Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (December Suppl., No. 2) : 2018 : S137-S147

ISSN-0972-3005

ANTI-SWARMING, ANTI-ADHERENCE AND ANTI-BIOFILM ACTIVITIES OF GARLIC-RELATED AQUATIC EXTRACTS: AN *IN VITRO* STUDY

NADA KHAZAL KADHIM HINDI, NEBRAS N. AL-DABBAGH AND ZAINAB ADIL GHANI CHABUCK

PhD in Microbiology, Department of Basic and Medical science, College of Nursing, University of Babylon, Babylon Province, Iraq
PhD in Microbiology, Department of Microbiology, College of Dentistry, University of Babylon, Babylon Province, Iraq.
PhD in Microbiology, Department of Microbiology, College of Medicine, University of Babylon, Babylon Province, Iraq.

(Received 8 September, 2018; accepted 27 October, 2018)

Keywords *: Antibacterial activity, Garlic, garlic oil, Vinegar and Vinegar-Garlic Extract combination.*

Absrtact– Allium vegetables, principally *Allium sativum* L. (garlic) display antibiotics activities against bacteria, especially strains which have become resistant to antibiotics. Evaluation of Antimicroborial Activity, Anti-adherence, Anti-Biofilm and Anti-Swarming of the Aquatic Extract of Garlic, Vinegar of Garlic and oil Garlic against yeast and fourteen bacterial pathogens. The study of antibacterial activities of aqueous extract of garlic, vinegar, garlic oil, and garlic-vinegar combination against nine gram-negative and five gram-positive bacterial and one yeast isolates, these are included *E. feacalis, S. epidermidis, S. aureus, S. pyogenes, S. pneumoniae, Ps. fluresence, Ps. aeruginosa, S. typhi, En. aerugenes, E. coli, K. pneumoniae, Acinetobacter, P. vulgaris, Proteus mirabilis* and *candida albicanis* were carried out. Antimicrobial activity of extract by well-diffusion method, agar wells-diffusion method, biofilm inhibition test by the using of tissue culture plate method, swarming and adherence inhibition assay were done for estimation and evaluation of the garlic extracts as bacterial inhibitor agents. The maximum effects of garlic extracts were observed in *S. typhi*. All tested microorganisms were highly sensitive to the combination of garlic-vinegar, while all of them were slightly sensitive to action of vinegar. All garlic extracts were inhibited bacterial motility, which show using swarming assays with all gradual different concentrations, inhibits bacterial cell adherence to epithelial cells of oral and can inhibit quorum sensing and biofilm formation. The extracts of garlic showed a wide range of activities and performs all the effects and activities of antimicrobial agents. From this work's results, we can propose that garlic canhave a beneficial role in the protection of consumers from the hazard of most infectious pathogens.

INTRUDUCTION

In history, it is known that garlic had been applied through out many centuries, worldwide by various civilizations and cultures to prevent infections. It could be used as raw juice, powders and capsules by means of dietary complements, so it differs from elements of food or conventional foods. Pasteur was the principal and first one who designated the antibacterial influence of garlic and onion liquids. The vegetables Allium, principally *Allium sativum L.* (garlic) display antibiotics activities against bacteria, especially strains which have become resistant to antibiotics (Whitemore and Naidu, 2000). Many and many researches about the antibacterial activities of garlic juice, steam-distilled oil, lyophilized powders, alcoholic, aqueous extracts and other commercial available preparations of garlic. The pharmacological effects of garlic on numerous fungal and bacterial species, it has been used in prevention of wound infection and food poisoning (Hsieh *et al.,* 2001; Singh and Singh, 2008).

Han *et al*. (1995) found that 1mg of allicin has an antibiotic activity equal to the activity of 15 IU of penicillin. Water- and oil- soluble organosulfur compounds in garlic are likely responsible for its

***Corresponding author's email**: nadakhazal@yahoo.com; nebras84@yahoo.com; zainabibz@gmail.com

therapeutic influence, also they are the cause of typical flavor and odor of it. Thiosulfinates of garlic also have a significant role in the antibacterial property of garlic. Hughes and Lawson (1991) revealed that; when thiosulfinates (as allicin) are separated from this extract, it will completely lose its antimicrobial activities. As many workers are trying to find plants that have been applied as alternative medicines since years (Lingham *et al*., 2012).

Consumers are gradually trying to avoid the intake of foods that are treated with different chemical materials. Natural replacements are required to attain human safety in a high level, regarding the food borne infectious pathogens (Rauha *et al*., 2000).

The natural decontaminators, as the organic acids, are checked for their activity as bactericidals. Uyttendaele *et al*. (2004) among foodstuffs, vinegar contains sterilizing actions. It has been prepared from alcoholic beverage fermentation, mainly wine (Nascimento *et al*., 2003). Acetic acid which is an organic acid, is responsible for the total acidity of vinegar. Acetic acid is a monocarboxylic acid. It has a sharp pungent flavor and odor; and it is safe for general usages and miscellaneous purposes. As it is used to inhibit bacteria causing spoilage from growth in meat; thus are applied by dipping and spraying techniques (Dincer and Baysal, 2004).

