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RESEARCH ARTICLE

SCREENING OF SPICES EXTRACT AND ANTIBACTERIAL ACTIVITIES AGAINST RED MEAT BACTERIAL (RMB) STRAIN *ESCHERICHIA COLI* AT AQUEOUS AND METHANOL EXTRACT

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ABSTRACT

Natural spices are widely used in a variety of food products. A number of studies have been made in the bactericidal properties of spices to evaluate their effectiveness in preventing or retarding spoilage caused by microorganisms. The preservative qualities of spices are due to the presence of some active antimicrobial principles contained in them. In present investigation screening of spices extract at various microorganism and antibacterial activities of spices extracts against red meat bacterial strain *Escherichia coli* at aqueous and methanol extract. Among the solvents tested, methanol extract recorded more inhibitory effect than aqueous extracts. The methanol extract of the turmeric extract recorded more mean inhibition zone (23.66 mm) in *Escherichia coli* (RMB-1) compared to other spices extracts.

Key words: Antibacterial, Spices, Solvent, Red meat.

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INTRODUCTION

In terms of global meat production over the next decade, there will be an increase from the current annual production of 267 million tons in 2006 to nearly 320 million tons by 2016. Almost exclusively, developing countries will account for the increase in production of over 50 million tons. Food spoilage is one of the most important issues facing the food industry. In fact, food-borne illnesses are a global problem, even in developing countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms. Many pathogenic microorganisms, including *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni, Candida* sp., *Zygosaccharomyces* sp., *Fusarium* sp., *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp. and *Salmonella* sp. have been identified as the causal agents of food-borne diseases or food spoilage (Betts *et al*., 1999; Solomakos *et al.,* 2008). Spices are defined as plant substances used to enhance flavour, they include leaves (mint and coriander), flower (clover), bulbs (garlic, turmeric), fruits (black pepper), stem (cinnamon), rhizomes (ginger and turmeric) (Shelef, 1983). Medicinal plants produce certain bioactive molecules which show both antibacterial and antifungal activities (Chopra *et al*., 1992; Bruneton, 1995). Agaoglu *et al.* (2007) stated that the inhibitory effect of cinnamon, clove and cumin against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Micrococcus luteus* and *Candida albicans.* Spices are their antimicrobial properties, mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Ebana, 1991). As reviewed by Lopez-Malo *et al*., 2006), some of antimicrobial components that have been identified in spices and herbs are: eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin from garlic, cinnamic aldehyde from cinnamon, allyl isothiocyanate from mustard, etc. Turmeric extracts are found to show antibacterial activity against methicillin resistant *Staphylococcus aureus* (Kim, and Rajagopa) 2001, Smith, 1998). Antimicrobial activity of cinnamon bark and oil was reported against many bacterial and fungal.

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MATERIALS AND METHODS

Plants used for the present study: Ten plants were collected from local area and specific part of the plant used for the present study. The details of the plants which were used for the present study were given below Table 1.

Table 1. Plants used for the present study

S.No.	Scientific name	Vernacular name		Family	Parts used
		Tamil name	English name		
1	*Syzygium aromaticum*	Krambu	Clove	Myrtaceae	Rhizome
2	*Cinnamomum zeylanicum*	Pattai	Cinnamon	Lauraceae	Bark
3	*Allium sativum*	Poondu	Garlic	Alliaceae	Bulb
4	*Piper nigrum*	Melagu	Pepper	Piperaceae	Grain
5	*Zingiber officinale*	Engi	Ginger	Zingiberaceae	Rhizome
6	*Curcuma longa*	Manjal	Turmeric	Zingiberaceae	Rhizome
7	*Cuminum cyminum*	Jeeragam	Cumin seed	Apiaceae	Seed
8	*Allium cepa*	Vengayam	Onion	Alliaceae	Bulb
9	*Pimpinella anisum*	Sombu	Aniseed	Apiaceae	Seed
10	*Solanum trylobatum*	Thuthuvalai	Thuthuvalai	Solanaceae	Leaves

Screening of plant extracts against the red meat bacteria associated with red meat

The well diffusion method was used for screening the aqueous extracts of ten plants at 2.5 and 5% per cent concentration against five red meat bacteria *Escherichia coli* (RMB-1), *Pseudomonas aeruginosa* (RMB-2), *Bacillus subtilis* (RMB-3), *Staphylococcus aureus* (RMB-4), *Klebsiella pneumoniae* (RMB-5) in nutrient agar medium. Among the ten spices, six were selected based on their inhibition zone.

