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In-Vitro evaluation of antimicrobial activity of *Allium sativum* and *Zingiber Officinale* against multi-drug resistant clinical pathogens.

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Abstract

Background: Clinically significant microbes have evolved over time and developed such mechanisms to protect themselves from synthetic drugs. This study aims to determine the antimicrobial activity of culinary condiments against multi-drug resistant clinical pathogens.

Methodology: The antimicrobial activity of *Allium sativum* (garlic) and *Zingiber Officinale* (ginger) was assessed against clinical pathogens such as E. coli, P. aeruginosa, and S. aureus with the aid of wet experiments, namely disc diffusion method, and well agar technique.

Results: It was observed that both *A. sativum* and *Z. officinale* in their neat form were very effective against tested pathogens ranges from 16 ± 0.623 mm to 36 ± 0.816 mm and 19 ± 0.707 to 20 ± 1.414 mm respectively as compared to the other concentrations with peptone water and distal water. However, extract *A. sativum* showed more antibacterial strength than *Z. officinale*.

Conclusion: Natural antimicrobial agents obtained from herbs and spices could be effective alternatives to treat various infections with minimum to no side effects.

Keywords

A. sativum, *Zingiber Officinale*, Multidrug Resistance, Antibacterial Activity.

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Introduction

Herbs and spices have been used from an ancient period as culinary condiments. Because of their exceptional aroma and the boost that adds a particular taste to the dish, it makes the food more tempting. Not only for culinary purposes but these condiments were also utilized to treat various ailments and help to protect the degeneration of cells that are responsible for osteoporosis and rheumatoid arthritis. They also possess anti-inflammatory, cleansing, and detoxifying attributes[1]. The most common herbs and spices including ginger, garlic, bay leaf, fennel, cilantro, onion, turmeric, coriander, black seeds, thyme, etc. offer several health benefits like relieving various gastrointestinal disturbances. Many of them contain medicinal properties and have been consumed as a traditional remedy for centuries[2].

The ginger belongs to the family Zingiberaceae and is scientifically called *Zingiber Officinale*[3]. Ginger is officially associated with tropical rainforest regions of the Indian subcontinent and extended towards Southern Asia countries with slight variations in the genetic makeup of *Z. officinale*[4]. Basically, a warm climate is crucial for the growth of *Z. officinale*, thus easily grown and cultivated in Taiwan, India, Nigeria, Bangladesh, and Jamaica. *Z. officinale* was reportedly used as a folk medicine to treat different digestive problems, nausea, bleeding, and rheumatism[5]. *Z. officinale* was also responsible for relieving toothache, treating alopecia, snake bite, and different respiratory conditions[6]. African locals consume *Z. officinale* to protect from mosquito bites[3], while in Arabs, *Z. officinale* is used as a medicine to increase the intimacy and treatment for sexual dysfunction[7]. On the other hand, garlic is scientifically named *Allium sativum* and the relative member of onion, leek, and shallot. The enchanted medicinal qualities of *A. sativum* to treat various sicknesses have been written on the ancient temples of Egypt. It is the oldest of all spices and cultivated in Central Asian states[8,9]. Nigerians used *A. sativum* to treat abdominal discomfort, diarrhea, and respiratory disorders[10]. *A. sativum* was reportedly found effective in treating hay fever, cold, and asthma in India and several countries in Europe[11]. The use of *A. sativum* in cooking is never getting old and is famous for its aroma, spiciness, and also the major components of most of the meat-related cuisines in modern cooking. Allicin is the compound present in the *A. sativum* responsible for fighting cancer and has anti-inflammatory and antimicrobial properties, thus protecting the host body from infectious agents[12,13]. The process of cooking removes allicin from *A. sativum*, causing subtleness in the spiciness, while raw crushed *A. sativum* has a substantial amount of allicin[14].

Essential oils and extracts of medicinal plants have phytochemicals; therefore, they are rich in antioxidants antimicrobials properties. Several researches were documented on the preservative quality of herbs and spices to prevent food from microbial spoilage[15-17]. Nowadays, choices have been inclined towards botanical preparation as plant-derived medicines were successfully treating several ailments with great effectiveness and less or no side effects as compare to synthetic drugs[18,19].

