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REGULAR ARTICLE

ANTIBACTERIAL EFFECT OF GARLIC (*ALLIUM SATIVUM*) AND GINGER (*ZINGIBER OFFICINALE*) AGAINST *STAPHYLOCOCCUS AUREUS, SALMONELLA TYPHI, ESCHERICHIA COLI* AND *BACILLUS CEREUS*

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ABSTRACT

Antibacterial activity of extracts of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) has been evaluated against four different bacteria namely *Escherichia coli*, *Salmonella* Typhi, *Staphylococcus aureus* and *Bacillus cereus.* Two methods were used to determine the antimicrobial activity of garlic and ginger extracts namely disk diffusion method and agar well diffusion method. Garlic extract exhibited excellent antibacterial activity against all four test organisms while ginger extract showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* only. In addition, agar well diffusion method showed higher zone in inhibition when compared with the zone of inhibition produced by the spice of same concentration against the test microorganism by disk diffusion method.

Antibiotic sensitivity of the four different bacteria was tested with commercially available antibiotics namely Ciprofloxacin; Oxytetracycline; Vancomycin; Streptomycin; Gentamicin; Tetracycline; Novobiocin; Amikacin and Penicillin G. Penicillin G produced the highest zone of inhibition of 40.00±0.00against *Staphylococcus aureus* and the lowest zone of inhibition of 0.00±0.00against *Escherichia coli.*

Keywords: antibacterial activity, *Escherichia coli*, *Salmonella* Typhi, *Staphylococcus aureus*, *Bacillus cereus*, garlic, ginger

INTRODUCTION

Herbs and spices parts of plants from indigenous or exotic origin are essential part of human diet as they improve taste, color and aroma of foods **(de Souza *et al*., 2005; Venugopal *et al*., 2009)**. In addition they act as preservatives in many foods; they also have antioxidant **(Karuppiah and Rajaram, 2012)** and antimicrobial properties **(Singh *et al*., 2008)**. Herbs have also been utilized in human and veterinary medicine **(Alsaid *et al*., 2010)**.

Ginger is used as a herb and also a spice especially in the East. It is a member of the family *Zinberaceae* and its scientific name is *Zingiber officinale* **(Karuppiah and Rajaram, 2012)**. Ginger is thick scaly rhizomes which are aromatic, thick lobed, branched, have a scaly structure and they possess a spicy lemon like scent. The rhizomes contain both aromatic and pungent components **(Singh *et al*., 2008)**.

Garlic belongs to a family of *Alliaceae* and its scientific name is *Allium sativum*. Other members of the family include onion, leek, shallot and leek. Garlic is widely used in culinary and medicine **(Karuppiah and Rajaram, 2012)**. It has a pungent hot flavor but mellows and improves with cooking. It has been utilized to fight infections such as cold, cough, asthma, diarrhea, flu, headache, sore throat, abdominal discomfort and respiratory tract infections **(Abubakar, 2009; Shobana *et al*., 2009)**.

Food borne pathogens are widely distributed in the environment and may be a significant cause of mortality and morbidity in the population **(Indu *et al*., 2006)**. *Escherichia coli* (EHEC), is a significant foodborne hazard in many countries around the world. Infection often causes haemorrhagic diarrhoea, and occasionally to kidney failure and death. *Salmonella* is another bacteria that is the cause of foodborne illness mainly from foods of animal origin throughout the world. *Staphylococcus aureus* and *Bacillus cereus* cause foodborne illness due to their ability to form heat stable toxins **(WHO, 2007)** .

The present study was aimed at determining the *in vitro* antibacterial activity of the widely used spices in Fiji namely garlic cloves and ginger rhizomes extract on the isolates of *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Bacillus cereus*. The inhibitory effects of these spices were compared with nine antibiotics namely Ciprofloxacin, Oxytetracycline, Vancomycin, Streptomycin, Gentamicin, Tetracycline, Novobiocin, Amikacin and Penicillin G.

MATERIAL AND METHODS

Microorganism

Bacillus cereus, Escherichia coli, Staphylococcus aureus and *Salmonella* Typhi cultures were obtained from the culture collection of School of Applied Sciences, Fiji National University, Fiji. The stock cultures were streaked onto individual agar plates and incubated at 37 °C for 24 h. After 24 h of incubation, cultures were transferred to nutrient broth and incubated at 37 °C for 24 h.

Preparation of spice extract

The fresh spices were obtained from the local market. The spices were cleaned and washed using sterile distilled water. Hundred grams of each spice was homogenized using a sterile blender and the extract was sieved using sterile cheesecloth. The extract was considered 100 % concentration of the extract. The concentrations of 75 %, 50 % and 25 % were prepared by mixing with appropriate volumes of sterile distilled water.

