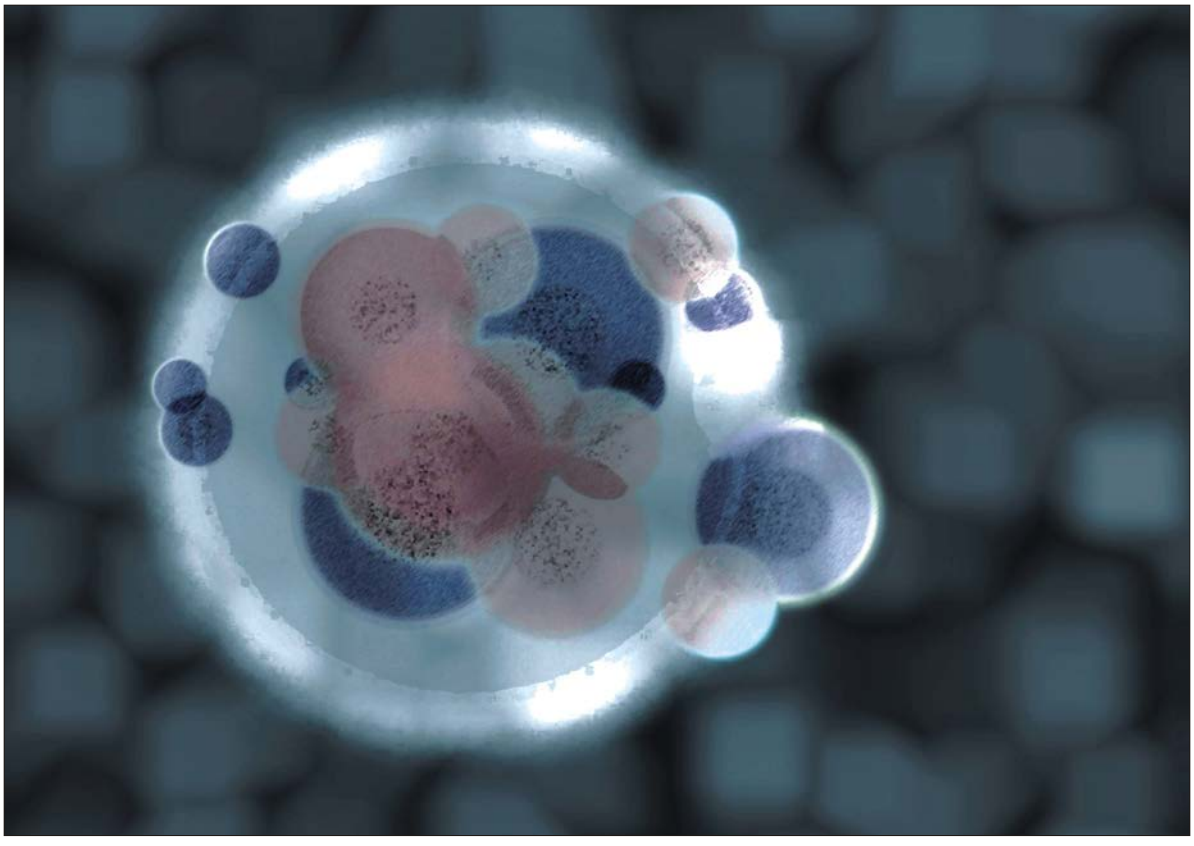

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Antibacterial Assay of *Cinnamomum cassia* (Nees and Th. Nees) Nees ex Blume Bark and *Thymus vulgaris* L. Leaf Extracts against Five Pathogens

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Abstract: In this study we investigate the antibacterial activities of the *Cinnamomum cassia* (Nees and Th. Nees) Nees ex Blume Bark and *Thymus vulgaris* L. leaf extracts. Five strains of bacteria, including *Bacillus subtilis*, *Enterobacter aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were used in the antibacterial tests. Results from the antibacterial tests demonstrated that both plant extracts had an excellent inhibitory effect. The MICs (Minimum Inhibitory Concentrations) of the both plant extracts were 250 μg mL^{-1} against all tested strains. These results suggest that *Cinnamomum cassia* and *Thymus vulgaris* are beneficial to human health, having the potential to be used for medical purposes and to be utilized as anti-bacterial additives in food products.

Key words: *Cinnamomum cassia*, *Thymus vulgaris*, plant extracts, antibacterial assay, MIC

INTRODUCTION

The use of medicinal plants as a source for relief and illness is ancient and well documented in the written documents of the early civilization in China, India and the near east, but it is without question an art as old as mankind (Mahesh and Satish, 2008).

