Antimicrobial Activity Of Garlic Juice (*Allium Sativum*), Honey, And Garlic -Honey Mixture On Some Sensetive And Multiresistant Microorganisms

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ABSTRACT: Garlic is well known for having strong antimicrobial effects. The antimicrobial effects of Chinese garlic juice alone, honey alone (Langaneza honey, Black Forest) and honey-garlic mixture (1/1v, 1/4 v, 4/1v) with different concentrations 100%, 50%, 20%, 10% against 8 microbial species, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus*; (Methicillin- Sensitive *Staphylococcus aureus* - MSSA) ATCC 25923, (Methicillin-Resistant *Staphylococcus aureus* -MRSA) ATCC 10442, *Enterococcus faecalis*; (Vancomycin -Sensitive *Enterococci*-VSE) ATCC 29212, (Vancomycin - Resistant *Enterococci*-VRE) ATCC 51299, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10291 were examined by broth Dilution Method. The results showed that garlic juice at 100%, 50%, 20% and 10% concentration have a very strong effect on the growth of all species of microbes comparing with control, and *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* were the most sensitive microbes. Moreover, Honey at 100%, 50%, 20% and 10% concentration have a very strong effect on the growth of all species of microbes but significantly less than the effect of garlic juice. Also when studying the effects of the garlic- honey mixture (1/1v, 1/4v, 4/1v) with different concentrations, it became clear that the garlic- honey mixture had a very noticeable effect on all kinds of microbes examined, and when comparing the effect of the honey alone and the garlic alone with the garlic- honey mixture, it became clear that all honey-garlic mixtures concentrations (1/1 v, 1/4 v, 4/1 v) were significantly the most effective comparing with garlic or honey alone.

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1- Introduction:

Garlic (*Allium sativum*) in the family Liliaceae is a perennial bulb-forming plant. It is world-wide known, as a dietary and medicinal purposes (**Palaksha** *et al*.,2010; **Daka,2011)** Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices (**Penecilla; Magno**;2011). Garlic has antimicrobial effect which has proved in many previous studies (**Elnima *et al*., 1983; Ayala-Zavala**; **González-Aguilar**,2010;. **Goncagul; Ayaz,2010**) Garlic has been found to exhibit antibacterial activity against a wide range of Gram negative and Gram-positive bacteria (**Chehregani *et al*., 2007**; **Pundir**; **Jain**,**2010**; **Bakht *et al*.**,**2011**; **Bakhshi *et al*.**,2012) including multidrug-resistant strains (**Ham *et al*., 2010; Gull *et al*., 2012**), and also possesses antifungal activity (Aala ***et al*., 2010**); antiparasitic (**Farkhondeh *et al*., 2010**), and antiviral activity **(Guo *et al*., 1993)** Garlic is a very important natural plant that used as alternative medicine for management of typhoid that caused by *sensitive and multi resistance isolates of Salmonella typhi.*(**Hannan *et al*., 2012**), and it can be used alongside conventional antibiotics to fight agents of nosocomial infections that are so prevalent in hospitals **(Abubakar,2009)**.

The most important chemical compounds of garlic which was thought to be responsible for antimicrobial activity are the organosulphur compound including allicin (**Hovana *et al*., 2011**). It showed better antibacterial activity than streptomycin and ampicillin (**Ilić *et al*., 2012**). Allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (**Durairaj *et al*., 2009**). Moreover, garlic is known to inhibit cell wall synthesis because it inhibits trans-peptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall and also activates lytic enzymes (**Eja *et al*., 2007**). Joe ***et al*** (2009) postulated that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group. The ability of garlic to inhibit the growth of both gram-positive and gram-negative bacteria shows that it has a broad spectrum of activity and can be used for formulation and development of newer broad