Antibacterial Activity

The antibacterial properties of ginger and garlic were studied by agar well diffusion method and disk diffusion methods (**Bauer *et al*., 1996**).

Agar well diffusion method

The selected bacterial strains were inoculated into 10 ml of sterile nutrient broth and incubated at 37 °C for 16-18 h. Using a sterile cotton swab, the nutrients broth cultures was swabbed on the surface of sterile agar plates. Agar wells were prepared with the help of sterilized cork borer with 6 mm diameter. Using a micropipette, 100 µL solution of ginger and garlic were added to different wells in the plates. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zone (in mm) were measured.

Disk diffusion method

Filter paper discs (Whatman no.1) of 6 mm diameter were prepared and sterilized. Using an ethanol dipped and flamed forceps these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganism. Hundred microlitres of ginger and garlic extract of various concentrations was aseptically transferred to these discs. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zone (in mm) were measured.

Antibiotic sensitivity testing

The test microorganisms were also tested for their sensitivity against the antibiotics Ciprofloxacin, Oxytetracycline, Vancomycin, Streptomycin, Gentamicin, Tetracycline, Novobiocin, Amikacin and Penicillin G by disc diffusion method. The cultures of test microorganism were enriched in sterile nutrient broth for 16-16 h at 37 °C. Sterile cotton swabs were used to transfer the test cultures aseptically on the surface of sterile nutrient agar plates. An ethanol dipped and flamed forceps was used to place the antibiotic disc aseptically over the seeded agar plates. The plates were incubated at 37 °C for 24 h and the diameters of inhibition zones were measured.

RESULTS AND DISCUSSION

Antibacterial activity using well diffusion method

The results of the antibacterial investigations using the well diffusion method are given in Table 1. It indicates that different bacterial species demonstrated different levels of sensitivities towards the tested samples of garlic and ginger extracts. The zone of inhibition of the garlic extract was higher than that of the ginger extract for the corresponding concentrations. The diameter for zone of inhibition for garlic extract ranged from 38.67±0.57 to 19.33±0.57 mm at various concentrations used. Maximum inhibitory effect was towards on *Staphylococcus aureus* and minimum inhibitory effect was towards on *E. coli*. The ginger extract had lower zone of inhibition that ranged from 6.67±0.57 mm to no inhibition. Maximum inhibitory effect was towards on *Bacillus cereus* and *Staphylococcus aureus*. *E. coli* and *Salmonella* Typhi were completely resistant to all the ginger extract samples tested.

In agar well method, the zone of inhibition were larger for all bacteria found to be sensitive than the disk diffusion method.

Table 1 Antibacterial activity of ginger and garlic using the well diffusion method

Sample tested	Concentration (%)	Zone of inhibition (mm)			
		BC	EC	SA	ST
Garlic	25	28.67±1.03	19.33±0.57	34.00±3.46	21.67±0.57
	50	31.00±0.00	24.00±0.00	30.33±0.57	24.33±1.15
	75	34.00±0.00	25.33±0.57	36.67±1.15	26.33±1.15
	100	36.67±0.52	26.00±0.57	38.67±0.57	27.33±0.57
Ginger	25	6.00±0.00	-	6.00±0.00	-
	50	6.00±0.00	-	6.00±0.00	-
	75	6.67±0.57	-	6.67±0.57	-
	100	6.67±0.57	-	6.67±0.57	-

Legend: BC=*Bacillus cereus*; EC=*Escherichia coli*; SA= *Staphylococcus aureus*; ST=*Salmonella* Typhi; Data are mean of three replicates; - No inhibition was observed

Antibacterial Activity using disk diffusion method

Results pertaining to the antibacterial investigations using the disk diffusion method are given in Table 2 and shows that diverse bacterial species demonstrated different levels of sensitivities towards the tested samples of garlic and ginger extracts. Once again, the zone of inhibition of the garlic extract was higher than that of the ginger extract for the corresponding concentrations. The diameter for zone of inhibition for garlic extract ranged from 26.00±1.73 to 7.33±1.54 at various concentrations used. The ginger extract had lower zone of inhibition which ranged from 6.67±0.57 to no inhibition. *E. coli* and *Salmonella* Typhi were completely resistant to all the ginger extract samples tested. The antimicrobial activity of ginger may be due to the considerable amounts of phenolic compounds present in ginger **(Singh *et al*., 2008)**. The solvent used in preparation of the spice extract plays a major role in the inhibitory effect of the spice **(Ekwenye and Elegalam, 2005)**. In the current study, water was utilized to prepare the extracts with various concentrations. This would have prevented the liberation of active organic compounds thus affected the results of this study. If ethanol had been utilized as a solvent, the results would have shown larger inhibitory zones for the microbes tested **(Shobana *et al*., 2009)**. In contrast, water extracts of garlic were reported to be more potent than ethanol and chloroform extracts against the tested microbes in a study **(Abubakar, 2009)**.

