Antimicrobial Activity of Some Plant Extracts on Microbial Pathogens Isolated from Hilla City Hospitals, Iraq

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Abstract

Medicinal plants such as 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 drug. The use of plants in treating ailments has been in practice since a long time ago.

The present study aimed to determine the antimicrobial activity of both aqueous and oil extracts of garlic and onion singly and mixed *in vitro*.

Antimicrobial 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 isolates and one yeast (*Candida albicans*) were determined.

Antimicrobial activity of these aqueous and 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).

Natural spices of Garlic and Onion (aqueous and oil extract) possess antimicrobial activity against our natural enemies like bacterial and fungal pathogens and further evaluation is necessary.

Keywords: plant extract, Garlic, Onion, Garlic-Onion combination, essential oil, antimicrobial activity.

الخلاصة

النباتات الطبية كالثوم والبصل (*Allium sativam* و *Allium cepa*) بالترتيب يمكن وصفها بأنها النباتات التي يكون فيها واحد أو أكثر من أعضاءها محتوياً على مواد يمكن إستخدامها لأغراض العلاج الكيميائي أو بادئات لتخليق دواء مفيد، إذ تم إستخدام تلك النباتات بمعالجة العديد من العلل وعرف ذلك التطبيق منذ وقت طويل. هدفت الدراسة لتحديد الفعالية ضد ميكروبية لمستخلصي الثوم والبصل المائي والزيتي بشكلية المفرد والمشترك ضد العزلات السريرية للبكتريا والفطر.

حضِر المستخلص المائي والزيتي بشكليه المفرد والمشترك لأبصال الثوم والبصل وكما موصوف بمصادر تحضير المستخلصات، كما عزلت مجموعة عزلات بكتيرية وفطرية سريرية أختيرت منها (٥ عزلات بكتيرية موجبة لصبغة كرام و٨ عزلات سالبة لصبغة كرام ، وعزلة فطر واحدة (Candida albicans) لتحديد الفعالية ضد ميكروبية لها.

بعد تطبيق طريقة نشر الحفر في الاكار لتحديد الفعالية ضد ميكروبية لمستخلصي الثوم والبصل المائي والزيتي بشكلية المفرد والمشترك تجاه العزلات الميكروبية (بكتريا وفطر) أعلاه، كل الميكروبات المختبرة كانت عالية الحساسية للمستخلص المشترك المائي والزيتي للثوم والبصل, بعدها مستخلص الثوم (المائي والزيتي), بينما كانت قليلة الحساسية لمستخلص البصل (المائي والزيتي).

وجد أن التوابل الطبيعية لمستخلصي الثوم والبصل المائي والزيتي المستخدمة بالدراسة الحالية تمتلك فعالية ضد ميكروبية واضحة تجاه الأعداء الطبيعيين كالبكتريا والفطريات الممرضة ومن الضروري إجراء تقييم أكثر لفعاليتها المضادة.

الكلمات المفتاحية: المستخلص النباتي ، الثوم ، البصل ، مزيج الثوم والبصل ، الزيت الاساسي ، الفعالية ضد الميكروبية.

Introduction

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

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

The Garlic plant (*Allium sativum*) and Onion (*Allium cepa*) belongs to the family Alliaceae and genus *Allium*, [7].Garlic and Onion possess many biological activities including antimicrobial, antioxidant, anticarcinogenic immunomodulatory and biotic action [7]. The biological and medical effects of Garlic and Onion are mainly due to their high content of organo-sulfur compounds, such as allin and 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 Gram positive and Gram negative 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 extract have the ability to prevent and manage viral, fungal and even helminthic infections [11,12]. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as *Escherichia coli* [11,12]. Onion has been widely reported to show their antimicrobial activities against Gram negative and Gram positive bacteria [13]. 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 evaluate the antibacterial activity of aquatic and oil extracts of garlic extract, onion & garlic-onion extract combination against some G+ve and G-ve bacteria and one fungus strain pathogens.

Materials and Methods

The Bacterial Isolates

The test isolates were obtained from samples taken from patients submitted to Teaching Hilla 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 ºC. The identification of studies organisms was confirmed by using conventional biochemical tests [15], the diagnosed as microorganisms were: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus viridans* (G+ve), and *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter aerugenes, Acinetobacter baumanni*, *Escherichia coli*, *Serratia marcescans* and *Salmonella typhi* (G-ve), and *Candida albicans* (fungus).