The potential of higher plants as source for drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Uniyal *et al*., 2006; Mahesh and Satish, 2008).

Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of countless therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics. Even now, contrary to common belief drugs from higher plants continue to play an important role in modern medicine. Medicinal plants represent a rich source of antimicrobial agents (Uniyal *et al*., 2006; Mahesh and Satish, 2008).

Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been thoroughly evaluated including cinnamon and thyme plants (Govindarajan *et al*., 2006; Mahesh and Satish, 2008).

Cinnamon (*Cinnamomum cassia*) is a medicinal plant that posses antimicrobial properties and shown to inhibit the growth of many pathogens such as *B. cereus* (Hoque *et al*., 2007) and *Helicobacter pylori* (Zarchil and Babaei, 2006). The oil of thyme and its different components are becoming increasingly popular as a naturally occurring antimicrobial and also as an antioxidant agent (Zarchil and Babaei, 2006). Thyme showed broad antibacterial activity by inhibiting the growth of both gram-positive and gram-negative bacteria.

Recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (Hoque *et al*., 2007). However, little study has been done on the effects of plant extracts including cinnamon and thyme against many food borne diseases and pathogens. The present study was conduced to screen the effect of cinnamon and thyme extracts against 5 common food borne pathogens.

MATERIALS AND METHODS

Plant materials and microorganisms: This study was done at Ohio University Zanesville, Ohio, USA between October 12th, 2008 and February 22nd, 2009. Medicinal plants were obtained from Medicinal Plant Teaching Laboratory at Ohio University Zanesville, Ohio, USA. These plants have previously been reported to have antibacterial activity against different bacterial pathogens. Five bacterial pathogens (*Bacillus subtilis*, *Enterobacter aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) were obtained from Microbiology Teaching Laboratory Collection at Ohio University Zanesville, Ohio, USA.

These bacteria were selected as these are common pathogens that cause many food borne diseases.

Preparation of extracts: One liter of an 80% ethanol extraction fluid was mixed with 100 g of powdered plant material. The mixtures were kept for 2-5 days in tightly sealed vessels at room temperature at 22°C, protected from sunlight and mixed several times daily. This mixture is filtered through muslin cloth.

The extracted liquid was filtered using Whatman No.1 filter paper and the filtrates were then evaporated to dryness under reduced pressure to remove the ethanol. Extract was then weighed and stored at 22°C for further use.

Antimicrobial assay: Inhibition of microbial growth was tested by using the paper disc agar diffusion method (Kirby-Bauer Method; Drago *et al*., 1999). In order to detect potential antimicrobial activity in the plant extracts, entire surface of 20 mL muller hinton agar plate was inoculated with the culture of bacteria and the paper discs (diameter 5 mm which absorbs 10-12 μg). The paper discs were soaked in each of the test solutions containing different extract solutions at varying concentrations (100, 250, 500, 750 μg mL^{-1}) as well as the standard antibiotic solution (Erythromycin) and the control-blank (sterile water discs) were placed separately in each quarter of the plate under aseptic conditions. Multiple plates were (three replications) done for each of the extract. The plates were then maintained at room temperature for 1 h allowing for diffusion of the solution. All plates were then incubated at 37°C for 24 h and the zones of inhibition were subsequently measured in mm in all three replicates using measuring scale and the average of the three measurements was calculated (Mukherjee *et al*., 1995).

Determination of minimum inhibitory concentration: MIC of cinnamon and thyme extracts was calculated using plate dilution method. Taking different concentrations (100, 250, 500 and 750 μg mL^{-1}) of each plant extract against 0.1 mL of 10^{-4} inoculum dilution prepared from 24 h incubated culture of the test microorganism into sterile a sterile plate followed by pouring of 20 mL Muller Hinton agar medium. In this study, the MIC was the lowest concentration of plant extracts that exhibited no growth of microorganism by visual reading. Each experiment was done in triplicates along with positive and negative controls (Ghosh *et al*., 2008).

RESULTS

The cinnamon extract at (250, 500 and 750 μg mL^{-1}) concentrations showed antibacterial activity against *Bacillus subtilis*, *Enterobacter aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The range of the zone of inhibition was 19 to 29 mm (Table 1). The thyme extract at the same concentrations also exhibited antibacterial activity against *Bacillus subtilis*, *Enterobacter aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The range of the zone of inhibition was 17 to 28 mm (Table 1). The MIC of cinnamon and thyme extracts against all tested strains were found to be 250 μg mL^{-1}.

