J. Food and Dairy Sciences, Mansoura Univ., Vol. 1 (5): 313 - 320, 2010

THE ANTIMICROBIAL ACTIVITY OF CUMIN, THYME AND ANISE EXTRACTS ON SOME PATHOGENIC AND FOOD SPOILAGE BACTERIA

Madkour, M. H. ; A. A. Neamatallah and M. A. Al-Ghamdi
Environmental Sci. Dept., Fac. of Meteorology, Environment and Arid Land Agric., King A . Aziz Univ., Saudi Arabia

ABSTRACT

The cold and hot water extracts of Cumin (*Cuminum cyminum*), Thyme (*Thymus vulgaris*) and Anise (*Piminella anisum*) were examined for their antimicrobial activity against some food borne pathogens such as *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus subsp. aureus* and food spoilage bacteria such as *Bacillus cereus* and *B. stearothermophilus*. The hot extraction method of the used plant parts gave a pronounced inhibition effect as well as bactericidal activity more than the cold extraction method.

It was found that hot water extracts of cumin and thyme have the strongest inhibition effect on *E coli*, *S. typhimurium* and *Staph. aureus* subsp. *aureus* when experimented by disc diffusion assay method. Moreover, *E. coli* was also strongly inhibited by the hot water extract of anise. On the other hand, *B. cereus* and *B. stearothermophilus* were slightly inhibited by hot extract of cumin and thyme. Whereas, Anise extracts show no inhibition effect on *Staph. aureus* subsp. *aureus*, *B. cereus* and *B. stearothermophilus*. The extracts which exhibit strong inhibition effect were examined for their bactericidal activity by using of kill curve method. It was found that cumin and thyme extracts have bactericidal effect on *E. coli*, *S. typhimurium* and *Staph. aureus* subsp. *aureus*. While, Anise extract has bactericidal effect only on *E. coli*.

INTRODUCTION

Herbs and spices are among the most important targets as natural antioxidants and antimicrobial agents (Huhtanen, 1980; Conner and Beuchat, 1984 and Deans and Richie, 1987, Baser *et al.* (2002), Azaz *et al.* (2002) and Singh *et al.*, 2005). Bacteria of public health including *Clostridium botulinum, B. cereus, Staph.. aureus, S. typhimurium, Vibrio parahaemolyticus* and *E. coli* known to be adversely affected by certain compounds presented in many herbs (Beuchat and Golden, 1989).

Oregano, thyme, sage, fennel, nutmeg, rosemary, calamus and cassia cortex showed antibacterial activity against *Streptococcus mutants* (You *et al*., 1993). Özcan and Erkmen (2001) reported that essential oils of nine plant spices (savory, laurel, oregano, basil, cumin, seafennel, myrtle, pickling herb, and mint) varied in their antimicrobial activity.

Agaoglu *et al*. (2007), Chaudhry and Tariq (2008) and Tayel and El-Tras (2009) studied the antimicrobial activity of twenty five herbs and some food additives such as cumin, cloves, crushed red pepper, fennel, and anise

against 188 bacterial strains such as *Staph. aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli* and *Enterococcus faecalis*.

Yasin and Abou-Taleb (2007), Bölükbasi and Erhan (2007) and Imelouane *et al*. (2009) found that maximum activity of thyme essential oil was observed against Gram negatives bacteria but this oil has poor activity on the growth of Gram positive bacteria.

Chanwitheesuk *et al*. (2005) studied the antimicrobial activity of different anise (*Pimpinella anisum*) extracts on several micro-organisms including bacteria, yeast, dermatophytic fungi, and plant pathogenic fungi by using disc diffusion assay method. They found that all extracts had strong antibacterial activity against *Vibrio cholera* bacterium, while *Pseud. aeruginosa* exhibited resistance.

Ates and Erdourul (2003) studied the antibacterial activities of the alcoholic, ethyl acetate, acetone and chloroform extracts of 5 plant species (Anise seed, Coriander seed, Liquorice root, Chinese Cinnamon bark and Juniper seed) against 13 bacterial species and strains by the agar diffusion assay method.