In a Bradley's study, showed that the addition of acetic acid and citric acid each of them will reduce the growth of Enterobacteriaceae (Bradley *et al*., 2011). Sengun and Karapinar (2005) and Makino *et al*. (2000) found the vinegar has inhibit the growth of many organisms such as *E. coli* and *Salmonella*. It also helps in cleaning carrots from *Salmonella Typhimurium* (Sengun and Karapinar, 2004). In a study by Frederick *et al*. (1994) indicated that addition of 2% acetic acid can reduce the incidence of *Salmonella* in pork. Finally, vinegar can be used alone or as a mixture as a natural flavoring additive in some salads.

In this paper we evaluated the of Antimicroborial Activity, Anti adherence, AntiBiofilm and Anti Swarming activities of the Aquatic Extract of Garlic, Vinegar of Garlic and oil Garlic against yeast and fourteen bacterial pathogens.

MATERIAL AND METHODS

For preparation of Aquatic Garlic Extract from fresh garlic (*Allium Sativum L.*) acorrding to (hindi, 2013).

Bacterial and Yeast Isolates

The total of 1 yeast *(Candida albicanis*), 14 bacterial isolates (clinical specimens) were used in the work. Bacterial isolates tabulated in the following table. Bacterial isolates were activated in three continual times on nutrient agar then stored at 4ºC as nutrient agar slant. The documentation of isolates were established by various biochemical tests (Forbes *et al*., 2007).

Table. Bacterial isolates

Gran negative bacteria	Gram positive bacteria
Salmonella typhi	*Staphylococcus aureus*
Escherichia coli	*Staphylococcus epidermidis*
Pseudomonas aeroginosa	*Streptococcus pneumoniae*
Pseudomonas fluroscences	*Streptococcus pyogenes*
Proteus merabilis	*Streptococcus fecalis*
Proteus vuligaris	
Klebsiellap neumoniae	
Enterobacter aerugenes	
Acinetobacter	

Antimicrobial activity test by Agar-well diffusion assay *(In vitro)*: (Hindi and Chabuck, 2013, Hindi *et al*, 2014**).**

Antibacterial activity assay: According to Forbes (2007), the antimicrobial activity of ciprofloxacine was detected by agar-disc diffusion (the test were performed in triplicates).

Biofilm Formation Assay: Semi quantitative microtiter plate test or Tissue culture plate method assay (TCP) designated by Christensen *et al*. (1985) was assumed as the gold standard method for detection of biofilm formation.

Table 2. Bacterial adherence and biofilm formation by method of TCP (Hindi *et al*., 2016).

Mean of OD value at 630 nm	Adherence	Biofilm formation
<0.120	non	Non
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Adherence test

Bacterial adherence to epithelial cell of the mouth is one of the chief and important virulence properties of these bacteria and can be identified using method designated by (Mataveki *et al*., 2004 and Avila-Compose *et al*., 2000).

Inhibition of motility (swarming) by plant extract: The method of Iwalokun *et al.* (2004), Plant extracts were separately applied in concentrations of (10%, 20%, 30%).

All the extracts involved in this study were used in each tests to detect the antibacterial properties of these extracts separately.

Statistical analysis

Bonferroni test (Danial, 1988) was used to analyse data; as ($P \leq 0.05$) to show significant differences between the types of extracts.

RESULT

Antimicrobial Activity Estimation

During the course of this work, the three designated antimicrobial procedures were compared. The vinegar alone, watery extract of garlic alone and a combination of vinegar along with garlic extract. Results revealed that the highest influences of the combined garlic extract with the vinegar extracts; while the other extracts separately had lower effects than the combination of extracts.

The used aqueous garlic extract were applied at 50% concentration by agar diffusion methods, and examined for their inhibitory activity to the yeast, Gram-ve and Gram+ve bacterial isolates. Entire tested organisms were inhibited by this extract concentration, with the maximum inhibition zone was detected against *S. typh*i.

On the other hand, the results showed a lowest activity of vinegar against microorganism. The highest inhibition zone was detected against *S. aureus* (15mm) and has no activity against yeast isolate. Furthermore, the activities of garlic extract and vinegar combination on the microorganisms showed high sensitivity of all bacterial isolates to this combination. *S. typh*i, *E. feacalis* and *P. aeruginosa* were greatly sensitive to vinegar garlic extract than other bacteria followed by *E. coli*, *E. aerugenes*, *P. Mirabilis*, *P. fluorescence*, *K. pneumoniae*, and *S. pneumoniae* with zones of inhibition were 50mm and 40mm respectively (Figure 1).

Moreover, inhibitory activities of garlic oil against yeast and different bacterial isolates were checked, as the results shown that all the tested bacterial isolates were greatly sensitive to this oil. The maximum inhibition zone was observed against *S. pneumoniae* followed by *K. pneumonia* with zones of inhibition as 35mm and 33mm respectively (Figure 2).

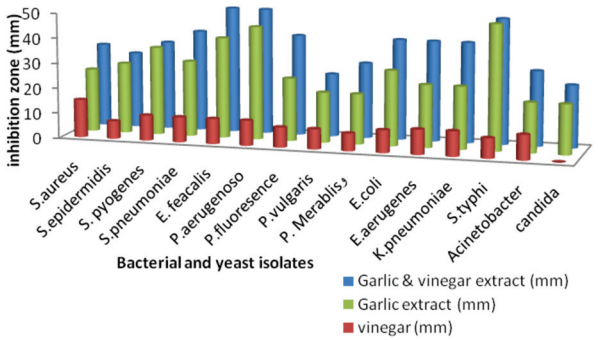

Fig. 1. Antimiriacrobial activity (inhibition zones (mm)) at 50% concentrations of the aqueous garlic extract by agar well method.

