

Antimicrobial Activities of Heated Extracts of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) on Some Selected Pathogens

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Abstract: Many of the spices used daily in our homes have been documented to be antimicrobial and have medicinal value as well. Spices such as garlic and ginger have been used as antimicrobial agents in their raw form for the treatment of wounds, injuries and joint pains. The present study investigated the antimicrobial activity of heated extracts (ethanolic and aqueous) of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on some selected pathogens using the agar-well diffusion assay method. Three different concentrations of the extracts (i.e. 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml) were prepared and used for the study. The antimicrobial activity of these heated extracts was tested against *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albican*, and *Proteus mirabilis* at different concentrations of the extracts. The results showed that neither the heated ethanolic extracts nor the heated aqueous extracts of *A. sativum* had antimicrobial activity against any of the test organisms. Similarly, heated aqueous extracts of *Z. officinale* showed no activity against any of the test organisms. Only the heated ethanolic extracts of *Z. officinale* showed some level of activity against *C. albicans* (8.5 – 11.0 mm) and *S. typhi* (7.0 – 14.5 mm). The findings of the study were attributed to the high temperatures used during the extraction phase, which may have caused the denaturing of some bioactive compounds resulting in the ineffectiveness of the extracts against the test microorganisms. It is therefore suggested that, garlic and ginger should not be heated or cooked if they are to be used for medicinal purposes.

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1. Introduction

In many developing countries, there are treasures of traditional medicine and traditions concerning naturally occurring drugs, based on the empirical knowledge of medicinal and toxic plants, gained by the ancestors and passed on from generation to generation by oral tradition (Martins *et al.*, 1998). Populations throughout Africa, Asia and Latin America use traditional medicine to help meet their primary health care needs. The usage of medicinal plants have been given much importance due to its less side-effects, better patient tolerance, cheap cost, easy accessibility and wide range of applications (Kumar *et al.*, 2012; Tepe *et al.*, 2004; WHO, 2002). In many developed countries, 70 to 80% of the population have used some form of alternative or complementary medicine in parallel to allopathic medicine particularly for treating and managing chronic diseases (WHO, 2008; WHO, 2002). According to WHO (2008), 80% of African populations use some form of traditional herbal medicine. In Ghana, about 70% of the population depends exclusively on traditional medicine for their health care (WHO, 2001). There are several widely used indigenous forest species which are harvested from the wild and used as an ingredient for

herbal medicines including species such as *Allium sativum* (garlic) and *Zingiber officinale* (ginger) (FAO, 1996).

Dating back over 6000 years, garlic is a native to central Asia and has long been a staple in the Mediterranean region as well as a frequent seasoning in Asia, Africa and Europe (Rajsekhar *et al.*, 2013). Garlic is a bulbous perennial herb; closely related to the onion. It has a tall, erect flowering stem that reaches 2 – 3 feet in height. The plant has pink or purple flowers that bloom in mid to late summer. The part used medicinally is the bulb. Formerly classified in the lily (Liliaceae) family, garlic, is now a member of the family Alliaceae and include two basic types- hard neck and soft neck. Hard neck garlics are characterized by hard, woody central stalks that extend down to the basal plate at the bottom of the bulb. Softneck garlics have a non-woody pseudostem formed from overlapping leaf sheaths and rarely send up a flower stalk, unless stressed by climatic conditions (The Herb society of America, 2006).

Garlic is therapeutically effective because of its oil and water soluble organosulfur compounds (Mukhtar and Ghori, 2012). Thiosulfinates is mainly responsible for its antibiotic activity because extracts of

garlic free of thiosulfates normally lose their antimicrobial capacity (Hughes and Lawson, 1991). Garlic is also claimed to help prevent heart diseases such as atherosclerosis, high cholesterol and high blood pressure; and certain types of cancer including stomach and colon cancers (Rajsekhar *et al.*, 2013).

Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice (Akintobi *et al.*, 2013). It is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species (Newall *et al.*, 1996). The ginger plant has a long history of cultivation known to originate in China and then spread to India, South-East Asia, West Africa and the Caribbean (Rajsekhar *et al.*, 2013). *Z. officinale* is an erect perennial plant growing from one to three feet in height; its stem is surrounded by the sheathing bases of the two ranked leaves. A club like spike of yellowish, purple lipped flowers has greenish yellow bracts which rarely flowers in cultivation (Tyler, 2002).

