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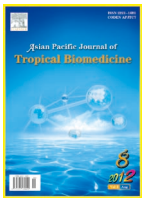

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Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens

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ABSTRACT

Objective: To evaluate the antibacterial properties of *Allium sativum* (garlic) cloves and *Zingiber officinale* (ginger) rhizomes against multi-drug resistant clinical pathogens causing nosocomial infection. **Methods:** The cloves of garlic and rhizomes of ginger were extracted with 95% (v/v) ethanol. The ethanolic extracts were subjected to antibacterial sensitivity test against clinical pathogens. **Results:** Anti-bacterial potentials of the extracts of two crude garlic cloves and ginger rhizomes were tested against five gram negative and two gram positive multi-drug resistant bacteria isolates. All the bacterial isolates were susceptible to crude extracts of both plants extracts. Except *Enterobacter* sp. and *Klebsiella* sp., all other isolates were susceptible when subjected to ethanolic extracts of garlic and ginger. The highest inhibition zone was observed with garlic (19.45 mm) against *Pseudomonas aeruginosa* (*P. aeruginosa*). The minimal inhibitory concentration was as low as 67.00 μg/mL against *P. aeruginosa*. **Conclusions:** Natural spices of garlic and ginger possess effective anti-bacterial activity against multi-drug clinical pathogens and can be used for prevention of drug resistant microbial diseases and further evaluation is necessary.

1. Introduction

The acquaintance with different ethnic groups has contributed to the development of research on natural products, to the increase in knowledge about the close relationship between the chemical structure of a certain compound and its biological properties, and to the understanding of the animal/ insect-plant interrelation[1]. For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. The microbial infections are the major cause of morbidity and mortality of developed and developing country, although a number of antimicrobial agents are available for the treatment and management of infectious diseases. In addition, misuse of the antibiotics which can lead to the development of antibiotic resistance is also a major health concern[2]. Therefore, there is a perpetual need to exploit new bioactive principles with high safety index.

Historically, medicinal plants have been a source of novel drug compounds. Plants derived products have made large contributions to human health and wellbeing. Green pharmacy may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new drug with novel mechanisms of action. Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very meager portion of this tremendous potential drug-repertoire has been scientifically screened[3]. A number of medicinal plants have been screened for antimicrobial activity in recent years[4] and efforts have been done to identify their active constituents[5]. The plants extracts possessing bioactivity are essentially evaluated for toxicity and the extracts are usually tested for short or long term toxicity in animal models[6,7]. Nontoxic extracts possessing good bioactive principles may provide potential antimicrobial leads.

Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species; its scientific name is *Zingiber officinale* (*Z. officinale*)[8]. It

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is an erect perennial plant growing from one to three feet in height; its stem is surrounded by the shealthing bases of the two ranked leaves. A clublike spike of yellowish, purple lipped flowers has greenish yellow brackswhich rarely flowers in cultivation[9]. Ginger is truly a world domestic remedy. It is also used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, less of appetite, and rheumatism, in short for the same purposes as in ancient china[8]. In nineteenth century ginger serves as a popular remedy for cough and asthma when the juice of fresh ginger was mixed with a little juice of fresh garlic and honey[8]. A paste of powdered dried ginger was applied to the temples to relieve headache and fresh ginger was mixed with a little honey, tapped off with a pinch of burnt peacock feathers to alley nausea.

Garlic [*Allium sativum* (*A. sativum*)] belongs to the family Alliaceae. Its close relatives include the onion, shallot, and leek. It has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. The head of garlic (the most commonly used plant part) comprises numerous discrete cloves whereas the leaves and stems are sometimes eaten, particularly while immature and tender. Garlic has been used as medicine in many cultures for thousands of years, dating as far back as the time that the Egyptian pyramids. It is also claimed to help prevent heart diseases including atherosclerosis, high cholesterol, high blood pressure, and to improve the immune system as well as protection against cancer[10]. A daily dose of 1 mL/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream. Garlic's ability to prevent these oxidation reactions may explain some of its beneficial effects in atherosclerotic cardiovascular diseases.

The present study was aimed at determining the *in vitro* antibacterial activity of garlic cloves and ginger rhizomes ethanolic extracts on the isolates of Gram-negative organisms and Gram-positive with the view to finding alternative means of treating infections caused by them.

2. Materials and methods

2.1. Collection of plant materials

The fresh forms of and garlic cloves (*A. sativum*), ginger rhizomes (*Z. officinale*) used in this study were collected on January 2010, from Erode vegetables market, Erode, Tamil Nadu. They were identified in the Botanical Survey of India, Coimbatore. The fresh forms of these plants were made into pieces, air-dried and made into powdered forms using a clean pestle and a mortar of microbiology laboratory of the department.