spectrum antibacterial substances (**Penecilla; Magno**;2011) and it provides scientific basis for its utilization in traditional and folk medicine (**Meriga *et al*., 2012**). **Victor and Igeleke(2012)** found that garlic has very strong antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi.* Joe and co-workers evaluated the antimicrobial activity of garlic extract against *Klebsiella pneumoniae, Staphylococcus aureus, Morganella morgani, Candida albicans, Escherichia coli* and *Proteus vulgaris*. The results showed excellent antibacterial activity of garlic extract against *P. vulgaris* and *M. morgani* and the garlic extracts showed excellent antimicrobial activity against almost of all pathogens tested better than ginger and pepper (**Joe *et al*., 2009**). **Al-Masaudi and Al-Bureikan(2012)** found that a combination of onion juice suspended in honey inhibits the growth of some microbe's showing stronger effect than that observed by honey alone or onion juice alone. **Omoya and Akharaiyi(2011)** proved that there is a synergistic effect of antimicrobial activity from the combination of ginger and honey against some clinical isolates. The synergistic effect of antimicrobial activity from the combination of garlic and ginger or garlic and lime, against isolates from carious teeth was noted (**Patel *et al*., 2011**). Allicin has even been noted to act synergistically with antibiotics and enhance the antimicrobial Activity of antibiotics against tested microbes (**Cai *et al*., 2007; Khodavandi *et al*., 2010**).

2- MATERIALS AND METHODS

Type of honey*:* one brand of commercial honey was obtained available in Saudi Arabia (Jeddah) used in current study called Black Forest honey, Langaneza, Germany.

Type of garlic: The garlic which used in this study was Chinese garlic **(***Allium sativum)* from local market in Jeddah, KSA.

Microbial strains: six strains of standard microbes; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus*; (Methicillin-Sensitive *Staphylococcus aureus* - MSSA) ATCC 25923, (Methicillin-Resistant *Staphylococcus aureus*-MRSA) ATCC 10442, *Enterococcus faecalis*; (Vancomycin-Sensitive *Enterococci* - VSE) ATCC 29212, (Vancomycin - Resistant *Enterococci* - VRE) ATCC 51299 and *Candida albicans* ATCC 10291. All strains are collected from the Microbiology lab at King Khaled National Guard Hospital and King Abdulaziz University in Jeddah, Saudi Arabia.

Preparation of *Allium sativum* extract:

Fresh garlic (*Allium sativum*) bulbs were purchased from local market. The bulbs were peeled, weighed (100 g) and cleaned. Cleaned cloves were surface-sterilized by immersing them into 70% (v/v) ethanol for 60s. Residual ethanol on surface was evaporated in sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract. The concentrated mother extract was further diluted to 50%, 20% and 10% by mixing with sterile Nutrient broth (Oxoid) (**Palaksha *et al*.,2010**; **Durairaj *et al*., 2009**)

Media used; Nutrient agar (Oxoid), Nutrient broth (Oxoid), and Blood agar (Oxoid) were used in this study.

Assay of antibacterial activity:

The antibacterial effect of the garlic, honey and garlic-honey mixture was determined by broth dilution method (**Al-Masaudi; Al-Bureikan**, **2010**)

The broth Dilution Method:

A- All five ml of different concentration (100, 50,20and 10%) of (garlic or honey or mixture) were prepared in Nutrient broth in test tubes. All the tubes were inoculated with 0.1 ml of Over Night culture of the test organisms **1, 5 × 10^6** cfu/ml. The tubes were incubated at 37^o C for 24 h and serial dilution were made using sterile Nutrient broth and counts were determined as cfu/ml using Nutrient agar plate count.

B- Garlic-honey mixture was prepared in deferent volume (1v/1v), (1v/4v) and (4v/1v). The garlic extract was considered as the 100% concentration of the extract. The concentrated mother extract was further diluted to 50%, 20% and 10% by mixing with sterile Nutrient broth then, every volume prepared with different concentration in Nutrient broth (100, 50, 20 and 10%) in test tubes (**Al-Masaudi and Al-Bureikan,2012)**.

3- RESULTS:

Table (1) illustrates the antimicrobial activity of garlic on all tested microorganisms that not shown any growth at 100%, 50% and 20% concentration of garlic. While when we use 10% concentration of garlic the result showed some clear microbial growth of *S. pyogenes* ، VSE ، VRE and *C. albicans* while MSSA, MRSA, *P. aeruginosa* and *E. coli* did not show any growth at this concentration. Despite the appearance of growth at a 10% concentration of garlic the growth was significantly less than the growth of the control sample for all species.

The table (2) illustrate that Langaneza Black Forest honey has antimicrobial activity against all microorganisms in different concentrations. *Staphylococcus aureus* (MSSA) was the most affected

microbe while *E. coli* and *Enterococcus faecalis* (VSE) were significantly the least affected microbes comparing with the growth of the control sample.