Plant collection and extraction

fresh garlic & onion *Allium sativum L.* & *Allium cepa* bulbs were collected from un Al-Hilla retail food store, 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 and 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 and onion were collected from a Al-Hilla retail food store [16].

***In Vitro* Antimicrobial activity testing using Agar well diffusion assay CLSI [17]**

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 1.5×108 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 5-15 minutes at room temperature to dry.

Media, In to make four wells (5 mm diameter) by cork borer and add (20µ) of the garlic extracts solutions or onion extracts solution or garlic- onion extracts also garlic oil, onion oil and 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] was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences among all study obtained results.

Results and Discussion

In the present study, the inhibitory effect of the studied extracts was evaluated for exploration of their antimicrobial activity against certain Gram negative, Gram positive bacteria and one fungus isolate, which were regarded as human pathogenic microorganisms. 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.

Figure 1and Figure 2 show antimicrobial inhibition zone of aqueous Garlic, Onion, Garlic- Onion combination extracts on fungal and bacterial species; Garlic-Onion combination extracts showed high degree of inhibition followed by Garlic and Onion extract against all studied microorganisms.

All tested organisms (Gram- positive and Gram- negative bacteria) and fungus 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. epidermidi*s (40 mm), and the minimum was against *Strep.* viridians (30 mm) (see 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 baumanni* (25 mm) and *E. aerugenes* (25 mm) (Fig.2). The maximum inhibition zone of Gram positive bacteria to Garlic extract were observed against *S. aureus* (30 mm), *S. epidermidi*s (30 mm),

& *Strep. pneumoniae* (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. aeroginos*a (25 mm), *Proteus mirabilis* (25 mm), and the minimum was against *E. aerugenes* (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. pneumoniae* (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).

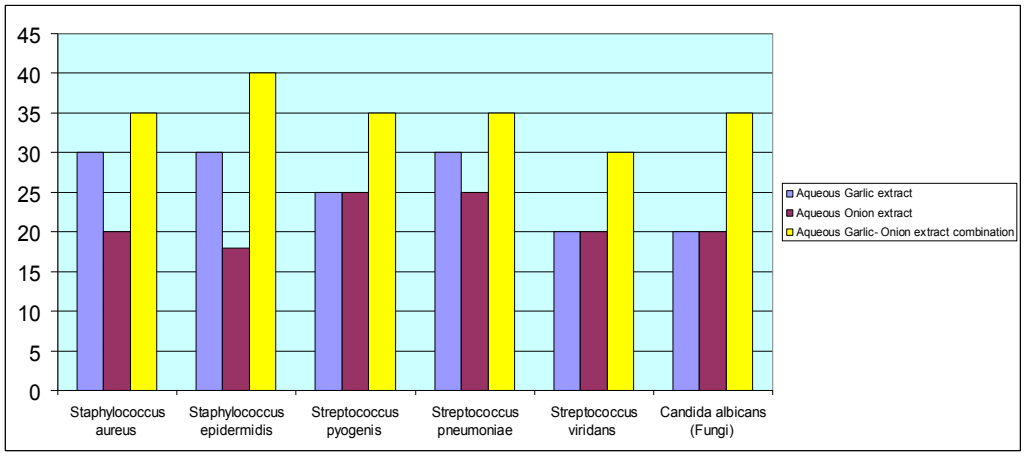

Figure (1): Antimicrobial inhibition zone diameters (mm) of aqueous Garlic, Onion, Garlic-Onion combination extracts on fungal and G+ve bacterial species, *in Vitro*.

