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ANTIMICROBIAL ACTIVITY OF SOME OF THE SOUTH-INDIAN SPICES AGAINST SEROTYPES OF *ESCHERICHIA COLI, SALMONELLA, LISTERIA MONOCYTOGENES* AND *AEROMONAS HYDROPHILA*

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ABSTRACT

Antibacterial activity of extracts of *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Allium cepa* (onion) and *Piper nigrum* (pepper) has been evaluated against 20 different serogroups of *Escherichia coli*, 8 serotypes of *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Garlic extract showed excellent antibacterial activity against all the test organisms, except *L. monocytogenes*. Nutmeg showed good anti-listerial activity, although activity against *E. coli* and *Salmonella* were serotype dependent. Both garlic and nutmeg extracts were effective against *A. hydrophila*. Extracts of ginger showed inhibitory activity against two serogroups of *E. coli:* as O8 (enterotoxigenic *E. coli*) and O88 only. Extracts of onion and pepper did not show any antibacterial activity against the test organisms.

Key words: spices extract, antibacterial activity, *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Aeromonas hydrophila*

INTRODUCTION

Food borne pathogens such as diarrheagenic serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila* are widely distributed in nature, causing considerable mortality and morbidity in the population. It has been reported that, worldwide, there are more than 1.3 billion cases of human salmonellosis annually, with three million deaths (19). Among the various diarrheagenic serotypes of *E. coli*, enterohemorrhagic *E. coli* O157:H7 is implicated in large number of food borne outbreaks in many parts of the world including developed nations (17). It is also reported that *E. coli* O157:H7 has low infective dose (8-9). *Listeria monocytogenes* has been isolated from various environments and is reported to cause 25% of all the deaths resulting from food-borne outbreaks in the United States annually (4). *Aeromonas* spp. represents a group of ubiquitous microorganisms in aquatic environments (18). These bacteria have broad host range and have often been isolated from humans with diarrhoea (15).

Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens (6,11). This shift in susceptibility greatly affects our ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments (14). They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also.

In the present study we have evaluated the antibacterial effect of the extracts of five widely used spices in South India such as *Allium sativum* (garlic), *Myristica fragrans* (nutmeg),

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Zingiber officinale (ginger), *Allium cepa* (onion) and *Piper nigrum* (pepper) against 20 different serogroups of *E. coli*, 8 serotypes of *Salmonella*, *L. monocytogenes* and *A. hydrophila*. The inhibitory effect of these spices was compared with that of 5 antibiotics (chloramphenicol, ciprofloxacin, nalidixic acid, streptomycin and tetracyclin) and the results are discussed.

MATERIALS AND METHODS

Microorganisms

Twenty serogroups of *E. coli*, both pathogenic and non-pathogenic, were included in the present study. The pathogenic serogroups included O8 (enterotoxigenic *E. coli*, ETEC) and O157 (enterohemorrhagic *E. coli*, EHEC). The non pathogenic serogroups were O86, O30, O1, O69, O80, O88, O91, O51, O25, O116, O78, O22, O101, O33, O173, O104, O165, and O63. The strains were isolated from the Cochin estuary in a previous investigation (12) funded by Department of Science and Technology, Govt. of India.

Among *Salmonella*, the serotypes *S. paratyphi, S. mgulani, S. bareily, S. enteritidis, S. senftenberg, S. typhimurium, S. weltevreden* and *S. worthington* were included in the present study. While *S. paratyphi, S. mgulani, S. typhimurium, S. weltevreden* and *S. senftenberg* were isolated from seafood, *S. worthington, S. bareily* and *S. enteritidis* were isolated from chicken.

L. monocytogenes and *A. hydrophila* cultures were obtained from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of spices extracts

The fresh spices were obtained from the local market. The spices were cleaned, descaled when necessary, and washed in sterile distilled water. In order to obtain the spice's extracts, about 100g of each washed spice were crushed with mortar and pestle. The extracts were sieved through a fine mesh cloth and sterilized using a membrane filter (0.45-micron sterile filter). This extract was considered as the 100% concentration of the extract. The concentrations, 75%, 50%, 25% and 10% were made diluting the concentrated extract with appropriate volumes of sterile distilled water.