Preparation of spice extracts (Ezhilan *et al*., 1994)

The spices plant parts were collected from local market and washed with distilled water, drained the water, shade dried and used for the preparation of aqueous, methanol, ethanol and ethyl acetate extracts.

Aqueous extract: Five hundred mg of shade dried plant parts were homogenized with pestle and mortar in ten ml sterile distilled water (1:20 w/v) and filtered through a double layered cheese cloth. The filtrate was collected and stored in refrigerator.

Methanol extract: Five hundred mg of shade dried plant parts were macerated using pestle and mortar with ten ml of methanol. After maceration, it was filtered through double layered cheese cloth. Then, the filtrate was collected and stored in refrigerator.

The method for testing antimicrobial properties of spice extracts

- Agar well diffusion method
- Minimum inhibitory concentration method

Agar well diffusion method: The Muller-Hinton agar medium was prepared and seeded with one ml cell suspension of the red meat bacterial strains *Escherichia coli* (RMB-1). The seeded, medium was poured in sterile petri plates allowed to solidified cork bore was used to form the well (6 mm size) and filled with spice extracts prepared at 200, 400, 600, 800 and 1000 µg levels in different solvents such as aqueous and methanol. The spice extracts were separately poured in to well. After the incubation period, the inhibition zones were measured.

Inhibitory effect of selected spices against red meat bacteria by Minimum inhibitory concentration (MIC) method: The tube dilution method was adopted for the evaluation of antibacterial activity of selected plant extracts *viz*., turmeric, cinnamon, clove, ginger, garlic and pepper. Nutrient broth was prepared and sterilized. The broth was poured into sterile test tubes. Cell suspension (10^6 cells ml^{-1}) of the test organism *viz*., *Escherichia coli* (RMB-1), were prepared and added. The methanol extract of selected plant extracts was added at different concentration *viz*., 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml. The tubes were incubated at 37°C for 24 hrs and observed the turbidity. The turbidity was matched with 0.5 McFarland standards (Baron *et al.,* 1994). The concentration at which no growth was recorded and considered as minimal inhibitory concentration of the extract to the organism.

RESULTS

Screening of plant extracts against red meat bacterial strains

The antibacterial activity of spice extract against the five red meat bacterial strains *viz., Escherichia coli* (RMB-1), *Pseudomonas aeruginosa* (RMB-2), *Bacillus subtilis* (RMB-3), *Staphylococcus aureus* (RMB-4) and *Klebsiella pneumoniae* (RMB-5) was tested at 2.5 and 5.0 per cent concentration and the results are presented in Table – 1 & Figure 1. Based on their antibacterial activity six spice extracts (turmeric, cinnamon, clove, ginger, garlic and pepper at 5%) were selected out of 10 extracts screened. Of the six spice extracts (turmeric, cinnamon, clove, ginger, garlic and pepper), turmeric extract exhibited higher inhibition zone in *Escherichia coli* (RMB-1), *Pseudomonas aeruginosa* (RMB-2) and *Klebsiella pneumoniae* (RMB-5) (18, 17 and 20.00 mm) followed by cinnamon, clove, garlic, ginger, and pepper extracts. Turmeric extract exhibited higher inhibition zone in *Bacillus subtilis* (RMB-3) followed by cinnamon, garlic, ginger, clove pepper extracts and *Staphylococcus aureus* (RMB-4).