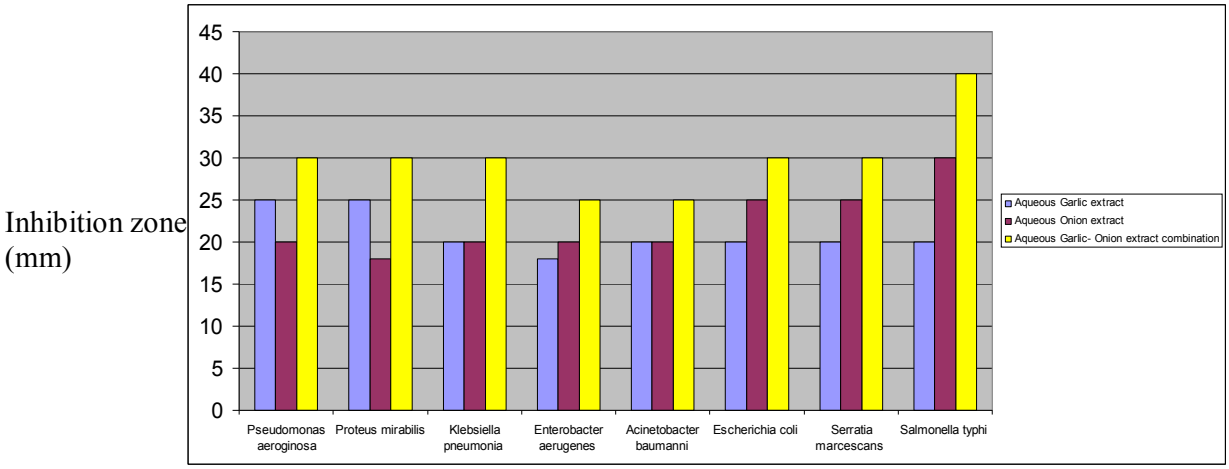

Figure (2): Antimicrobial inhibition zone diameters (mm) of aqueous Garlic, Onion, Garlic-Onion combination extracts on G-ve bacterial species, *in vitro*.

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*. 2007, [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. enterica serovar* Enteritidis. Celiini *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 enterotoxicogenic strains of *E. coli, Enterococcus, Shigella dysenteriae, S. flexneni,* and *S. sonnei* cells, were all found to be sensitive to allicin [29]. This result concerning Garlic agrees with earlier observations of Muhsin *et al*. [30], who founded out that *E. coli* and *S. aureus* showed promising sensitivity to Garlic water extract. 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 enterotoxicogenic 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 Esimone *et al*. 2010 [32],which has shown

that Garlic extract active against microbial pathogens such as *C. albicans s*pecies, *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], whom were studied the antibacterial effect of Garlic aqueous on certain gram- positive pathogens (*S. aureus, S. saprophyticus, Strep. pneumoniae* and *Strep. faecalis*) and gram-negative bacteria (*E. coli, E. cloacae, K. pneumoniae, P. mirabilis, P. aeruginosa* and *A. haemlyticus*).

On the other hand, The Onion bulbs contains numerous organic sulfur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide and cycloalliin; 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 via cell membrane deeply 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 [1], which showed the susceptibility of the tested bacteria especially *P. aeruginosa* and *S. typhi* to 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 microbes.

Figure 3 and Figure 4 reveal antimicrobial 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* (23 mm) (fig.3), while the maximum inhibition zone of Gram- negative bacteria to same oil was observed against *A. baumanni* (40 mm), and the minimum was against *S. marcescans* (18 mm) (fig.4). In otherwise, the maximum inhibition zone of Gram-positive bacteria to Garlic oil alone were observed against *Strep. pyogenis* (30 mm), and *Strep. pneumonia* (30 mm), and the minimum was against *S. aureu*s (18 mm) (fig.3), while the maximum inhibition zone of Gram- negative bacteria to same oil was observed against *S. typhi* (30 mm), and the minimum was against *Escherichia coli* (12 mm) (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*e (10 mm), while *Strep. 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* (20 mm), while *E. aerugenes and P. aeroginosa* not affected by Onion oil (fig.4); For the antifungal activity, *C. albicans* showed high efficient antifungal activity for Garlic- Onion combination oil (35 mm) comparing with Garlic & Onion oil individually (25, 20 mm) inhibition zone, respectively (fig.3).

