IN VITRO ANTIMICROBIAL ACTIVITY OF GARLIC, ONION, GARLIC-ONION COMBINATION (AQUATIC AND OIL) EXTRACT ON SOME MICROBIAL PATHOGENS IN BABYLON PROVINCE, IRAQ.

Hiba Jassim Hamza

MSc. Medical science Department, College of Nursing, Babylon University, Babylon Province, Iraq.

ABSTRACT

Background: Medicinal plants Garlic and Onion (Allium sativum and Allium cepa) respectively, can be described as those plants in which one or more of its organs contain substances that can be used for chemotherapeutic purposes or precursors for the synthesis of useful drugs. The use of plants in treating ailments has been in practice since a long time ago. Aims: The study aims at determining the antibacterial activity of aquatic garlic, onion and garlic-onion combination extract, then garlic oil, onion oil and garlic-onion oil combination. Methods: The antibacterial effects of aquatic garlic, onion and garlic-onion combination extract, then garlic oil, onion oil and garlic-onion oil combination against five Gram-positive and eight Gram-negative bacterial & one yeast isolates including Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus viridans, Streptococcus pneumoniae, Pseudomonas aeruginosa, Enterobacteraerogenes, Klebsiellapneumoniae, Escherichia coli, Salmonella typhi, Proteus mirabilis, Serratiamarcescans, Acinetobacterbaumanni, and Candida albicans (fungi) all of them were studied. Results: Antibacterial activity of these aqueous & oil extracts by well-diffusion method were characterized by inhibition zones of five Gram-positive, eight Gram-negative and one yeast pathogenic microbes. All organisms tested were highly sensitive to garlic-onion combination (aqueous and oil extract), then garlic (aqueous and oil extract), whereas all organisms tested were slightly sensitive to onion (aqueous and oil extract). Conclusion: Natural spices of Garlic and Onion (aqueous and oil extract) possess...
antimicrobial activity against our natural enemies like bacterial pathogens and further evaluation is necessary.

**Keywords:** Garlic, Onion, Garlic-Onion Extract combination, essential oil, antibacterial activity.

**INTRODUCTION**

It is believed that the history of herbal medicine began with the earliest man. The first written herbal record was in 2800BC and herbal medicine is practiced today in countries around the world ([1,2]). Some of the advantages of herbs over the formulated drugs are that they typically have fewer side effects and may be less safe to use over time; they are inexpensive compared to formulated drugs and they are readily available ([3]).

Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains.

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants ([4]). One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds ([5,6]).

The plant Garlic (*Allium sativum*) and Onion (*Allium cepa*) belongs to the family Alliaceae and genus *Allium*, they cultivated abundantly in India ([7]). Garlic and Onion possess many biological activities including antimicrobial, antioxidant, anticarcinogenic immunomodulatory & biotic action. The biological and medical effects of Garlic and Onion are mainly due to their high content of organo-sulfur compounds, such as allin&allicin and their breakdown products. flavonoids, abundant in Onion but practically absent in Garlic ([8]). *Allium* vegetables, particularly garlic (*Allium sativum L.*) exhibit a broad antibiotic activity against both Grampositive and Gramnegative bacteria ([9]). 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 ([10]).

Besides that, garlic may have the ability to prevent and manage viral, fungal and even helminthic infections. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as *Escherichia*
Onion have been widely reported to show their antimicrobial activities against Gram negative and Gram positive bacteria \(^{(11,12)}\). Onion is used as antiseptic, antihelminthic, antispasmodic, carminative, diuretic, cholagogge, diaphoretic and expectorant. It is used also for coughs, the flu, parasites, wound, burns, dog bites, bee stings, ear aches, athletes' foot, warts, baldness, toothaches, intestinal infections, kidney infections, contaminated blood and heart failure. Raw onion can completely sterilize the mouth and throat \(^{(14)}\).

This paper aimed to evaluated the antibacterial activity of aquatic & oil extracts of garlic extract, onion & garlic- onion extract combination against fourteen (14= 13 bacterial & 1 fungal) pathogens.

