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ANTIBACTERIAL ACTIVITY OF MEDICINAL HERB AND SPICE EXTRACTS

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Abstract

Purpose: This study aimed to test a variety of naturally occurring, medicinal and potentially food-compatible herb and spice extracts for their antimicrobial potential against a group of food borne bacterial pathogens.

Design/methodology/approach: A total of 5 herbs and spices (garlic, thyme, cinnamon, marjoram and clove) collected from different markets in Alexandria were tested using the broth dilution method for determination of minimum inhibitory concentration (MIC), disc diffusion method and synergy assay using the checkerboard method on four types of food borne bacterial isolates obtained from food samples referred to the Central Lab of the High Institute of Public Health (HIPH).

Findings: All the selected aqueous plant extracts exhibited antibacterial activities against all tested organisms with varying degrees. Garlic extract showed the maximum activity with MIC values ranging from 18.75 to 37.5 mg/ml. Garlic extract also caused inhibition of all tested bacterial isolates using the disc diffusion method. *Staphylococcus aureus* and *Shigella* were the most susceptible to crude aqueous extracts.

Originality/value: The use of herbs and spices can provide an adequate degree of protection against food borne pathogens in processed foods.

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INTRODUCTION

Food poisoning is a major problem in developing countries, with serious public health, trade and economic implications. It is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of sickness outbreaks caused by some pathogenic and spoilage microorganisms in foods. The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern (Meng *et al.*, 1998; Stermitz *et al.*, 2000).

More than 90 per cent of the cases of food poisoning each year are caused by *Staphylococcus aureus (S. aureus)*, *Salmonella, Clostridium perfringens (C. perfringens)*, *Campylobacter, Listeria monocytogenes (L. monocytogenes)*, *Vibrio parahaemolyticus (V. parahaemolyticus)*, *Shigella, Bacillus cereus (B. cereus)*, and *Escherichia coli* (*E. coli*) (Food and Drug Administration, 2007). These bacteria are commonly found in many raw foods. Normally a large number of food-poisoning bacteria must be present to cause sickness. Therefore, sickness can be prevented by controlling the initial number of bacteria present, preventing the small number from growing, destroying the bacteria by proper cooking and avoiding contamination (Wagner, 2011).

In Egypt, the organisms most commonly associated with travellers' diarrhoea are *E. coli*, *Salmonella*, *Campylobacter*, *Shigella*, *Vibrio cholerae* and *Giardia*. (Harris, 2011; Haberberger *et al.*, 1991). Since the beginning of May 2011 a mass outbreak of *E. coli* infection began in Germany with more than 1,500 residents in nine nations infected by entero-haemorrhagic *E. coli*, which can cause the deadly haemolytic-uremic syndrome. The source of the outbreak remains unknown, although the majorities of those affected either lived in Germany, particularly in or around the northern city of Hamburg, or have travelled there recently (CDC, 2011).

Consumers are concerned with the safety of foods containing synthetic preservatives. Therefore, there has been increasing interest in using natural antibacterial compounds, such as extracts of spices and herbs for food preservation (Smid and Gorris, 1999).

Medicinal herbs are being increasingly studied by pharmacological researchers, and many such herbs have a long history of medicinal use (Sinclair, 1998). These herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components. This has resulted in a better understanding of their physiological, therapeutic and clinical actions (Merken, 2001; Zheng and Wang, 2001). Antimicrobial agents can also be derived from herbs and over 1000 plants exhibit antimicrobial effects (Nychas, 1995).

Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smid and Gorris, 1999; Souza *et al.*, 2005). Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives. In the last few years, medicinal plant extracts have been developed and proposed for use in food as natural antimicrobials (Hoque *et al.*, 2007).

This study was conducted to investigate the antibacterial activity of medicinal herb and spice extracts on food borne bacteria.

MATERIALS AND METHODS

This experimental study was carried out during a 12 month period from April 2010 to April 2011. A total of 5 herbs and spices collected from different markets in Alexandria, namely: garlic, thyme, cinnamon, marjoram and clove, were tested on four types of food borne bacterial isolates representing Gram-positive and Gram-negative bacteria obtained from food samples referred to the Central Lab of the HIPH for investigation.

PURIFICATION AND CONFIRMATION OF THE SELECTED FOOD BORNE BACTERIAL ISOLATES

The selected bacterial isolates were inoculated separately onto the surface of blood and MacConkey's agar plates and incubated at 37°C for 24 hours for subculture and purification. Isolated colonies were identified according to the methods described by Forbes *et al.* (2007).