To fight against the problem of multidrug resistance that has currently becomes the most challenging issue for the scientist. Traditional herbs and spices that have germicidal characteristics might be favorable to combat pathogens. In the present study, the antimicrobial strength of *Z. officinale* and *A. sativum* extracts have been evaluated towards clinical pathogens.

Methodology

Samples Collection

The test organisms were the bacterial isolates, collected from Al Mustafa Hospital Karachi. Two gram-negative and one-gram positive organisms, namely Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. The cultures were obtained and isolated from the patient's clinical samples suffering from the urinary tract, pus, and throat infection, respectively. The cultures were further confirmed by the gram staining and biochemical test[20].

Preparation of herbal extract

Fresh 100 grams of *Allium sativum* (garlic cloves) and *Zingiber Officinale* (ginger rhizome) were obtained from the local vegetable market. They were cleaned, peeled and sliced, and homogenized using a clean and sterile mortar pestle under aseptic conditions. Obtained extracts were then filtered with Whatman filter. The extract was considered as 100% concentration of the extract. The concentrations of 25 %, 50 %, and 75 % were prepared by adding appropriate amounts of distilled water and peptone water[21].

Antibiotic Sensitivity via disc diffusion method

Pure cultures of *S. aureus*, *E. coli*, and *P. aeruginosa* maintained in brain heart infusion agar were subjected to antibiotic susceptibility via disc diffusion method[22]. Using a sterile wire loop, the test organism was inoculated in 5 ml nutrient broth and was compared with the McFarland index. This bacterial suspension was swabbed on the surface of sterile MHA plates to make a uniform lawn. Subsequently, antibiotic discs were aseptically placed on MHA plates seeded with the respective test microorganism. The plates were incubated at 37°C for 24 hours. The inhibition zone was observed on the very next day.

Antimicrobial activity of *Z. officinale* and *A. sativum* extracts via well diffusion technique.

Pure cultures of *S. aureus*, *E. coli*, and *P. aeruginosa* maintained in brain heart infusion agar were subjected to the antimicrobial activity of *Z. officinale*, and *A. sativum* extracts via agar well diffusion method[23]. With the help of a swab, lawning was done on the MHA plates with the test strains. Afterward, the wells were prepared using a sterile cork borer of approximately 6 mm diameter. The 50 µL extracts of different concentrations from 25 %, 50 %, 75 %, and 100 % were added to the wells with the help of a micropipette. The plates were incubated overnight, and the zone of inhibition was measured after incubation.

Phenotypic Characterization of Biofilm Formation

The test strains were also screened phenotypically by Congo red agar method. The agar plates were prepared by adding 0.8 g Congo red and 36 g of Saccharose to 1 liter of blood agar. After inoculation of the test organisms, the macroscopic characteristics of the isolates were observed in CRA plates after incubation[24].

Statistical analysis

All experiments were performed in triplicates, and outcomes of the study were analyzed using SPSS version 22.0. The results were presented as Mean ± SD.

Results

Antimicrobial activity of *A. sativum* and *Z. officinale* was observed in this study against the clinical isolates, namely *S. aureus*, *E. coli*, and *P. aeruginosa*. The antibiotic sensitivity pattern are determined as shown in table 1, and their biofilm production can be seen in table 2. *S. aureus* and *P. aeruginosa* were sensitive to all the six tested antibiotics with inhibition zone ranges between 11 mm to 22 mm and 15 mm to 30 mm, respectively. In the case of *S. aureus*, the most effective antibiotic was levofloxacin, while for *P. aeruginosa*, the highest inhibition zone was produced by Ciprofloxacin. On the other hand, *E. coli* showed resistance towards Erythromycin, Ampicillin, Cefotaxcin, and Levofloxacin. However, Piperacillin and Cefotixin have successfully inhibited the growth of *E. coli* with 19 mm and 11 mm zone of inhibition, respectively.

Table 1: Antibacterial sensitivity pattern of clinical isolates.