Soliman and Badeaa (2002) studied the effect of essential oil of 12 medicinal plants against *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*.

The aim of this study was to investigate the bacteriostatic and the bactericidal effect of cumin, thyme and anise extracts against some food borne pathogens and food spoilage bacteria..

MATERIALS AND METHODS

Organisms:

1. *Escherichia coli* DSM 5212
2. *Salmonella typhimurium* DSM 5569
3. *Staphylococcus aureus* subsp. *aureus* DSM 20231
4. *Bacillus cereus* DSM 2302
5. *B. stearothermophilus* DSM 297

The aforementioned bacterial strains were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. These strains were sub-cultured on nutrient agar then stored at 4^{o}C. All cultivations of *E. coli*, *S. typhimurium*, *B. cereus* and *Staph. aureus* subsp. *aureus* strains were carried out at 37^{o}C for 24 h, and at 55^{o}C for 48 h for *B. stearothermophilus* using nutrient broth.

Herb samples: All herb samples of Cumin (*Cuminum cyminum*), Thyme (*Thymus vulgaris*) and Anise (*Piminella anisum*) were obtained from local market in Jeddah, Saudi Arabia.

Preparation of herbs extracts:

The collected herbs were milled using laboratory miller to fine powder. The cold extraction was done at room temperature by mixing the fine powder of them with distilled water at room temperature (20%) to give high concentrations in the case of cold extraction. The concentrated extracts were

then sterilized by filtration using Sartorious membrane filters, 0.2 μm, (Germany). The sterilized extracts were diluted by the growth medium to advise level. On the other hand, the hot extraction was done by placing a certain weight of herbs in cloth bags. The weight of herbs was selected to give the required expected concentrations(1, 3 and 5%). The bags containing herbs were submerged in the nutrient broth medium (or distilled water in case of agar diffusion procedure) and autoclaved at 121^{o}C for 15 min. The test of kill curve was done in these previous medium where hot and cold herbs extracts were placed (Schoenknecht *et al*., 1995).

Antibacterial activity determination:

1. Disc diffusion assay:

The effect of both cold and hot herb extracts of Cumin, Thyme and Anise were applied to agar plates, using an impregnated filter paper disk. The paper discs were sterilized separately and impregnated under sterile conditions when cold extraction was done at room temperature. However, the filter paper discs were impregnated and sterilized during the hot extraction at 121^{o}C/15 min (Davidson and Parish, 1989).

2. Inhibition or kill curve: This test involves inoculation of one of the tested bacteria into the liquid medium, in which one of the tested herbs was added, followed by incubation and periodic sampling to determine growth or survival (Schoenknecht *et al*., 1995)

RESULTS AND DISCUSSION

The antibacterial effect of both cold and hot extracts of cumin, thyme and anise was studied using the disc diffusion assay. Different concentrations (1%, 5% and 10%) of tested materials were used. The obtained results of 1% concentration were presented in Table (1), samples with no inhibitory effect in Table (1), did not show any further response when their concentration were raised.

Table (1): Antibacterial effect of hot and cold water extracts of cumin, thyme and anise at 1% concentration on some food borne pathogens and food spoilage bacteria.

		Bacterial strains				
Type of herb	Type of extract	*E. coli*	*S. typhimurium*	*Staph. aureus subsp aureus*	*B. cereus*	*B. stearothermophilus*
Cumin	H	+++	+++	+++	++	+
	C	+	+	+	-	-
Thyme	H	+++	+++	+++	++	++
	C	+	+	±	-	-
Anise	H	+++	++	-	-	-
	C	+	+	-	-	-

H	Hot extraction	C	Cold extraction
-	No inhibition.	+	Very slight inhibition.
++	Slight inhibition	+++	Complete inhibition

From Table (1), it could be seen that hot extraction was more effective and give higher inhibition when compared with the cold extraction.