Table 2 Antibacterial activity of ginger and garlic using the disk diffusion method

Sample tested	**Concentration (%)**	**Zone of inhibition (mm)**			
		BC	EC	SA	ST
Garlic	25	19.00±0.00	14.67±0.57	17.67±0.57	13.67±.15
	50	23.33±0.57	9.67±0.57	19.33±1.54	8.67±0.57
	75	22.33±0.57	7.33±1.54	25.33±0.57	13.33±5.77
	100	26.00±1.73	17.33±0.57	14.67±0.57	17.33±0.57
Ginger	25	6.00±0.00	-	6.00±0.00	-
	50	6.00±0.00	-	6.00±0.00	-
	75	6.67±0.57	-	6.67±0.57	-
	100	6.67±0.57	-	6.67±0.57	-

Legend: BC=*Bacillus cereus*; EC=*Escherichia coli*; SA= *Staphylococcus aureus*; ST=*Salmonella* Typhi; Data are mean of three replicates; - No inhibition was observed

Allicin has been found to be the active ingredient in garlic and it works as an antimicrobial agent by inhibiting DNA and protein synthesis moderately and inhibiting RNA synthesis completely as a primary target **(Shobana *et al*., 2009; Rahman *et al*., 2011)**. Garlic is also rich in anionic components such as nitrates, chlorides and sulfates and other water soluble components found in plants and these components may have antimicrobial properties **(Shobana *et al*., 2009)**.

Previous authors have described the antibacterial activity of garlic extract against microorganisms. Bulbs belonging to the Allium genus had the most antibacterial activity against *Streptococcus mutans* **(Ohara *et al*., 2008)** and against *Streptococcus agalactiae* **(Alsaid *et al*., 2010)**. In addition, garlic was shown to have antimicrobial activity against *Streptococcus olaris, Streptococcus mitis, Staphylococcus aureus* **(Silva and Fernandes, 2010; Daka, 2011)**; *Escherichia coli, Salmonella typhi, Shigella flexineri, Proteus mirabilis* **(Shobana *et al*., 2009)**; and *Vibrio parahaemolyticus, Escherichia coli* and *Staphylococcus aureus* **(Vuddhakul *et al*, 2007)**. Few studies have shown some bacteria to be resistant towards garlic extract and these include *Escherichia coli* and *Staphylococcus aureus* **(Esimone *et al*., 2010)**.

In ginger, the gingerol related components have been found to have antimicrobial activities **(Rahman *et al*., 2011)**. There are several reports of the inhibitory effect of ginger in the form of extract against several bacteria **(Nanasombat *et al*., 2005; Joe *et al*., 2009; Patel *et al*., 2011)**. Moderate to good antimicrobial properties of ginger were shown in previous studies **(Ibrahim *et al*., 2003; Singh *et al*., 2008; Venugopal *et al*., 2009)**. Ginger has been reported to decrease 3 log cycles of the microorganisms in beef sausages after two to three months of frozen storage when used as an antimicrobial agent at the concentration of 1% **(Sediek *et al*., 2012)**.

However, some studies have reported as ginger having weak antimicrobial effects **(Indu *et al*., 2006; Eruteya and Odunfa, 2009; ; Esimone *et al*., 2010; Silva and Frenandes, 2010)** and it compares well with this study where *E. coli* and *Salmonella typhi* were resistant towards all ginger extract samples. Similar results were reported where ginger did not show any antibacterial activity against the multidrug resistant bacteria viz: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* Typhi **(Adeshina *et al*., 2011)**. Study by **Vuddhakul *et al*., (2007)** also showed no antibacterial activity of ginger to *Vibrio parahaemolyticus, Escherichia coli* and *Staphylococcus aureus.*

Antibiotic sensitivity testing

Table 3 Diameters of inhibition zones of antibiotics with *Staphylococcus aureus, E coli, Bacillus cereus* and *Salmonella typhi*.