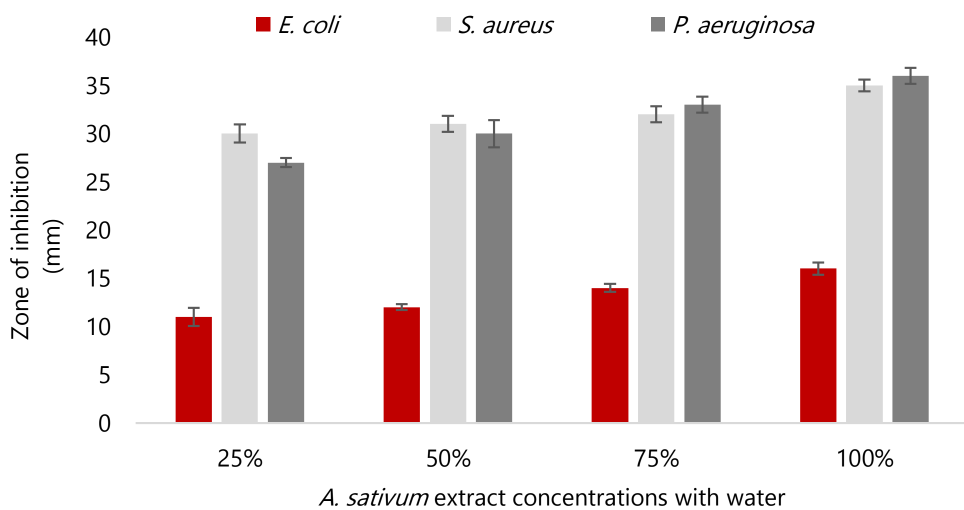
Antibiotics	Tested bacteria Zone of inhibition recorded in millimeter (mm)		
	P. aeruginosa	***E. coli***	***S. aureus***
Erythromycin (E)	NA	R	14 ± 0.0
Ampicillin (AMP)	24 ± 0.0	R	15 ± 0.0
Piperacillin (TZP)	28 ± 0.0	19 ± 0.0	21 ± 0.0
Azithromycin (AZI)	17 ± 0.0	NA	11 ± 0.0
Levofloxacin (LEV)	26 ± 0.0	R	22 ± 0.0

Cefoxitin (FOX)	NA	11 ± 0.0	11 ± 0.0
Vancomycin (VAN)	15 ± 0.0	NA	NA
Ciprofloxacin (CPFX)	30 ± 0.0	NA	NA
Ceftriaxone (CTX)	NA	R	NA

NA: Not Available, R: Resistant.

Table 2: Biofilm production of tested clinical strains.

Bacterial strain	Source	Biofilm production on Congo red agar
P. aeruginosa	Pus	Negative
S. aureus	Throat swab	Highly positive
E. coli	Urinary Tract Infection	Negative



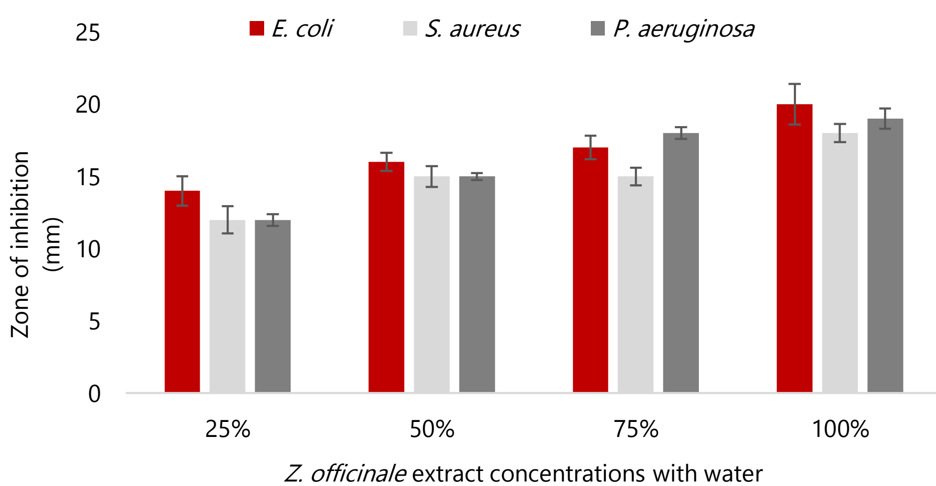


Figure 1a & b: Antibacterial effect of variable concentrations of extracts against clinical pathogens in water.

