Original Article

# *In vitro* Antibacterial Activity of Aquatic Garlic Extract, Apple Vinegar and Apple Vinegar - Garlic Extract combination

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## ABSTRACT

**Background**: Garlic (*Allium sativum*) has had an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family. Garlic is used in traditional medicine for infectious disease and some other cases.
**Aims**: The study aims at determining the antibacterial activity of of aquatic garlic extract, apple vinegar and apple vinegar - garlic extract combination against some bacterial isolates.
**Methods**: The antibacterial effects of aqueous garlic extract, apple vinegar, and apple vinegar- garlic extract combination against 9 Gram-positive and 5 Gram-negative bacterial isolates, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococcus feacalis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluresence, Enterobacter aerugenes, Klebsiella pneumoniae, Escherchia coli, Salmonella typhi, Proteus mirabilis, Proteus vulgaris, and Acinetobacter*, all of them were studied.
**Results**: Antibacterial activity of aqueous garlic extract at 50% concentrations by well-diffusion method was characterized by inhibition zones of 5 Gram-positive and 9 Gram-negative pathogenic bacteria. The maximum zone of inhibition of aqueous garlic extract was observed in *Salmonella typhi* and the minimum was observed for *Proteus sp*. All organisms tested were highly sensitive to apple vinegar- garlic extract combination, whereas all organisms tested were slightly sensitive to apple vinegar.
**Conclusions**: In summary, the aqueous garlic extract showed a wide spectrum activity and appears to satisfy all of the criteria for antibacterial agents. These results suggest that garlic can be used to protect food and reduce the risk of contamination from pathogenic microorganisms.

**Keywords**: Garlic, Apple Vinegar, Apple Vinegar - Garlic Extract combination, and antibacterial activity.

## INTRODUCTION

Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Allium vegetables, particularly garlic (*Allium sativum L.*) exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria[1]. From the published research articles it is clear that the raw juice of garlic was effective against many common pathogenic bacteria and against the strains that have become resistant to antibiotics [2]. The pharmacological effects and antimicrobial properties of garlic have been reviewed by Singh and Singh[3]. In India, garlic has been used to prevent wound infection and food spoilage[4]. Han *et al*.,[5] reported that the antibiotic activity of 1mg of allicin, is equated to that of 15 IU of penicillin. Recent reports also demonstrated the inhibitory activity of garlic aqueous extracts on numerous bacterial and fungal species[6]. Therapeutic effect of garlic is possible because of its oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic. Thiosulfinates play an important role in the antibiotic activity of garlic. Hughes and Lawson[7] showed that the antimicrobial activity of garlic is completely abolished when the thiosulfinates (e.g., allicin) are removed from the extract. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents[8]. Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals, there has been increasing interest in the quest for natural alternatives. Researchers are looking at plants that have been used as alternative therapies for generations[9].

Natural alternatives are needed to achieve a high level of safety with respect to foodborne pathogenic microorganisms[10]. The natural sanitizers, such as organic acids, have been investigated because of their bactericidal activity.

Uyttendaele *et al*.,[11] Among the natural products, vinegar, also known as acetic acid, contains sanitizing properties. Vinegar is an acidic liquid that is made from the fermentation of an alcoholic beverage mainly wine[12]. The total acidity of vinegar is expressed as acetic acid which is the major organic acid in vinegar.

In this paper evaluated the antibacterial activity of aquatic garlic extract, apple vinegar and apple vinegar - garlic extract combination against fourteen bacterial pathogens.

## METHODS

### Plant collection

Aqueous Garlic Extract (AGE) preparation fresh garlic (*Allium Sativum L.*) bulbs were collected from a retail food store (Al- Hilla) 2012. Then, the garlic bulbs were cleaned, peeled, sun dried, and cut into small pieces; it was then ground using an electric blender and placed in clean container. Aqueous extract was soaked 50 gram of garlic powder by 100 ml distillated water, and allowed to stand for 72 hr, and sterilized by filtration (using Millipore 0.45 filter paper). This extract was considered as the 50% concentration of the extract. Preparation of apple vinegar, and apple vinegar-garlic extract was dissolved 50 gram of garlic powder by 100 ml of apple vinegar (weakly acidity), and sterilized by filtration.

### Bacterial Isolates

A total of 9 Gram negative, 5 Gram positive and 1 yeast isolates (isolated from clinical samples) were used in this study. The bacterial isolates represented by; *S. aureus, S.epidermidis, S. pyogenes, E. feacalis, S.pneumoniae, P.aeruginosa, P.fluresence, E. coli, S. typhi, E.aerugenes, K. pneumoniae, Proteus mirabilis, P.vulgaris,* and *Acinetobacter*. These bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slant at 4 ºC. The identification of these organisms was confirmed by using conventional biochemical tests[13].