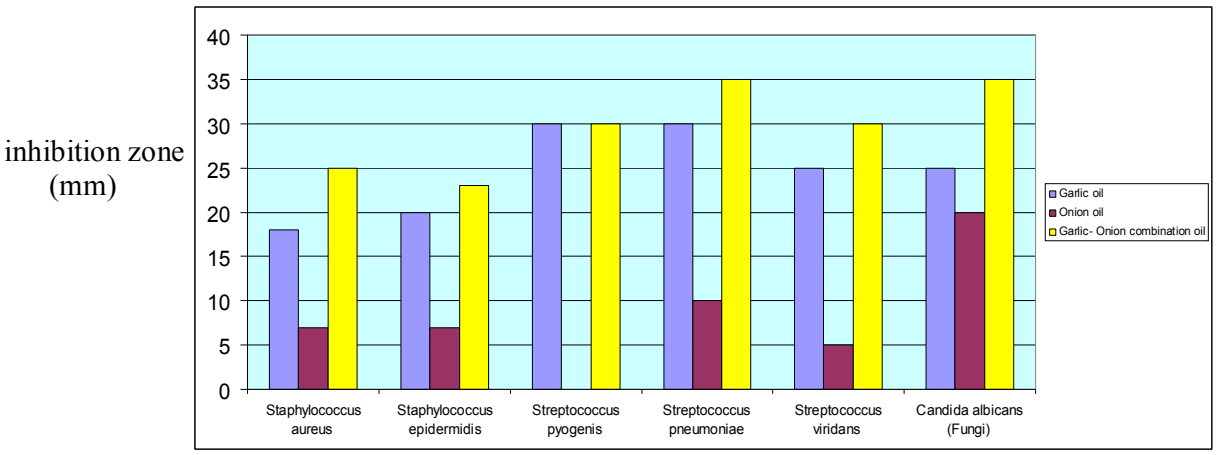

Figure (3): Antimicrobial inhibition zone diameters (mm) of Garlic, Onion, & Garlic- Onion combination oil on fungal and G+ve bacterial species, *in vitro*.

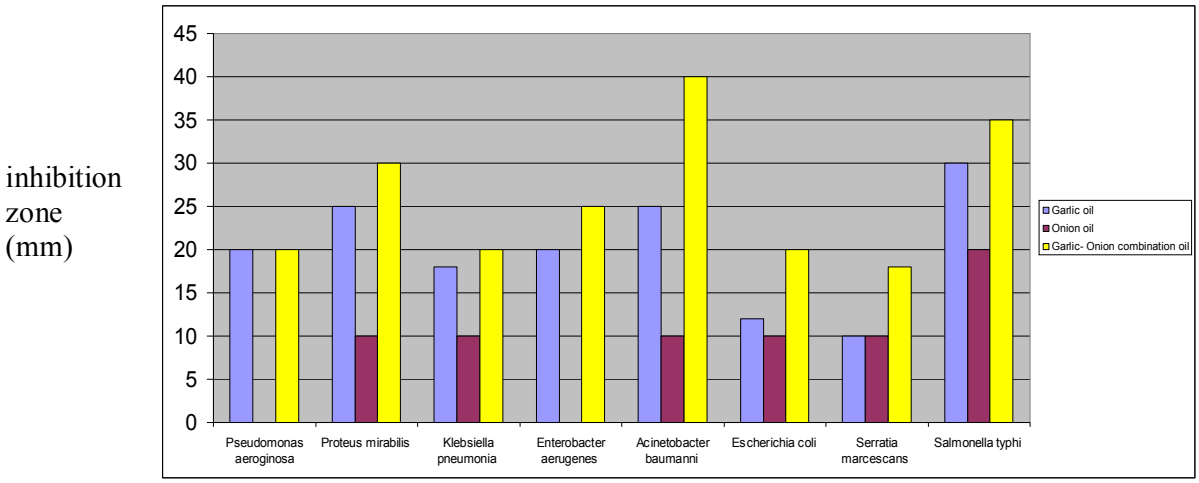

Figure (4): Antimicrobial inhibition zone diameters (mm) of Garlic, Onion & Garlic- Onion combination oil on G-ve bacterial species, *in vitro*.

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 Onion aqueous 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, relative with oil extract, is agreed with Benkeblia [39] that showed the essential oils extracts of common onions and garlic were found to inhibit bacterial and yeast growth, and inhibition of Garlic oil was strong than those of Onions oil extracts. Garlic oil possessed moderate antibacterial activity in this study, which enhanced 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 and 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 [42].

Conclusion

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 and oil extracts). Garlic and Onion have activity on both (G+ve and G-ve) bacteria and fungi.

The synergistic effect of Garlic-Onion combination extract and 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

1- Adeshina, G.O.; Jibo, S.; Agu, V.E. and Ehinmidu, J.O. Antibacterial activity of fresh juices of *Allium cepa* and *Zingiber officinale* against multidrug resistant bacteria. Intl. J. Pharma. Bio. Sci. 2011; 2: (2).