Cumin and thyme extracts had a broad spectrum of inhibition on *E. coli*, *S. typhimurium, Staph. aureus* subsp. *aureus* and *B. cereus*. These results are in agreement with those of Shetty *et al*. (1994) and Imelouane *et al*. (2009). Anise shows complete inhibition only on *E. coli* and Slight inhibition on *S. typhimurium*. This is in contradiction with results reported by Agaoglu *et al*. (2007), who found that Anise shows no inhibition effect with *Staph. aureus subsp. aureus*, *B. cereus* and *B. stearothermophilus*.

The herbs with the highest inhibition effect were then examined using the inhibition or kill curve method (Schoenknecht *et al*., 1995) to confirm the results, and to determine the exact effect of them on the tested microbial strains.

Fig. (1) shows the effect hot extracts of cumin, thyme and anise on *Escherichia coli* after incubation at 37^{o}C for 24 h. It is clear that all of them had bactericidal effect.

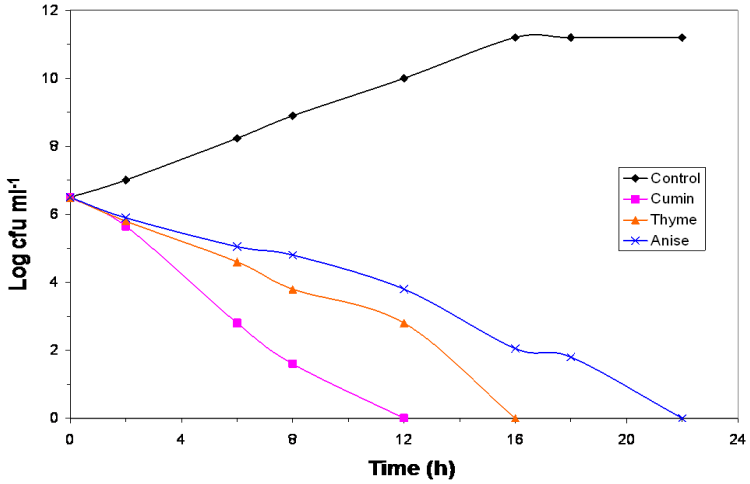

Fig. 1: Effect of cumin, thyme and anise hot water extracts on growth of *E. coli*.

The bactericidal effect of cumin hot water extract was achieved faster than thyme and anise. All *E. coli* cells were killed after 12, 16 and 22 h by cumin, thyme and anise, respectively. These results are in agreement with those of Burt (2003), Di-Pasqua *et al*. (2005), Yasin and Abou-Taleb (2007), Chaudhry and Tariq (2008) and Imelouane *et al*. (2009).

Fig. (2) shows the effect of cumin and thyme hot extracts on *Staphy. aureus* subsp. *aureus*. Both of them had bactericidal effect. Thyme was more effective than cumin. Thyme eliminated all the previous strain cells after 8 h incubation followed by cumin, which required 18 h. These results are in agreement with results obtained by Shetty *et al*. (1994) and Imelouane *et al*. (2009).

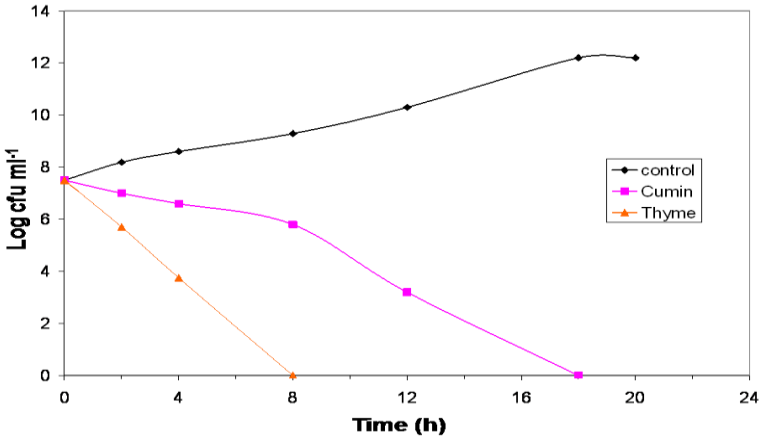

Fig.2: Effect of cumin and thyme hot water extracts on growth of *Staph. aureus* subsp. *aureus*.