Garlic extract was made in a different way due to the difficulty to filter the crushed material. One hundred grams of the descaled and cleaned garlic were taken and surface sterilised using ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber, and the garlic was homogenised aseptically using a sterile mortar and pestle. The extract was aseptically squeezed out using sterile cheesecloth.

Antibacterial activity testing using agar well method (cup plate method)

The selected strains of bacteria were inoculated into 10 mL of sterile nutrient broth, and incubated at 37ºC for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter (23). Using a micropipette, 100 microlitres of different concentrations of spices extracts (100%, 75%, 50%, 25% and 10%) were added to different wells in the plate. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded. The inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity.

Antibacterial sensitivity testing using filter paper method

Filter paper discs of 7 mm diameter were prepared and sterilised. Using an ethanol dipped and flamed forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganisms (23). One hundred microlitres of the various spices' extract (100%) were aseptically transfered to these discs. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity. Diameters between 12 and 16 mm were considered moderately active, and these with >16mm were considered highly active.

Antibiotic sensitivity testing

The test microorganisms were also tested for their sensitivity against the antibiotics chloramphenicol (30 mcg), ciprofloxacin (5 mcg), nalidixic acid (30 mcg), streptomycin (10 mcg) and tetracycline (30 mcg) by the disk diffusion method (3).

The cultures were enriched in sterile nutrient broth for 6-8 hours at 37ºC. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over the seeded MHA plates sufficiently separated from each other to avoid overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 hours and the diameter of the inhibition zones was measured in mm. All the media used in the present investigation were obtained from Hi-media Laboratories Ltd., Mumbai, India.

RESULTS AND DISCUSSION

Among the five spices tested, three (garlic, nutmeg and ginger) showed antibacterial activity. The result of the antibacterial activity against serogroups of *E. coli* is given in Table 1.

Garlic extract showed excellent antibacterial activity at all concentrations (100%, 75%, 50% and 25%) to all serogroups of *E. coli* tested in the present investigation, and the activity was a linear function of concentration. Different serogroups

Table 1. Antibacterial activity of different concentrations of garlic, nutmeg and ginger extracts on serogroups of *Escherichia coli* by agar well method.

E. coli Serogroups	Diameter of inhibition zone (in mm) against various concentrations of spices extract														
	Garlic extract					Nutmeg extract					Ginger extract				
	100%	75%	50%	25%	10%	100%	75%	50%	25%	10%	100%	75%	50%	25%	10%
O86	20	15	11	0	0	13	10	0	0	0	0	0	0	0	0
O30	28	21	16	10	0	14	11	0	0	0	0	0	0	0	0
O1	18	14	10	0	0	13	12	0	0	0	0	0	0	0	0
O69	25	19	15	9	0	15	14	0	0	0	0	0	0	0	0
O80	27	20	16	10	0	15	11	0	0	0	0	0	0	0	0
O88	26	21	15	8	0	17	11	0	0	0	18	13	0	0	0
O157*	25	19	14	9	0	16	14	10	0	0	0	0	0	0	0
O91	25	20	14	10	0	0	0	0	0	0	0	0	0	0	0
O51	29	22	17	11	0	13	0	0	0	0	0	0	0	0	0
O25	30	24	18	13	0	14	0	0	0	0	0	0	0	0	0
O116	26	19	14	8	0	13	10	0	0	0	0	0	0	0	0
O78	25	20	13	9	0	10	0	0	0	0	0	0	0	0	0
O22	30	22	17	11	0	0	0	0	0	0	0	0	0	0	0
O101	28	21	15	10	0	10	8	0	0	0	0	0	0	0	0
O33	25	18	11	0	0	12	10	0	0	0	0	0	0	0	0
O173	27	22	14	8	0	18	16	12	0	0	0	0	0	0	0
O104	24	18	11	0	0	19	16	14	10	0	0	0	0	0	0
O165	24	19	10	0	0	15	10	0	0	0	0	0	0	0	0
O8**	24	18	12	0	0	0	0	0	0	0	10	0	0	0	0
O63	25	20	14	9	0	0	0	0	0	0	0	0	0	0	0