Various concentrations of *A. sativum* and *Z. officinale* were prepared by using distilled water and peptone water separately to analyze the antibacterial strength against tested clinical isolates. As shown in Figure 1a, *A. sativum* with water at 25 %, 50 %, & 75 % concentrations demonstrated remarkable results against all 3 clinical strains. At 25 %, the highest ZOI was recorded against *S. aureus*, i.e., 29.57 mm; at 50 %, the highest inhibition zone was 31 mm against *S. aureus*, and at 75 %, 32.55 mm ZOI against *P.aeruginosa* was recorded. As compared to *A. sativum*, *Z. officinale*, with water produced promising effects with ZOI ranges between 12.4 mm to 17.66 mm. The highest ZOI was 14.25 mm at 25 %, 16 mm at 50 % against *E.coli*, and 17.66 mm at 75% against *P. aeruginosa* (Figure 1b). Besides, both *A. sativum* and *Z. officinale* in their undiluted form (neat) produced greater antibacterial activity towards tested bacteria compared to diluted ones. *A. sativum*, in its neat form, produced ZOI up to 36 mm against *P. aeruginosa*, followed by 34.55 mm towards *S. aureus*, and 16 mm against *E. coli* (Figure 1a and b).

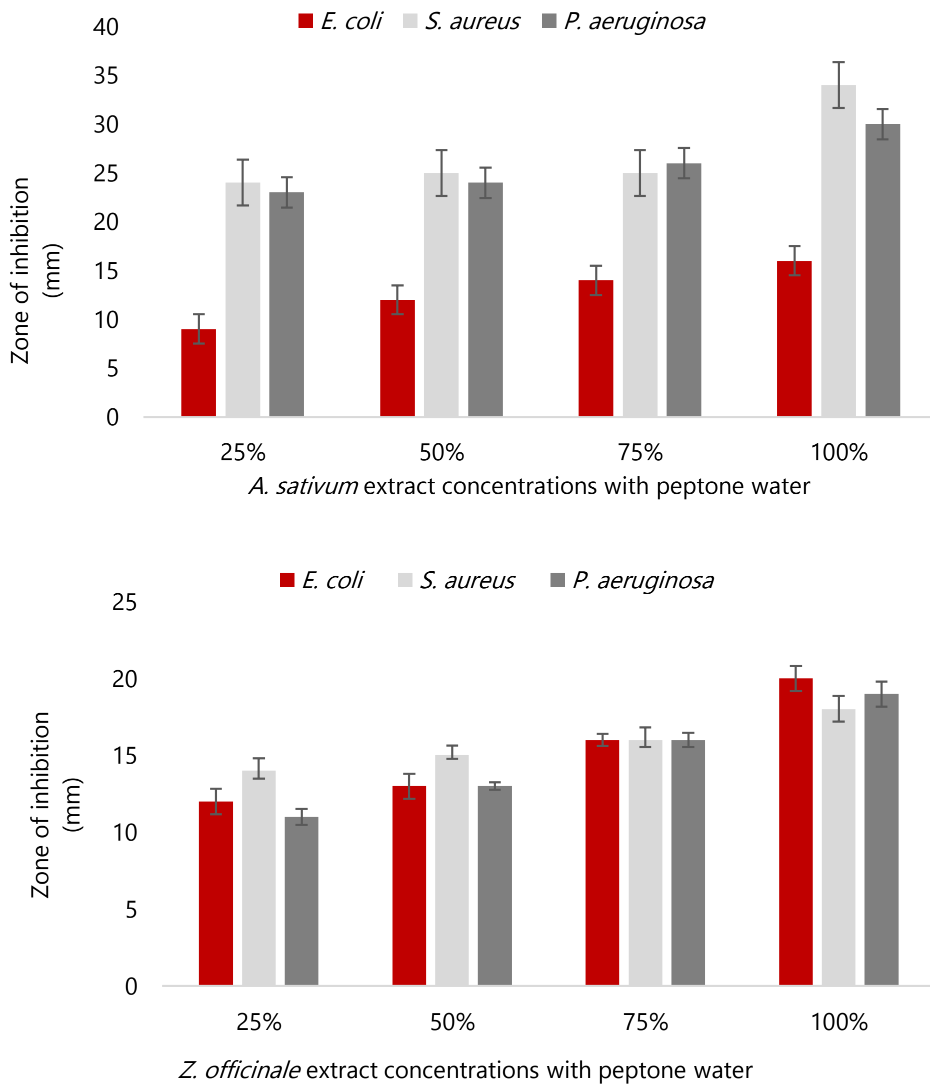
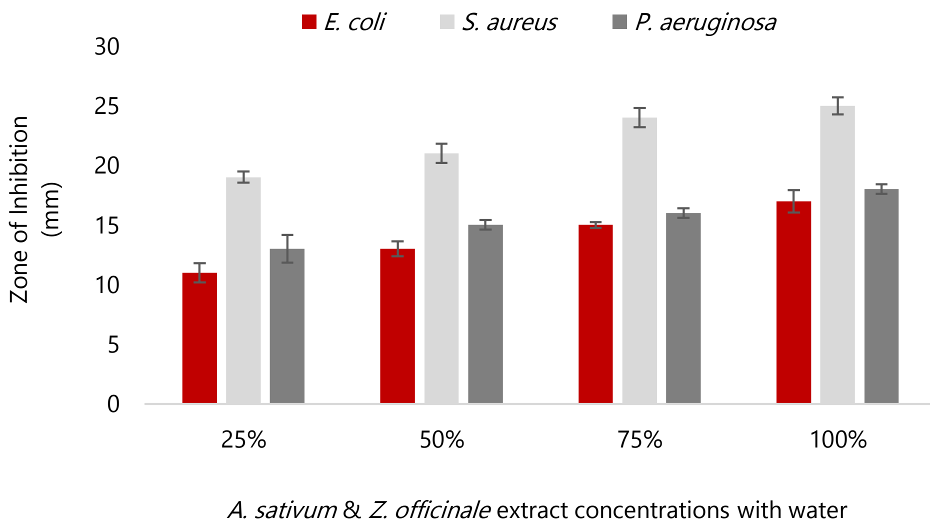