Fig. (3) illustrates the effect of cumin, thyme and anise hot water extracts on growth of *S. typhimurium*. It is clear that thyme and cumin extracts have both bactericidal effect and reach their bactericidal effect at 8 h and 12 h of incubation. Anise shows strong inhibition activity with the same previous strain. Similar results were presented by Di-Pasqua *et al*. (2005) and Imelouane *et al*. (2009).

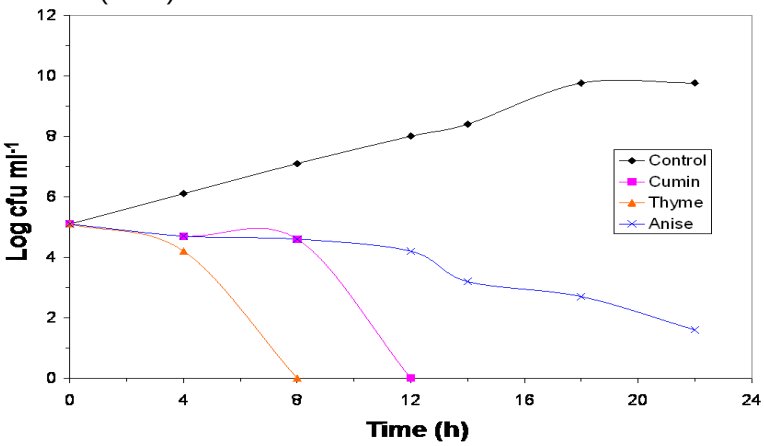

Fig. 3: Effect of cumin, thyme and anise hot water extracts on growth of *S. typhimurium*.

The effect of cumin and thyme hot water extracts on growth of *B. cereus* was presented in Fig. (4). Cumin had inhibition effect, whereas thyme had only slight inhibition effect. Same trend was reported by Imelouane *et al.* (2009).

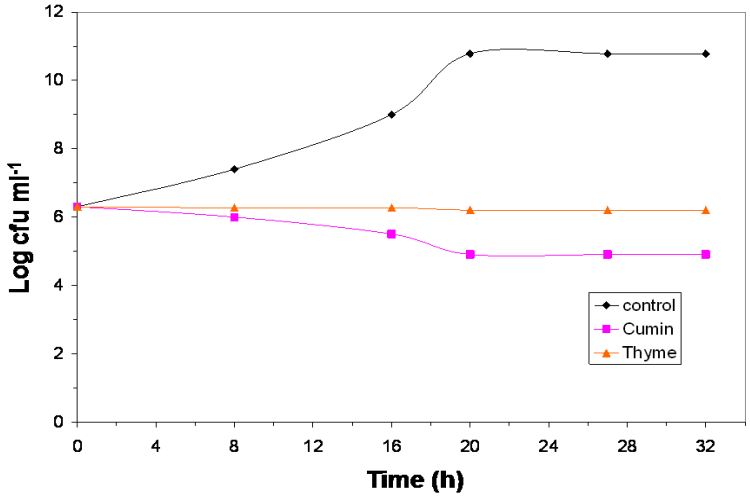

Fig. 4: Effect of cumin and thyme hot water extracts on growth of *B. cereus*.

Fig. (5) shows that cumin and thyme hot water extracts had inhibition effect on growth of *B. stearothermophilus*, where cumin extract was more effective than thyme extract. This is in accordance with Imelouane *et al*. (2009) who reported that gram positive bacteria are less sensitive than gram negative bacteria.

It is possible to recommend the use of cumin, thyme and anise as additives for appropriate foodstuffs or in the preservation of some foodstuffs. This is particularly important to get the benefit of the combined effect of both bactericidal activity of the abovementioned plant extracts and thermal treatments.