### *In vitro* Antibacterial activity testing using Agar well diffusion assay NCCLS[14]

Loopfull growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing $1.5\times10^8$ CFU / ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) and sabouraud dextrose agar (for yeast) surface of plates and the plates were left for one 5 -15 minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and add 20µ of the garlic extracts solutions or apple vinegar solution or garlic extracts-vinegar (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

### Statistical analysis

Bonferroni test recommended by Danial[15] was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences between results of garlic extract, apple vinegar, and garlic extract & apple vinegar combination.

## RESULTS & DISCUSSION

### Determination of antimicrobial activity

Throughout these stages, the three described antibacterial methods were compared. The water extract of garlic alone, apple vinegar alone & apple vinegar along with garlic extract, were found to have the highest potencies out of water extract of garlic along with vinegar extracts while the rest of each the extracts alone had lower than extract combination, from time immemorial man has been using various parts of the plants against common ailments with varying degree of success. The knowledge of drug has developed together with the evolution of scientific and social progress. Drugs derived from plants are effective, easily available, and less expensive and rarely have side effects. The practitioners of traditional and indigenous medicine rely mainly on medicinal plants and herbs for preparation of therapeutic substance. Initial screening for the potential antibacterial and antifungal compounds from plants may be performed by using the crude extracts.

The agar well diffusion assay is most commonly used to determine antibacterial susceptibility against Gram positive and Gram negative bacteria. In this study used aqueous garlic extracts (AGE) by agar well diffusion assay. In the present investigation at 50% concentration of AGE was tested for their inhibitory activity on 9 Gram-negative and 5 Gram-positive bacterial isolates. All test organisms (Gram positive and Gram negative bacteria) were sensetive and

inhibited by AGE at 50% concentration. All test organisms (Gram positive) were sensetive and inhibited by AGE at 50% concentration. The maximum inhibition zone of Gram positive bacteria was observed against *E. feacalis* (40 mm), and the minimum was against *S. aureus*, (Table 1). The maximum inhibition zone of Gram negative bacteria was observed against *S.typh*i (50 mm), and the minimum was against *Proteus mirabilis*, *P.vulgaris & Acinetobacter* (20 mm), (Table 2).

Regarding the result of antibacterial activity of apple vinegar they were studies, the results of agar diffusion of vinegar showed that a low activity of apple vinegar against bacterial isolates. The maximum inhibition zone of Gram positive bacteria of apple vinegar was observed against *S. aureus* (15mm), (Table 1), while the maximum inhibition zone of Gram negative bacteria of apple vinegar was observed (10 mm) and has no activity against *S. typhi* (Table 2), due to using apple vinegar (weakly acidity).

According to Malicki *et al*.,[16] organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by its undissociated forms. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria[17]. Various researchers have proved the antibacterial effect of organic acids on different types of pathogenic bacteria.

On the other hand, the activities of garlic extract combination with apple vinegar (VGE) on the bacterial isolates were studies. The results of garlic extract combination with vinegar (VGE) on bacterial isolates revealed that all bacterial isolates were highly sensitive to VGE.

The results of VGE of Gram positive bacteria *(E. feacalis*) were higher sensitive to VGE than other bacteria followed by, *S. pneumoniae* with inhibition zones were 50 mm and 40 mm respectively (Table 1)

Also, the results of VGE of Gram negative bacteria (*E. feacalis*, *P. aeruginosa*, and *S.typhi)* were higher sensitive to VGE than other bacteria followed by *P. fluorescence*, *P. Mirabilis*, *E. aerugenes*, *K. pneumoniae*, and *E. coli* with inhibition zones were 50 mm and 40 mm respectively (Table 2).

Statistical analysis showed significant differences between effect of garlic and garlic–apple vinegar extract combination on bacterial isolates, there were no significant differences between vinegar and garlic –vinegar extract combination on bacterial isolates and there were no significant differences between vinegar and garlic extract on bacterial isolates at level ($P \leq 0.05$).

The results of VGE combination on the bacterial isolates were; synergistic effect towards all organisms. These results were in agreement with Qin *et al*.,[18] who pointed out that the bacterial effect of vinegar is strong on bacteria but weak on fungi. Both water garlic extract and apple vinegar pickled garlic extract had strong antimicrobial activity against both bacteria and fungi.

