± 0.38) mm at 60%, (10 ± 0.40) mm at 80% and (10.3 ± 0.41) mm at 100% conc. of aqueous extract which is almost similar with this study result.

This study results were also in consistent with study conducted by Madhumita and C.Ramalingam (2011).[12] They proved that aqueous bark extract of *Cinnamomum verum* has antibacterial activity against *Staphylococcus aureus,Bacillus cereus, Enterococcus fecalis* and *Escherichia coli, Proteus mirabilis*. For *Staphylococcus aureus* the diameter of ZOI were between 0 to 16mm and for *E.coli* between 0mm to 17.5mm. According to that study the sensitivity of aqueous extract against bacteria was *E.coli* > *S.aureus*. But according to this study *S.aureus* was more active and it begins to be active from 20% conc. whereas *E.coli* shows activity at high conc. from 80% and 100%. Odhav et al. (2002), suggested that the mechanism of antibacterial action of spices involve the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and destruction of electron transport systems and cell wall disruption. The antimicrobial activity of aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulphates in addition to many other compounds naturally present in plants (Darout , 2000).The antibacterial activity of cinnamon might be due to the presence of cinnamaldehyde compound which inhibits the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell death (Wendakoon and Sakaguchi, 1995).[2]

Conclusion:

The aqueous extracts of *Cinnamomum zeylanicum* were found to be effective against two important food spoilage bacteria like *E.coli* and *Staphylococcus aureus*. This is an absolutely in vitro study. Whether the extract of cinnamon does act as an antimicrobial agent in using particularly during infection- it is yet to be studied by MIC estimation process.Furthermore the chemical compound of extract which is thought to have antimicrobial activity has yet to be isolated and identified.So further extensive studies are needed in order to find out the rational use of cinnamon as an antimicrobial agent among the patients suffering from infection particularly with *E.coli* and *Staphylococcus aureus.*

From the above studies we can come to the conclusion that the extract of cinnamon could be used in food preservation to prevent spoilage of food caused by *E.coli* and *S. aureus* as because cinnamon is found safer when consumed along food.

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