The identified isolates were as follows: *S. aureus* (3), *Salmonella* species (5), *Shigella* species (5) and *E. coli* (3). Isolates were maintained at 4°C on nutrient agar slants for one month. The purity of each was checked monthly by streaking on blood agar plates. Long-term preservation was performed by stabbing into soft agar deeps. Means were calculated for different isolates of the same species to obtain results.

HERBS:

1. Thyme: Dried leaves of *Thymus capitatus*, commercially known as "Zaatar"
2. Sweet marjoram: Dried leaves of *Origanum majorana*, commercially known as "Bardakoush"
3. Cinnamon: Dried bark of *Cinnamomum zeylanicum* commercially known as "Kerfa"
4. Garlic: Fresh bulb of *Allium sativum*, commercially known as "Tom"
5. Clove: Dried flower bud of *Eugenia caryophyllata*, commercially known as "Koronfel".

PREPARATION OF HERBAL EXTRACTS

Thirty grams of thyme, marjoram, cinnamon or clove were boiled in 100 ml of distilled water (DW) for 10 minutes. The extract was filtered, followed by centrifugation at 4000 rpm for 5 minutes. The supernatant was collected and sterilised by filtration through a sterile syringe membrane filter with 0.20–0.45 μm pore (Chawdhry and Tariq, 2006). Each herbal extract was sterilised by filtration to prevent the formation of volatile oils, the main components of herbal extracts which can form during sterilisation by autoclaving (Bradley, 1992; Youngken, 1950).

GARLIC EXTRACTION

Garlic cloves were separated and peeled. Thirty grams of garlic cloves were chopped and homogenised in 100 ml of sterile DW. The extract was centrifuged and the supernatant was collected and sterilised by filtration through the membrane filter (Iwalokun *et al.*, 2004).

ANTIMICROBIAL ASSAYS

Each of the five selected herbs and spices were tested on each type of isolated food borne bacteria by the following methods:

BROTH DILUTION METHOD DETERMINATION OF MIC

The broth dilution method for the determination of MIC was performed as previously described by Sivagurunathan *et al.* (2008). In brief, bacterial suspensions were prepared by isolating and picking one or two colonies of test organism using a sterile inoculating loop and inoculating into 5 mls of sterile DW matched with 0.5 McFarland turbidity standards.

Equal volumes of each bacterial isolate culture (0.1ml) containing approximately 10^8 CFU/ml were applied onto 2 ml of nutrient broth supplemented with 300, 150, 75, 37.5 or 18.75 mg/ml of herbal extract using the two-fold broth dilution method. An extra concentration of 600 mg/ml was performed using marjoram only. Cultures were then incubated at 37°C for 24 hours. MIC was determined as the lowest concentration of plant extract that completely suppressed the growth of the microorganism (which was determined by the tube showing no turbidity), the tested bacteria were exposed to broth without the extracts as a control. The test was performed in duplicate.

DISC DIFFUSION METHOD

The paper disc diffusion technique was applied to determine the antimicrobial activities of the tested plant extracts as previously described by Tayel and El-Tras (2009). Sterile paper discs (6 mm in diameter) were placed on the surface of inoculated Mueller Hinton Agar (MHA) plates, and then 335 µl from every dilution of each herbal extract were pipetted onto the surface of each disc. Plates were then incubated at 37°C for 24 hours and diameters of the inhibition zones were recorded in mm. Inoculated plates with the tested bacteria were exposed to non-impregnated discs as a control.

SYNERGY ASSAY: CHECKERBOARD METHOD

The checkerboard method was performed to obtain the fractional inhibitory concentration (FIC) index of extract combinations as previously described by Gutierrez *et al.* (2008) and Hongbin *et al.* (2008). The experiment was done on *S. aureus* as a representative of Gram-positive bacteria and *Shigella* as a representative of Gram-negative bacteria. Dilutions of herbal extracts were prepared using the nutrient broth as described in the broth dilution method above. The concentration range of each antimicrobial agent in combination ranged from 1/32 times the