Zasshi *et al*.,[19] who showed that the bacteriostatic and bactericidal activities of vinegar products against *E. coli*, the bactericidal activities of vinegars were independent of bacterial inoculums sizes, but was dependent of growth phase. Bacteria of logarithmic growth phase were more sensitive than those of stationary phase.

Medina *et al*.,[20] found that the vinegar and aqueous extracts of virgin olive oil showed the strongest bactericidal activity against *Salmonella Enteritidis.*

In Thailand, wood vinegar was not only utilized in agriculture, it was also used

to treat skin infection and dandruff, due to the vinegar has antibacterial and antifungal activities against dermatitis bacteria and gastrointestinal tract disorder. In addition, they presented antioxidant activity. Since, antioxidant agent may play an important role in anti-inflammatory caused by wounds or skin diseases. Therefore, the growth of *S. aureus, S. epidermidis, S. faecalis, E. coli and P. acnes* were inhibited by vinegars[21]. Although, vinegar contained high amount of organic acids, especially acetic acid which their antimicrobial effect was extremely weak. Other components such as phenolic compounds, ketonic compounds and neutral compounds might be key compounds in bio-efficacies Phenolic compounds such as phenol and cresols have been well known to have antimicrobial property and the relationship between the structures and antimicrobial activities of substituted phenols were already reported[22,23], therefore phenolic would be the components contribute to the antibacterial and antifungal activities.

Phenolic compounds and antioxidant property of phenolic compounds is responsible for fungal growth inhibition activity. Antioxidant activity of phenolic compounds could change due to the change in para position by pH modification[24]. The electron donating ability of alkyl groups in the para position stabilizes the antioxidant capacity of phenolic compounds and this electron donation ability of para or ortho group responsible for inhibition of lipid oxidation. The inhibition of lipid oxidation originates the fungal growth inhibition[24]. The antioxidant ability of phenolic compounds depends on the number and presence of hydroxyl group and methoxyl group[25]. It concluded that the antifungal property of vinegar ingredients especially phenolic compounds depends on the pH of the vinegar.

Eja *et al*.,[26] showed that the bacteriostatic and bacteriocidal activities of garlic extract towards *E. coli* and *S. enterica Enteritidis. Escherichia coli* were more sensitive than *S. enterica*. Garlic reduced the viable cells of *S. enterica serovar Enteritidis.*

Celiini *et al*.,[27] showed the garlic extracts 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, garlic extracts are also effective against *Helicobacter pylori*, garlic extracts can also prevent the formation of Staphylococcus enterotoxins A, B, and C1 and also thermo nuclease[28]. Cavalito and Bailey[29] were the first to demonstrate that the antibacterial action of garlic is mainly due to allicin. The sensitivity of various bacterial and clinical isolates to pure preparations of allicin is very significant. Interestingly, various bacterial strains resistant to antibiotics such as methicillin resistant *S. aureus* as well as other multidrug-resistant entero toxicogenic strains of *E. coli, Enterococcus, Shigella dysenteriae, S. flexneni,* and *S. sonnei* cells were all found to be sensitive to allicin[30].

Most recently the University of East London have shown that aqueous extracts of allicin when formulated into a simple cream are able to kill vast swathes of the so called “superbug” MRSA (methicillin resistant *Staphylococcus aureus*).This nasty bacterium is forever changing its structure and developing resistance to many Pharmaceutical antibiotics[31].

This resullts agrees with earlier observations Muhsin *et al*.,[32] who founded out that *E. coli* and *S. aureus* showed promising sensitivity to water extract of garlic.

Ankri and Mirelman[33] showed that the Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *E. coli*; antifungal activity, particularly against *Candida albicans*; antiparasitic activity, and antiviral activity.

The main antimicrobial effect of allicin is attributed to the action of biological active ingredient of allicin. which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action[34]. Allicin interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. Also, as lipid synthesis is affected, other parts of the cell are interfered with. The main effect being that the phospholipid biolayer of the cell wall cannot form correctly in both Gram positive and Gram negative bacteria. and due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, the action of allicin or diallyl thiosulphinic acid or diallyl disulphide, the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group[35]. Garlic is rich in anionic components such as nitrates, chlorides and sulfates as well as other water soluble components common in most plants which may be responsible for its antibacterial activity[36].

All these things contribute to the bacteria cannot grow in the presence of allicin or AGE**.** The diameter of the inhibition zone obtained against commonly used antibiotics is presented in the AGE showed a higher inhibition zone (the bacterial isolates very sensetive), when compared to the activity with the commercially used antibiotics[37].

