INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 12, p. 314-319, 2014

RESEARCH PAPER OPEN ACCESS

Study antibacterial effects of cinnamon extract on *Staphylococcus aureus* and *Escherichia coli*

Masood Khakzadihe[1*], Hadi eslami[2], Ali toupchi[3], Pouyan Houshmandpour[3], Morteza Alizadeh[3]

[1]*Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

[2]*Young Researchers and Elite Club, Tabriz branch, Islamic Azad University, Tabriz, Iran*

[3]*Department of Phatobiology, College of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz , Iran*

Key words: Cinnamon, *Staphylococcus aureus, Escherichia* coli, antibacterial activity.

http://dx.doi.org/10.12692/ijb/5.12.314-319 Article published on December 20, 2014

Abstract

Cinnamon (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India. For this research 95% ethanol was used as solvent for extraction of cinnamon. Nine experimental tubes were selected and 0.5 ml of Dimethyl sulfoxide was poured for all tubes. 0.5 ml of extract was added to the first tube, and then by 0.5 ml transferring, serial dilutions were prepared. We were designed 4 disks per culture plate for each dilution that one of them was control disk. For this research *Staphylococcus aureus* (PTCC1431) and Escherichia *coli* (PTCC1399) were used. In staphylococcus aureus cultures there were inhibition zones around disks with cinnamon extract. This inhibition zones were on 1/2, 1/4, 1/8 Dilutions. Also in Escherichia coli cultures there were inhibition zones on 1/2, 1/4, 1/8 dilutions.

* **Corresponding Author:** Masood Khakzadihe ✉ Masood_Khakzadihe@ymail.com

Introduction

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria (Snyder 1997). Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor. The antimicrobial properties of some spices and their components have been documented (Shelef 1983, Hirasa and Takemasa 1998, Nevas *et al.* 2004, Kim *et al.* 1994). *Cinnamon* (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India(Dragland *et al.* 2003). The flowers, which are arranged in panicles, have a greenish colour and have a distinct odour. The fruit is a purple one-centimeter berry containing a single seed. Its flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition (Dragland *et al.* 2003, Friedman *et al.* 2002). In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. *Cinnamon* is high in antioxidant activity. The essential oil of *Cinnamon* also has antimicrobial properties, which aid in the preservation of certain foods (Jakhetia *et al.* 2010). "*Cinnamon*" has been reported to have remarkable pharmacological effects in the treatment of type II diabetes. *Cinnamon* has traditionally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold and aid digestion. The *Cinnamon* is having essential oils, resinous compounds, Cinnamic acid, Cinnamaldehyde and Cinnamate. Essential oil such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, b-caryophyllene, E-nerolidol, and cinnamyl acetate was reported by Tung *et al.* Some other constituents are Terpinolene, α-Terpineol, α-Cubebene, and α-Thujene.(Jakhetia *et al.* 2010) Several articles have been written about the effects of cinnamon for example In Al-Kassie (2010) research, effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks has been studied. Also Mahfuzul Hoque *et al.* (2008) Ethanol, aqueous extracts, and essential oils of Cloves (*Syzygium aromaticum*), and Cinnamon (*Cinnamomum cassia*) were analyzed for determination of antibacterial activity against 21 food borne pathogens. According to these researches we decided to design this research to investigate the antibacterial effects of cinnamon extract on two pathogen bacteria include staphylococcus aureus and Escherichia coli.

Materials and methods

Extraction

Cinnamon (*Cinnamomum verum*) were purchased from the local market of Azerbaijan Shargi and Tabriz city then identified from the Department of botany and Agricultural, Islamic Azad University, Tabriz branch, Iran. After preparation were crushed using a ball mill and powder was obtained. In this study, 95% ethanol was used as solvent for extraction. After mixing the solvent and cinnamon (18gr leaf powder and 100 ml solvent), it made 48 hours in the darkroom, and then extracted with rotary device. Extract was filtered by filter paper (125 mm, produced by Whatman Company) after extraction.

Disc diffusion method

The antimicrobial activity of the *C. verum* extract was determined by the disc diffusion method. For this research, nine experimental tubes were selected and 0.5 ml of Dimethyl sulfoxide (DMSO-Merck©) was poured for all tubes. 0.5 ml of extract was added to the first tube, and then by 0.5 ml transferring, serial dilutions were prepared. At the end 0.5 ml was poured out from last tube. After all, three Blank discs (6.4mm diameter, produced by Padtanteb Company) placed in each tube and after half an hour, disks were removed from the tubes and placed in an incubator with 37±2 degrees Celsius for 20 minutes, for drying. After drying, disks were ready to use. For control group we were used Blank discs with solvents that they were dried in same method.