Figure 2a & b: Antibacterial effect of variable concentrations of extracts against clinical pathogens in peptone water.

A. sativum with peptone water at various concentrations also showed antibacterial strength against selected bacterial strength with minimum ZOI 9 mm was noted against *E. coli* at 25 %, and the highest inhibition zone was 26 mm at 75% against *P. aeruginosa*. While *Z. officinale* with peptone water showed decent results and retard the growth of all selected bacterial strains. The ZOI was ranged between 11 mm to 16 mm, as shown in Figure 2a and 2b.



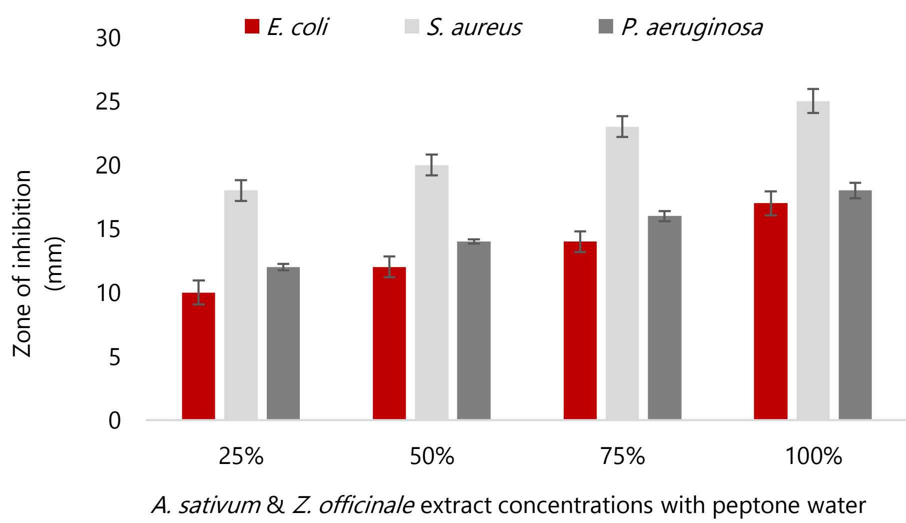


Figure 3a & b: Antibacterial effect of variable concentrations of extracts against clinical pathogens in water and peptone water.

The combination of *Z. officinale* and *A. sativum* with water and peptone water separately had more or less similar effects on clinical stains; thus, no significant difference between (ginger + water), (ginger + peptone water), (garlic + water), and (garlic + peptone water) was observed. Likewise, the combination of undiluted *A. sativum* and *Z. officinale* demonstrated an antagonistic effect on the tested bacteria. As *A. sativum* alone produced ZOI more than 30 mm against *P.aeruginosa* and *S. aureus.* It might be possible that *Z. officinale* blocks or interfere with the interaction of *A. sativum* and bacteria (Figure 3a and 3b).