Inhibitory effect of aqueous extracts of the selected spices against red meat bacterial strain *Escherichia coli* (RMB-1) by Agar well diffusion method: The results on the inhibitory effect of aqueous extract at various concentrations (0, 200, 400, 600, 800 and 1000 µg) of the selected spices against *Escherichia coli* (RMB-1) are presented in Table - 3. Among the six spice extracts prepared in various concentrations of aqueous, turmeric exhibited more inhibitory activity against *Escherichia coli* (RMB-1) compared to other spices extracts. The aqueous extract at 1000 µg all the six plants recorded more mean inhibitory effect than 0, 200,400, 600 and 800 µg. The mean inhibition zone recorded among the various spice extracts and various concentrations differed. Turmeric recorded more inhibition zone (14.20 mm) followed by cinnamon (13.46 mm), clove (12.46 mm), garlic (11.46 mm), ginger (10.73 mm) and pepper (9.73 mm).

Table 2. Screening of spices extract against red meat bacterial strains associated with red meat

Name of the spices	Aqueous Concentration (%)	*Diameter of inhibition zone (mm)				
		Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
Clove	2.5	7.00	7.33	8.00	8.00	9.33
	5.0	16.00	14.66	15.33	17.00	18.33
Cinnamon	2.5	7.33	7.00	8.66	9.00	10.00
	5.0	17.00	15.00	16.00	18.33	19.00
Garlic	2.5	7.00	7.33	8.00	8.33	9.66
	5.0	15.00	13.00	15.00	16.00	17.33
Ginger	2.5	7.66	7.33	8.00	9.33	10.00
	5.0	14.33	12.66	15.00	16.00	16.66
Pepper	2.5	6.00	7.00	7.66	8.00	8.33
	5.0	13.33	12.00	15.00	13.00	14.33
Turmeric	2.5	8.00	8.33	9.66	10.00	11.00
	5.0	18.00	17.00	17.66	19.00	20.00
Cumin seed	2.5	6.33	6.66	7.00	7.33	8.00
	5.0	11.00	11.00	10.33	11.66	12.00
Onion	2.5	5.00	5.33	6.00	6.33	6.66
	5.0	9.00	8.33	9.00	9.66	10
Aniseed	2.5	5.00	5.66	6.33	7.00	7.33
	5.0	8.33	8.00	10.00	10.33	11.00
Thuthuvalai	2.5	5.33	5.66	6.00	7.33	8.00
	5.0	7.33	8.00	8.66	9.66	10.66