For Antibiogram method we were used Mueller-

Hinton agar culture that produced by Merck Company. 24 hour BHI-broth culture was used for this research. For Mueller-Hinton agar culture, we were used McFarland half. We were designed 4 disks per culture plate for each dilution that one of them was control disk (dried extract-free solvent). Cultures were placed in an incubator at 37 degrees Celsius for 18 hours. We were used these levels for each bacteria individually. For this research *Staphylococcus aureus* (PTCC1431) and Escherichia *coli* (PTCC1399) were used.

Results

In staphylococcus aureus cultures there were inhibition zones around disks with cinnamon extract. This inhibition zones were on 1/2, 1/4, 1/8 Dilutions. Also in Escherichia coli cultures there were inhibition zones on 1/2, 1/4, 1/8 dilutions. Results are given in table 2.

Table 1. Serial dilution of extract by DMSO.

Tube number	1	2	3	4	5	6	7	8	9
Dilution	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2. Results of disk diffusion by cinnamon extract on Staph.aureus and E.coli.

Num	Staphylococcus aureus		Escherichia coli	
	Dilution	Inhibition zone mean ± St(cm)	Dilution	Inhibition zone mean ± St (cm)
1	1/2	1.8±0.685	1/2	1.1±0.511
2	1/4	1.1±0.510	1/4	0.5±0.10
3	1/8	0.9±0.085	1/8	Neg*
4	1/16	Neg*	1/16	Neg
5	1/32	Neg	1/32	Neg
6	1/64	Neg	1/64	Neg
7	1/128	Neg	1/128	Neg
8	1/256	Neg	1/256	Neg
9	1/512	Neg	1/512	Neg
10	Control	Neg	Control	Neg

*Neg: Negative.

Discussion

Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor (Mohaghegi *et al.* 2011, Reichard 2011). In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste (Reichard 2011) Ethanol, CH3CH2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom (Reichard 2011).

Escherichia coli is a Gram negative rod (bacillus) in the family Enterobacteriaceae (Quinn *et al.*1994, Naderinasab *et al.* 1997, Tabatabayi and Firouzi 2011). Few microorganisms are as versatile as *Escherichia coli*. An important member of the normal intestinal microflora of humans and other mammals, it can be a highly versatile, and frequently deadly, pathogen. Several different *E. coli* strains cause diverse intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes (Naderinasab *et al.* 1997, Kaper *et al.* 2004). *Escherichia coli* typically colonizes the gastrointestinal tract of some mammals and human infants within a few hours after birth. Usually, *E. coli* and its host coexist in good health and with mutual

benefit for decades. These commensal *E. coli* strains rarely cause disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached (Kaper *et al.* 2004, Tadjbakhsh 1997, Tabatabayi and Firouzi 2011). Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 µm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters (Farrell *et al.* 2002, Kloos and Lambe 1991). To date, there are 32 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially colonise the human body (Quinn *et al.*1994, Kloos and Lambe 1991). *S. aureus* is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immunocompetent people and animals in the community. This bacterium is found naturally on the skin and in the nasopharynx of the some animals and human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening (Shulman and Nahmias 1972, Tadjbakhsh 1997, Tabatabayi and Firouzi 2011). The excessive use of antibiotics has led to the emergence of multiple drug resistant *S. aureus* strains (Lowy 1998). Penicillin was introduced for treating *S. aureus* infections in the 1940s, and effectively decreased morbidity and mortality. However, by the late 1940s, resistance due to the presence of penicillinase emerged (Eickhoff 1972). The staphylococci are very capable of evolving resistance to the commonly used antimicrobial agents, such as, erythromycin (Klein and Finland 1963), ampicillin (Klein and Finland 1963), and tetracycline (Lowy 1998).

In Sana Mukhtar and Ifra Ghori (2012) research, spices such as garlic turmeric and cinnamon have been used as antimicrobial agents in their raw form for the treatment of wounds and injuries and joint pains etc. The Mukhtar and Ghori (2012) study was conducted to investigate the antibacterial activity of garlic, cinnamon and turmeric. Different concentrations of extracts were prepared by using two solvents water and ethanol. The antibacterial activity was tested against *Bacillus subtilus* (DSM 3256) and *E.coli* (ATCC25922) at different concentration of extracts of spices by using disc diffusion method. The cinnamon ethanolic extracts are equally effective against both Gram negative and Gram positive bacteria (Mukhtar and Ghori 2012). Study of Mukhtar and Ghori shows antibacterial effects of cinnamon on *E.coli* so their studies are similar to our results.