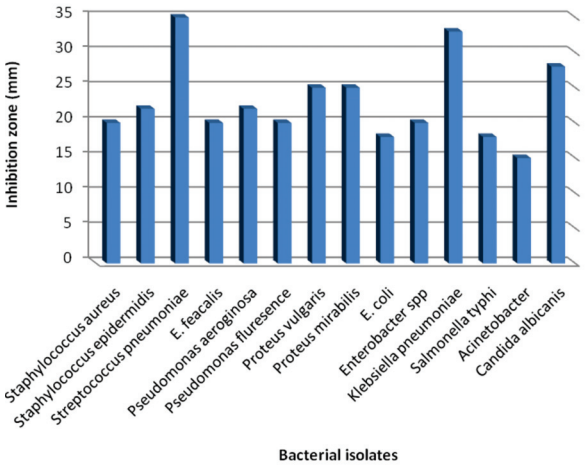

Fig. 2. Antimiriacrobial activity Garlic oil (inhibition zones (mm)) by agar well method.

Statistical analysis of data, exhibited significant differences between the inhibitory effects of garlic and the combination of garlic-vinegar extract on the yeast and different bacterial isolates. While, no significancy were obtained between the effects of vinegar and the combination of garlic -vinegar extract on microbial isolates, also there was no significant difference between garlic extract and vinegar on bacterial and yeast isolates at level ($P \leq 0.05$).

Statistical analysis indicated significant differences between the effects of combination extracts and garlic oil on bacterial and yeast isolates, there were no significant differences between garlic-vinegar extract combination and garlic oil on microbial isolates, there were no significant differences between garlic oil and garlic extract combination on microbial isolates and there were significant differences between vinegar and garlic oil on microbial isolates at level ($P \leq 0.05$).

Additionally, minimum inhibitory concentration (MIC) of Aquatic Extract of Garlic, Vinegar of Garlic and oil Garlic against fungal and bacterial isolates were determined (Table 3). The MIC values of Aquatic Extract of Garlic, Vinegar of Garlic and oil Garlic for Gram negative bacteria were ≤ 2560µg / mL, while it was ≤ 1280 µg /mL against most Gram positive bacteria. The value of MIC were increased ≤) 5120 µg /mL) against *E. aerugenes* and *C.albicans* at the these extracts.

MIC values of Vinegar Gram positive bacteria were 640 ≤ µg /mL, while it was ≤ 1280 µg/mL against most Gram negative bacteria MIC value was increased ≤) 2560 µg /mL) *E. aerugenes* and *C.albicans* at the these extracts.

In contrast, antibacterial activity of garlic extract was compared to ciprofloxacin, (Figure 3) summarized response to this drug, which showed less effect than the garlic extract.

Conversely, anti-adherence and Anti-biofilm activities of vinegar, aquatic garlic extract, vinegar-garlic extract and garlic oil against Gram negative bacteria were studied, the results shows that the garlic extracts and its vinegar reduce and inhibit the growth with adhesion of bacterial isolate on glass plate (Table 3).

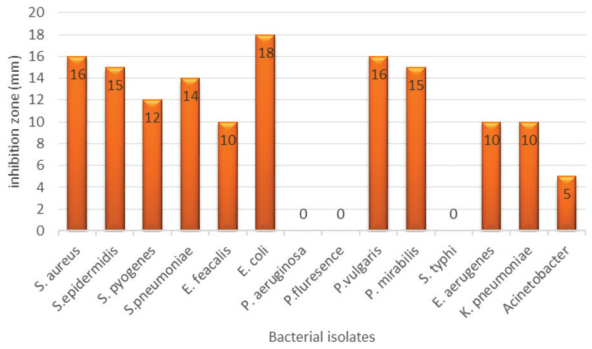

Fig. 3. Effect of ciprofloxacin (inhibition zones (mm) against different types of bacteria.

Furthermore, the result shows that the adhesion of epithelial cell for all used bacterial types are high, (Table 4).

On the other hand, Anti-swarming action of the aquatic extract of garlic, vinegar, and vinegar-garlic extract and garlic oil against Gram negative bacteria

Table 3. Effect of Aquatic Extract of Garlic, Vinegar of Garlic and oil Garlic on the bacterial and yeast isolates by determination of MIC of the extract

Microorganism	Concentration MIC (µg /ml)			
	Vinegar of Garlic	Garlic Extract	Oil Garlic	Vinegar
S. aureus	1280≥	1280≥	1280≥	640≥
S.epidermidis	1280≥	1280≥	1280≥	640≥
S. pyogenes	1280≥	1280≥	1280≥	640≥
S.pneumoniae	1280≥	1280≥	1280≥	640≥
E. feacalis	1280≥	1280≥	1280≥	640≥
E. coli	2560≥	2560≥	2560≥	1280≥
P. aeruginosa	2560≥	2560≥	2560≥	1280≥
P.fluresence	2560≥	2560≥	2560≥	1280≥
P.vulgaris	2560≥	2560≥	2560≥	1280≥
P. mirabilis	2560≥	2560≥	2560≥	1280≥
S. typhi	2560≥	2560≥	2560≥	1280≥
E. aerugenes	5120≥	5120≥	5120≥	2560≥
K. pneumoniae	2560≥	2560≥	2560≥	1280≥
Acinetobacter	2560≥	2560≥	2560≥	1280≥
C. albicans	5120≥	5120≥	5120≥	2560≥

Table 4. Anti biofilm and anti-adherence activity of aquatic garlic extract, vinegar, vinegar-garlic extract and garlic oil against Gram negative bacteria.