MIC (1/32 × MIC) to 4 × MIC. Plates consisted of columns and rows of epindorf tubes. A 500 µl of each dilution of extract "A" were added to the tubes in a vertical orientation and 500 µl of each dilution of extract "B" were added to the tubes in a horizontal orientation so that the plate could contain various concentration combinations of the two extracts. Then each tube was inoculated with 100 µl of bacterial suspension (10^8 CFU/ml) as prepared in the broth dilution method above. Plates were then incubated at 37°C for 24 hours. FIC was calculated by dividing the MIC of the herbal extract "A" in combination by the MIC of extract "A" alone FIC_A=(MIC_A combination / MIC_A alone) and the same was done for extract "B" FIC_B=(MIC_B combination / MIC_B alone). The FIC index was obtained by adding FIC_A to $FIC_{B.}$ .The results were interpreted as synergy (FIC ≤ 0.5), addition (0.5 < FIC ≤ 1), indifference (1 < FIC ≤ 4) or antagonism (FIC > 4).

STATISTICAL ANALYSIS

The data of the study was tabulated and statistically analysed as previously described by Armitage *et al.* (2002) with the following statistical tests:

- Repeated measures ANOVA.
- Kruskal-Wallis test.

RESULTS

All the aqueous plant extracts exhibited antibacterial activities against all the tested organisms by varying degrees. The MIC for the selected herbal extracts ranged from 18.75 – 600 mg/ml as shown in Table 2. Garlic extract showed the maximum activity with MIC values ranging from 18.75 to 37.5 mg/ml, followed by cinnamon and thyme extracts with MIC values ranging from 75 to 150 mg/ml against all the tested strains. The remaining herbs (clove and marjoram) showed the minimum inhibitory activity with MIC values ranging from 150

Garlic + Cinnamon	Cinnamon + Marjoram
Garlic + Clove	Cinnamon + Thyme
Garlic + Marjoram	Clove + Marjoram
Garlic + Thyme	Clove + Thyme
Cinnamon + Clove	Marjoram + Thyme

Table 1. The 10 different combinations of extract used in the synergy assay

to 600 mg/ml, and all these observed differences were found to be statistically significant ($P = 0.00$) (Table 2).

As shown in Table 3, garlic was found to have an inhibitory effect against all the tested strains using the disc diffusion experiment. The aqueous extracts of cinnamon and marjoram exhibited the lowest antibacterial activity. Cinnamon had no inhibitory effect on *Salmonella* or *Shigella,* while an inhibition zone of 27 mm was obtained for both *S. aureus* and *E. coli*. While marjoram had no inhibitory effect on *Salmonella* or *E. coli*, an inhibition zone of 25 and 32 mm was obtained for both *S. aureus* and *Shigella* respectively. Good inhibition zones were obtained for clove and thyme (26.0–45.0 mm and 15.0–39.0 mm respectively). The extract which showed the best antibacterial activity in the disc diffusion method was that of garlic (48.0–62.0 mm). The observed differences for clove and thyme were found to be statistically significant ($P = 0.038$) each (Table 3).

Table 4 shows the results of herbal combinations against the Gram-positive bacterium, *S. aureus* used in the synergy assay. This preliminary synergistic study of herbs showed promising results for marjoram, thyme, clove and cinnamon. The majority of combinations displayed additive effects at very low concentrations. Synergistic effects were found when marjoram and thyme were combined. Whereas additive effects were found in the following combinations: cinnamon and thyme, cinnamon and marjoram, cinnamon and clove, marjoram and clove, marjoram and garlic, clove and thyme. Indifference effects were found in the following combinations: garlic and clove, garlic and cinnamon, garlic and thyme. No antagonistic effects were observed. All the observed differences in FIC were found to be highly statistically significant ($P = 0.000$) (Table 4).

Table 5 shows the results of herbal combinations against the Gram-negative bacterium *Shigella* using the synergy assay. The results show that all thyme combinations showed an additive effect, except the combination of thyme and garlic that showed an antagonistic effect. No synergistic effects were observed. Additive effects were observed in the following combinations: cinnamon and thyme, cinnamon and marjoram, marjoram and thyme, clove and thyme. Indifference effects were observed in the following combinations: garlic and cinnamon, cinnamon and clove, garlic and marjoram, marjoram and clove, clove and garlic. The observed differences in FIC were found to be statistically significant ($P = 0.000$) (Table 5).