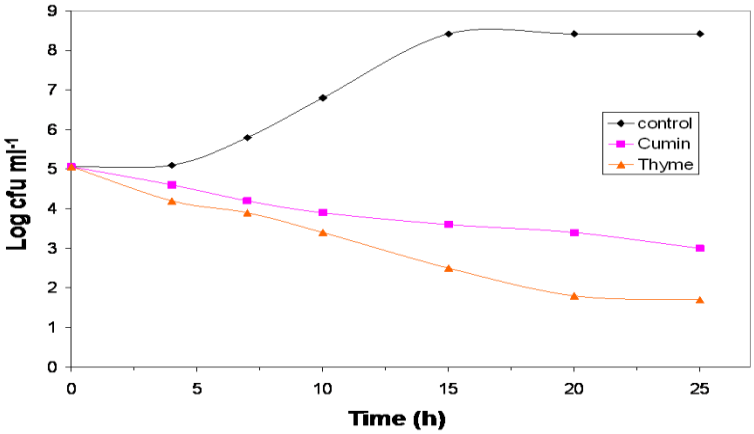

Fig. 5: Effect of cumin and thyme hot water extracts on growth of *B. stearothermophilus*.

REFERENCES

Agaoglu, S.; Dostbil, N. and Alemdar, S. (2007). Antimicrobial activity of some spices used in the meat industry. Bull. Vet. Inst. Pulawy, 51: 53-57.

Ates, D.A. and Erdourul, O.T. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol., 27: 157-162.

Azaz, K.; Demirci, F.; Satil, F.; Kürkcüoglu, M. and Baser, K.H.C. (2002). Antimicrobial activity of some Satureja essential oils. Zeitschrift Naturforschung, 57: 817-821.

Baser, K.H.C; Özek, T.; Kirimer, N. and Tümen, G. (2002). A comparative study of the essential oils from *Satureja wiedemanniana*. Zeitschrift Naturforschung, 56: 731-738.

Beuchat, L.R. and Golden, D.A. (1989). Antimicrobials occurring naturally in foods. Food Technol., 43: 134-143.

Bölükbasi, S.C. and Erhan, N.K. (2007). Effect of Dietary Thyme (*Thymus vulgaris*) on Laying Hens Performance and *Escherichia coli* (E. coli) Concentration in Feces. International Journal of Natural and Engineering Sciences, 1(2): 55-58.

Burt, S.A. (2003). Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett. Appl. Microbiol., 36: 162-7.

Chanwitheesuk A.; Teerawutgulrag, A.; Wongchanapiboon, T.; Kilburn, J.D. and Rakariyatham, N. (2005). Antimicrobial Activity of Anise (*Pimpinella anisum*) Seed Extracts. Chiang Mai J. Sci., 32(1): 53-59.

Chaudhry, N.M.A. and Tariq, P. (2008). In vitro antibacterial activities of kalonji, Cumin and poppy seed. Pak. J. Bot., 40(1): 461-467.

Conner, D.E. and Beuchat, L.R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. J. Food Sci., 49: 429-435.

Davidson, P.M. and Parish, M.E. (1989). Methods for testing the efficacy of food antimicrobials. Food Technol., 43(1): 148-155.

Deans, S.G. and Richie, G. (1987). Antibacterial properties of plant essential oils. Intl. J. Food Microbiol., 5: 165-172.

Di-Pasqua, R.; De-Feo, V.; Villani, F. and Mauriello, G. (2005). In vitro antimicrobial activity of essential oils from Mediterranean Apiaceae, Verbenaceae and Lamiaceae against food borne pathogens and spoilage bacteria. Annals Microbiol., 55(2): 139-143.

Huhtanen, C.N. (1980). Inhibition of Clostridium botulinum by spice extracts and aliphatic alcohols. J. Food Sci., 43: 195-199.

Imelouane, B., Amhamdi, H.; Wathelet, J.P.; Ankit, M.; Khedid, K. and El-Bachiri, A. (2009). Chemical composition of the essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. Int. J. Agric. Biol., 11: 205–208.

Özcan, M. and Erkmen, O. (2001). Antimicrobial activity of the essential oils of Turkish plant spices. Eur. Food Res. Technol., 212: 658–660

Schoenknecht, F.D.; Sabath, L.D. and Thornsberry, C. (1995). Susceptibility tests, special tests. In "Manual of Clinical Microbiology" ed. Lennette E, 4th ed., pp.1000-1001, Am. Soc. for Microbiology, Washington, DC.