Bacteria	Biofilm formation				Adherence			
	garlic extract	vinegar-garlic extract	vinegar	garlic oil	garlic extract	vinegar-garlic extract	vinegar	garlic oil
S. typhi	*Moderately	High**	Moderately	High	Moderately	High	Moderately	High
P. aeroginosa	Moderately	High	Moderately	High	Moderately	High	Moderately	High
P.fluresence	Moderately	High	Moderately	High	Moderately	High	Moderately	High
P.vulgaris	Moderately	High	Moderately	High	Moderately	High	Moderately	High
P.mirabilis	Moderately	High	Moderately	High	Moderately	High	Moderately	High
K. pneumoniae	Moderately	High	Moderately	High	Moderately	High	Moderately	High
E. aerugenes	Moderately	High	Moderately	High	Moderately	High	Moderately	High
Acinetobacter	Moderately	High	Moderately	High	Moderately	High	Moderately	High
E. coli	Moderately	High	Moderately	High	Moderately	High	Moderately	High

*Moderately (0.120-0.240) ** High (>0.240)

at 10%, 20%, and 30% concentration of each extract (Table 5), (Table 6), and (Table 7) respectively. The aquatic garlic extract, vinegar, vinegar-garlic extract and garlic oil have no Anti-swarming activity of against Gram negative bacteria at 10% concentration, whereas aquatic garlic extract, vinegar, vinegar-garlic extract and garlic oil have an anti-swarming activity of against Gram negative bacteria at 20% and 30% concentration.

DISSCUSSION

Drugs derived from active components of plants are effective, less expensive, easily available, and without secondary effects. The specialists of indigenous and traditional medicine principally depend on medicinal plants to prepare therapeutic materials. The crude extracts of plants may be used directly against antibacterial and antifungal activities.

The method of agar-well diffusion is the most ordinarily used to decide the antimicrobial susceptibilities.

The combinations outcomes on microbial growth have a synergistic effect against all microorganisms. These were match with (Qin *et al*., 2009) who found that the antimicrobial activity of vinegar is strong on bacterial cells but it is weak on fungi. Vinegar and extract of stored garlic both of them had heavy antimicrobial activity against both fungi and bacteria.

Zasshi and his group (1997) pointed out, as the microbiocidal and microbiostatic actions of vinegar yields on growth of *E. coli*, as bactericidal influence of vinegar was independent on the size of bacterial inoculums, but it depends on the phase of growth. Bacteria at log phase of growth were more susceptible, than those at the stationary phase.

In Thailand, vinegar was utilized in treat dandruff and other skin infection, due to the vinegar has antimicrobioial effect against gastrointestinal tract disorder and dermatitis bacteria, also it has an antioxidant activity. As, the antioxidant agents may play a vital role as anti-inflammatory caused by skin diseases and wounds. Vinegars inhibited growth of *P. acnes*, *S. epidermidis, S. aureus, S. faecalis, and E. coli* (Rakmai, 2009). Other elements as ketonic, neutral and phenolic compounds could be principal composites in biological efficacies. The phenolic compounds as cresols or phenol are well recognized to have antimicrobial features and contribute to the

Table 5. Anti-swarming activity of aquatic garlic extract, vinegar, vinegar-garlic extract and crude garlic oil against Gram negative bacteria at 10% concentration

Bacteria	Swarming 10%			
	garlic extract	vinegar	Garlic vinegar *extract*	crude Oil garlic
S. typhi	motile	motile	motile	motile
P. aeroginosa	motile	motile	motile	motile
P.fluresence	motile	motile	motile	motile
P.vulgaris	motile	motile	motile	motile
P. mirabilis	motile	motile	motile	motile
K. pneumoniae	No motile	No motile	No motile	No motile
E. aerugenes	No motile	No motile	No motile	No motile
Acinetobacter	motile	motile	motile	motile
E. coli	motile	motile	motile	motile

Table 5. Anti-swarming activity of aquatic garlic extract, vinegar, vinegar-garlic extract and crude oil against Gram negative bacteria at 20% concentration

Bacteria	Swarming 20%			
	Garlic extract	vinegar	Garlic-vinegar extract	crude Oil garlic
S. typhi	No motile	No motile	No motile	No motile
P.aeroginosa	No motile	No motile	No motile	No motile
P.fluresence	No motile	No motile	No motile	No motile
P.vulgaris	No motile	No motile	No motile	No motile
P. mirabilis	No motile	No motile	No motile	No motile
K.pneumoniae	No motile	No motile	No motile	No motile
E. aerugenes	No motile	No motile	No motile	No motile
Acinetobacter	No motile	No motile	No motile	No motile
E. coli	No motile	No motile	No motile	No motile

antimicrobial effect (Velmurugan, 2009). The phenolic compounds antioxidant ability depends on the presence of methoxyl, alkyl and hydroxyl groups (Lopez, *et al*., 2003).