Diameter of inhibition zone (mm)	Pathogenic bacteria			
	Staphylococcus aureus	*Escherichia coli*	*Bacillus cereus*	*Salmonella Typhi*
CIP	26.67 ± 1.15	37.00 ± 1.73	26.67 ± 1.52	34.33 ± 2.88
OT	30.00 ± 0.00	24.33 ± 0.57	29.33 ± 1.15	28.00 ± 0.00
VA	14.67 ± 0.57	24.67 ± 0.57	16.33 ± 0.57	10.67 ± 1.15
S	17.33 ± 0.57	14.67 ± 1.15	18.00 ± 0.00	18.00 ± 0.00
CN	11.67 ± 0.57	14.00 ± 1.73	17.00 ± 0.00	15.33 ± 2.88
TE	30.00 ± 0.00	25.33 ± 0.57	27.33 ± 0.57	25.33 ± 2.31
NV	30.00 ± 0.00	13.33 ± 1.15	16.67 ± 1.15	20.67 ± 0.57
AK	14.00 ± 1.00	16.00 ± 1.73	9.33 ± 1.15	20.67 ± 0.57
P	40.00 ± 0.00	-	9.33 ± 0.57	11.33 ± 1.15

Legend: CIP-Ciprofloxacin; OT-Oxytetracycline; VA-Vancomycin; S-Streptomycin; CN-Gentamicin; TE-Tetracycline; NV-Novobiocin; AK-Amikacin; P-Penicillin G; Data are mean of three replicates; - No inhibition was observed

Table 3 represents the antimicrobial activity of commercial antibiotics against *Escherichia coli, Staphylococcus aureus, Salmonella* Typhi and *Bacillus cereus*. The control experiment showed that Penicillin G had the widest zone of inhibition against *Staphylococcus aureus* showing 40.00 ±0.00 mm zone of inhibition. This indicates that Pencillin G is the right antibiotic for treating infection caused by *Staphylococcus aureus*. This was followed by

Ciprofloxacin against *Escherichia coli* with 37.00±0.00 mm zone of inhibition. Lowest zone of inhibition was produced by Penicillin G against *Escherichia coli* with 0.00±0.00 mm zone of inhibition. Hence, Penicillin G is not appropriate for infections caused by *Escherichia coli*. Penicillin G also produced lower zone of inbition against Bacillus cereus and Salmonella Typhi. This indicates that it may not be ideal broad spectrum antibiotic against certain bacterial infections. However, Ciprofloxacin, Oxytetracycline and Tetracycline produced higher zone of inhibition of more than 20mm for all the four bacteria tested hence these antibiotics can be used as a broad spectrum drug against many bacterial infections.

CONCLUSION

Garlic can be utilized for the development of broad spectrum antibiotics as it has wide spectrum antibacterial activity. It can also be used as a potential inhibitor of food pathogens and prevent food poisoning. Shelf life of processed foods could be increased if garlic is used in the preservation of foods. In comparison, ginger has weaker antibacterial and may be used along with garlic as an antibacterial agent. However, further studies needs to be conducted to determine if better antibacterial activity is achieved by combining the two spices.

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REFERENCES

ABUBAKAR, E. L. M. M. 2009. Efficacy of crude extracts of garlic (*Allium sativum Linn*.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia* and *Pseudomonas aeruginosa*. In *Journal of Medicinal Plants research*, vol.3, 2009, p. 179-185.

ADESHINA, G. O. - JIBO, S. - AGU, V. E. - EHINMIDU, J. O. 2011. Antibacterial activity of fresh juices of *Allium cepa* and *Zingiber officinale* against multidrug resistant bacteria. In *International Journal of Pharma and Bio Sciences*, vol. 2, 2011, p. B289-B295.

ALSAID, M. - DAUD, H. - BEJO, S. K. - ABUSELIANA, A. 2010. Antibacterial activities of some culinary spice extracts against *Streptococcus agalactiac* and its prophylactic used to prevent streptococcal infection in red hybrid Tilapia (*oreochromis* sp.). In *World Journal of Fish and Marine Sciences 2*, vol. 6, 2010, p. 532-538.

DAKA, D. 2011. Antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus*: an in vitro study. In *African Journal of Biotechnology*, vol. 10, 2011, p. 666-669.

DE SOUZA, E. L. - STAMFORD, T. L. M.- LIMA, E. D - O. - TRAJANO, V. N. FILHO, J. M. B. 2005. Antimicrobial effectiveness of spices: a approach for use in food, In *Brazilian Archives of Biology and Technology: An International Journal*, vol. 48, 2005, p. 549-558.

EKWENYE, U. N. - ELEGALAM, N. N. 2005. Antibacterial activity of ginger (*Zingiber officinale Roscoe*) and garlic (*Allium sativum L.*) extracts on *Escherichia coli* and *Salmonella* typhi, In *International Journal of Molecular Medicine and Advance Science*, vol. 1, 2005, p. 411-416.

