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The Antibacterial Effect of Ginger and Garlic Extracts on Some Pathogenic Bacteria Isolated from Patients with Otitis Media

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Abstract

*Newly obtained rhizomes of Zingiber officinale (Ginger) and Cloves of Allium sativum (Garlic) were put together, leaved nearly at 25^{0}C to permit air-drying, milled to fine powder and then these powders would be extracted (each alone) using water and ethanol as solvents for the extraction. After that, the extracts were examined for its antibacterial (inhibitory) effect toward some clinical isolates (patient with otitis media) of Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogens (G+ve) and Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis (G-ve). In this study, the antibacterial (inhibitory) effect of the extracts of both ginger and garlic has been determined toward six clinical bacterial isolates (mentioned previously). Two kinds of extracts for ginger and two kinds of extracts for garlic have been obtained (involving watery extract and ethanolic extract) and then examined separately and in combination of these extract. In the present study, some antibiotics (cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin) were used to compare their effect with the effect of the extracts obtained. Disc diffusion method (*Kirby-Bauer *method) was used to determine the antibacterial activity of extracts. The test isolates showed variable susceptibility to the garlic and ginger extract (aqueous and ethanolic) and to other antibiotics (cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin). The outcomes of susceptibility experiment depicted that ethanolic extract of garlic and ginger (each alone and in combination) showed more inhibitory effect than aqueous extract and also the combination of ethanolic extract of both ginger and garlic resulted in inhibitory effect greater than each extract alone. Both ginger and garlic extract have antibacterial activity (especially the ethanolic extract) against some pathogenic G+ve and G-ve bacteria.*

Keywords: Ginger, garlic, extract, pathogenic bacteria, otitis media.

Introduction

Ginger (ginger rhizome) is the root of the *Zingiber officinale* plant, which can be utilized as a medication or as pleasant condiments. Ginger derived its name from the genus (*Zingiber officinale)* and the family (Zingiberaceae). In addition to ginger, there are other important followers to this plant family including turmeric, cardamom and galangal[1]. *Zingiber officinale* creates sets of flower sprouts (pink and white) that developed into yellow flowers. As a result of the beautiful appearance and the habituation of plant to hot weather, *Zingiber officinale* is usually utilized as scenery across subequatorial homes[2].

Fully developed *Zingiber officinale* roots are fibrous and approximately arid. The syrup from ancient ginger rhizomes is highly strong and usually utilized like condiments in Indian prescription, and is as typical component of cooking in many Asian countries also this return to its pleasant relish which makes the taste of many food dishes an extremely delicious. *Zingiber officinale* plays role as powerful food maintenance. In limited studies, ginger was found to has better effectiveness than placebo in relieving disgust produced by sea dizziness, morning dizziness, despite ginger is not reported to be preferred on placebo in relieving surgical sickness. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity[3]. Reported pharmacological activities of ginger include antipyretic, analgesic, ant tissues in addition to hypersensitive effects[4].

Ginger extract also showed a hepatoprotective effect in rats[5]. Besides that, Ginger was found to have free radical remover activity (prevent oxidant formation); so prevents lipid oxidation. This finding can explain the well documented gastro-protective effects[6]. Additionally, ginger extract has radiopreventive activity which could be useful in preventing gamma radiation from generating adverse reactions during the course of cancer therapy in laboratory animal[7]. Moreover, ginger displayed chemopreventive and antineoplastic effects; also ginger shows to have in the future role in cancer inhibition, although additional studies are required to assess this property of ginger [8]. Patients with diabetes mellitus (cataract) might have developed glycation end-yield for this case ginger is chosen because of its antiglycating property[9].

Garlic is the common name of the genus *Allium sativum*. The other closely related species including the shallot, the onion and

others. *Allium sativum* can be considered as a national product of many centralized Asian countries. Moreover, garlic had been utilizes for longer period of time in the areas of Mediterranean, likewise a condiment in more than one continent including Europe Africa, as such Asia. Garlic had been well identified for antique Egyptians, as well as utilized in cooking and in remedial goals. *Allium sativum* follows to the family *Alliaceae*. Plants of *Allium sativum* can be developed in a close proximity to each other, giving up proper area to enhance the maturation of the bulbs, and are simply developed in vessels of appropriate deepness. Garlic does well in loose, dry; well drained soils in sunny locations. There are various kinds or subdivisions of *Allium sativum*, more obviously softneck and hardneck garlic. Hardneck garlic has been usually developed in neerly cool wheather while softneck garlic is usually well developed in close vicinity to the equability line. There are several works had been made on laboratory animals and human being that proves the beneficial cardiovascular property of (garlic)[10]. When both ginger and garlic used as a natural supplement, this considered as a healthier choice for many diseases (Alzheimer's disease as example)[11].