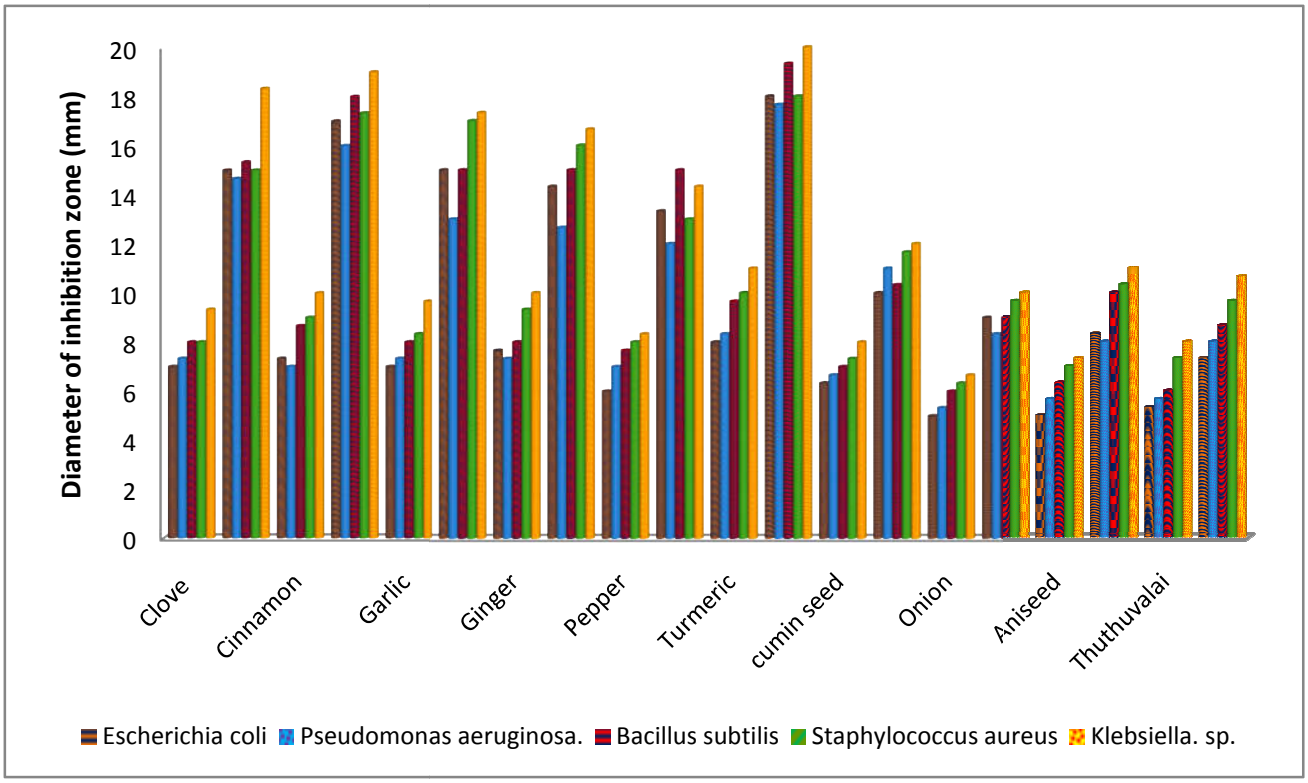

Figure. 1. Screening of spices extracts against red meat bacterial strains

Table 3. Inhibitory effect of aqueous extracts of the selected spices against red meat bacterial strain Escherichia coli (RMB-1) by Agar well diffusion method

Name of the spices	Aqueous extracts (5%) of different concentration (µg)											
	*Diameter of inhibition zone (mm)											
	0	200	Growth inhibition (%)	400	Growth inhibition (%)	600	Growth inhibition (%)	800	Growth inhibition (%)	1000	Growth inhibition (%)	Mean
Turmeric	-	11.00	12.22	12.00	13.33	14.00	15.55	16.00	17.77	18.00	20.00	14.20
Cinnamon	-	10.00	11.11	11.66	12.95	13.33	14.81	15.33	17.03	17.00	18.88	13.46
Clove	-	9.33	10.36	10.00	11.11	12.66	14.06	14.00	15.55	16.33	18.14	12.46
Garlic	-	8.33	9.25	9.66	10.73	11.00	12.22	13.33	14.81	15.00	16.66	11.46
Ginger	-	7.66	8.51	9.00	10.00	10.00	11.11	12.66	14.06	14.33	15.92	10.73
Pepper	-	7.00	7.77	8.33	9.25	9.00	10.00	11.00	12.22	13.33	14.81	9.73
Mean	-	8.88	-	10.10	-	11.66	-	13.72	-	15.66	-	-

Table 4. Inhibitory effect of methanol extracts of the selected spices against red meat bacterial strain Escherichia coli (RMB-1) by Agar well diffusion method

Name of the spices	Methanol extracts (5%) of different concentration (µg) *Diameter of inhibition zone (mm)											
	0	200	Growth inhibition (%)	400	Growth inhibition (%)	600	Growth inhibition (%)	800	Growth inhibition (%)	1000	Growth inhibition (%)	Mean
Turmeric	-	14.00	15.55	17.00	18.88	19.00	21.11	21.33	23.70	23.66	26.28	18.99
Cinnamon	-	13.00	14.81	16.33	17.03	18.00	20.00	20.00	22.22	22.00	24.44	17.86
Clove	-	12.33	13.70	14.00	15.55	16.66	18.51	18.66	20.73	20.33	22.58	16.39
Garlic	-	12.00	13.33	13.66	15.17	15.33	17.03	18.00	20.00	19.66	21.84	15.78
Ginger	-	11.66	12.95	13.00	14.44	15.00	16.66	17.66	19.62	18.00	20.00	15.06
Pepper	-	11.00	12.22	12.00	13.33	14.66	16.28	16.33	18.14	17.66	19.62	14.33
Mean	-	12.33	-	14.33	-	16.44	-	18.66	-	20.21	-	-

Table 5. Inhibitory effect of spices extract against Escherichia coli (RMB) by Minimal Inhibitory Concentration (MIC) method