**MATERIAL AND METHODS**

The bacterial isolates

The test isolates were obtained from samples taken from patients submitted to Al-Hilla Teaching Hospital. A total of (8) Gram negative, (5) Gram positive and (1) yeast isolates. These bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slant at 4 °C. The identification of these organisms was confirmed by using conventional biochemical tests \(^{(15,43)}\), then diagnosed as *S.aureus*, *S.epidermidis*, *Strep.pyogens*, *Strep.pneumoniae* & *Strep.viridans* (G+ve), and *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis*, *E. aerugenes*, *Acinetobacterbaumanni*, *Escherichia coli*, *Serratiamarcescans* & *Salmonella typhi* (G-ve), and *Candida albicans* (fungi).

**Plant collection**

Aqueous Garlic & onion Extract preparation fresh garlic & onion (*Allium sativum L.* & *Allium cepa*) bulbs were collected from a retail food store (Al-Hilla) 2014, then the Garlic & Onion bulbs were cleaned, peeled, sun dried, and cut into small pieces; then ground using an electric blender and placed in clean container. Aqueous extracts were soaked 50 gram of garlic & onion powder by (100 ml) distillated water, and allowed to stand for (72 hr), and sterilized by filtration (using Millipore 0.45 filter paper). These extracts were considered as the 50% concentration of the extract. Oil of garlic & onion were collected from a retail food store (Al-Hilla) 2014 \(^{(16)}\).

**In Vitro Antibacterial activity testing using Agar well diffusion assay NCCLS**\(^{(17, 44)}\)

Loop full 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 1.5×10^8 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 (20µl) of the garlic extracts solutions or onion extracts solution or garlic- onion extracts also garlic oil, onion oil& garlic- onion oil (The plates were performed in triplicates). All plates of the tested organisms were 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(18,45) was used for statistical analysis (P≤ 0.05) to show if there is any significant differences between results of Aqueous Garlic extract, Aqueous onion Extract, Aqueous Garlic-onion extract combination, garlic oil, onion oil& garlic-onion oil combination.

**RESULTS&DISCUSSION**

In the present study, the inhibitory effect of different extracts (Aqueous Garlic, Aqueous onion, Aqueous garlic-onion combination, garlic oil, onion oil & garlic-onion oil combination) was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria, fungus which was regarded as human pathogenic microorganism. Measurement of antimicrobial activity using Agar well diffusion Method; the antimicrobial potential of both the experimental plants was evaluated according to their zone of inhibition against various pathogens. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones.

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism (19,20). These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial
compounds [21-22]. Therefore, medicinal plants are finding their way into pharmaceuticals, nutraceuticals and food supplements.

Fig. (1&2) show antimicrobial inhibition zone of aqueous Garlic, Onion, Garlic-Onion combination extracts on fungal and bacterial (G+ve & G-ve) species; Garlic-Onion combination extracts showed high degree of inhibition followed by Garlic & Onion extract against all the microorganisms studied.

All test organisms (Gram- positive and Gram- negative bacteria) & fungi were sensitive and inhibited by Garlic- Onion combination extract, Garlic & Onion at (50%) concentration of each extract. The maximum inhibition zone of Gram positive bacteria to Garlic-Onion combination extract was observed against S. epidermidis (40 mm), and the minimum was against Strep. viridians (30 mm) (fig.1) while The maximum inhibition zone of Gram negative bacteria to same extract was observed against Salmonella typhi (40 mm) and the minimum were against Acinetobacter baumannii (25 mm) & E. aerogenes (25 mm) (fig.2 ).

The maximum inhibition zone of Gram positive bacteria to Garlic extract were observed against S. aureus (30 mm), S. epidermidis (30 mm), & Strep. pneumonia (30 mm), and the minimum was against Strep. viridians (20 mm) (fig.1), while The maximum inhibition zone of Gram negative bacteria to same extract were observed against P. aeruginosa (25 mm), Proteus mirabilis (25 mm), and the minimum was against E. aerogenes (18 mm) (fig.2 );

Regarding the result of antibacterial activity of Onion extract they were studies, the results of agar diffusion showed that a low activity of Onion extract against bacterial isolates.also the maximum inhibition zone of Gram positive bacteria to Onion extract were observed against Strep. pyogenes (25 mm), Strep. pneumonia (25 mm) and the minimum was against S. epidermidis (18 mm) (fig.1), while The maximum inhibition zone of Gram negative bacteria to same extract were observed against Salmonella typhi (30 mm), the minimum was against Proteus mirabilis (18 mm) (fig.2 ); For the antifungal activity, Candida albicans showed high efficient antifungal activity for Garlic- Onion combination extract (35 mm) comparing with Garlic & Onion extract individually (20 mm) inhibition zone to each one (fig.1).
Fig. (1): Antimicrobial inhibition zone diameters (mm) of aqueous Garlic, Onion, Garlic-Onion combination extracts on fungal and G+ve bacterial species, *In Vitro*.