2.2. Test isolates and their multiple antibiotic resistances (MAR) index

The test organisms were bacterial isolates obtained from Government hospital, Erode. They were isolated from clinical samples submitted by patients having suspected urinary tract and pus infections. Two Gram positive and five Gram negative organisms included in this study are indicated in Table 1. The isolates were subjected to Gram's staining and other biochemical tests according to standard procedures and identified as *Escherichia coli (E. coli)*, *Enterobacter* sp., *Pseudomonas aeruginosa (P. aeruginosa)*, *Proteus* sp., *Klebsiella* sp., *Staphylococcus aureus (S. aureus)* and B*acillus* sp[11]. The cultures were maintained in the department laboratory. The antibiotic susceptibility patterns of the test organisms were performed as per standard procedure and their MAR index was calculated by the ratio of number of antibiotics ineffective over the organisms to the number of antibiotics exposed[12,13]. The antibiotics used in the study were: ampicillin (A-10 μg), amoxicillin (Ac-30 μg), amikacin (Ak-30 μg), cephalothin (Ch-30 μg), chloramphenicol (C-30 μg), ciprofloxacin (Cf-5 μg), ceftriaxone (Ci-30 μg), clindamycin (Cd-2 μg), co-trimoxazole (Co-1.25 μg), erythromycin (E-15 μg), gentamycin (G-10 μg), kanamycin (K-30 μg), methicillin (M-5 μg), novobiocin (Nv-30 μg), nalidixic acid (Na-30 μg), norfloxacin (Nx-10 μg), ofloxacin (Of-5 μg), penicillin G (P-10 units), rifampicin (R-5 μg), streptomycin (S-10 μg), tetracyclin (T-30 μg) and vancomycin (Va-30 μg). The reference strains used for the study include *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *Enterococcus faecali (E. faecalis)* ATCC 29212, *Klebsiella pneumoniae (K. pneumoniae)* ATCC 12658, *Proteus mirabilis (P. mirabilis)* ATCC 35491, *Enterococcus cloacae* (*E. cloacae*) ATCC 10699 and *Bacillus subtilis* (*B. subtilis*) ATCC 31578.

2.3. Extraction of plant material

The extraction was carried out according to the method of Fatope *et al*[14]. In this, 20 g of the powdered plant samples were percolated at room temperature with 400 mL and 300 mL 95% ethanol for garlic cloves and ginger rhizomes ,respectively in 400 mL beakers (thus achieving 1:20 and 1:15 ratio, respectively). The beakers were covered with foil paper, shaken and left to stand for 2 weeks with regular shaking. After two weeks the suspensions were filtered and the filtrates were concentrated using Rotary-evaporating machine at 40 ℃. The extracts were labeled accordingly and stored in the refrigerator for further analysis.

2.4. Preparation of sensitivity discs

Discs of 6 mm in diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in Bijou bottles. The discs were then sterilized by autoclaving at 121 ℃ for 15 min after which they were allowed to cool.

Stock solutions of these garlic cloves and ginger rhizomes ethanolic crude extracts (that were recovered) were prepared by dissolving 0.5 g (*i.e.* 50 mg) of each of the two plant extracts in 5 mL of dimethyl sulphoxide. Therefore, each stock solution had a concentration of 10 000 mg/mL. from these stock four different concentrations of each the plants extract were prepared. These are 25 μg/mL, 50 μg/mL, 100 μg/mL, and 200 μg/mL which finally yielded disc potencies of 0.25 μg/disc, 0.5 μg/disc, 1.0 μg/disc and 2.0 μg/disc respectively. This was followed by introducing 100 sterile discs into each concentration. The discs were allowed to

absorb the solution and kept for further analysis. Each paper disc was capable of absorbing 0.01 mL[15].

2.5. Bioassay

The bioassay was carried out using the procedure described by Cheesbrough[16]. Using a sterile wire-loop 3-5 well isolated colonies of the test organism were touched and emulsified into about 3 mL of physiological saline. Turbidity of the suspension of test organism was compared with Mcfarland turbidity standard. Using a sterile swab stick, the test organism was inoculated onto sterile prepared nutrient agar media. The inoculated plates were then allowed to stay for about 3-5 min for the surface of the agar to air-dry. Prepared discs of the four different concentrations (*i.e.* 25 μg/mL, 50 μg/mL, 100 μg/mL and 200 μg/mL) for each of the garlic cloves and ginger rhizomess ethanolic extracts were then placed on the inoculated nutrient agar media. Within 30 min of discs application, the plates were inverted and incubated aerobically at 37 ℃ for 24 h. Ciprofloxacin was used as control. After overnight incubation, the plates were observed or examined for zones of inhibitions. The zones of inhibition were measured in mm using a plastic ruler (HiMedia, Mumbai). The end of inhibition is where the growth starts.