Name of spices	Methanol extracts (5%) Spices extract of different concentration (ml)							
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6
Turmeric	++	+	-	-	-	-	-	-
Cinnamon	+++	++	+	-	-	-	-	-
Clove	+++	+++	++	+	-	-	-	-
Garlic	+++	++	++	+	+	-	-	-
Ginger	+++	+++	++	+	+	+	-	-
Pepper	+++	+++	+++	++	++	+	+	-

+++ = More growth; ++ = Moderate growth; + = Growth; - = No growth

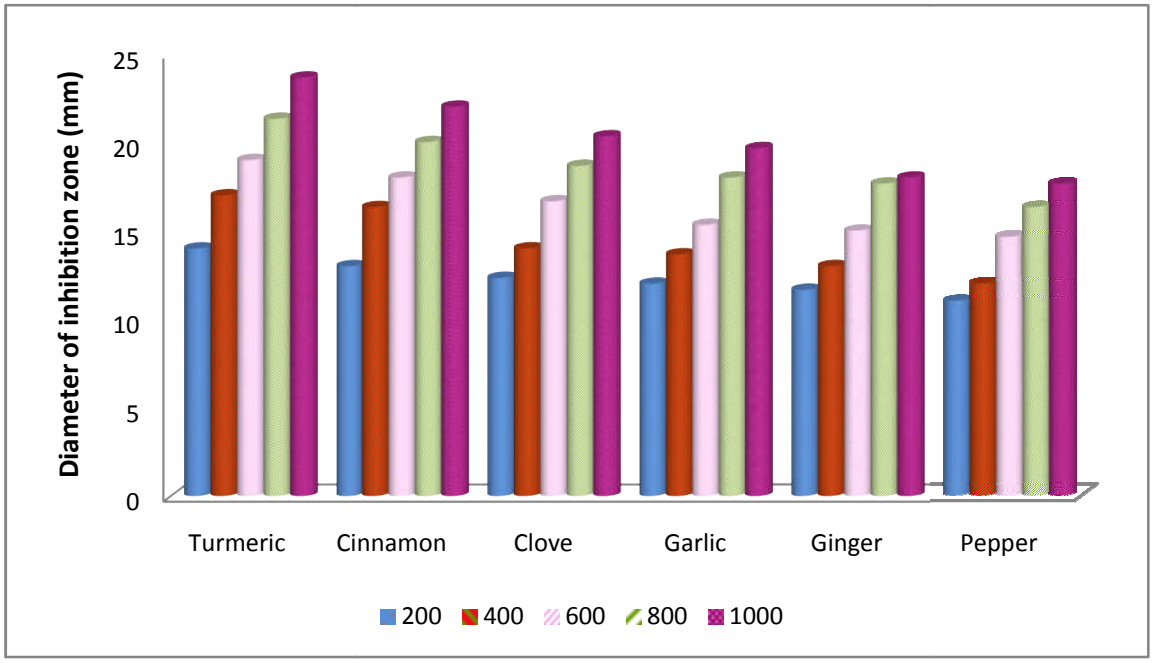

Figure 2. Inhibitory effect of methanol extracts of the selected spices against red meat bacterial strain Escherichia coli (RMB-1) by Agar well diffusion method

Among the concentration tested, 1000 µg concentrations of all the six species recorded significantly more mean inhibition zone (15.66 mm) followed by 800 µg (13.72 mm), 600 µg (11.66 mm), 400 µg (10.10 mm) and 200 µg (8.88 mm) than others.

Inhibitory effect of methanol extracts of the selected spices against red meat bacterial strain *Escherichia coli* (RMB-1) by Agar well diffusion method

The results on the inhibitory effect of methanol extract at various concentrations (0, 200,400, 600, 800 and 1000 µg) of the selected spices against *Escherichia coli* (RMB-1) are presented in Table - 4 and Figure - 2.