Table 2. Mean minimum inhibitory concentration (mg/ml) of tested herbal extracts against food borne bacterial isolate

Bacterial types	*S. aureus*	*Shigella*	*Salmonella*	*E. coli*	Median	Mean	S.D	*P* value
Herbal extracts	MIC in mg/ml							
Garlic	**18.75**	**18.75**	**37.50**	**18.75**	18.8	23.4	9.4	
Cinnamon	**75.00**	**75.00**	**150.00**	**150.00**	112.5	112.5	43.3	
Clove	**150.00**	**150.00**	**300.00**	**300.00**	225.0	225.0	86.6	0.00**
Thyme	**75.00**	**75.00**	**150.00**	**150.00**	112.5	112.5	43.3	
Marjoram	**300.00**	**300.00**	**600.00**	**600.00**	448	450.0	173.2	
Median	150	150	75	75				
Mean	243.8	247.5	123.8	123.8				
S.D	222.6	218.0	109.0	109.0				
***P* value**	0.00**							

**P value < 0.01 highly statistically significant

DISCUSSION

Antibiotic resistance has been an emerging problem worldwide in the last two decades. This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products (Walsh, 2000; Cohen, 2002). Herbs and their essential oils are potentially useful sources of antimicrobial compounds (Friedman *et al.*, 2002; Rančić *et al.*, 2005). The antimicrobial property of these herbs may differ depending on whether they are fresh, dried, or extracted. In order to use the herbs to control food borne bacteria, it is essential that antibacterial effects of crude herbal extracts against several food borne bacterial isolates be investigated.

In this study the *in vitro* antibacterial activity of the aqueous extracts of five different herbs (*Thymus capitatus*, *Origanum majorana*, *Allium sativum*, *Cinnamomum zeylanicum* and *Eugenia caryophillata*) wase tested against four food borne bacterial isolates (*S. aureus*, *E. coli*, *Shigella* and *Salmonella*). The present study has used established methods such as the broth dilution and disc diffusion techniques to establish the antibacterial activity of herbal extracts (Hongbin *et al.*, 2008; Miyasaki *et al.*, 2010).

The results suggest that four selected food borne bacteria were sensitive to the five tested herbal extracts with varying degrees. The variation in antimicrobial potentiality of examined plants could

Table 3. Antibacterial activity of tested aqueous herbal extracts against food borne bacterial isolates measured as the mean diameter of inhibition zones

Herbs	Organisms		Aqueous extract concentration (mg/ml) 100	50	25	12.5	6.25	Median	Mean ± SD	-value
Garlic	***S. aureus***	Inhibition Zones (mm)	62	48	37	27	16	37.00	38.00±17.90	0.665
	Shigella		48	39	33	28	0	33.00	29.60±18.15	
	Salmonella		49	40	36	29	0	36.00	30.80±18.67	
	E. coli		60	54	55	45	38	45.50	40.50±21.29	
	Median		54.505	44.00	34.50	28.5	8.00			
	Mean Mean ± SD		54.7± 7.3	45.2±7.1	27.8±15.0	32.3±8.5	13.5±17.9			
	P value				0.07					
Cinnamon	***S. aureus***	Inhibition zones (mm)	27	24	14	0	0	14.00	13.00±12.81	0.087
	Shigella		0	0	0	0	0	0.00	0.00±0.00	
	Salmonella		0	0	0	0	0	0.00	0.00±0.00	
	E. coli		27	0	0	0	0	0.00	5.40±12.08	
	Median		13.5	0.00	0.00	0.00	0.00			
	Mean ± SD		13.5±15.88	6.00±12.00	3.5±7.00	0.00±0.00	0.00±0.00			
	P value				0.31					
Clove	***S. aureus***	Inhibition zones (mm)	45	35	0	0	0	0.00	16.00±22.19	0.364
	Shigella		38	34	0	0	0	0.00	14.40±19.77	
	Salmonella		0	0	0	0	0	0.00	0.00±.000	
	E. coli		26	0	0	0	0	0.00	5.2±11.63	
	Median		32.00	17.00	0.00	0.00	0.00			
	Mean ± SD		27.25±19.7	17.25±19.9	0.00±0.00	0.00±0.00	0.00±0.00			
	P value				0.03*					

Table 3. Antibacterial activity of tested aqueous herbal extracts against food borne bacterial isolates measured as the mean diameter of inhibition zones