Fig. (2): Antimicrobial inhibition zone diameters (mm) of aqueous Garlic, Onion, Garlic-Onion combination extracts on G-ve bacterial species, *In Vitro*.

Allicin is an organo-sulfur 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 *S. aureus*(MRSA) \(^{(23)}\). *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agents (free radicals)\(^{(24)}\). Eja *et al.*, \(^{(25)}\) showed that the bacteriostatic and bactericidal 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. entericas*erovar*Enteritidis*. Celiiniet *et al*. 1996\(^{(26)}\), 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\(^{(27)}\).

Cavalito and Bailey\(^{(28)}\), 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 enterotoxigenic strains of *E. coli, Enterococcus, Shigelladsenteriae, S. flexneni,* and *S. sonneicells,* were all found to be sensitive to allicin\(^{(29)}\). This results about Garlic agrees with earlier observations of Muhsinet al.\(^{(30)}\), who founded out that *E. coli* and *S. aureus* showed promising sensitivity to water extract Garlic. Ankri and Mirelman\(^{(31)}\), 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 enterotoxigenic strains of *E. coli;* antifungal activity, particularly against *Candida albicans;* antiparasitic activity, and antiviral activity. The antimicrobial activity of Garlic extract obtained in this study is similar to that reported by Esimoneet al. 2010\(^{(32)}\), which has shown that Garlic extract active against microbial pathogens such as *Candida albicans* species, *E. coli* and *S. aureus,* so this results agreed with those of Hindi 2013\(^{(33)}\); the aqueous Garlic extract showed a wide spectrum activity and appears to satisfy all of the criteria for antibacterial agents, also this results agreed with those of Zakaria and Astal(2003)\(^{(34)}\), they studied antibacterial effect of Garlic aqueous on certain gram-positive pathogens (*S.aureus,* *S.saprophyticus,* *Strep.pneumoniea and Strep.faecalis*) and gram-negative bacteria (*Escherichia coli, Enterobacter cloacae, Klebsiellapneumoniae, Proteus mirabilis, P.aeruginosa* and *Acinetobacterhaemlytics*).

On other hand, The Onion bulbs contains numerous organic sulfur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteinesulfoxide and cycloalliiin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed
mainly of sulfur compounds, so the antibacterial activity of Onion attributed to its content; Alkaloids act through penetration into cell membrane inside the cell and interacts with DNA, while flavones act through its conjugation with bacterial adhesions at the bacterial cell surface and form complex with bacterial cell wall \(^{(35,36,37)}\), also the results of this study agreed with those of Kabelik, (1970), which showed that Extract of Allium cepa bulb was active against Candida albicans \(^{(38)}\). the results of this study agreed with those of Adeshina et al. 2011 \(^{(11)}\), which showed the susceptibility of the test bacteria especially P. aeruginosa and S. typhi the onion juice is encouraging because of the health crisis caused by these organisms all over the world.

Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms.

Fig. (3&4) revealantimicrobial inhibition zone (Garlic , Onion & Garlic-Onion combination) oils on fungal and bacterial (G+ve & G-ve) species. Garlic-Onion combination oil showed high degree of inhibition followed by Garlic then Onion individually oil against all the microorganisms studied. All test organisms (Gram- positive and Gram- negative bacteria) & fungi were sensitive and inhibited by Garlic-Onion combination oil and then by Garlic oil but showed that a low activity of Onion oil against bacterial isolates.