*Enterohemorrhagic *E. coli*, **Enterotoxigenic *E. coli*.

responded differently to the garlic extract at different concentrations. At 100% concentration *E. coli* serogroup O1 was least sensitive (18 mm) and serogroup O22 and O25 were more sensitive (30 mm). Both enterohemorrhagic *E. coli* (EHEC, serogroup O157) and enterotoxigenic *E. coli* (ETEC, serogroup O8) were highly sensitive to garlic extract.

Table 2 represents the antibacterial activity of the spices extracts against different serotypes of *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Garlic extract showed high antibacterial activity against *Salmonella* serotypes and *A. hydrophila* at 100% and 75% concentrations. Fifty percent concentration of the extract also showed moderate antibacterial activity against these strains. However, garlic extract was unable to inhibit the growth of *L. monocytogenes* at all concentrations. Different serotypes of *Salmonella* responded differently to the garlic extract. Highest inhibition was noticed against *S. bareily*.

The results agree with observations of previous researchers (1,7,10,16,21). Antibacterial activity of garlic powder on *E. coli* O157:H7 was also reported (20). However, garlic extract did not show any inhibitory effect on the growth of *L. monocytogenes*. Our results were also comparable to those of other authors (16,26), who had reported reduced activity of garlic extract on *L. monocytogenes*, suggesting that Gram positive organisms may be better equipped naturally to prevent the action of garlic extract.

The antibacterial activity of garlic is reported to be due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphide (2). It is postulated that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase *via* the inactivation of thiol group. Our results revealed differences in the sensitivity of different serogroups of *E. coli* to garlic extract, suggesting that mechanisms of resistance are developing in this organism. Garlic can be used as a potent inhibitor of food pathogens. Use of garlic would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods.

Results of the antibacterial effect of nutmeg (*Myristica fragrans*) extract against 20 serogroups of *E. coli* revealed that most of them were sensitive to the extract at 100% and 75% concentrations (Table 1). At 10% concentration, the extract did

Table 2. Antibacterial activity of different concentrations of garlic, nutmeg and ginger extracts on various serotypes of *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila* by agar well method.

Pathogenic Bacteria	Diameter of inhibition zone (in mm) against various concentrations of spices extract														
	Garlic extract					Nutmeg extract					Ginger extract				
	100%	75%	50%	25%	10%	100%	75%	50%	25%	10%	100%	75%	50%	25%	10%
S. paratyphi	27	20	14	9	0	0	0	0	0	0	0	0	0	0	0
S. mgulani	28	22	17	11	0	0	0	0	0	0	0	0	0	0	0
S. bareily	30	24	19	13	0	0	0	0	0	0	0	0	0	0	0
S. enteritidis	29	22	17	10	0	0	0	0	0	0	0	0	0	0	0
S. senftenberg	28	20	14	8	0	0	0	0	0	0	0	0	0	0	0
S. typhimurium	25	17	12	0	0	0	0	0	0	0	0	0	0	0	0
S. weltevreden	27	19	11	0	0	21	17	14	10	0	0	0	0	0	0
S. worthington	28	21	18	12	0	20	15	12	0	0	0	0	0	0	0
A. hydrophila	27	21	17	10	0	15	0	0	0	0	0	0	0	0	0
L. monocytogenes	0	0	0	0	0	22	20	18	0	0	0	0	0	0	0

not show any antibacterial activity. While the EHEC was sensitive to 100% concentration of the nutmeg extract, ETEC was not affected. The diameter of inhibition zone was maximum against *E. coli* serogroup O104. The growth of serogroups O22, O8, O63 and O91 was not inhibited by even 100% concentration of the nutmeg extract.