The main constituents of ginger are sesquiterpenoids, with zingiberene as the main component. Other components include β -sesquiphellandrene bisabolene and farnesene which are also sesquiterpenoids (β -sesquiphellandrene, cineol and citra) (Opdyke, 1974; O'Hara *et al.*, 2006; Rajsekhar *et al.*, 2013). The volatile oil gingerol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation (Longe *et al.*, 2005; Omoya and Akharaiyi, 2012). Fresh ginger has been used for cold-induced diseases such as, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite, and rheumatism. Ginger is truly a world domestic remedy (Foster, 2009).

Several studies have shown that, extracts of garlic and ginger have antibacterial activity against many Gram-positive and Gram-negative bacteria (Yusha'u *et al.*, 2008; Mukhtar and Ghori 2012; Omoya 2012; Akintobi *et al.*, 2013). Other studies have confirmed that they are not only effective against bacteria but also possess antiviral and antifungal activity (Sivam *et al.*, 1997; Ankri and Mirelman, 1999; Whitmore and Naidu, 2000; Ross *et al.*, 2001).

Spices are normally used either in the process of cooking or after it, to garnish or to mask undesired flavor (Rajsekhar *et al.*, 2013). It is however not known whether the heat used during the cooking process has any effects on the antimicrobial potency on spices. Therefore, there is the need for detailed scientific study to investigate whether the heat used during cooking has any effect on the efficacy of these spices against microorganisms. It is against this backdrop that the present study sought to investigate the antimicrobial

activity of heated extracts (ethanolic and aqueous) of garlic and ginger on some selected pathogenic organisms namely, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albican*, and *Proteus mirabilis*.

2. Materials and Methods

2.1. Collection and Identification of Plants

Samples of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) were obtained from the madina market in Accra, Ghana. They were placed in separate clean mesh bags and transported immediately to the laboratory of the Centre for Scientific Research into Plant Medicine (CSRPM) in Mampong-Akuapem, Ghana. The samples were identified and authenticated at the plant Development Unit of the Centre by comparing them with known samples deposited at the herbarium of the department. The evaluation of antimicrobial properties of *A. sativum* and *Z. officinale* was done in the microbiology laboratory of the CSRPM.

2.2. Test Microorganisms

The microorganisms used to test the antimicrobial activity of ethanolic and aqueous extracts of *A. sativum* and *Z. officinale* were *Staphylococcus aureus* (ATCC25923), *Salmonella typhi* (ACTCC194300), *Klebsiella pneumonia* (ATCC33495), *Escherichia coli* (ATCC25922), *Candida albican* (ATCC10231), and *Proteus mirabilis* (ATCC49565). Standard strains of these microorganisms were obtained from the microbiology laboratory of CSRPM.

2.3. Preparation of Heated Extracts from Garlic and Ginger

The plant materials were washed with clean water, chopped, and partially allowed to air-dry in the shade at room temperature for three days in order to remove excess moisture and for colour development.

2.3.1. Heated Ethanolic Extract Preparation

A 400 g of *Z. officinale* and 400 g of *A. sativum* were cold macerated with 1000 ml each of 70% ethanol for four days. Both were kept on an orbital mixer for continuous agitation of the active compounds. The ethanolic extracts were concentrated using rotary evaporator (LABOROTIA 4000, Germany) at a temperature of 65°C. The concentrates obtained were reconstituted in 500 ml distilled water and re-concentrated to ensure complete removal of residual ethanol. The resulting concentrates were poured into flasks and lyophilized using a Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 gr. Germany) for 24 hours. The dried powders were stored in air-tight containers and refrigerated until needed.

2.3.2. Heated Aqueous Extracts Preparation

Three hundred (300 g) of *Z. officinale* and *A. sativum* each were boiled in 300 ml of water at 100 °C for about 45 minutes. The resulting extracts were

concentrated at reducing temperature, 45 ° C for 60 minutes to simmer. The extra time and heat softened up the woody material to enable the therapeutic ingredients to be drawn into the liquid. The resulting extracts were lyophilized and stored in a refrigerator until needed.