Discussion

The antimicrobial activity of *A. sativum* and *Z. officinale* was evaluated against *S. aureus*, *E. coli*, and *P. aeruginosa*. Both *A. sativum* and *Z. officinale* demonstrated antibacterial properties, but *A. sativum* was more effective as compared to *Z. officinale*. From the previous years, herbs and spices have been explored extensively by researchers in terms of their medicinal, antimicrobial, antioxidant, and anti-cancerous properties. Therefore, the extract of *A. sativum* and *Z. officinale* can prevent the host body from several communicable and non-communicable illnesses. Medicinal plants are the pool enriched with bioactive substances that have gained the interest of scientists in real application of these compounds on an industrial scale, including cosmetics, pharmaceutics, and in food business[3,25].

Both freshly crushed and freeze *A. sativum* have shown its germicidal characteristics in wet methods reported. It has also been reported in results of different studies[26,27] that supports our study. Major bioactive compounds in *A. sativum* have a different mode of action, such as allicin has a partial effect on DNA while completely inhibiting RNA production[28]. On the other hand, organosulfur and phenolic compounds also play an important role in the antimicrobial activity of *A. sativum* documented in various researches[29-32]. In a study, the minimal inhibitory concentrations of *A. sativum* and *Z. officinale* against *E. coli* were 65.50 µg/mL and 75.60 µg/mL, for *P. aeruginosa* were 58.50 µg/mL and 67.00 µg/mL, and *S. aureus* was 78.90 µg/mL and 68.45 µg/mL respectively[33].

Bioactive compounds, for example, tanins, saponins, flavonoids, essential oils, and phenolic compounds, are believed to possess substantial antimicrobial strength[34]. The noteworthy point is that the raw form of these extracts also contains remarkable bactericidal, fungicidal, and virucidal activity more than commercially marketed antimicrobial agents to which resistance has been developed. Similarly, the active compounds in *Z. officinale* were assumed to be zingiberol, zingiberine, and bisabolene. Basically, the crystal of gingerone accounts for *Z. officinale*'s acidity and produces a bacteriostatic effect on *S. aureus* and *B. subtillus*, and also cures dermatological problems[35]. In vivo effect cannot be anticipated on the basis of In-vitro outcomes of these spices. Well, the diffusion technique was used to determine the antibacterial strength, but this activity could be influenced by certain factors like solubility, diffusibility, viscosity, and volatilization of extracts[34]. Thus there is a need for further investigation of these culinary condiments to retrieve curative knowledge that could be the possible replacement of synthetic drugs with least or no side effects.

Conclusion

Hence it is inferred that *A. sativum* and *Z. officinale*, in their neat form, showed the highest antimicrobial efficacy as compared to other diluted concentrations. *A. sativum* proved to be more competitive against selected bacterial strains than *Z. officinale*. Moreover, all the tested concentrations of *A. sativum* and *Z. officinale* produced a satisfactory zone of inhibition towards tested clinical strains and could be applied in making different formulations to use as a medicinal drug.

Conflicts of Interest

The authors have declared that no competing interests exist.

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References

1. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol. 2002;40(8):1091-1097.