Table 2 represents antibacterial activity of nutmeg extract against serotypes of *Salmonella***,** *Aeromonas hydrophila* and *Listeria monocytogenes*. When tested against eight serotypes of *Salmonella*, *S. worthington* and *S. weltevreden* were found to be sensitive. The inhibition zone obtained for both organisms was 20 mm and 21 mm respectively at 100% concentration, 15 mm and 17 mm at 75% concentration. The inhibitory effect against *Aeromonas hydrophila* was obtained at 100% concentration. Other concentrations did not show any activity. *Listeria monocytogenes* was found to be highly sensitive to nutmeg extract, although the growth of this organism was not affected by garlic extract. The inhibition zone obtained was high (22 mm) at 100% concentration, 20 mm at 75% and 18 mm at 50% concentration.

Results of the antibacterial activity of nutmeg extract showed interesting observations. Although the antibacterial activity of nutmeg extract on growth of *E. coli* serogroups was lower when compared to garlic extract, it had significant inhibitory effect on *L. monocytogenes*, the growth of which was otherwise unaffected by all other spices used in the present study. Except *S. weltevreden* and *S. worthington*, growth of other *Salmonella* serotypes was not

Table 3. Diameters of inhibition zones of antibiotics and spices extracts against *Escherichia coli* serogroups.

E. coli serogroups	Diameter of inhibition zone (mm)							
	Antibiotics					Spices extracts		
	Cf	C	T	S	Na	Nutmeg (100%)	Garlic (100%)	Ginger (100%)
O86	27	28	16	15	21	13	20	0
O30	0	26	19	13	0	14	28	0
O1	20	29	11	14	0	13	18	0
O69	26	28	21	12	22	15	25	0
O80	17	26	7	14	0	15	27	0
O88	28	29	11	15	22	17	26	18
O157*	25	22	8	9	21	16	25	0
O91	26	25	21	14	24	0	25	0
O51	21	29	10	0	12	13	29	0
O25	25	25	16	13	22	14	30	0
O116	19	31	18	18	0	13	26	0
O78	24	28	13	13	22	10	25	0
O22	25	27	20	14	18	0	30	0
O101	0	26	11	13	0	10	28	0
O33	22	26	19	13	18	12	25	0
O173	28	28	17	15	22	18	27	0
O104	25	29	20	13	22	19	24	0
O165	23	24	22	14	24	15	24	0
O8**	25	27	22	14	24	0	24	10
O63	0	28	10	13	0	0	25	0

*Enterohemorrhagic *E. coli* (EHEC). **Enterotoxigenic *E. coli* (ETEC);
Cf – Ciprofloxacin, C – Chloramphenicol, T – Tetracycline, S – Streptomycin, Na – Nalidixic acid.

inhibited by the nutmeg extract. *E. coli* serogroups also showed variation in their sensitivity to nutmeg extract. The antililsterial activity of nutmeg extract is significant, as *L. monocytogenes* was otherwise not sensitive to any of the spices extracts used in present study. Considering the availability of nutmeg pods as an agricultural by-product in South India, efforts should be undertaken to extract the active component from the nutmeg pods for further use in medication. Literature on the antibacterial activity of *Myristica fragrans* is reduced, probably due to the limited distribution of this plant species.

Ginger extract was also found to have moderate antibacterial properties against *E. coli* serogroups O8 and O88. However, our results compare well with previous observations (5). Ginger extract did not show any antibacterial activity against all other serogroups of *E. coli*, *Salmonella*, *L. monocytogenes* and *A. hydrophila*. These results are contradictory to the observations of others authors (22,24), who had reported moderate activity of ginger extract on *S. paratyphi* and *S. enteritidis*. Extracts of onion and pepper also did not show any antibacterial activity.

Table 4. Diameters of inhibition zones of antibiotics and spices extracts serotypes of *Salmonella, Listeria monocytogenes* and *Aeromonas hydrophila*.