2.3.3. Preparation of Ethanolic and Aqueous Stock Solution for the Bioassays

In the preparation of the stock solutions for the bioassays, different concentrations (2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml) of the lyophilized ethanol products were reconstituted using 20% Dimethyl sulfoxide (DMSO) for the determination of the antimicrobial activity assay. Quantities of concentrations (i.e. 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml) of lyophilized aqueous products were reconstituted in sterile distilled water respectively for the antimicrobial activity determination. The stock solutions were stored in a refrigerator until needed.

2.4. Preparation of Culture Media

All media were prepared and sterilized according to manufacturer's instructions. The media used for this study were obtained from the Oxoid Limited, England. Sterility control plates of each media and diluents were made by incubating them overnight at 37 °C.

2.5. Inocula Preparation

Using a sterile wire-loop, 3-5 well isolated colonies of each sub-cultured organisms were suspended in test tubes containing 5 ml of sterilized bacteriological peptone and incubated at 37 °C for 3-6 hours to attain the McFarland Standard. The turbidity of the actively growing broth cultures was adjusted with sterile bacteriological peptone to attain turbidity optically comparable to that of 0.5 McFarland standards (approximately 1.2×10^8 CFU/ml).

2.6. Antimicrobial Activity Assay

The agar well diffusion method was used to investigate the antimicrobial properties of the crude extracts as described in the National Committee for

Clinical Laboratory standards (2000). Within 15 minutes after adjusting the turbidity of the inocula suspension, a sterile swab stick was used to inoculate the inocula onto dried surface of sterile prepared Mueller Hinton agar plates and Sabouraud 4% glucose agar with bacteria and fungi respectively. In each case, streaking was repeated two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. The inoculated plates were then allowed to stay for about 3-5 minutes for the surface of the agar to air-dry.

While the plates were drying, the various concentrations of the extracts were filtered using micro filters. A sterilized cork borer of an internal diameter of about 4 mm was then used to punch five holes in the inoculated medium and the various concentrations of the extracts were dispensed into the respective labeled holes. 0.3 mg/ml Chloramphenicol was used as control for bacteria, while 20 mg/ml Miconazol for fungi.

Duplicates of each plate were made and the procedure was repeated for the other organisms. The plates were kept in refrigerator for about 4 - 5 hours for complete diffusion of the extracts and incubated at 37°C for 24 - 48 hours. After the incubation period, the diameter of each zone of inhibition was measured with a sterilized ruler and the results recorded.

3. Results

Table 1 and Table 2 show the antimicrobial activities of heated ethanolic and aqueous extracts of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against selected human pathogens (namely, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albican*, and *Proteus mirabilis*) respectively.

From Table 1, both heated ethanolic and aqueous extracts of *A. sativum* showed no antimicrobial activity against *S. aureus*, *S. typhi*, *K. pneumoniae*, *E. coli*, *C. albican*, and *P. mirabilis*. Only the control showed zones of inhibition for most of the organisms.

Table 1: Antimicrobial activities of heated ethanolic and aqueous extracts of garlic (*A. sativum*) against some selected human pathogen

Samples	Zones of inhibition exhibited by various organisms against different concentrations (mm)							
	Heated Ethanolic extracts				Heated Aqueous extracts			
	A	B	C	D	A	B	C	D
<i>Allium sativum</i>								
<i>S. aureus</i> (ATCC25923)	0	0	0	0	0	0	0	0
<i>K. pneumoniae</i> (ATCC33495)	0	0	0	0	0	0	0	11.5
<i>P. mirabilis</i> (ATCC49565)	0	0	0	13.5	0	0	0	15.5
<i>E. coli</i> (ATCC25922)	0	0	0	25.1	0	0	0	25.5
<i>S. typh</i> (ACTCC194300)	0	0	0	33.0	0	0	0	30.5
<i>C. albican</i> (ATCC10231)	0	0	0	23.5	0	0	0	21.5

Concentration of the extracts: A = 2.5mg/ml; B = 5.0mg/ml; C = 10.0mg/ml; D = Control

In Table 2, heated aqueous extracts from *Z. officinale* had no activities against the organisms except the heated ethanolic extracts which had activity against *S. typhi* and *C. albican*. Chloramphenicol,

however, showed zones of inhibition for most organisms for *P. mirabilis*, *E. coli*, *S. typhi* and *C. albican*.

