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Short Communication

Antimicrobial activity of some common spices against certain human pathogens

M. Melvin Joe*, J. Jayachitra and M. Vijayapriya

Department of Microbiology, Faculty of Agriculture, Annamalai University Annamalainagar – 608 002, Tamil Nadu, India.

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Antibacterial activity of *Allium sativum* (garlic), *Zingiber officinale* (ginger) and *Piper nigrum* (pepper) extracts has been evaluated against *Klebsiella pneumoniae, Staphylococcus aureus, Morganella morgani, Candida albicans, Escherichia coli* and *Proteus vulgaris.* Among ten extracts evaluated for antimicrobial activity garlic extract showed excellent antibacterial activity against *P. vulgaris* and *M. morgani* and the garlic extracts showed excellent antimicrobial activity against almost of all pathogens tested. The ginger extract, however, showed only a moderate antimicrobial activity against *P. aureus*, whereas the pepper extract showed the least activity against the test organisms.

Key words: Antimicrobial activity, pathogens, *Allium sativum, Zingiber officinale, Piper nigrum,* parts per million (ppm).

INTRODUCTION

Spices have been used for many centuries by various cultures to enhance flavour and aroma of our foods as our ancestors have recognized the usage of spices in food preservation and in treatment of clinical ailments and there are several reports on development of antibiotic resistance in diverse bacterial pathogens (Gold and Mellering, 1996). This shift in susceptibility of pathogens to antibiotics greatly affects its ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes many centuries. At present it has been estimated that about 80% of the world population rely on botanical preparations as medicine to meet the needs as they are considered safe and provided to be effective against certain ailments (Hora and Nair, 1944).

The spices have a unique aroma and flavour which are derived from compounds known as phytochemicals or secondary metabolites (Avato et al., 2002). The phytochemicals are antimicrobial substances present in the spices which are capable of attracting benefits and repel harmful organisms; they also serve as photoprotectants and responds to environmental changes. Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids are found associated with the spices (Chang, 1988).

*Corresponding author. E-mail: mel_vin@sify.com.

In the present study, we have evaluated the antimicrobial effect of the extracts of the three widely used spices in South India such as garlic (*Allium sativum*), ginger (*Zingiber officinale*) and pepper (*Piper nigrum*).

MATERIALS AND METHODS

Test microorganisms

The pathogenic organisms which are isolated from patients were either obtained from the Vivek Institute, Nagarcoil, or brought through the Institute of Microbial Technology (IMTECH), Chandigarh, India. They were maintained as pure cultures in respective specific agar slants with periodic subculturing every 4 - 8 days. The different pathogenic strains used in the present study are *Klebsiella pneumoniae, Staphylococcus aureus, Morganella morgani, Candida albicans, Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris.*

Preparation of spices extracts

The fresh spices samples used in the present study were obtained from the local market. They were collected and surface sterilized with 0.1% $HgCl_2$. Spices were dried in hot air oven at 35 - 40°C for 2 to 3 days and are powdered using a blender. In order to obtain the spices extract, about 100 g of each species were macerated well and soaked in ethanol (95%) for 30 min. The distillate was prepared using soxhellet apparatus and different concentrations (1000, 1500, and 2000 ppm) were made using distilled water.

Antimicrobial sensitivity test using filter paper method (Baur et al., 1966)

Filter paper discs of 5 mm diameter were prepared and sterilized by dipping them in 95% ethanol using sterile forceps. These discs were dipped aseptically in respective spices extract of appropriate concentration and placed over Muller – Hinton Agar plates seeded with respective pathogens. The plates were incubated in an upright position at 37°C for 24 h. The diameter of inhibition zones formed was measured in mm and the results were recorded. Discs with 7 mm diameter are considered as having no antibacterial activity. Diameter between 7 and 12 were considered as moderatively active and those with > 12 mm were considered as highly active.

Antibiotic sensitivity testing

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

Statistical analysis

Values are mean ±SD (standard deviation) of three replicates. All experiments were performed at least, three times (unless indicated otherwise) and were highly reproducible. Therefore, data from one replicate is presented below.

RESULTS AND DISCUSSION

Among the three spices tested, all the spices showed antimicrobial activity. The results of the antibacterial activity of ginger against the microorganism were given in Table 1.

Garlic extract showed excellent antibacterial activity at all concentrations, that is, (1000, 1500 and 2000 ppm) and the activity was a linear function of concentration. It has been found that different microorganisms responded differently to garlic extract at different concentrations. At 2000 ppm, the isolate *Proteus vulgaris* followed by *Morganella morgani* and *Escherichia coli* were more sensitive and the isolate *Pseudomonas aeruginosa* showed no activity. While *Staphylococus aureus*, *Klebsiella Pneumoniae* and *Candida albicans* showed moderate antibacterial activity against the extracts at all concentrations. The results agree with observations of previous researchers (Arora and Kaur, 1999; Elnima et al., 1983). The antibacterial activity of garlic is reported due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphate (Avato et al., 2000). 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. It has been found that garlic can be used as a potent inhibitor of food pathogens and would increase the shelf life of processed foods.