ERUTEYA, O. C. - ODUNFA, S. A. 2009. Antimicrobial properties of three spices used in the preparation of *suya* condiment against organisms isolated from formulated samples and individual ingredients. In *African Journal of Biotechnology*, vol. 8, 2009, p. 2316-2320.

ESIMONE, C. O. - OKOYE, F. B. C. - ODIMEGWU, D. C. - NWORU, C. S. - OLEGHE, P. O. - EJOGHA, P. W. 2010. In vitro antimicrobial evaluation of lozenges containing extract of garlic and ginger. In *International Journal of Health Research*, vol. 3, 2010, p. 105-110.

IBRAHIM, S. A. - DHARAMAVARAM, S. R. - SEO, C. W. - SHAHBAZI, G. 2003. Antimicrobial activity of *Bifidobacterium Longum* (CNCFB2259) as influenced by spices. In *Internet Journal of Food Safety*, vol. 2, 2003, p. 6-8.

INDU, M.N.- HATHA, A.A.M.- ABINOSH, C.- HARSHA, U.- VIVEKANANDAN, G. 2006. Antimicrobial activity of some South Indian spices against serotypes of *Eschenchia coli*, *Samonella*, *Listeria monocytogenes* and *Aeromonas Hydrophila.* In *Brazilian Journal of Microbiology*, vol. 37, 2006, p. 153-158.

JOE, M. M. - JAYACHITRA, J. - VIJAYPRIYA, M. 2009. Antimicrobial activity of some common spices against certain human pathogens. In *Journal of medicinal Plants Research*, vol. 3, 2009, p. 1134-1136.

KARUPPIAH, P. - RAJARAM, S. 2012. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against clinical pathogens. In *Asia Pacific Journal of Tropical Biomedicine*, vol. 2, 2012, p. 597-601.

NANASOMABAT, S. - LOHASUPTHAWEE, P. 2010. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. In *KMITL Science and Technology Journal*, vol. 5, 2010, p. 527-538.

OHARA, A. - SAITO, F. - MATSUHISA, T. 2008. Screening of antibacterial activities of edible plants against *Streptococcus mutans*. In *Food Science and Technology Research*, vol. 14, 2008, p. 190-193.

PATEL, R. V. - THAKER, V. T. - PATEL, V. K. 2011. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment, In *Asian Pacific Journal of Biomedicine*, vol. 1, 2011, p. S58-S61.

RAHIMAN, A. - PARVEZ, A. K. - ISLAM, R. - KHAN, M. H. 2011. Antibacterial activity of natural spices on multiple drug resistant *Eschericha coli* isolated from drinking water, Bangladesh. In *Annals of Clinical Microbiology*, vol. 10, 2011, p. 1-4.

SEDIEK, L. E. L. - ABOZEID, W. M. M. - ALKHALIFAH, D. H. - FARAG, S, E, A. 2012. Efficacy of ginger extract (*Zingiber officinale*) and gamma irradiation for quality and self stability of processed frozen beef sausage, In *Life Science Journal*, vol. 9, 2012, p. 448-461.

SHOBANA, S. - VIDHYA, V.G. - RAMYA, M. 2009. Antibacterial activity of garlic varieties (*ophioscordon* and *Sativum*) on enteric pathogens. In *Current Research Journal of Biological Science*, vol. 1, 2009, p. 123-126.

SILVA, N. C. C. - FERNANDES JUNIOR, A. 2010. Biological Properties of Medicinal Plants: A review of their antimicrobial activity. In *The Journal of Venomous Animals and Toxins Including Tropical Disease*, vol. 16, 2010, p. 402-413.

SINGH, G. - KAPOOR, I. P. S.- SINGH, P. - DE HELUANI, C. S. - DE LAMPASONA, M. P. - CATALAN, C. A. N. 2008. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. In *Food and Chemical Toxicology*, vol. 46, 2008, p. 3295-3302.

VENUGOPAL, A. - DASANI, S. - RAI, S. 2009. Antibacterial effect of herbs and spices extract on *Escherichia coli*. In *Electronic Journal of Biology*, vol.5, 2009, p. 40-44.

VUDDHAKUL, V. - BHOOPONG, P. - HAYEEBILAN, F. - SABHADHIRASAKUL, S. 2007. Inhibitory activity of Thai condiments on pandemic strain of *Vibrio parahaemolyticus*. In *Food Microbiology*, vol. 24, 2007, p. 413-418.

WHO, 2007. Foodborne Disease Outbreak Guidelines. [cit. 2012-11-27]. Available on internet: http://www.who.int/foodsafety/publications/foodborne_disease/FERG_Nov07pdf.