Shetty, R.S.; Singhal, R.S. and Kulkarni, P.R. (1994). Antimicrobial properties of cumin. World J. Microbiol. and Biotechnol., 10: 232-233.

Singh, G.; Marimuthu, P.; Carola, S.; Heluani, D.E. and Catalan, C. (2005). Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. J. food Sci., 70(2): 141-148.

Soliman, K.M., Badeaa R.I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol., 40(11): 1669-75.

Tayel, A.A. and El-Tras, W.F. (2009). Possibility of fighting food borne bacteria by Egyptian folk medicinal herbs and spices extracts. J. Egypt Public Health Assoc., 84 : 1-2.

Yasin, Nessrien M.N. and Mohamed Abou-Taleb (2007). Antioxidant and antimicrobial Effects of marjoram and thyme in coated refrigerated semi fried mullet fish fillets. World J. Dairy and Food Sci., 2(1): 01-09.

You, Y.S.; Park, K.M.; Kim, Y.B. (1993). Antimicrobial activity of some medical herbs and spices against *Streptococcus mutans*. Korean J. Applied Microbiol. Bioengineering, 21(2): 187-191.

تـأثير مستخلصـات الكمـون، الزعتـر والينسـون علـى نمـو بعـض سـلالات البكتريـا الممرضه والمسببه لفساد الأغذية

محمد حسين مدكور،عبد اللطيف بن عبد القادر نعمة الله ومنصور بن أحمد الغامدي

قسم العلوم البيئية - كلية الأرصاد والبيئة و زراعة المناطق الجافة – جامعة الملك عبد العزيز – السعودية

تـم دراسـة التـأثير المضـاد للميكروبـات للمستخلصـات المائيـة لكـل مـن الكمـون والزعتـر والينسون المستخلصين علي البارد وعلي الساخن وذلك علي ثلاثـة سلالات مسببة للامراض التـي يمكـن أن تنتقـل أو تحـدث نتيجـة تنـاول الغـذاء وهـي *Escherichia coli, Salmonella typhimurium,* والـ *Staphylococcus aureus* subsp. *aureus* وكذلك على سلالتين من سـلالات الفسـاد الغـذائي وهمـا *Bacillus cereus, Bacillus stearothermophilus* وقد وجد أن الاستخلاص علي الساخن للتوابل يعطي التأثير الفعـال المثبط والقاتل بدرجـة أكبر مـن الاستخلاص علي البارد. وقد وجد ان مستخلص الكمون والزعتر علي الساخن لها تأثير مثبط قوي وذلك باستخدام تقنية الانتشار بواسطة الاقراص Disk diffusion assay وذلك علي كل من الـ *E. coli* والـ *S. typhimurium* والـ *Staphy. ~~lococcus~~ aureus* subsp. *aureus*. وفوق ذلك أمكن تثبيط الـ *E. coli* تثبيطا كليا بواسطة مستخلص الينسون علي الساخن. ومن ناحيـه اخرى أظهر مستخلص الكمون والزعترتثبيطا ضـعيفا علـى كل من الـ *Bacillus cereus* والـ *B. stearothermophilus*. بينما لم تظهر مستخلصـات الينسون أي تـأثير مثبط علـي كل من *Staphy. aureus* subsp. *aureus* والـــ *B. cereus* والـــ *B. stearothermophilus*. المستخلصات ذات التأثير المثبط القوي تم اختبارها باستخدام طريقـة منحني القتل وذلك لتحديد أي الأنواع له تأثير قاتل. وقد وجد أن مستخلصـات الكمون والزعتر لهـا تأثير قاتل على كل من *E. coli* والـ *S. typhimurium* وكذلك *Staph. aureus* subsp. *aureus* . بينما كان لمستخلص الينسون تأثير قاتل *E. coli* فقط.

قام بتحكيم البحث

أ.د / احمد عبد العزيز الرفاعى	**كلية الزراعة – جامعة المنصورة**
أ.د / حمدى عبد اللطيف حسانين	**كلية الزراعة – جامعة بنها**