The maximum inhibition zone of Gram- positive bacteria to Garlic-Onion combination oil was observed against Strep. pneumoniae (35 mm), and the minimum was against S. epidermidis (23mm)(fig.3), while the maximum inhibition zone of Gram- negative bacteria to same oil was observed against Acinetobacter baumannii (40 mm), and the minimum was against Serratiamarcescans (18 mm) (fig.4). In otherwise, the maximum inhibition zone of Gram- positive bacteria to Garlic oil alone were observed against Strep. pyogenis (30mm), and Strep. pneumonia (30mm), and the minimum was against S. aureus (18 mm)(fig.3), while the maximum inhibition zone of Gram- negative bacteria to same oil was observed against Salmonella typhi (30 mm), and the minimum was against Escherichia coli (12mm) (fig.4); Regarding the result of antibacterial activity of Onion oil they were studies, the results of agar diffusion showed that a low activity of Onion oil against bacterial isolates, also the maximum inhibition zone of Gram- positive bacteria to Onion oil was observed against Strep. pneumonia (10 mm), while Streptococcus pyogenis not affected by Onion oil (fig.3); and the
maximum inhibition zone of Gram negative bacteria to same oil were observed against *Salmonella typhi* (20mm), while *Enterobacteraerugen*es & *P. aeruginosa* not affected by Onionoil (fig.4); For the antifungal activity, *Candida albicans* showed high efficient antifungal activity for Garlic-Onion combination oil (35mm) comparing with Garlic & Onion oil individually (25,20mm) inhibition zone, respectively (fig.3).

Fig. (3): Antimicrobial inhibition zone diameters (mm) of Garlic, Onion & Garlic-Onion combination oil on fungal and G+ve bacterial species, *In Vitro*.

Fig. (4): Antimicrobial inhibition zone diameters (mm) of Garlic, Onion & Garlic-Onion combination oil on G-ve bacterial species, *In Vitro*. 
Statistical analysis showed no significant differences between effect of aqueous (Garlic and Garlic-Onion combination) extracts on microbial isolates, there were no significant differences between aqueous (Onion and Garlic-Onion combination) extracts on microbial isolates, and there were no significant differences between Onionaqueous and Garlic aqueous extracts on microbial isolates at level($P \leq 0.05$).

On the other hand, Statistical analysis showed no significant differences between effect of crude oil (Garlic and Garlic-Onion combination) on microbial isolates, there were significant differences between crude oil (Onion and Garlic-Onion combination) on microbial isolates, and there were significant differences between crude Onion oil and crude Garlic oil on microbial isolates at level($P \leq 0.05$).

The result of this study which relative with oil extract agreed with Benkeblia 2004 (39) that showed the essential oils extracts of common onions and garlic were found to inhibit bacterial and mould growth, and inhibition of Garlic oil was strongest than those of Onions oil extracts. Garlic oil possessed moderate antibacterial activity in this study, which enhanced and highest when Garlic extract was heated for (45 min.) at 121 °C, (39). *E. coli* was less sensitive to Garlic extracts than *E. aerogenes* which is in agreement with previous observations (40).

Garlic oil extract has been found to possess antibacterial property against several bacteria including *S. typhimurium, S. typhi, E. coli, Bacillus cereus, S.epidermidis,* and *S. aureus* (40). Since such antifungal and antibacterial essential oils have penetration action, these may especially be used to control seed-borne fungal pathogens and human pathogenic bacteria. The volatility, ephemeral nature and biodegradability of these compounds of angiosperms will be especially advantageous, if they are developed as pesticides and antibiotics (41). Ross *et al.* 2001 showed that essential oil of *Allium* genus exhibit considerable antimicrobial activity when tested in liquid cultures, especially when precautions to minimize volatilization loss are applied. Reduced antimicrobial activity in the presence of tryptone&cysteine suggests that essential oil activity may be underestimated in microbial culture media and reduced within the enteric environment and is compatible with evidence that the mode of action garlic compounds depend upon SH reactions. In contrast, Cavallito et al. 1944 (42), suggested that the earlier evaluation of antimicrobial activity of essential oil of *Allium* using agar plate method, which indicated negligible antimicrobial activity, failed to take account of the hydrophobic and volatile nature of essential oils.
CONCLUSIONS
The results obtained in this study showed an explanation for the relatively higher therapeutic efficacy of plant materials (spices). Both Garlic and Onion have antibacterial activity (especially when combined together, aqueous & oil extracts). Garlic and Onion have activity on both(G+ve and G-ve) bacteria and fungi.

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.

The synergistic effect of Garlic-Onion combination extract & Garlic-Onion oil combination were prevent the pathogenic organism grow their resistance against antibiotic. 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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REFERENCES