The property of phenolic compounds as an antioxidant is responsible for inhibition activity of fungal growth. Eja *et al.* (2007) and Celiini *et al.* (1996) revealed that the microbiocidal effect of extract garlic against *Streptococcus*, *Helicobacter pylori, Staphylococcus, Proteus, Escherichia, Clostridium, Klebsiella, Bacillus, Salmonella* and *S. enterica Enteritidis.* Also, the extracts of garlic can stop the thermo nuclease and Staphylococcal enterotoxins A, B, and C1 formation (Gonzalez-Fandos *et al*., 1994). Cavallito and Bailey (1994) who demonstrate that the antimicrobial effect of garlic extract due to active component of garlic is allicin, some strains of bacteria resistant to methicillin but its sensitive to allicin (Koch *et al*., 1996). These results match with Muhsin *et al.* (2007) who established that *S. aureus* and *E. coli* were sensitive to garlic extracts.

Ankri and Mirelman (1999) showed that the Allicin displays an antibacterial effect against a wide variety of bacterial types, fungi, parasite and virus, it is the principal, active element of fresh homogenized crushed garlic.

The Allicin antimicrobial activity is by inhibition of synthesis of RNA, DNA and protein syntheses. Allicin interferes with lipid synthesis and RNA production (primary target of allicin action is the RNA). If RNA produced in less amount, or it cannot be produced; thus severely disturbance of protein synthesis will be occur. It may be paused at any stage contributed to the deficiency of tRNA, mRNA, and rRNA. When amino acids, subsequently proteins failed to be made, thus development and growth of the this organism will not happen as they are necessary for all structures of cell and growth.

Concerning, the lipid synthesis is also affected, with its effect mainly, inhibiting the formation of cell wall phospholipid biolayer in both Gram-stained bacterial types, and because of its reaction chemically with various enzymes through thiol groups, such as thioredoxin reductase, alcohol dehydrogenase and RNA polymerase. The action of diallyl disulphide or diallyl thiosulphinic acid or

Table 5. Anti-swarming activity of aquatic garlic extract, vinegar, vinegar-garlic extract and crude garlic oil against Gram negative bacteria at 30% concentration

Bacteria	Swarming 30%			
	garlic extract	vinegar	Garlic-vinegar extract	crude Oil garlic
S. typhi	No motile	No motile	No motile	No motile
P. aeroginosa	No motile	No motile	No motile	No motile
P.fluresence	No motile	No motile	No motile	No motile
P.vulgaris	No motile	No motile	No motile	No motile
P. mirabilis	No motile	No motile	No motile	No motile
K. pneumoniae	No motile	No motile	No motile	No motile
E. aerugenes	No motile	No motile	No motile	No motile
Acinetobacter	No motile	No motile	No motile	No motile
E. coli	No motile	No motile	No motile	No motile

allicin, the antifungal and antibacterial activities of extracted garlic juice are because of the inhibition of succinic dehydrogenase via the thiol group inactivation. Anionic components of garlic is chlorides, sulfates and nitrates and other water soluble components which could be responsible for its antimicrobial action (Astal, 2004).

All of these elements lead to the inability of the bacteria to mature in the existence of garlic or allicin**.** Thus the diameter size of the zone of inhibition obtained with garlic extract showed a higher zone, as it is compared to the activity of the marketable antibiotics (Iwalokun *et al*., 2004).

Yamada and Azuma (1991) and Mojabi (2012), who instituted that, garlic extract has a strong antifungal and anticandidal properties, as it is attributed to the growth inhibition of fungi, and it can decrease the germination of spores and development of hyphae.

Antibiotics are commonly prescribed for therapy of bacterial infections, but numerous of these pathogenic bacteria developed different degrees of resistance. Natural plant products may provide new various sources of antibacterial agents; from all these results, that the medical importance of plant extracts is equivalent to the daily used antibiotics.

Durairaj (2009) reported that the aqueous garlic extract has ability to inhibite the growth of *E. coli, S. typhi* and *S. aureus.* Combined effects can avert the development of resistance to antibiotic.

The first and decisive step in the microbial sequence of events in pathogenicity is the adhesion, which leads to colonization, exposure to sub-lethal doses of antimicrorial materials can weaken the ability to adhere (Sharma and Sabris, 2010). The effectivenes of polyphenolic compounds on the enzymatic activity such as glucosyltransferase which are very important as a virulence parameter which allows the bacterial adherence and colonization (Gregoire *et al*., 2007), garlic extracts revealed lessening in formation of biofilm.

There are bacterial specificity in the attachment to the tissue surface of host cell, as *E. coli* have fimbriae Type-I and P-pili (pap) for adhesion to Intestinal and Urethral epithelial tissues and Upper urinary tract, *Vibrio, Neisseria* and *Pseudomonas* have pili Type IV, which comprises a protein subunit with phenylalanine, (methylated amino acid), near or at its amino terminus. The "N-methylphenylalanine pili" have been recognized as elements of virulence factor in the pathogenesis of *P. aeruginosa* to cause lung infection in patients with cystic fibrosis. The treatment of bacteria with garlic extract show significant inhibition of bacterial adherence to the epithelial host cells, thus we can conclude that the aquatic extract of garlic extract can prevent pathogenic bacteria to cause Pyelonephritis, Urethritis and Diarrhea.