2.6. Minimal inhibitory concentration (MIC)

Serial tube dilution technique was used to determine MIC[17]. The garlic cloves and ginger rhizomes crude extracts were serial diluted in the range from 100 to 1 000 μg/mL. The tubes were inoculated with 100 μL of bacterial culture at a concentration of 10^6 cells/mL. Standard antibiotic ciprofloxacin was included in the assay for comparison. Nutrient broth with the inoculums only was used as growth control. All experiments were carried out in triplicates. The tubes were incubated aerobically at 37 ℃ or 12-18 h. The growths of inoculum were decrease in next dilution was taken as MIC value.

3. Results

The MAR index value of the test organisms are reported in Table 1. The MAR value is a ration of the number of effective antibiotics to that of effective antibiotics tested against the different number of isolates. Due to the over-usage of antibiotics[18], mutation and environmental stress the antibiotics resistant organisms have become a major challenge in hospital acquired infections. This often causes difficulties for the treatment of microbial diseases.

In the present investigation, the garlic cloves extracts exhibited high degree of inhibitory activity against most of the seven tested organisms followed by ginger rhizomes extracts. Among the clinical pathogens *P. aeruginosa*, *E. coli* and *Bacillus* sp. were the most susceptible followed by *Proteus* sp., *S. aureus* and *Enterobacter* sp. and *Klebsiella* sp. were the least inhibited by the both garlic and ginger extracts. The diameter of zone of growth inhibition varied between 7 mm and 19 mm (in garlic) and 4 and 16 mm (in ginger) (Table 2). The garlic cloves ethnaolic extract showed highest diameter of zone of inhibition of 19.45 mm against *P. aeruginosa* followed by *E. coli* (18.50 mm) and *Bacillus sp.* (16.5 mm). The garlic cloves ethanolic extract showed almost

Table 1
MAR index value of antibiotic resistance bacterial isolates used in this study.

Clinical isolates	MAR value[1]	No. of ineffective antibiotics	Name of ineffective antibiotics[2]
E. coli	0.50	11	A, Ac, Ci, G, Of, P, C, Cd, Ak, Ch, T
Enterobacter sp.	0.64	14	S, T, Nv, C, Va, E, M, A, Ac, Na, Nx, K, Co, Cf
P. aeruginosa	0.32	7	A, Ac, Ci, G, Of, P, C
Proteus sp.	0.32	7	A, Ac, Ci, G, Of, P, C
Klebsiella sp.	0.36	8	A, Ac, Ci, G, Of, P, C, Cd
S. aureus	0.64	14	S, T, Nv, C, Ch, M, A, Ac, Na, Nx, P, Ci, G, Ch
Bacillus sp.	0.41	9	Na, E, Va, T, Of, Nx, Ci, C, Ak

Note: [1]Multiple antibiotic resistance (MAR) value near to 1 indicates the antibiotics are ineffective; [2]Antibiotics abbreviations: Ampicillin (A-10 μg), Amoxicillin (Ac-30 μg), Amikacin (Ak-30 μg), Cephalothin (Ch-30 μg), Chloramphenicol (C-30 μg), Ciprofloxacin (Cf-5 μg), Ceftriaxone (Ci-30 μg), Clindamycin (Cd-2 μg), Erythromycin (E-15 μg), Gentamycin (G-10 μg), Kanamycin (K-30 μg), Methicillin (M-5 μg), Novobiocin (Nv-30 μg), Nalidixic acid (Na-30 μg), Norfloxacin (Nx-10 μg), Ofloxacin (Of- 5 μg), Penicillin G (P-10 units), Streptomycin (S-10 μg), Tetracyclin (T-30 μg) and Vancomycin (Va-30 μg).

Table 2
Antibacterial zone of inhibition (mm, in diameter) of garlic cloves ethanolic extracts against clinical isolates.

Clinical isolates	Zone of inhibition in diameter (mm)			
	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
E. coli	10.50±0.21	15.00±0.40	17.00±0.58	18.50±0.29
Enterobacter sp.	8.50±0.42	11.00±0.20	12.00±0.34	13.50±0.50
P. aeruginosa	13.50±0.70	15.50±0.89	17.10±0.55	19.45±0.68
Proteus sp.	9.50±0.26	11.50±0.50	12.00±0.60	13.65±0.48
Klebsiella sp.	0.00±0.00	7.50±0.50	9.00±0.60	11.50±0.48
S. aureus	10.30±0.42	12.65±0.45	13.52±0.56	14.55±0.20
Bacillus sp.	11.00±0.32	13.50±0.55	14.52±0.66	16.55±0.25

Table 3
Antibacterial activity of ginger rhizomes ethanolic extracts against clinical isolates.