Of all the six spices extracts prepared in methanol at various concentrations turmeric exhibited more inhibitory activity against *Escherichia coli* (RMB-1) compared to other spices extracts. The methanol extract at 1000 µg of all the six plants recorded more mean inhibitory effect than 0, 200,400, 600 and 800 µg. The mean inhibition zone recorded among the various plant extracts and various concentrations differed. Turmeric recorded more inhibition zone (18.99 mm) followed by cinnamon, clove, garlic, ginger and pepper (17.86, 16.39, 15.78, 15.06 and 14.33 mm). Among the concentrations tested, 1000 µg concentrations of all the six spices showed significantly more mean inhibition zone (20.21mm) followed by 800 µg (18.66 mm), 600 µg (16.44 mm), 400 µg (14.33 mm) and 200 µg (12.33 mm) than others.

Inhibitory effect of spice extracts against *Escherichia coli* (RMB) by Minimal Inhibitory Concentration (MIC) method

The minimal inhibitory concentration of methanol extract of the selected spices *viz.,* turmeric, cinnamon, clove, garlic, ginger and pepper against *Escherichia coli* (RMB-1) was studied and the results were given in Table - 5. Among the six plant extracts prepared in methanol solvents, turmeric exhibited more inhibitory activity against *Escherichia coli* (RMB) compared to other five plant extracts. The increase in the level of concentration of plant extracts from 0.0 to 1.6 ml increased the inhibitory effect of the red meat. Among the extracts, the MIC of pepper was 1.6 ml followed by ginger (1.4 ml), garlic (1.2 ml) clove (1.0 ml), cinnamon (0.8 ml) and turmeric (0.6 ml) against *Escherichia coli* (RMB-1). Of all the six plant extracts, turmeric extract exhibited highest inhibitory activity than others.

DISCUSSION

Antibacterial Activity of Spice Extracts against Red Meat Bacteria

Rhee *et al*. (2003) also found that mustard to have similarly high inhibitory activity on *Escherichia coli*, but in acidic products. Clove and cinnamon showed strong activity towards *Escherichia coli* and *Bacillus cereus*, but relatively less towards *Staphylococcus aureus* at 0.5% and 1% concentrations. Papachan Karur Sofia *et al.* (2007) reported that two and three per cent clove concentrations showed inhibitory zones of up to 25.6 mm by the well method. Ali *et al*. (2007) reported antibacterial activity of water, petroleum ether, ethyl acetate, ethanolic and methanolic black pepper extracts against *Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*. Gur *et al*. (2006) reported that the ethanolic extract of turmeric was effective in the extraction of antimicrobial active substances as compared to water and hexane and also turmeric extract was effective against both the test bacteria *Bacillus subtilis* and *Escherichia coli.* Sana Mukhtar *et al.* (2012) reported that the ethanol extracts of turmeric and cinnamon showed better results as compared to the aqueous, cinnamon ethanolic extract showed maximum zone of 17 mm against *Escherichia coli* (ATCC 25922 and 16mm against *Bacillus subtilis* DSM 3256. Indu *et al*. (2006) suggested that the garlic extract was effective against different serotypes of *Escherichia coli.* The present study also showed that the antibiotic Nalidixic acid was effective against both the *Bacillus subtilis* and *Escherichia coli*. The cinnamon extract posses effective antibacterial properties against *Bacillus subtilis* and *Escherichia coli.* In the present, the inhibitory effect of aqueous and methanol extract of the selected spices against the red meat bacterial strains were tested. The mean inhibition zone recorded among the various spice extracts and various solvents differed. Among the solvents tested, methanol extract recorded more inhibitory effect than aqueous extracts. The methanol extract of the turmeric extract recorded more mean inhibition zone (23.66 mm) in *Escherichia coli* (RMB-1) compared to other spices extracts.

Inhibitory effect of spice extracts against five red meat bacterial strains (Minimum inhibitory concentration)

Iram Gull *et al*. (2012) reported that the minimum inhibitory concentration (MIC) was determined by making the dilutions of different extracts of garlic and ginger ranging from 100 mg/ml to 0.01 mg/ml. The MIC values of different garlic and ginger extracts all tested strains were susceptible to garlic aqueous, methanol and ethanol extract but the most effective was garlic aqueous extract. From all MIC values of different garlic extracts, lowest MIC values for *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus epidermidis and Salmonella typhi* were 0.1 mg/ml, 0.09 mg/ ml, 0.1 mg/ml, 0.2 mg/ml, 0.2 mg/ml, 0.09 mg/ml and 0.02 mg/ml respectively with garlic aqueous extract except Shigella which showed the lowest MIC value (0.07mg/ml) with a garlic methanol extract. Ethanol and methanol extract of ginger had a lower MIC in comparison to the ginger aqueous extract against tested bacterial strains. In the present study, the methanol extract of the selected species exhibited more MIC activity of the red meat bacterial strain of *Escherichia coli* (RMB-1 was compared with control.