Thyme	***S. aureus***	**Inhibition zones (mm)**	35	34	0	0	0	0.00	13.80±18.90	**0.366**
	Shigella		39	26	0	0	0	0.00	13.00±18.39	
	Salmonella		15	0	0	0	0	0.00	3.00±6.71	
	E. coli		0	0	0	0	0	0.00	0.00±0.00	
	Median		25	13.00	0.00	0.00	0.00			
	Mean ± SD		22.25±18.2	15.00±17.6	0.00±0.00	0.00±0.00	0.00±0.00			
	***P* value**				0.03*					
Marjoram	***S. aureus***	**Inhibition zones (mm)**	25	20	19	0	0	19.00	12.8±11.90	**0.100**
	Shigella		32	28	0	0	0	0.00	12.00±16.49	
	Salmonella		0	0	0	0	0	0.00	0.00±0.00	
	E. coli		0	0	0	0	0	0.00	0.00±0.00	
	Median		12.50	10.00	0.00	0.00	0.00			
	Mean ± SD		14.25±16.7	12.00±14.2	4.75±9.5	0.00±0.00	0.00±0.00			
	***P* value**				0.23					

P value < 0.05 statistically significant

Herbal combinations	1st	2nd	3rd	4th	Mean ± SD	Results	*P* value
Cinnamon+ Marjoram	1	0.75	1.1	1.06	0.978±0.157	Addition	
Cinnamon+ Thyme	1	0.55	0.48	1.1	0.783±0.312	Addition	
Cinnamon+ Clove	0.55	0.5	0.49	0.62	0.54±0.59	Addition	
Cinnamon+ Garlic	1.5	1.5	1.24	1.12	1.34±0.191	Indifference	
Marjoram +Thyme	0.31	0.37	0.56	0.5	0.43±0.115	Synergism	0.000**
Marjoram +Clove	0.31	0.62	1.1	1	0.758±0.363	Addition	
Marjoram + Garlic	0.5	0.62	0.56	0.53	0.553±0.512	Addition	
Clove + Thyme	1	0.75	0.62	0.5	0.718±0.214	Addition	
Clove + Garlic	1	1.25	1.1	1.1	1.113±0.103	Indifference	
Garlic + Thyme	2	2.5	2	1.1	1.9±0.583	Indifference	

Results are interpreted with reference to FIC scale as synergy (FIC ≤ 0.5), addition (0.5 < FIC ≤ 1), indifference (1 < FIC ≤ 4) or antagonism (FIC > 4)
***P* value < 0.01 highly statistically significant.

Table 4. Mean fractional inhibitory concentration values of herbal extracts combinations against *S. aureus*

be attributed to their disparate contents of biocidal agents. This is in accordance with previous studies which found that bacterial strains had varying degrees of sensitivity against combinations of phytocompounds present in herb extracts (Suree and Pana, 2005; Davidson, 2001). In addition, various researchers recorded that medicinal and herbal plants and spices such as oregano, thyme, and garlic, can be successfully used alone or in combination with other preservation methods. They were found to exert direct or indirect effects to extend the shelf life of foodstuff and were considered antimicrobial against a variety of Gram-positive and Gram-negative bacteria. However, their efficacy depended on the pH, the storage temperature, the amount of oxygen, the essential oil concentration and active components. (Burt *et al.*, 2007; Du and Li, 2008; Gutierrez *et al.*, 2008a; Holley and Patel, 2005; Viuda-Martos *et al.*, 2008).

The present study suggests that sterile garlic extract has biostatic properties, since low MIC were required for growth inhibition of all

Herbal combinations	1st	2nd	3rd	4th	Mean ± SD	Results	*P* value
Cinnamon+ Marjoram	1	0.74	0.61	0.59	0.735±0.1888	Addition	
Cinnamon+ Thyme	1	0.56	0.7	0.62	0.720±0.1953	Addition	
Cinnamon+ Clove	1	1	1.2	1.12	1.080±0.0980	Indifference	
Cinnamon+ Garlic	1	1.5	1.24	2.12	1.465±0.4820	Indifference	
Marjoram + Thyme	0.5	0.51	0.62	0.5	0.533±0.5850	Addition	0.000
Marjoram + Clove	0.74	1.25	2.12	2.1	1.553±0.6766	Indifference	
Marjoram + Garlic	0.74	1.25	2.12	2.1	1.553±0.6766	Indifference	
Clove+ Thyme	1.1	0.74	0.74	0.62	0.800±0.2078	Addition	
Clove+ Garlic	1.2	0.7	1.2	1.1	1.050±0.2380	Indifference	
Garlic+ Thyme	4.2	4.2	4.06	4	4.115±0.1012	Antagonism	