Clinical isolates	Zone of inhibition in diameter (mm)			
	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
E. coli	8.50±0.12	12.00±0.30	13.50±0.48	15.50±0.30
Enterobacter sp.	0.00±0.00	4.00±0.20	5.00±0.14	5.50±0.40
P. aeruginosa	10.40±0.60	13.50±0.59	14.10±0.55	14.45±0.66
Proteus sp.	10.50±0.26	11.50±0.40	12.50±0.50	13.55±0.45
Klebsiella sp.	0.00±0.00	0.00±0.00	5.00±0.30	7.50±0.50
S. aureus	9.30±0.32	11.55±0.55	12.52±0.50	13.55±0.20
Bacillus sp.	10.00±0.30	12.50±0.45	12.52±0.56	16.55±0.25

similar zone of inhibition of 13.50 mm in diameter against *Proteus* sp., *Enterobacter* sp. and *S. aureus* (Table 2). Taura *et al*[19] reported the activity of ethanolic extracts of garlic cloves on some gram negative bacteria.

The ginger rhizomes ethanolic extracts demonstrated antibacterial activity against five clinical isolates with zone of growth inhibition ranging from 4 mm to 16 mm. The maximum zone of inhibition was showed against *Bacillus* sp. (16.55 mm) followed by *E. coli* (15.50 mm) and *P.aeruginosa* (14.45 mm) (Table 3). The minimum diameter of zone of growth inhibition was recorded against *Klebsiella* sp. (5 mm) and *Enterobacter* sp. (4 mm) (Table 3). However, ginger has been used widely as herbal medicine. In particular, its gingerol-related components have been reported to possess antimicrobial and antifungal properties, as well as several pharmaceutical properties[20]. The garlic and ginger is popular as plants and food ingredient for flavouring and adding acidity, its juices have been reported to exhibit antibacterial activity against wide range of microbes including *K. pneumoniae*, *Shigella flexnerii*, *E. coli* ATCC 25922 and *Vibrio cholerae*[21, 22].

Table 4
MIC of cured ethanolic extracts of both garlic cloves and ginger rhizomes.

Clinical isolates	MIC range (μg)	
	Garlic	Ginger
E. coli	65.50	75.60
Enterobacter sp.	110.80	185.50
P. aeruginosa	58.50	67.00
Proteus sp.	89.00	70.20
Klebsiella sp.	160.20	185.58
S. aureus	78.90	68.45
Bacillus sp.	80.10	74.50

The MIC values of crude extracts and garlic cloves were 65.50, 110.80, 58.50, 89.00, 160.20, 78.90 and 80.10 μg/mL against *E. coli*, *Enterobacter* sp., *P. aeruginosa*, *Proteus* sp., *Klebsiella* sp., *S. aureus* and *Bacillus* sp., respectively (Table 4). The MIC were as low as 58.50 μg/L of an extracts against gram negative bacteria is suggestive of best antibacterial potential of the bioactive principles of garlic extracts. In case of MIC values of ginger rhizomes crude extracts was comparatively high (67.0 μg/mL) against *P. aeruginosa* (Table 4). Hence the crude extracts of garlic cloves may yield potential molecules in the treatment of infections caused by the pathogenic bacteria.

4. Discussion

The growing population concern about health problems has recently led to the development of natural antimicrobials to control microbial diseases. Medicinal plants and spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for controlling common health complications. Natural plant product based antimicrobials drug discovery attained paramount importance as newly discovered drugs are likely to be effective against multi drug resistant microbes.

According to earlier reports garlic has traditional dietary and medicinal applications as an anti-infective agent[23]. *In vitro* evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria[24], fungi and viruses[25] supports these applications. Allicin, the active ingredient of garlic, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target[26]. Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity[27-32].

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids[33]. It is interesting to note that even crude extracts of these plants showed good activity against multidrug resistant strains where modern antibiotic therapy has limited effect. The effect of these spices on these organisms in vivo cannot be predicted from this study. And though paper disk assays a practical approach to study potential antibacterial compounds, using the size of inhibition zone to indicate relative antibacterial activity is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization; and thus the results could be affected. Thus, there is a need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some plants is preserved and also to provide scientific evidence for their efficacies.

To conclude, there is wide body of scientific evidence to show that garlic and ginger has great potential in the treatment of many microbial diseases. We prophesize that this plants has an extraordinary potential to yield biologically active materials which could be valuable in the treatment of many microbial diseases and this should be fully explored in proper approach. However, it is necessary to isolate the active constituents, and determine their toxicity, side effects and pharmaco-kinetic properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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