Proteus mirabilis showed high ability to enhance host infection during rapid swarming; as it enhances cell migration through urinary tract and cause many infections. This phenomena had been studied in different genera *Serratia*s pp., *Salmonella* spp., *E. coli, Aeromonas* spp., *Bacillus spp, Yersinia* spp., *Pseudomonas* spp., *Vibrio* spp. and *P. mirabilis* **(**Stickler, 1999**)**.

Swarming behavior and swarm cell differentiation are the results of many global control mechanisms and a complex sensory transduction. *Proteus mirabilis* swarming requires the sensing with intracellular, cell-to-cell and integration of a variety of environmental signals and encompasses a regulated gene expression leading to physiological and morphological changes (Fraser, 1999). In this paper, we got suggestion that compounds in garlic extracts serve as environmental stimulus to affect swarming of *P. mirabilis* and other gram negative bacteria.

The result shows the effects of crud extract of garlic extracts on the adherence and growth of pathogenic bacteria on the human buccal epithelial cells. Garlic extracts completely inhibited the adherence and growth of pathogenic bacteria on these cells. A lot of studies showed the activity of compound within the garlic extracts.

Pathogenic biofilm has been associated with persistency of infections becuse their deveioped high resistancy to many antibacterial agents, whereas biofilms formation in commensal bacteria often support immune system of the host. The supervisory biofilm formation of both commensal and disease-causing bacteria is important in bacteria-associated diseases.

Regarding flavonoids, it had been showed to obstruct biofilm foundation of *Streptococcus mutans* (Koo, 2003), Aeromonashydrophila (Kappachery, 2010), and Escherichia coli O157:H7 (Vikram, 2010).

"Other study demonstrated that certain natural flavonoid, phloretin, is a nontoxic inhibitor of biofilms in EHEC O157:H7 but it is not harmful to commensal E. coli. Also, importantly, we confirmed that it shows anti-inflammatory chriteria in both the *in vivo* and *in vitro* models of inflammatory colitis. All these revealed that phloretin is a good biofilm

inhibitors of *E. coli* with many beneficial activities (Lee *et al*., 2011).

Moreover, the biofilm is considered as a protection for the microbes against attacks by phagocytes, antibiotics, disinfectants, many protective proteins of host immune system's and signaling fragments (Jefferson, 2004).

Many pathogens including *Salmonella* spp., *E. coli*, *Listeria monocytogenes*, *Enterobacter* spp., *Staphylococcus aureus*, and *Shigella* spp. exploit a biofilm production strategy to persist and cause disease. Tamayo *et al*. (2010) institute that pathogenic *Vibrio cholera* in both intact and dispersed biofilms massively competed planktonic populations. Wilson *et al*. (2007) reported that *S. enterica* serovar Typhimurium samples displayed greater virulence in a model of mouse infection, along with matrix extracellular accumulation, which is compatible with the biofilm.

Most of the test microorganisms are food born pathogenic bacteria, thus, extracts of garlic and its vinegar are obstacle elements in many processed caned foods and work a protective valuable activity against pathogenic microbes through out the ingestion of contaminated foods.

Based on this work, we can be postulated that the aquatic extracts of garlic holds potent antimicrobial properties, inhibit adherence, inhibit biofilm formation, and inhibit swarming. These extracts have activities against Gram negative and positive bacteria, and inhibits bacterial groups causing dental carries, UTI anddiarrhea, therefore I suggest that the consumption of garlic extracts may inhibit bacterial adhesion of many bacterial types and can prevent bacterial diseases. Therefor such type of research may open the door for further exploration of orally administered vaccines which exploit bacterial adhesions.

CONCLUSIONS

Garlic extracts exhibit obvious antimicrobial properties against both bacterial types negative and positive, and fungi. The synergism of vinegar-garlic extract combination has ability to subside the growth of infectious pathogens which have antibiotics resistance.

ACKNOWLEDGMENTS

I'm grateful to the College of Nursing/ University of Babylon, for providing the necessary facilities, that are needed for effective achievement of the work.

REFERENCES

Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1(2) : 125-129.

Astal, Z.E. 2004. The inhibitory action of aqueous garlicextract on the growth of certain pathogenic bacteria. *Eur. Food Res. Technol.* 218 : 460-464.

Avila-Campos, M.J. Simionato, M.R. Cai, S., Mayer, M. Delorenzo, J.L. and Zelant, F. 2000. Virulence Factors of Actinobacillus actinomycetecomitans: other Putative Factors. *Pesq. Odont. Bras.* 14(1): 5-11.

Bradley, E.M. Williams, J.B. Schilling, M.W. Coggin, P.C. and Crist, C. 2011. Effects of sodium lactate and acetic acid derivatives on the quality and sensory characteristics of hot-boned pork sausage patties. *Meat Sci.* 88 : 145-150.

Cavallito, C.J. and Bailey, H.J. 1944. Allicin, the antibacterial principle of Allium sativum I. Isolation, physical properties and antibacterial action. *J Am Chem Soc.* 66 : 1950-1951.