Allium sativum was utilized to inhibit gangrene formation through the first and Second World War. In new researches, it is reported that garlic extract has been proved to be efficient toward *Streptococcus mutans*, garlic extract mouth wash may be utilized as a modern line in inhibiting dental caries formation[12]. Garlic is highly documented in exhibiting powerful antimicrobial activities. Garlic can provide proper management for bacterial growth ranging from disinfectant, antiseptic, bacteriostatic and even bactericidal characteristics. Besides that, garlic may have the ability to prevent and manage viral, fungal and even helminthes infections. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as Escherichia coli[13]. Recently, it has approved that there is a possibility of using garlic in preserving meats from bacterial spoilage and this related to the antibacterial activity of garlic. Upon treatment of meat with garlic, bacterial numbers were significantly diminished in comparison with the non-treated meat when both meats were kept in refrigerator at 4°C[14].

The present study was targeted for the determination of the antibacterial property of both garlic and ginger extracts (aqueous and ethanolic extracts) on both G+ve and G-ve bacteria isolated from otitis media patient and compares their effect with the effect of some antibiotics.

Material and Methods

The test bacterial isolates: The test isolates were obtained from samples taken from patients submitted to the consultative ENT department of Al-Hilla Teaching Hospital and having suspected otitis media. All bacterial organisms were solated and diagnosed to the species level by using different available procedures including Gram's stain and other phenotypic methods following standardized methods, then diagnosed as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogens* (G+ve) and *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* (G-ve).

Extraction of plant material: The fresh ginger rhizomes and garlic cloves were collected, cleaned, peeled, sliced and dried at room temperature. After drying, pieces of *Allium sativum* and *Zingiber officinale* were grinded to fine particles in isolated manner utilizing a suitable grinder. Ten grams of the powder *Allium sativum* and Ten grams of *Zingiber officinale* was weighed and macerated in 50 milliliters of D.W, 96% ethyl alcohol, each alone. The containers were left at 25^0C for 3 days (72 hrs). After that, the suspensions were filtered and the filtrates (extracts) were delivered into sterile, clean containers with suitable labeling and kept at 4^0C until used for additional assay.

Production of discs (disks of the extracts): By using whatman filter paper No. 1, Discs of 5 mm in diameter were produced by using of a paper borer. After that, the prepared disks were put in suitable containers. Then, the discs were subjected to autoclave in order to sterilize the disks (adjusting the conditions of autoclave to be 121^0C for 15 mins) and left to become cold. Later on, the discs were allowed to suck up the extract filtrate and maintained for later assay. The produced discs (each one) have the ability to absorb about 0.01ml[15].

Antimicrobial susceptibility test by Kirby-Bauer method: The antibiotics susceptibility procedure for bacterial spices has been done through using a method that depends on the ability of disc to permit the penetration of antibiotics through the medium which is also called Kirby-Bauer method [15]. The overall steps of the procedure should be produced through entirely sterilized status. Plates of Mueller Hinton agar have been inoculated; each alone, by the test bacterial isolates -10^7 CFU (compared with Mcfarland turbidity standard), then the bacterial suspension was uniformly distributed overall the area of the plate. After a while, sterilized discs (measuring five milliliters in diameter) were put under sterilized conditions in various extracts (for about 1 min), then fixed on Mueller Hinton plates (petridishes) inoculated previously by suspension of the bacteria. After this step, all plates had been put aside (at 25^0C for about 15 mins). After that, all cultured plates were placed in the incubator at 36°C for 18-20 hrs; the area of inhibition has been examined and calculated in millimeters (mm).

The organisms under the experiment were evaluated, as well, for their susceptibility toward some antimicrobials including: cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin, again by disc diffusion procedure. The cultures of test organisms were reactivated by culturing in sterile nutrient broth for 16 hrs at 37°C. After incubation and turbidity comparison with Mcfarland turbidity standard, sterile cotton swabs were used to transfer the bacterial cultures aseptically and swabbed over Mueller Hinton agar petridishes. A sterilized forceps was

used to fix the antibiotic disc aseptically over the cultured petridishes. Then, the petridishes were placed in the incubator at 37°C for 20-22 hours and subsequently all diameters of inhibition zones could be determined.

Results and Discussion

Bacterial isolates: The bacterial isolates those were isolated in the present study listed below in table-1.