2- Gerber,C. History of herbal medicine, [cited 2010 Oct13]. Available from: http://herbs.lovetoknow.com/History_Herbal_Medicine.

3- Grunnet, J. Advantages and disadvantages of herbal medicine, [cited 2010 Oct12]. Available at: http://herbs.lovetoknow.com/Advantages_and_Disadvantages_of_Herbal_Medicine

4- Farnsworth, N.R. and Loub, W.D. Information gathering and data bases that are pertinent to the development of plant-derived drugs in Plants: The potentials for extracting protein, medicines, and other useful chemicals. Workshop proceedings. OTA-BP-F-23.U.S. Congress, Office of Technology Assessment, Washington D.C.1983; pp. 178-195.

5- Janovska, D.; Kubikova, K. and Kokoska, L. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci. 2003; 21:107-111.

6- Grover, A.; Bhandari, B.S. and Rai, N. Antimicrobial activity of medicinal plants-*Azadirachta indica* A. Juss, *Allium cepa L.* and *Aloe vera L.* Intl. J. Pharm. Tech. Res. 2011; 3:(2).

7- Raman, S.G.; Kumar, D.S.; Harani, A.; Parthiban, N.; Prasad, M.S.; Venkateshwarlu, G. and Reddy, A.H.P. Comparison studies of phytochemical screening and antibacterial activities of *Allium cepa* and *Allium sativum* bulb extract. Asian J. Pharmaceut. Health Sci.2011; 1:(3).

8- Corzo-Martinez, M.; Corzo, N. and Villamiel, M. Biological properties of

Onion and Garlic. Trends Food Sci. Technol. 2007; 18: 609-615.
9- Whitemore, B.B. and Naidu, A.S. Thiosulfinates. In: Naidu, A.S. Ed., Natural food antimicrobial systems. Boca Raton, FL: CRC Press. 2000; 265-380.
10- Kumar, A. and Sharma, V.D. Inhibitory effect of garlic *Allium sativum L.* on enterotoxigenic *Escherichia coli*. Indian J. Med. Res. 1982; 76: 66-70.
11- Shuford, J.A.; Steckelberg, J.M. and Patel, R. Effects of fresh garlic extract on *C. albicans* biofilms, *Antimicrob. Agents Chemother*. 2005; 49 (1): 473.
12- Abdulzahra, D.M. and Mohammed, H.F. The antibacterial effect of Ginger and Garlic extracts on some pathogenic bacteria isolated from patients with otitis media. Int. Res. J. Med. Sci., 2014; 2:(5).
13- Sridhar, T.M.; Josthna, P. and Naidu, C.V. *In vitro* antibacterial activity and phytochemical analysis of *Solanum nigrum L.*- An important antiulcer medicinal plant. J. Exper. Sci. 2011; 2(8): 24-29.
14- Mehrabian, S. and Larry, H. Antimicrobial activity of *Allium sativum*, *Allium cepa*, *Allium porrum* against entricpathogenes (*Enterobacteracea*). 2005
15- Forbes, B.A.; Sahm, D.F. and Weissfeld A.S. Bailey and Scotts' Diagnostic Microbiology *12th ed. Elsevier* 2007.
16- Onyeagba, R.A.I.; Ugbogu, O.C.; Okeke, C.U. and Iroakasi, O. Studies on the antimicrobial effects of garlic (*Allium sativum L.*), ginger (*Zingiber officinale roscoe*) and lime (*Citrus aurantifolia L.*). Afr. J. Biotech. 2004; 3 (10): 552-554.
17- CLSI (National Committee for Clinical Laboratory Standards). Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M100-S12.Wayne. PA, NCCLS 2002.
18- Danial W.W. Biostatistics a foundation for analysis in health sciences, *4thed.* John Wilely and Sons Inc. 1988.
19- Kelmanson, J.E.; Jager, A.K. and Vaan Staden, J. Zulu medicinal plants with antibacterial activity. J. Ethanopharmacol. 2000; 69: 241-246.
20- Ahmad, I. and Beg, A.Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multiple drug resistant human pathogens. J. Ethanopharma. 2001; 74: 113-123.
21- Guleria, S. and Kumar, A. Antifungal activity of some himalayan medicinal plants using direct bioautography. J. Cell Mol. Bio. 2006; 5: 95-98.
22- Zakaria,Z.; Sreenivasan ,S. & Mohamad, M. Antimicrobial Activity of Piper ribesoides root extract against *S. aureus*. J. App. Biol. Sci. 2007; 1(3): 87-90
23- Cutler, R.R.& Wilson, P. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin resistant *S. aureus*, *Brit. J. Biomed. Sci*.2004; 61 (2): 71–74.
24- Vaidya, V.; Keith, U. and Ingold, D.A. Garlic source of the ultimate antioxidants-sulfenic Acids. *Angewandte Chemie*. 2009; 121 (1):163-166.
25- Eja, M.E. and Asikong, B.E. A comparative assessment of the antimicrobial effects of garlic *Allium sativum* and antibiotics on diarrheagenic organisms. South east Asian J Trop Med Public Health. 2007; 38 (2): 343-8.
26- Celiini, L.D.; Campli, B.; Masulli, M.; Bartolomeo, S. and Aliocati, N. Inhibition of *Helicobacter pylori* by garlic extract *Allium sativum*. *FEMS* Immunol. Med. Micrbiol. 1996; 13: 273-277.
27- Gonzalez-Fandos, F.; Garcia-Lopez, M.; Sierra, M. and Otero, A. Staphylococcal growth and enterotoxins (A-D) and thermonuclease synthesis in the presence of dehydrated garlic J. Appl. Bacteriol. 1994; 77: 549-552.
28- Cavallito, C.J. and Bailey, H.J. Allicin, the antibacterial principle of *Allium sativum L.* isolation, physical properties and antibacterial action. J Am Chem Soc. 1944; 66: 1950-1951.
29- Koch, H.P. and Lawson, L.D. Garlic, the science and therapeutic application of *Allium sativum L.* and related species, in: Retford D.C. Ed. Williams and Wilkins, Baltimore. 1996. 1-233.
30- Muhsin, A.J. & Al-Mossawi, A. Susceptibility of some multiple resistant bacteria to garlic extracts. *Afr J Biotech*. 2007; 6 (6):771-776.
31- Ankri, S. and Mirelman, D. Antimicrobial properties of allicin from