Garlic can be used as a potent inhibitor of food pathogens. Use of garlic would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods.

Deresse[37] and Shobana[38] who clarified that the *S. aureus, E. coli, S.typhi,* and *Proteus mirabilis* were susceptible to garlic extracts, while *E.aerogenes* was not susceptible to the aqueous extract of garlic.

This indicates that AGE has the potential of a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. In this study the variation in the size of the inhibition zone among the different group of bacteria & yeast. This may be due to the lipid content of the membranes of the different groups of the microorganisms and the permeability of allicin and other garlic constituents. There are a greater number of studies showing antimicrobial activity of garlic against bacteria, fungi, virus and human intestinal protozoan parasites. Modern antimicrobial garlic research started with the classic studies of Cavalitto and Bailey[29]. De *et al*.,[39] observed the antimicrobial activity *in vitro* against *Bacillus subtilis, Escherichia coli* and *saccharomyces cerevisiae.* Arora and Kaur[8] observed a significant bactericidal effect of garlic extract against *Staphylococcus*

*epidermidis*, *Salmonella typhi* and various yeasts. Even bacteria resistant to antibiotic agents were sensitive to extracts of garlic[35].

Mojabi[40] and Yamada & Azuma[41] who found that the garlic extract has a strong anticandidal activities, and responsible for inhibition of fungal growth, and can reduce both germination of spores and growth of hyphae.

AGE showed very good activity against most organisms. Antibiotics were used for therapy, but many of the pathogenic bacteria are resistant. Natural products of higher plants may offer a new source of antibacterial agents and from this result it is clear that the medicinal value of AGE is comparable to the present day antibiotics.

The lowest concentration of garlic extract that completely inhibits the growth of the organism. This may be due to the garlic preparations, different concentrations of the active compounds present in the extract and their interactions in the culture media.

Durairaj[42] reported that the aqueous extracts of garlic to possess potent bacteriostatic principle against bacteria and the garlic extract inhibited the growth of *S. aureus*, *E. coli*, and *S. typhi*. Synergistic use can prevent the pathogenic organism grow their resistance against antibiotic.

## CONCLUSION

From this study and the earlier reports it is clear that, garlic appears to satisfy all of the criteria for antibacterial agents, being cheap and safe. The historical view that garlic was 'cure all' may not be unjustified. Since the introduction of antibiotics there has been tremendous increase in the resistance of many bacterial pathogens. Scientists advance in their search for new bacterial targets to attack, bacteria evolve and as a result a large number of bacterial species have become resistant to antibacterial drugs. Hence, search for new antimicrobials is very important in recent times. Because garlic was known to act synergistically with antibiotics, and resistance has not been reported for garlic, more dose-response preclinical studies and eventually clinical studies should be done to assess the use of an antibiotic/garlic combination for bacteria that are difficult to eradicate. In view of the strong antibiotic properties and the complete absence of development of resistance further investigation upon the principles of the antimicrobial activity of juices from *Allium* species merits consideration. The synergistic effect of vinegar- garlic extract combination was prevent the pathogenic organism grow their resistance against antibiotic.

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**Table 1. Antibacterial activity of Gram positive bacteria at 50%concentrations of garlic extract, apple vinegar, and apple vinegar - garlic extract combination by agar well method**

| Bacterial isolates | garlic extract (mm) | apple vinegar (mm) | apple vinegar - garlic extract combination (mm) |
|---|---|---|---|
| *S. aureus* | 25 | 15 | 33 |
| *S. epidermidis* | 28 | 7 | 30 |
| *S. pyogenes* | 35 | 10 | 35 |
| *S. pneumoniae* | 30 | 10 | 40 |
| *E. feacalis* | 40 | 10 | 50 |

**Table 2. Antibacterial activity of Gram negative bacteria at 50%concentrations of garlic extract, apple vinegar, and apple vinegar - garlic extract combination by agar well method**

| *Bacterial isolates* | garlic extract (mm) | apple vinegar (mm) | apple vinegar - garlic extract combination (mm) |
|---|---|---|---|
| *P. aeruginosa* | 45 | 10 | 50 |
| *P. fluresence* | 25 | 8 | 40 |
| *P. vulgaris* | 20 | 6 | 25 |
| *P. merablis* | 20 | 7 | 30 |
| *E. coli* | 30 | 9 | 40 |
| *E. aerugenes* | 25 | 10 | 40 |
| *K. pneumoniae* | 25 | 10 | 40 |
| *S. typhi* | 50 | 0 | 50 |
| *Acinetobacter* | 20 | 10 | 30 |