Tables (3, 4, 5) illustrate that garlic - honey mixture (1v/1v, 4v/1v, 1v/4v) at concentrations 100%, 50%, 20%, and 10% have antimicrobial activity on all tested microorganisms. The results showed that garlic-honey mixtures (1v/1 v, 4v/1 v, 1v/4 v) at all concentrations of 100%, 50%, 20%, and 10% were significantly have the stronger antimicrobial effect on all tested organisms comparing to garlic alone or honey alone.

Table 1. Effect of garlic on different microbial organisms by dilution method.

Total plate count of organisms with different concentrations of garlic.					
Organism	**Cfu/ml**	**Different concentration of garlic**			
		100%	**50%**	**20%**	**10%**
S.pyogenes	**$2{,}02 \times 10^8$**	**0**	**0**	**0**	**6.1×10^6**
MSSA	**$5{,}8 \times 10^8$**	**0**	**0**	**0**	**0**
MRSA	**$5{,}2 \times 10^8$**	**0**	**0**	**0**	**0**
VSE	**$4{,}8 \times 10^8$**	**0**	**0**	**0**	**9.8×10^5**
VRE	**$2{,}8 \times 10^8$**	**0**	**0**	**0**	**7.1×10^5**
E. coli	**$1{,}35 \times 10^9$**	**0**	**0**	**0**	**0**
P.aeruginos	**$5{,}2 \times 10^8$**	**0**	**0**	**0**	**0**
C. albicans	**$1{,}04 \times 10^7$**	**0**	**0**	**0**	**4.8×10^5**

MSSA, (*Methicillin- Sensitive Staphylococcus aureus)*; MRSA, (*Methicillin-Resistant Staphylococcus aureus)*; VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

Table 2. Effect of honey on different microbial organisms by dilution method.

Total plate count of organisms with different concentrations of honey.					
Organism	Cfu/ml	L.B.F Langaneza Black Forest honey			
		100%	50%	20%	10%
S.pyogenes	$2{,}02 \times 10^8$	0	1.04×10^4	2.3×10^5	2.4×10^7
MSSA	$5{,}8 \times 10^8$	0	0	0	2.4×10^3
MRSA	$5{,}2 \times 10^8$	0	0	0	4.8×10^4
VSE	$4{,}8 \times 10^8$	0	8.2×10^5	1.83×10^6	1.17×10^8
VRE	$2{,}8 \times 10^8$	0	2.3×10^5	1.29×10^6	2.4×10^7
E. coli	$1{,}35 \times 10^9$	0	0	0	2.35×10^8
P.aeruginosa	$5{,}2 \times 10^8$	0	0	1.6×10^3	4.1×10^7
C. albicans	$1{,}04 \times 10^7$	0	0	2.3×10^6	3.3×10^6

MSSA, (*Methicillin- Sensitive Staphylococcus aureus)*; MRSA, (*Methicillin-Resistant Staphylococcus aureus)*; VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

Table 3. Effect of garlic - honey mixture (1/1 v) on different microbial organisms by dilution method.

Total plate count of organisms with different concentrations of garlic - honey mixture (1/1 v).					
Organism	Cfu/ml	different concentrations garlic - honey mixture (1/1 v)			
		100%	50%	20%	10%
S.pyogenes	$2{,}02 \times 10^8$	0	0	0	0
MSSA	$5{,}8 \times 10^8$	0	0	0	0
MRSA	$5{,}2 \times 10^8$	0	0	0	0
VSE	$4{,}8 \times 10^8$	0	0	0	0
VRE	$2{,}8 \times 10^8$	0	0	0	0
E. coli	$1{,}35 \times 10^9$	0	0	0	0
P.aeruginosa	$5{,}2 \times 10^8$	0	0	0	0
C. albicans	$1{,}04 \times 10^7$	0	0	0	0

MSSA, (*Methicillin- Sensitive Staphylococcus aureus)*; MRSA, (*Methicillin-Resistant Staphylococcus aureus)*; VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

Table 4. Effect of garlic - honey mixture (4/1 v) on different microbial organisms by dilution method.