2. Panpatil VV, Tattari S, Kota N, Nimgulkar C, Polasa K. In vitro evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic. J pharmacogn phytochem. 2013;2(3):143-148.
3. Dilis V, Trichopoulou A. Antioxidant intakes and food sources in Greek adults. J Nutr. 2010;140(7):1274-1279.
4. Everett TH. The New York botanical garden illustrated encyclopedia of horticulture. Taylor & Francis; 1982:1-3204.
5. Afzal M, Al-Hadidi D, Menon M, Pesek J, Dhami MS. Ginger: an ethnomedical, chemical and pharmacological review. Drug Metab Pharmacokinet. 2001;18(3-4):159-190.
6. Duke JA, Ayensu ES. Medicinal Plants of China. 1300 Strichzeichnungen. Reference Publ., Inc. Algonac. Michigan. 1985;2(705):1-398.
7. Qureshi S, Shah AH, Tariq M, Ageel AM. Studies on herbal aphrodisiacs used in Arab system of medicine. Am J Chinese Med. 1989;17(01n02):57-63.
8. Jognne B, Linda AA, David PJ. Herbal medicines-A guide for Health care professionals. Pharmaceutical Press. 1996.
9. Block E. Allium botany and cultivation, ancient and mdern. Garlic and other alliums: the lore and the science. 2010;2010:1-32.
10. Bradley PR, editor. A Handbook of Scientific Information on Widely Used Plant Drugs: Companion to Vol. 1 of the British Herbal Pharmacopoeia. Brit Herb Med Assoc. 1992;1992:1-239.
11. Imo C, Za'aku JS. Medicinal properties of ginger and garlic: A review. Curr Trends Biomed. Eng Biosci. 2019;18:47-52.
12. Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol. 2005;15(10):929-934.
13. Lau BH, Lam F, Wang-Cheng R. Effect of an odor-modified garlic preparation on blood lipids. Nutr Res. 1987;7(2):139-149.
14. Jabar MA, Al-Mossawi A. Susceptibility of some multiple resistant bacteria to garlic extract. Afr j biotechnol. 2007;6(6): 771-776.
15. Patil SD, Kamble VA. Antibacterial activity of some essential oils against foodborne pathogen and food spoilage bacteria. Int J Pharm Biosci. 2011;2:143-150.
16. Batool R, Imran M, Kandhro AH, Salahuddin N, Uddin MKH. Resistance Patterns among Multidrug-Resistant Tuberculosis Patients: A Multi-Center Study from Pakistan. Int. j. endorsing health sci. re. 2017;5(4):7-10.
17. Keskin D, Toroglu S. Studies on antimicrobial activities of solvent extracts of different spices. J Environ Biol. 2011;32(2):251-265.
18. Mehboob A, Abbas T. Synergistic Effect of the Combination of Commercial Essential Oils with Standard Antibiotics: In vitro Evaluation. Biological Sciences-PJSIR. 2020;63(3):242-252.
19. Sachindra NM, Airanthi MK, Hosokawa M, Miyashita K. Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds. J Food Sci Technol. 2010;47(1):94-99.
20. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press. 2005:1-379.
21. Chand B. Antibacterial effect of garlic (*Allium sativum*) and ginger (*Zingiber Officinale*) against staphylococcus aureus, salmonella typhi, escherichia coli and bacillus cereus. J Microbiol Biotechnol. 2021;2021:2481-2491.
22. Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr. 2008;63(1):15-20.
23. Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A, Gharsallah N. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arab J Chem. 2019;12(8):3075-3086.
24. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989;42(8):872-874.
25. Nirmala K, Prasanna Krishna T, Polasa K. Protective effect of ginger against Benzo (a) pyrene induced DNA damage. Int J Cancer Res. 2007;3(1):13-24.
26. Rees LP, Minney SF, Plummer NT, Slater JH, Skyrme DA. A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World J Microbiol Biotechnol. 1993;9(3):303-307.
27. Weber ND, Andersen DO, North JA, Murray BK, Lawson LD, Hughes BG. In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. Planta Med. 1992 Oct;58(05):417-423.
28. Eja ME, Asikong BE, Abriba C, Arikpo GE, Anwan EE, Enyi-Idoh KH. A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organisms. Southeast Asian J Trop Med Public Health. 2007;38(2):343.
29. Oyedemi SO, Afolayan AJ. Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of Schotia latifolia Jacq. Asian Pac j trop med. 2011;4(12):952-958.
30. Raja RD, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected

ethnomedicinal plants from South India. Asian Pac j trop med. 2011;4(5):375-378.

31. Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of Mentha arvensis L. Asian Pac j trop med. 2011;4(3):196-200.
32. Nweze EI, Ezute S, Emeka NC, Ogbonna CC, Eze C. Bacteria etiological agents causing respiratory tract infections in children and their resistance patterns to a panel of ten antibiotics. Asian Pac j trop med. 2012;2(1):18-23.
33. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber Officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. 2012;2(8):597-601.
34. Aboaba OO, Efuwape BM. Antibacterial properties of some Nigerian species. Bio Res Comm. 2001;183-188.
35. Joe MM, Jayachitra J, Vijayapriya M. Antimicrobial activity of some common spices against certain human pathogens. J Med Plant Res. 2009;3(12):1134-1136.