Results are interpreted with reference to FIC scale as synergy (FIC ≤ 0.5), addition (0.5 < FIC ≤ 1), indifference (1 < FIC ≤ 4) or antagonism (FIC > 4)
**P* value < 0.01 highly statistically significant

Table 5. Mean fractional inhibitory concentration values of herbal extracts combinations against *Shigella.*

the selected food borne bacteria. *S. aureus*, *Shigella* and *E. coli* were the most susceptible, whereas *Salmonella* was least susceptible. The MIC of garlic extract against *S. aureus*, *Shigella and E. coli* was 18.75 mg/ml, while for *Salmonella*, it was 37.5 mg/ml (Table 2). Iwalokun *et al.* (2004) previously examined the antimicrobial effects of aqueous garlic extract against multidrug-resistant Gram-positive and Gram-negative bacterial isolates including: *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Salmonella typhi*, *P. aeruginosa*, *E. coli*, *Shigella* species and *Proteus* species with similar results to the present study. The broth dilution method was characterized by MIC ranges of 15.6–48.3 mg/mL for Gram-positive bacteria and 22.9–37.2 mg/mL for Gram-negative bacteria. In the present study, observed zones of growth inhibition on MHA plates seeded with Gram-positive and Gram-negative bacterial isolates also showed that the isolates exhibited susceptibility to garlic. Many studies have found that the antibacterial action of garlic is mainly due to allicin (Ankri and Mirelman, 1999; Uchida *et al.*, 1975). Bakri and Douglas (2005) were the first to demonstrate the sensitivity of various bacterial and clinical isolates to pure preparations of allicin. Allicin is the most abundant thiosulfinate found in garlic and is generated when the enzyme alliinase reacts with its substrate alliin.

The present study found that four selected food borne bacteria had variable susceptibility to cinnamon extract. *S. aureus* and *Shigella* were more susceptible than *Salmonella* and *E. coli*. The MIC of cinnamon extract against *S. aureus* and *Shigella* was 75 mg/ml while it was 150 mg/ml in the case of *Salmonella* and *E. coli*. At the concentrations used in the disc diffusion experiment, cinnamon had an inhibitory effect against two of the tested strains (*S. aureus* and *E. coli*). The limited inhibitory activity of cinnamon extract in the disc diffusion assay can be explained by the low water solubility of the essential oil and its components (the active components of the extract). This limits their diffusion through the agar medium in the disc diffusion method with only water-soluble components able to diffuse into the agar. The hydrocarbon components either remain on the surface of the medium or evaporate (Griffin *et al.*, 2000).

The antibacterial activity of sterile clove extract determined using the broth dilution technique showed inhibition of the selected food borne bacteria. The MIC of clove extract against *Salmonella* and *E. coli* were 300 mg/ml each, while a MIC of 150 mg/ml was observed for *Shigella* and *S. aureus* (Table 2). In the disc diffusion experiment, clove had an inhibitory effect against three of the tested strains (*S. aureus*, *Shigella*, *E. coli*), while inhibition of *Salmonella* required a higher MIC (300 mg/ml). The results of the present study were similar to those previously reported by Lopez *et al.* (2005), who found that clove oil was active against food borne Gram-positive bacteria (*S. aureus*, *B. cereus*, *Enterococcus faecalis* and *L. monocytogenes*) and Gram-negative bacteria (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa*).

The present study found that thyme extract inhibited Gram-positive as well as Gram-negative bacteria. Once again *Salmonella and E. coli* were least susceptible to thyme extract (MIC = 150 mg/ml). However, *S. aureus* and *Shigella* were more susceptible to inhibition with a MIC of 75 mg/ml. The observed inhibition zones showed that thyme had an inhibitory effect against three of the tested bacteria (*S. aureus*, *Shigella* and *Salmonella*), whereas *E. coli* required higher MIC (150 mg/ml).

These results differ to those previously reported by Satta *et al.* (1999), who examined the antimicrobial activity of thyme oils and its components against strains of food-derived spoilage and pathogenic bacteria. The broth dilution method was characterized by MIC ranges of 450-900 µg/mL which were significantly lower than the MICs required in the present study. The presence of some variation between the results of this study and the latter study may be attributed to the use of whole aqueous extract in our study instead of the pure essential oil. Differences between herbs may also be attributed to the place from which the herbs have been bought and the storage conditions (moisture, heat, light); all these factors affect the nature and concentration of the active ingredients of the herbs.