Total plate count of organisms with different concentrations of garlic - honey mixture (4/1 v).					
Organism	Cfu/ml	different concentrations garlic - honey mixture (4/1 v)			
		100%	50%	20%	10%
S.pyogenes	$2{,}02 \times 10^8$	0	0	0	0
MSSA	$5{,}8 \times 10^8$	0	0	0	0
MRSA	$5{,}2 \times 10^8$	0	0	0	0
VSE	$4{,}8 \times 10^8$	0	0	0	0
VRE	$2{,}8 \times 10^8$	0	0	0	0
E. coli	$1{,}35 \times 10^9$	0	0	0	0
P. aeruginosa	$5{,}2 \times 10^8$	0	0	0	0
C. albicans	$1{,}04 \times 10^7$	0	0	0	0

MSSA, (*Methicillin- Sensitive Staphylococcus aureus)*; MRSA, (*Methicillin-Resistant Staphylococcus aureus)*; VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

Table 5. Effect of garlic - honey mixture (1/4 v) on different microbial organisms by dilution method.

Total plate count of organisms with different concentrations of garlic - honey mixture (1/4 v).					
Organism	Cfu/ml	different concentrations garlic - honey mixture (1/4 v)			
		100%	50%	20%	10%
S.pyogenes	$2{,}02 \times 10^8$	0	0	0	0
MSSA	$5{,}8 \times 10^8$	0	0	0	0
MRSA	$5{,}2 \times 10^8$	0	0	0	0
VSE	$4{,}8 \times 10^8$	0	0	0	0
VRE	$2{,}8 \times 10^8$	0	0	0	0
E. coli	$1{,}35 \times 10^9$	0	0	0	0
P.aeruginosa	$5{,}2 \times 10^8$	0	0	0	0
C. albicans	$1{,}04 \times 10^7$	0	0	0	0

MSSA, (*Methicillin- Sensitive Staphylococcus aureus)*; MRSA, (*Methicillin-Resistant Staphylococcus aureus)*; VSE, (*vancomycin -sensitive enterococci*); VRE, (*vancomycin - resistant enterococci*)

4- Discussion:

From our results in table (1) we noted that garlic juice has a strong antimicrobial activity on all tested microorganisms at 100%, 50% and 20% concentration of garlic, while when we use 10% concentration of garlic the result showed some clear microbial growth with some microbes. Despite the appearance of growth at a concentration 10% of garlic for some microbes but that growth is less than the growth of the control sample significantly for all species which is agree with many previous studies (**Goncagul; Ayaz,2010**; **Victor and Igeleke,2012**; **Rees *et al*.,1993** ; **Onyeagba *et al*.,2004; Khalid *et al*.,2011; Rahman *et al*.,2011**). Garlic is very important natural plant that used as alternative medicine for management of typhoid that caused by *sensitive and multi resistance isolates of Salmonella typhi* (**Hannan *et al*., 2012**), and it can be used alongside conventional antibiotics to fight agents of nosocomial infections that are so prevalent in hospitals **(Abubakar,2009)**. Also, our results proved that garlic has been found to exhibit antibacterial activity against a wide range of Gram negative and Gram-positive bacteria and this is agreement with (**Bakhshi *et al*.**,2012; Safithri ***et al***.,2011). From our results, MSSA, MRSA, *P. aeruginosa* and *E. coli* did not show any growth at 10% concentration of garlic and this is not in agreement with (**Gull *et al*., 2012**), who found that garlic aqueous extract exhibited highest antibacterial activity against all tested bacteria except *E. coli*. Also, our results were not in agreement with **Kivanc; Kunduhoglu (1997),** who found that garlic juice did not inhibit *B. subtilis* and *P. aeruginosa*. From table (1) we noted that garlic juice has clear effect on multidrug-resistant strains including MRSA and VRE, which is totally in agreement with (**Palaksha** *et al*.,2010; **Gull *et al*., 2012**; **Cutler**; **Wilson**,**2004**) The most important chemical compounds of garlic which was thought to be responsible for antimicrobial activity are the organosulphur compound including allicin ((**Hovana *et al*., 2011**) It showed better antibacterial activity than streptomycin and ampicillin (**Ilić *et al*., 2012**). Allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (**Durairaj *et al*., 2009**). Moreover, garlic is known to inhibit cell wall synthesis because it inhibits transpeptidation enzymes involved in the cross-linking

of the polysaccharide chains of the bacterial cell wall and also activates lytic enzymes (**Eja *et al*., 2007**). This ability of garlic to inhibit the growth of both gram-positive and gram-negative bacteria shows that it has a broad spectrum of activity and can be used for formulation and development of newer broad spectrum antibacterial substances (**Penecilla; Magno**;2011), and it provides scientific basis for its utilization in traditional and folk medicine (**Meriga *et al*., 2012**). Garlic also has antifungal activity on *C. albicans* which is agreed with (**Bakhshi *et al*.**,2012), but not agrees with (**Pundir**; **Jain**,**2010**) who found that the garlic extract was not effective against *Penicillium oxalicum*. **Joe *et al*(2009)** reported that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group. All our results from fresh garlic juice agree with (**Hovana *et al*., 2011**) who proved that garlic when used in its raw form has better antibacterial activity, either directly or as an adjuvant in a solvent.