Celiini, L. Di Campli, B., Masulli, M. Di Bartolomeo S Aliocati, N. 1996. Inhibition of Helicobacter pylori by garlic extract (*Allium sativum*). *FEMS Immenol. Med. Micrbiol.* 13 : 273-277.

Christensen, G.D., Simpson, W.A., Younger, J.A. Baddour, L.M., Barrett, F.F. and Melton, D.M. 1985. Adherence of cogulase negative Staphylococci to plastic tissue cultures:a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol.* 22: 996–1006.

Danial, WW. *Biostatistics a foundation for analysis in health Sciences.* 1988; 4thed. John Wilely and Sons,Inc.

DinCer, A.H. and Baysal, T. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology.* 30 : 197–204.

Durairaj, S., Srinivasan, S. and Lakshmanap, P. 2009. *In vitro* Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature *Electronic Journal of Biology.* 5(1) : 5-10.

Eja, M.E. and Asikong, B.E. 2007. (Nigeria) A comparative assessment of the antimicrobial effects of garlic (Allium sativum) and antibiotics on diarrheagenic organisms: *Southeast Asian J Trop Med Public Health*. 38(2): 343-348.

Forbes, B.A. Sahm, D.F. and Weissfeld, A.S. Bailey and Scotts' Diagnostic Microbiology, 2007; 12th. ed. Elsevier China.

Fraser, G.M. and Hughes, C. 1999. Swarming motility. *Curr. Opin. Microbiol.* 2 : 630–635.

Frederick, T.L., Miller, M.F., Thompson, L.D., Ramsey, C.B. 1994. Microbiological properties of pork cheek meat as affected by acetic acid and temperature. *J Food Sci.* 59: 300–305.

Gonzalez-Fandos, F., Garcia-Lopez, Mi Sierra Mi Otero A 1994. Staphylococcal growth and enterotoxins (A-D) and thermonuclease synthesis in the presence of dehydrated garlic. *J. Appl. Bacteriol.* 77: 549-552.

Grgoire, S., Singn, A.P., Vorsa, N. and Koo, H. 2007. Influence of Cranberry phenolics on glucan synthesis by glucosyltransterase and Streptococcus mutans acidigenicity. *J. Appl. Microbiol.* 103 : 1960-1968.

Han, J., Lawson, L. and Han, G. 1995. A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Annals of Biochemistry.* 225: 157-160.

Hindi, N. K. H. and Chabuck, Z. A. G. 1995. 2013. Antimicrobial Activity of Different Aqueous Lemon Extracts *Journal of Applied Pharmaceutical Science.* 3 (06) : 074-078.

Hindi, N. K. H., AL-Mahdi, Z.K.A. and Chabuck, Z. A. G. 2014. Activity of the Aquatic Extractof Fresh, Dry Powder Ginger, Apple Vinegar Extract of Fresh Ginger and Crud Oil of Ginger (*Zingiber officinale*) Against Different Types of Bacteria in Hilla City, IRAQ. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6(5): 414-417.

Hindi, N.K.H., Yasir, A. A. AL-Mahdi Zka, Jebur, M.H. 2016. Evaluation of Anti Bacterial Activity: Anti adherence, Anti Biofilm and Anti Swarming of the Aquatic Extract of Black Raisins and Vinegar of Black Raisins in Hilla City, Iraq. *International Journal of PharmTech Research.* 9(9).

Hindi, N.K. H. 2013. In vitro Antibacterial Activity of Aquatic Garlic Extract, Apple Vinegar and Apple Vinegar- Garlic Extract combination. *American Journal of Phytomedicine and Clinical Therapeutics*. 1(1) : 042-051.

Hsieh, P.C., Mau, J.L. and Huang, S.H. 2001. Antimicrobial effect of various combination of plant extracts. *Food Microbiology.* 18: 35-43.

Hughes, B.G. and Lawson, D.L. 1991. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* (Elephant garlic), and *Allium cepa* (onion), garlic compounds and commercial garlic supplement products. *Phytother Res.* 5 : 154-158.

Iwalokun, B.A. Ogunledun, A. and Ogbolu, D.O. 2004b. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. *Journal of Medicinal Food.* 7(3): 327-333.

Iwalokun, B.A., Olukosi, Y.A., Olaya, J.A. and Fashada, O. 2004a. Comparative biochemical and molecular evaluation of swarming of Proteus and effects of anti-swarming agent. *African. J. Biotech.* 3(1): 99-104.

Jefferson, K.K. 2004. What drives bacteria to produce a biofilm? *FEMS Microbiology Letters*. 236: 163-173.

Kappachery, S., Paul, D. Yoon, J. and Kweon, J.H. a potential agent to prevent biofouling of reverse osmosis membrane. *Biofouling.* 2010.

Koch, H.P. and Lawson, L.D. 1996. Garlic, the science and therapeutic application of *Allium sativum* L. and related species, in: Retford D.C. (Ed.), Williams and Wilkins, Baltimore, pp. ; 1-233.

Koo, H. 2003. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. *J. Antimicrob. Chemother*. 52: 782–789.