The antibacterial activity of sterile marjoram extract determined using the broth dilution technique showed that the extract inhibited Gram-positive as well as Gram-negative bacteria. However *Salmonella* and *E. coli* were less susceptible to the marjoram extract (MICs ≈ 600 mg/ml) compared with *Shigella* and *S. aureus* (MICs = 300 mg/ml). The observed inhibition zones showed that marjoram had an inhibitory effect against *S. aureus and Shigella*. Ben *et al.* (2001) concluded from their study that the essential oil of marjoram possessed antibacterial activity. It was stated that the leaves of marjoram had antimicrobial activity against *Bacillus anthracis*, *Proteus vulgaris*, *Salmonella stanley*, *Salmonella newport*, *Streptococcus agalactiae*, *and Aspergillus fumigates* (Farooqi and Sreeramu, 2004).

Combinations of herbal extracts were assessed for synergistic activity to assess whether lower doses could be used in the single extract without compromising their antimicrobial activity. In addition, antibacterial agents in combination prevent or minimize the likelihood of the emergence of drug-resistant strains as microbial tolerance is less likely to develop against substances having more than one type of mode of action (Eliopoulos and Eliopoulos, 1988; Gutierrez *et al.*, 2008). Synergies are known to occur in essential oil combinations and it is a field with countless opportunities to find potent antimicrobial blends. These blends may be the key to implementing essential oils in food preservation without simultaneous organoleptic effects (Nguefack *et al.*, 2012).

The checkerboard method was used to assess the efficacy of the five herbal extract combinations. The experiment was done on *S. aureus* as an example of the Gram-positive bacteria and *Shigella* as an example of the Gram-negative food borne bacterial isolates. FIC indices were calculated and interpreted as synergy, addition, indifference or antagonism. Interestingly, all marjoram combinations showed an additive effect against *S. aureus*, except the combination of marjoram and thyme, which showed a synergistic effect. In addition, all the combinations between cinnamon, clove and thyme with each herb extract, except for garlic, showed an additive effect. Gutierrez *et al.* (2008) found that the majority of essential oil combinations with reference to the FIC scale produced an additive effect, thus confirming the data from the present study. It is interesting to note that all garlic combinations showed an indifference effect except the combination of garlic with marjoram. This was in agreement with results reported by Betoni *et al.* (2006), who found that garlic showed limited synergistic capacity when tested against *S. aureus*. Herbal combinations against *Shigella* also produced some interesting findings with all thyme combinations showing an additive effect, except the combination of thyme and garlic, which showed an antagonistic effect. Again, all garlic combinations showed an indifference effect except the combination of garlic with thyme.

Based on the findings of the present study, the antibacterial property of the herbs tested can be defined in the following order: garlic > cinnamon > thyme > clove > marjoram. These herbs may be selected for use as potentially useful antibacterial agents in food products, depending upon the desired flavour of the product.

Data from the present and previous studies suggest that herbs and spices are considered to have good potential as antibacterial compounds and could be recommended for use, preferably in combinations. These findings may lead to many beneficial effects such as reduced antibiotic usage, which in turn would decrease the development of antibiotic resistance by pathogenic microorganisms. Furthermore, herbs and spices could be used to control microbial contamination in food, improve the shelf life of food, eliminate undesirable pathogens and/or delay microbial spoilage (Abou-taleb and Kawai, 2008; Fisher and Phillips, 2008; Gaysinsky and Weiss, 2007; Gutierrez *et al.*, 2008; Lopez-Malo Vigil *et al.*, 2005; Patrignani *et al.*, 2008).

CONCLUSIONS

The aqueous extracts of garlic, thyme, cinnamon, marjoram and clove showed considerable antibacterial activity against the tested food borne bacterial strains; so it can be concluded that the use of herbs and spices can provide an adequate degree of protection against food borne pathogens in processed foods. Of all tested bacterial isolates *S. aureus* and *Shigella* were the most susceptible to crude aqueous extracts, while *Salmonella* was the most resistant.

RECOMMENDATION

The use of herbal extracts in combination is recommended to reduce the concentration of herb extract and to widen the spectrum of activity.

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