garlic. *Microbes Infect*. 1999; 1(2):125-219.
32- Esimone, C.O.; Okoye, F.B.C.; Odimegwu, D.C.; Nworu, C.S.; Oleghe, P.O. and Ejogha, P.W. *In Vitro* antimicrobial evaluation of Lozenges containing extract of Garlic and Ginger. Int J. Health Res, 2010; 3(2): 1 05
33- Hindi, N.K.K. *In Vitro* antibacterial activity of aquatic Garlic extract, Apple vinegar and Apple vinegar-Garlic extract combination. Am. J. Phytomed. & Clinic. Therapeutics. 2013; (1): 042-051.
34- Zakaria, Y. and Astal, A.L. Effect of storage and temperature of aqueous garlic extract on the growth of certain pathogenic bacteria J. Al Azhar Univ. 2003; 6(2): 11-20.
35- Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbiol. Rev.1999; 12(4): 564-582.
36- El-Nima, E.I.; Ahmed, S.A.; Mekkawi, A.G. and Mossa, J.S. The antimicrobial activity of Garlic and Onion extracts Pharmazie. 1983; 38 (11): 747-748.
37- Benkeblia, N.; Dahmouni, S.; Onodera, S. and Shiomi, N. Antimicrobial activity of phenolic compound extracts of various onions (*Allium cepa L.*) Cultivars and Garlic (*Allium sativum L.*). J. Food Technol.2005; 3 (1): 35-40.
38- Kabelik, J. Antimikrobielle Eigen's chaften des Knob Lauchs pharmazie.1970; pp. 25:266.
39- Benkeblia, N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm.-Wiss. U.-Technol. 2004; 37: 263-268
40- Arora, D.S. and Kaur, J. Antimicrobial Activity of Spices. Intl. J. Antimicrobl. Agents.1999*; 12*: 257-262.
41- Ross, Z.M.; O'Gara; E.A.; Hill, D.J.; Sleightholme, H.V. and Maslin, D.J. Antimicrobial properties of Garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with Garlic oil sulfides and Garlic powder. Appl. Environ. Microbiol. 2001; 67: (1) 475-480.
42- Cavallito C.J. and Bailey, J.H. Allicin,the antimicrobial principle of *Allium sativum L* isolation, physical properties ,and antibacterial action. J. Am. Chem. Soc. 1944; 67:1032.