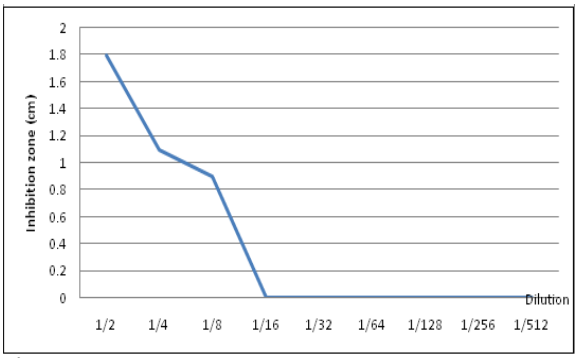

Fig. 1. Staph. aureus culture, inhibition zone differences of cinnamon disks in various dilutions.

In other research conducted by Mahfuzul Hoque *et al.* (2008) Ethanol, aqueous extracts, and essential oils of Cloves (*Syzygium aromaticum*), and Cinnamon (*Cinnamomum cassia*) were analyzed for determination of antibacterial activity against 21 food borne pathogens: *Listeria monocytogenes* (5 strains), *Staphylococcus aureus* (4 strains), *Escherichia coli* O157: H7 (6 strains), *Salmonella* Enteritidis (4 strains), *Vibrio parahaemolyticus* and *Bacillus cereus* and 5 food spoilage bacteria: *Pseudomonas aeroginosa*, *P. putida*, *Alcaligenes faecalis*, and *Aeromonas hydrophila* (2 strains). In their research for cinnamon, the ethanol extract was active only against *Staphylococcus aureus* strains except *S. aureus* JCM 2874 and *V. para. haemolyticus* with zones of inhibition ranging from 10.0 to 11.4 mm. The EOs from clove or cinnamon have antimicrobial properties and shown to inhibit all test organisms. The EOs of clove and cinnamon showed maximum inhibition for *A. hydrophila* NFRI 8282 (32.0mm) and *B. cereus* IFO 3457 (46.5 mm), respectively, with zones of inhibition larger than those observed against the antibiotic, gentamycin. (Mahfuzul *et al.* 2008).

In a research conducted by Thakare (2004) pharmacological screening of some medical plants as antimicrobial and feed additives have been studied.

In Thakare study Ethanol extracts of different medicinal plants including *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Piper nigrum* (Black Pepper), *Cinnamomum cassia* (Cinnamon), *Thymus vulgaris* (Thyme), *Laurus nobilis* (Bay leaf), and *Syzgium aromaticum* (Clove) were tested using the disc diffusion method for their antimicrobial activity against the common poultry pathogens *E. coli*, *S. typhimurium*, *E. faecium*, and *E. faecalis*. Thakare results suggest that cinnamon and thyme have antibacterial activity *in vitro* (Thakare 2004).

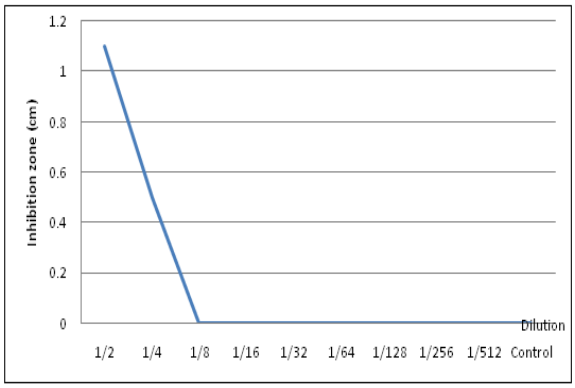

Fig. 2. E.coli culture, inhibition zone differences of cinnamon disks in various dilutions.

In Mohamed Al-Kassie (2010) research, effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks has been studied. This study was conducted to determine the effect of the addition of different percent levels of thyme and cinnamon, added to a standard diet, on the gastro intestinal tract. Total bacteria count, *coli form* bacteria, *lactobacilli* bacteria and fungal counts were determined in different region crop, jejunum and large intestine. The data showed that the additive thyme and cinnamon has statistical effect the decrease in the total bacteria count, *coli form* in group, jejunum and large intestine compared with the control. Different levels of thyme and cinnamon used as antimicrobial balance in gastro intestinal tract for broiler chicks (Al-kassie 2011).

Acknowledgment

We would like to express our deepest appreciations to the laboratory technician of veterinary college of Islamic Azad University, Tabriz branch; Mohamad-hassan Rasoulifard that assisted us in this experiments.

References

Al-Kassie GAM. 2011. The Effect of Thyme and Cinnamon on the Microbial Balance in Gastro Intestinal Tract on Broiler Chicks, International Journal of Poultry Science **9(5),** 495-498.

Dragland S, Senoo H, Wake K. 2003. Several culinary and medicinal herbs are important sources of dietary antioxidants. Nutr. **133(5),** 1286-1290.