Table 1. Determination of antimicrobial activity of ginger against pathogens by disc diffusion method Ginger-ethanol extraction method.

Microorganism	Zone of inhibition in mm		
	1000*	1500*	2000*
C. albicans	8 ±1.0	10 ±0.25	15 ± 1.5
E. coli	8±1.5	9 ±0.5	12 ± 1.0
K. pneumoniae	-	-	5 ± 0.5
M. morgani	7±0.5	8 ±0.5	10 ± 0.5
P. aeruginosa	10±0.5	13 ±0.86	21 ± 2.5
P. vulgaris	-	6 ±0.5	9 ± 0.5
S. aureus	7±0.5	8±	11 ± 1.5

* Concentration in ppm.
Values are mean ± SD of three replicates from one representative of each experiment was carried out in 3 times and similar results were obtained each time, within a column different letters.

Table 2. Determination of antimicrobial activity of garlic against pathogens by disc diffusion method Garlic-ethanol extraction method.

Microorganism	Zone of inhibition in mm		
	1000	1500	2000
C. albicans	8 ± 1.5	12.5 ± 0.25	13.5 ± 1.25
E. coli	11.5 ± 1.25	14.5 ± 1.5	20 ± 3.0
K. pneumoniae	9.5 ± 0.5	12 ± 1.25	16.5 ±2.5
M. morgani	12 ± 1.0	16 ± 2.0	22 ± 2.02
P. aeruginosa	-	7 ± 1.0	8 ± 1.0
P. vulgaris	13 ± 1.0	20 ± 2.0	23 ± 2.25
S. aureus	9±0.5	12 ± 1.5	14 ± 2.5

* Concentration in ppm.
Values are mean ± SD of three replicates from one representative of each experiment was carried out in 3 times and similar results were obtained each time, within a column different letters.

Results pertaining to the antimicrobial activity of ginger were presented in Table 2. It has been found that the extract exhibited maximum inhibitory effect against *P. aeruginosa* while it showed no effect against *K. pneumoniae* while the antimicrobial activity against *E. coli* and *C. albicans* were found to be moderate. Our results compare well with the findings of Patumaraj (2000), who reported the antimicrobial activity of ginger against *S. aureus, C. albicans* and *Salmonella* sp and are on par with the findings of Indu et al. (2006). The antimicrobial activity of ginger may be attributed to the fact that it contains antimicrobial substances such as zingiberol, zingiberine and bisabolene (Michael derrida, 1999). The rhizome of ginger contains pungent vanillyl ketones including gingerol and paradole, etc (Dougles and Miller, 1999). Gingerol is a mixture of crystal gingerone and it is the major cause of acidity of ginger and plays a role in

Table 3. Determination of antimicrobial activity of Black pepper against pathogens by disc diffusion method Black pepper-ethanol extraction method.

Microorganism	Zone of inhibition in mm		
	1000*	1500*	2000*
C. albicans	-	6 ± 1.0	8.5 ± 0.25
E. coli	12.5 ± 1.25	14 ± 1.5	16 ± 2.0
K. pneumoniae	8.5 ± 1.25	11 ± 0.5	12 ± 1.50
M. morgani	-	-	-
P. aeruginosa	7.25 ± 0.25	8.5 ± 0.5	9 ± 1.0
P. vulgaris	-	-	6 ± 0.5
S. aureus	11.5 ± 1.75	13.25 ± 1.25	15.5 ± 2.25

* Concentration in ppm.
Values are mean ± SD of three replicates from one representative of each experiment was carried out in 3 times and similar results were obtained each time, within a column different letters.

inhibiting bacteria such as *S. aureus, Trichomonas vasmalis* and help to cure bacterial vaginosis and skin diseases (Michael derrida, 1999).

The results obtained from antimicrobial activity of pepper were presented on Table 3. It has been found that pepper exhibited moderate antimicrobial activity against *E. coli* and *S. aureus* and no activity against M. *morgani* and *Proteus vulgaris*. This was concluding with the findings of Al Delaimy (1999). *Shigella* spp. the anti-microbial component present in pepper is prescribed for diseased such as diarrhea, cholera, flatulence, etc. Pepper can actively prevent bacteria such as *E. coli* (Arun, 2001).

Natural products of plant origin have played significant role in the search of new drugs such as quinone from cinchona (Hora and Nair, 1944).

The results of the present study are quite encouraging as almost all spices exhibited antimicrobial activity against most of the pathogens, but the antimicrobial activity varies widely, depending on the type of spices, test medium and microorganism. This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

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