Table 2: Antimicrobial activities of heated ethanolic and aqueous extracts of ginger (*Z. officinale*) against some selected human pathogen

Samples	Zones of inhibition exhibited by various organisms against different concentrations (mm)							
	Heated Ethanolic extracts				Heated Aqueous extracts			
	A	B	C	D	A	B	C	D
<i>Zingiber officinale</i>								
<i>S. aureus</i> (ATCC25923)	0	0	0	0	0	0	0	0
<i>K. pneumonia</i> (ATCC33495)	0	0	0	0	0	0	0	0
<i>P. mirabilis</i> (ATCC49565)	0	0	0	17.5	0	0	0	16.5
<i>E. coli</i> (ATCC25922)	0	0	0	29.5	0	0	0	31.5
<i>S. typh</i> (ACTCC194300)	7.0	10.5	14.5	30.0	0	0	0	34.5
<i>C. albican</i> (ATCC10231)	8.5	11.0	11.0	19.0	0	0	0	24.0

Concentration of the extracts: A = 2.5mg/ml; B = 5.0mg/ml; C = 10.0mg/ml; D = Control

4. Discussion

The result of this study revealed that, both heated aqueous and ethanolic extracts of *A. sativum* had no antimicrobial activity against all the test organisms. On the other hand, the heated extracts of *Z. officinale* had no activities against the test organisms except for its heated ethanolic extract which exhibited an activity against *S. typhi* and *C. albican*.

This result is unusual because other studies have proven the antimicrobial potency of *A. sativum* on some pathogens. According to Uchida *et al.* (1975), Yusha'u *et al.* (2008), Mukhtar and Ghorri (2012), Omoya (2012), and Akintobi *et al.* (2013), various garlic and ginger preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*. Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic. Sivam *et al.* (1997), Ankri and Mirelman (1999), Whitmore and Naidu (2000), and Ross *et al.* (2001) have also confirmed that they are not only effective against only bacteria but also possess antiviral and antifungal activity.

However, the result of this study could have been because of the heat applied during the extraction phase of both the ethanolic and aqueous extracts which may have caused the denaturing of some bioactive compounds in the spices used. According to Gupta and Ravishankar (2005), the paste of commercial garlic showed antimicrobial activity only at 4 °C and 8 °C (about 1 log CFU/g reduction), while fresh ginger paste showed antimicrobial activity only at 8 °C indicating that antimicrobial activity of garlic and ginger are temperature dependent.

Apart from temperature, it is also believed that, geographical location of a plant, temperature, and seasonal variation of an area may have influence over the yield of medicinal plants, hence, the low or no inhibition zones observed. The antimicrobial activity of a plant is due to specific photochemical or essential oils present in it (Avato *et al.*, 2000). Sagdic (2003) reported that the main factors that determine the antimicrobial activity of plants are the type and composition of the plant, the amount or concentration of the active ingredient used, pH, temperature and the type of microorganism. Other factors that determine how effective a herb will be may include the type of environment (climate, bugs, soil quality) in which a plant grew, when it was harvested, stored and processed, species or variety, and whether or not there are contaminants such as heavy metals and pesticides in the plant (Sagdic, 2003).

From this study, it is possible that the compounds in the extracts which could have given activity may have been evaporated during the evaporation and boiling processes of the extracts. This may have interfered with the efficacy of the plant extracts and for that matter the outcome of the study. This is a clear indication that bioactive components of garlic and ginger are sensitive to heat and therefore should not be cooked when using them for medicinal purposes.

5. Conclusion

The study revealed that both heated extracts of garlic and ginger had no antimicrobial activity against all the test organisms except heated ethanolic extract of ginger which exhibited an activity against *S. typhi* and *C. albican*. This was attributed mainly to the high temperatures used during the extraction process of both

the ethanolic and aqueous components of the spices. In comparison, some of the bioactive compounds in ginger had strong resistance to the heat and hence, were able to exhibit some level of antibacterial activity against two of the test organisms (*S. typhi* and *C. albican*). It is however recommended that, both spices should not be heated or cooked if they are to be used for medicinal purposes. Also, further studies need to be conducted to determine if better antibacterial activity is achieved by combining the two spices.

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