Eickhoff TC. 1972. Therapy of staphylococcal infection. In: Cohen, J.O., ed. The Staphylococci. Wiley, New York, 517-541.

Farrell DJ, Morrissey I, Bakker S, Felmingham D. 2002. Molecular characterization of macrolide resistance mechanisms among Streptococcus pneumonia and Streptococcus pyogenes isolated from the PROTEKT 1999–2000 study. The Journal of Antimicrobial Chemotherapy, **50(Suppl. S1),** 39–47. http://dx.doi.org/10.1093/jac/dkf806

Friedman M, Henika PR, Mandrell RE. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. Journal of Food Protection. **65(10),** 1545-1560.

Hirasa K, Takemasa M. 1998. Spices Science and Technology. Marcel Dekker Inc. New York, USA, 1-232.

Jakhetia V, Patel R, Khatri P, Pahuja N, Garg S, Pandey A, Sharma S. 2010. Cinnamon: A pharmacological Review. Journal of Advanced Scientific Research, **1(2),** 19-23.

Kaper JB, Nataro JP, Harry L, Mobley T. 2004. Pathogenic Escherichia coli. Nature reviews microbiology **2,** 123-140.

http://dx.doi.org/10.1038/nrmicro818

Kim J, Marshall MR, Wei C. 1994. Antibacterial activity of some essential oil components against five food borne pathogens. Journal of Agricultural and Food Chemistry. **43,** 2839-2845. http://dx.doi.org/10.1021/jf00059a013

Klein JO, Finland M. 1963. The new penicillins. New England Journal of Medicine. **269,** 1019-1025. http://dx.doi.org/10.1056/nejm196311072691907

Kloos WE, Lambe DW. 1991. Staphylococcus. In: Barlows, A., Hausler, W.J., Herrmann, K.L., Isenberg, H.D., Shadomy, H.J., eds. Manual of Clinical Microbiology, 5th ed. ASM, Washington D.C., 222-237.

Lowy FD. 1998. Is Staphylococcus aureus an intracellular pathogen? Trends Microbiol **8,** 341-344. http://dx.doi.org/10.1016/s0966-842x(00)01803-5

Mahfuzul HM, Bari ML, Juneja VK, Kawamoto S. 2008. Antimicrobial activity of Colves and Cinnamon extracts against food borne pathogens and spoilage bacteria, and Inactiveation of listeria monocytogenes in ground chicken meat with their essential oils. Rep. National Food Research Institute, **72(9),** 21.

Mohagheghi S, Poorazarang MA, Elhamirad H, Dezashibi AH, Hematyar ZN. 2011. Extraction of phenolic compounds from potato peel (Ramus variety) with solvent and ultrasound-assisted methods and evaluation of its antioxidant activity in soybean oil. Journal of Food Science and Technology**, 8(1),** 81-91.

Mukhtar S, Ghori I. 2012. Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric Escherichia coli ATCC 25922 and Bacillus DSM 3256. International journal of applied biology and pharmaceutical technology **3(2),** 131-137.

Naderinasab M, Rashed T, Nazem M. 1997. Laboratory bacteriology. Emam Reza Press. Mashhad, Iran, 24- 29.

Nevas M, Korhonen AR, Lindtrom M, Turkki P, Korkeala H. 2004. Antibacterial efficiency of Finnish spices essential oils against pathogenic and spoilage bacteria. Journal of Food Protection **67,** 199-202.

Quinn PJ, Carter ME, Markey BK, Carter GR. 1994. Clinical Veterinary Microbiology. Mosby-year Book Europe Limited, 95-102.

Reichard C. 2011. Solvents and Solvent Effects in Organic Chemistry, 4th ed, Wiley-VCH. http://dx.doi.org/10.1021/ja203600j

Shelef LA. 1983. Antimicrobial effects of spices. Journal of Food Safety, **6(29),** 44.

Shulman JA, Nahmias AJ. 1972. Staphylococcal infections: clinical aspects. In: Cohen, J.O., ed. The Staphylococci. Wiley, New York, 457-482. http://dx.doi.org/10.1099/00222615-7-2-312

Snyder OP. 1997. Antimicrobial effects of spices and herbs. Hospitality Institute of Technology and Management. St. Paul, Minnaesota. http://www.hitm.com/Documents/Spices.html.

Tabatabayi H, Firouzi R. 2011. Disease of animals due to bacteria. 3rd ed, University of Tehran press.

Tadjbakhsh H. 1997. General Bacteriology. 4th ed, University of Tehran press.

Thakare M. 2004., Pharmacological screening of some medical plants as antimicrobial and feed. Blacksburg, Virginia, July **16,** 1-73.