Pathogenic bacteria	Diameter of inhibition zone (mm)							
	Antibiotics					Spices extracts		
	Cf	C	T	S	Na	Nutmeg (100%)	Garlic (100%)	Ginger (100%)
A. hydrophila	25	26	19	15	20	15	27	0
L. monocytogenes	0	0	0	0	0	22	0	0
S. paratyphi	24	29	22	13	0	0	27	0
S. mgulani	30	31	21	16	24	0	28	0
S. bareily	27	28	16	16	24	0	30	0
S. enteritidis	32	31	26	18	29	0	29	0
S. senftenberg	27	28	22	17	26	0	28	0
S. typhimurium	28	31	23	16	24	0	25	0
S. weltervreden	26	28	16	15	22	21	27	0
S. worthington	22	28	19	14	0	20	28	0

Cf – Ciprofloxacin, C – Chloramphenicol, T – Tetracycline, S – Streptomycin, Na – Nalidixic acid.

When tested by paper disc method, the antibacterial activity of the spices was less evident. Although garlic extract (100%) maintained good antibacterial activity against all the test organisms except *L. monocytogenes*, nutmeg extract showed activity against *E. coli* serogroups O173, O104 and O165. Nutmeg extract (100%) showed activity against *S. weltevreden, S. worthington*, *A. hydrophila* and *L. monocytogenes.*

The results obtained for onion extract disagree with the observations of Srinivasan *et al.*, 2001 (23), who reported moderate antibacterial activity of onion extract against *E. coli, S. paratyphi* and *S. typhimurium*. Good antibacterial activity of onion extract on the growth of *S. enteritidis* was also reported by Suresh *et al.*, 2004 (24). In our study, the various concentrations of onion extract failed to inhibit the growth of all the test organisms. The differences may be due to a difference in the variety of the onion used in this study. Extracts of green pepper were found to be ineffective in controlling the growth of the various test organisms. Again, little literature on the antibacterial activity of *Piper nigrum* is available.

The diameter of the inhibition zone obtained against various spices at 100% concentration by agar well method was compared to those obtained against commonly used antibiotics (Tables 3 and 4). Garlic extract presented higher diameter of inhibition zones than ciprofloxacin, chloramphenicol, tetracycline, streptomycin and nalidixic acid. While *L. monocytogenes* was resistant against these antibiotics, nutmeg extract at 100% concentration had strong antilisterial activity, suggesting its possible use in treatment of infections caused by *L. monocytogenes.*

Natural products of plant origin have played significant role in the search for therapeutic drugs, such as quinine from *Cinchona* (14). Search for new antimcrobials is very important in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria (13,25). Enhanced animal husbandry practices have added further gravity to this problem. The results of this study are quite encouraging, considering the closeness of western ghats, a biodiversity hotspot in South India, to our institution.

RESUMO

Atividade antimicrobiana de condimentos do sul da India sobre *Escherichia coli, Salmonella, Listeria monocytogenes* e *Aeromonas hydrophila*

Avaliou-se a atividade antimicrobiana de extratos de alho (*Allium sativum*), noz-moscada (*Mysritica frangrans*), gengibre (*Zingiber officinale*) cebola (*Allium cepa*) e pimenta do reino (*Piper nigrum*) sobre 20 sorotipos de *Escherichia coli*, 8 sorotipos de *Salmonella*, *Listeria monocytogenes* e *Aeromonas hydrophila*. O alho apresentou atividade antimicrobiana excelente sobre todos os microrganismos testados, excepto *L. monocytogenes*. A noz-moscada apresentou boa atividade antilisteria, emboara atividade sobre *E. coli* e *Salmonella* tenha sido sorotipo-dependente. Tanto alho como noz-moscada foram eficientes contra *A. hydrophila*. O extrato de gengibre

apresentou atividade inibitória sobre dois sorotipos de *E. coli*: 08 (enterotoxigenico) e 088. Os extratos de cebola e pimenta do reino não apresentaram nenhuma atividade contra os microrganismos testados.

Palavras-chave: extrato de condimentos, atividade antibacteriana, *Escherichia coli, Salmonella, Listeria monocytogenes*, *Aeromonas hydrophila*

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