Table 1: Antibacterial activity of specific concentration (250 μg mL^{-1}) of medicinal plant extracts (IZD in mm)

Medicinal plant	Antibacterial activity				
	B. subtilis	*E. aeruginosa*	*E. coli*	*S. aureus*	*S. epidermidis*
C. Cassia	20.2	20.7	21	21	21
T. vulgaris	19.2	19.6	20	20	20
Distilled water	0	0	0	0	0

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Valero and Salmeron, 2003; Zarchil and Babaei, 2006). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Kone *et al*., 2004; Ghosh *et al*., 2008). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings (Mahesh and Satish, 2008).

This study shows that extracts of cinnamon and thyme produce effective antibacterial activity against both gram negative and gram positive food borne pathogens. Results of this study confirmed the observation of earlier studies by Zarchil and Babaei (2006).

The cinnamon extract was found to be effective against *Escherichia coli* and *Staphylococcus aureus*. This effect is in agreement with other recent studies (Yuste and Fung, 2004; Hoque *et al*., 2007). In this study the thyme extract showed effective antibacterial activity against all tested pathogens including *Escherichia coli* and *Staphylococcus aureus*. This result supports the findings of Dursun *et al*. (2003), Nevas *et al*. (2004), Solomakos *et al*. (2008) and Friedman *et al*. (2006). This study is the first to report the antimicrobial activity of cinnamon and thyme against *Bacillus subtilis*, *Enterobacter aeruginosa* and *Staphylococcus epidermidis* collectively.

The current results are very promising and suggest that *Cinnamomum cassia* and *Thymus vulgaris* are beneficial to human health, having the potential to be used for medical purposes and to be utilized as anti-bacterial additives in food products.

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REFERENCES

Drago, L., B. Mombelli, G. Ciardo, E. De Vecchi and M.R. Gismondo, 1999. Effects of three different fish oil formulations on *Helicobacter pylori* growth and viability: *In vitro* study. J. Chemother., 11: 207-210.

Dursun, N., N. Liman, I. Ozyazgan, I. Gunes and R. Saraymen, 2003. Role of thymus oil in burn wound healing. J. Burn Care Rehabil., 24: 339-395.

Friedman, M., P.R. Heinka, C.E. Levin and R.E. Mandrell, 2006. Antimicrobial wine formulations active against the foodborne pathogens *Escherichia coli* O157:H7 and *Salmonella enterica*. J. Food Sci., 71: 245-251.

Ghosh, S., E. Subudhi and S. Nayak, 2008. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. Int. J. Integrat. Biol., 2: 27-31.

Govindarajan, R., M. Vijayakumar, M. Singh, C.H.V. Rao, A. Shirwaikar, A.K.S. Rawat and P. Pushpangadan, 2006. Antiulcer and antimicrobial activity of *Anogeissus latifolia* medicinal plants: An expanding role in development. J. Ethnopharmacol., 106: 57-61.

Hoque, M.M., M.B. Inatsu, V.K. Juneja and S. Kawamoto, 2007. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. J. Food Sci. Technol., 72: 9-21.

Kone, W.M., K.K. Atindehou, C. Terreaux, K. Hostettmann, D. Traore and M. Dosso, 2004. Traditional medicine in North Cote-d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol., 93: 43-49.

Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci., 4: 839-843.

Mukherjee, P.K., R. Balsubramanian, K. Saha, M. Pal and B.P. Saha, 1995. Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizome extract. J. Indian Drugs, 32: 274-276.

Nevas, M., A.R. Korhonen, M. Lindstrom, P. Turkki and H. Korkeala, 2004. Antibacterial efficiency of finnish spice essential oils against pathogenic and spoilage bacteria. J. Food Prot., 67: 199-202.

Solomakos, N., A. Govarisa, P. Koidisb and N. Botsoglou, 2008. The antimicrobial effect of thyme essential oil, nisin and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. Food Microbiol., 25: 120-127.

Uniyal, S.K., K.N. Singh, P. Jamwal and B. Lal, 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed., 2: 1-14.

Valero, M. and M.C. Salmeroj, 2003. Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. Int. J. Food Microbiol., 85: 73-81.

Yuste, J. and D.Y. Fung, 2004. Inactivation of *Salmonella typhimurium* and *Escherichia coli* O157: H7 in apple juice by a combination of nisin and cinnamon. J. Food Prot., 67: 371-377.

Zarchil, M.A. and A. Babaei, 2006. An investigation of thyme effect on *Helicobacter pylori*. Middle East J. Scientific Res., 1: 54-57.