Table-1
Bacterial isolates obtained in the study

Isolates No.	Description
1	*Staphylococcus aureus*
2	*Staphylococcus epidermidis*
3	*Klebsiella pneumonia*
4	*Streptococcus pyogens*
5	*Pseudomonas aeruginosa*
6	*Proteus mirabilis*

Antibacterial determination for test isolates: The results obtained of the antibacterial activity using the disk diffusion method are summarized in table- 2. As demonstrated from this table that various bacterial species showed various levels of sensitivities towards the tested samples of garlic extracts and ginger extracts. The extracts of *Zingiber officinale* and *Allium sativum* with respect to solvents and their effect (zone of inhibition in mm) on different bacterial isolates are shown in table-2. The components associated with the susceptibility of the test organism to ginger are not fully determined but may be related to phytochemicals (such as flavonods and gingerol) and/or secondary metabolites that may be present in ginger[16].

The inhibition zones that obtained by the garlic and ginger extracts toward the bacterial isolates were measured as a determination of inhibitory effects of these plants. All results obtained above in table-2 were resulted by disk diffusion method (Kirby-Bayer). In addition to that, ethanolic disks were used to all test isolates (as a control) and all these disks with no value (no zones of inhibition have been occurred).

The results in table-2 revealed that the aqueous extract of ginger and garlic (each alone) gave no effect (against *Streptococcus pyogens* and *Pseudomonas aeruginosa,*) and low level of inhibition on other isolates. Moreover, the combination of aqueous extract of them gave slightly higher level of inhibition than that of each extract alone.

On the other hand, the ethanolic extract of ginger and garlic (each alone) showed variable level of inhibition (ranging from 9mm to 20mm) and this indicated that the ethanolic extraction gives better extraction than that of aqueous one. Additionally, the combination of ethanolic extract of both ginger and garlic produced broader zone of inhibition than that of each extract alone, this mean that there is a potentiation-like effect between both extract. The important thing is that *Streptococcus pyogens* was completely resistant to all extracts.

Allicin is an organosulfur compound found in garlic (the active ingredient), it shows inhibitory effect on some pathogenic bacteria. The best known and well studied effect of Allicin was illustrated by controlling and killing activity to *Staphylococcus aureus* (MRSA)[17]. *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agents (free radicals)[18].

Besides that, the behavior of all test isolates toward some antibiotics was also determined to compare the degree of inhibition that obtained with the ginger and garlic extract with that produced by the antibiotics. The antibiotic susceptibility pattern was summarized in table-3.

It was clear from the table above that all bacterial isolates were entirely insensitive to cloxacillin and clindamycin (an exception was the test isolate 3 which showed high level of sensitivity to clindamycin- 30mm). The test isolates exhibited a variable level of sensitivity to the other four antibiotics and as illustrated in the table-2 and table-3, we can see that the effect (zones of inhibition) of ethanolic extract of both ginger and garlic is approximately similar to that of cefoxitin and tobramycin while cefepime showed broader zone of inhibition for all isolates except isolates 3 and 4.

Table-2
The Yield of Extract of *Zingiber officinale* and *Allium sativum* with respect to solvents on different bacterial isolates (zone of inhibition in mm)

Isolates No.	Ginger Aqueous Extract	Ginger Ethanolic Extract	Garlic Aqueous Extract	Garlic Ethanolic Extract	Ginger+Garlic (Aqueous Extract)	Ginger+Garlic (Ethanolic Extract)
1	7	16	8	10	10	20
2	7	10	0	10	7	15
3	6	13	11	20	12	23
4	0	0	0	0	0	0
5	0	14	0	9	0	19
6	6	13	7	10	7	16

Table-3
The antibiotic-susceptibility pattern for the test isolates

Isolate No.	Cloxacillin	Cefoxitin	Cefepime	Clindamycin	Tobramycin
1	0	17	24	0	7
2	0	19	23	0	0
3	0	11	0	30	19
4	0	0	0	0	20
5	0	0	25	0	20
6	0	20	25	0	8

Conclusion

The results obtained in this study showed an explanation for the relatively higher therapeutic efficacy of plant materials (spices). Both garlic and ginger have antibacterial activity (especially when combined together). Garlic and ginger have activity on both G+ve and G-ve bacteria (except *Streptococcus pyogens)*. There are several advantages for the use of spices (that derived from plant origins) as dietary supplement or alternative medicine manifested by reduction the chance for developing antibiotic-resistant bacteria that resulted from the frequent use of antibiotics (misuse, abuse), beside decreasing the cost of treatment (drug administration) and also minimizes the development of adverse drug reactions. It is recommended for further in the future studies that should focus more on other advantages of spices especially the clinical applications in order to obtain low cost treatment and also prevention of recurrent infection.

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