Conclusion

Among the ten plant extracts screened (aqueous solvent), higher inhibition zone was observed in five plant extracts namely, turmeric, cinnamon, clove, garlic, ginger, and pepper against five red meat bacteria and selected for further studies. Among the aqueous and methanol extracts of spice products tested in an agar well diffusion methods against *Escherichia coli* (RMB-1), were effectively inhibited in turmeric extract, compared to other spice extracts. The methanol extract of clove recorded MIC of 0.6 µl was found to be the best among the spice extracts towards *Escherichia coli* (RMB-1), were inhibited by turmeric extract at 0.6 µl MIC.

REFERENCES

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

Ali, M.A., N. M. Alam, M. S. Yeasmin, A. M. M. Khan and A. Sayeed. 2007. Antimicrobial screening of different extracts of Piper longum Linn. Res. *J. Agri. Biol. Sci*, 3: 852-857.

Betts, G. D., P. Linton and R. J. Betteridge. 1999. Food spoilage yeasts: Effects of pH, NaCl and temperature on growth. *Food Control*, 10: 27–33.

Bruneton, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. (Lavisior, Paris).

Chopra. R.N., S.L. Nayer and I.C. Chopra. 1992. Glossary of Indian Medicinal Plants, 3rd Edn. New Delhi. *Council of Scientific and Industrial Research*, 7 – 246.

Ebana, R. U. B., B.E. Madunagu, E.D. Ekpe and I. N. Otung. 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus aurantiofolia*. *J. App. Bacteriol.*, 71: 398 – 401.

Gur, S., D.T. Balik and D.T. Gur. 2006. Antimicrobial activity and some fatty acids of turmeric, ginger root and linseed used in the treatment of infectious diseases. *World journal of agricultural sciences*, 2: 439 - 442.

Indu, M. N., A. A. M. Hatha, C. Abirohsh, C. Harssha, G. Vivekanandan. 2006. Antimicrobial activity of some of the south –Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. *Braz. J. Microbiol*., 210 – 217.

Iram, G., S. Mariam Halima Shaukat, M.A. Shahbaz , Zahoor Qadir Samra and A.M. Athar. 2012. Inhibitory effect of

Allium sativum and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. *Annals of Clinical Microbiology and Antimicrobials*, 11-13.

Kim, J.W and S.N. Rajagopal. 2001. Antimicrobial activities of *Lactobacillus crispatus* ATCC 33820 and *Lactobacillus gasseri* ATCC 33323. *J Microbiol*., 39: 146 - 148.

Papachan Karur Sofia, Rajendra Prasad, Virendra Kumar Vijay and Ashok Kumar Srivastava. 2007. Evaluation of antibacterial activity of Indian spices against common food borne pathogens. *International Journal of Food Science and Technology*, (42): 910 - 915.

Rhee, M.S., S.Y. Lee, H. Richard, R.H. Dougherty and K. Dong-Hyun. 2003. Antimicrobial effects of mustard flour and acetic acid against *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica*. *Applied Environmental Microbiology*, 69: 2959 - 2963.

Sana Mukhtar and Ifra Ghori. 2012. Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *Escherichia coli* ATCC 25922 and *Bacillus subtilis* DSM 3256, 3: 131 – 136.

Shelef, L. A. 1983. Antimicrobial effects of spices. *J. food Saf.*, 6: 29 - 44.

Smith - Palmer, A., J. Stewart and L. Fyfe. 1998. Antimicrobial properties of plant essential oils and essences against five important food - borne pathogens. *Lett. Appl. Microbiol.*, 26: 118 - 122.

Solomakos, N., A. Govaris, P. Koidis and N. Botsoglou. 2008. The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage. *Meat Science*, 80: 159 – 166.

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