Lee, J., Regmi, S.C., Kim, J. and Cho, M.H. 2011. Phloretin Inhibits *Escherichia coli* O157:H7 Biofilm Formation and Ameliorates Colon Inflammation in Rats Infect. *Immun.* 79(12) : 4819-4827.

Lingham, T., Besong, S., Ozbay, G. and Lingham, J.L. 2012. Antimicrobial Activity of Vinegar on Bacterial Species Isolated from Retail and Local Channel Catfish (*Ictalurus punctatus*). *J Food Process Technol.* S11.

Lopez, M., Martinez, F., Del Valle, C., Ferrit, M. and Luque, R. 2003. Study of pheolic compounds asnatural antioxidants by fluorescence method. *Talanta.* 60: 609-616.

Makino, S., Cheun, H., Tabuchi, H. and Shirahata, T. 2000. Antibacterial activity of chaff vinegar and its practical application. *Journal of Veterinary Medical Science*. 62: 893-889.

Mateveki, L.L., Aspiras, M., Ellen, R. and Lepine, G. 2004. Two Epithelial Cell Invasion Related Loci of the Oral Pathogen *A. actinomycetecomitans*. *Oral. Mic. & Immun.* 9(7) : 16.

Mojabi, K.B. Shari Karagah, T. and Karimi, H. 2012. Effecacy of Different Concentrations of Garlic Extract in Reduc-tion of Oral Salivary Microorganisms. *Archives of Iranian Medicine*. 15 : 12.

Muhsin, A. and Al-Mossawi, A. 2007. Susceptibility of some multiple resistant bacteria to garlic extract African *Journal of Biotechnology.* 6(6): 771-776.

Nascimento, M.S., Silva, N., Catanozi, M.P., Silva, K.C. 2003. Effects of different disinfection treatment on the natural microflora of lettuce. *J Food Protect.* 66: 1697-1700.

Qin, Y.Z., Hong, W., Wen, C., Yan-Jun, Z. and Wei, W. 2009. Antimicrobial Activity of Vinegar and Garlic Extracts 2009; [J];China Condiment; -06

Rakmai, J. 2009. Chcmical Dctcrminations, Antimicrobial and Antioxidant Activitics of Thai Wood Vincgars, 2009.

Rauha, P., Remes, S., Heinonen, M., Hopia, A. and Kahkonen, M. 2000. Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol.* 56 : 3-12.

Sengun, I.Y. and Karapinar, M. 2005. Effectiveness of household natural sanitizers in the elimination of Salmonella typhimurium on rocket (*Eruca sativa* Miller) and spring onion (*Allium cepa* L.). *Int J Food Microbial*. 98: 319–323.

Sengun, I.Y. and Karapinar, M. 2004. Effectiveness of lemon juice, vinegar and their mixture in the elimination of *Salmonella typhimurium* on carrots (*Daucus carota* L.). *Int J Food Microbiol.* 96 : 301-305.

Sharma, S. and Sabois, S. 2010. Study of anti-adhesive properties of fruit juices and plant extract on urine tract pathogens. *Asian J. Exp.Biol. Sci.* 2: 100-103.

Singh, V.K. and Singh, D.K. 2008. Pharmacological Effects of Garlic (*Allium sativum* L.). *Annu Rev Biomed Sci.* 10: 6-26.

Stickler, D.J. and Hughes, G. 1999. Ability of Proteus

mirabilis to swarm over urethral catheters. *Eur. J. Clin. Microbiol. Infect. Dis.* 18 : 206–208.

Tamayo, R., Patimalla, B. and Camilli, A. 2010. Growth in a biofilm induces a hyperinfectious phenotype in Vibrio cholerae. *Infection and Immunity.* 78 : 3560-3569.

Uyttendaele, M., Neyts, K., Vanderswalmen, Y., Notebaert, E. and Debevere, J. 2004. Control of Aeromonas on minimally processed vegetables by decontamination with lactic acid, chlorinated water and thyme essential oil solution. *Int J Food Microbiol.* 90 : 263-271.

Velmurugan, N., Han, S.S. and Lee, Y.S. 2009. Antifungal Activity of Neutralized Wood Vinegar with Water Extracts of Pinus densiflora and Quercus serrata Saw Dusts. *Int. J. Environ. Res.* 3(2) :167-176.

Vikram, A., Jayaprakasha, G.K., Jesudhasan, P.R., Pillai S.D. and Patil, B.S. 2010. Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids. *J. Appl. Microbiol.* 109 : 515–527.

Whitemore, B.B. and Naidu, A.S. In: Naidu AS (Ed), *Natural food antimicrobial systems*. Boca Raton, FL: CRC Press, pp. 2000; 265-380.

Wilson, J.W., Ott, C.M., Honer, Z.U. and Bentrup, K. Ramamurthy, R., Quick, L. and Porwollik, S. 2007. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proceedings of the National Academy of Sciences of the United States of America.* 104: 16299-16304.

Yamada, Y. and Azuma, K. 1991. Evaluation of the invitro antifungal activity of allicin. *Antimicrob Agents Chemother.* 11 : 743-749.

Zasshi, K., Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y. and Ohta, M. 1997. Antibacterial actin of vinegar against food-borne pathogenic bacteria including Escherichia coli O157:H7 (Part 1). *Examination of Bacteriostatic and Bactericidal Activities.* 71(5) : 443-450.