Honey is known to contain phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose, and has high osmolarity and low pH. These elements acting alone or synergistically may contribute significantly to the antimicrobial activity of honey. Our results in table (2) demonstrate the activity of different concentrations of Langaneza Black Forest honey against eight microorganisms. The results showed that honey has antimicrobial effect on all tested microorganisms at different concentrations which were in full agreement with the study performed by (**Aurongzeb**; **Azim,2011**). Antibacterial and antifungal activities of honey are well documented and characterized. These antimicrobial properties have been related to oligosaccharides, glycopeptides and peptides present in honey. Honey glucose oxidase provides a continuous and slow release of hydrogen peroxide at a level which is antibacterial but not tissue-damaging. Hydrogen peroxide produced by glucose oxidase plays important roles in inflammation, wound healing etc (**Aurongzeb**; **Azim,2011**). The results of the diffusion method exhibited a contrast in the sensitivity of microbes. It was recommended to use the dilution method instead of diffusion method because it gave the real effect at different concentrations of honey (**Al-Masaudi and Al-Bureikan,2012**).

The application of blends was based on the concept of the effectiveness of combination therapy (synergism) in the treatment of certain bacterial infections involving drug resistant organisms (**Eja *et al*.**, ***2011***). However, synergic treatment of ailments has been a long aged practice in both orthodox and traditional medicines. In Sub-saharan Africa, especially West Africa, including Ghana and Nigeria, herbal medicines are believed to be more effective when taken in combination (**Eja *et al*.**, ***2011***).

Our results in tables (1,2,3) showed that garlic-honey mixtures (1/1 v, 4/1 v, 1/4 v) at all concentrations of 100%, 50%, 20%, and 10% were significantly have the best antimicrobial effect on all tested organisms without any growth and this effect was significantly has stronger antimicrobial activity on all tested organisms than garlic alone and honey alone which is agree with Al-Masaudi and Al-Bureikan (2012), who found that a combination of onion juice suspended in honey inhibits the growth of some microbe's showing stronger effect than that observed by honey alone or onion juice alone. Also, agree with **Omoya and Akharaiyi (2011) and Patel *et al* 2011)**, who proved that there is a synergistic effect of antimicrobial activity from the combination of ginger and honey against isolates from carious teeth and on some clinical isolates. Also, our results agree with **Al-Jabri *et al* (2005)** who found that the combination of honey and bovine milk had stronger antimicrobial effect than honey alone or bovine milk alone. **Mboto *et al*. (2009)** were found that a combination of medicinal plants like *G. kola* and *V. amygdalina extracts* suspended in Honey inhibits the growth of some microbe's showing stronger effect than that observed by honey alone or medicinal plants alone. On the other hand, the results agree with many studies which confirm that garlic act synergistically with antibiotics and enhancement the antimicrobial Activity of antibiotics by Allicin against tested microbes (**Bakhshi *et al*.**,2012; **Cai *et al*., 2007**). Also, agree with **Patel *et al*(2011)** who detected the synergistic effect of antimicrobial activity from the combination of garlic and lime against isolates from carious teeth was noted and agree with the study which observed the highly antimicrobial activity for combination of garlic, onion, ginger, pepper and coriander seed on different microbes (**Rahman *et al*.,2011**). On the other hand, these results of mixtures on microbes not agree with **Eja *et al* (2011)** who found that the combination of garlic and utazi (*Gongronema latifolium*)may not always yield the desired effect on both Gram positive and Gram negative bacteria and the antagonistic effect is more evident when the combination is used on Gram negative organisms, e.g. *E.coli*. and not agree with **Shaista *et al*(2009)** who evaluated The effect of five stabilizers on microbes isolates and found that *E.coli* and *Salmonella typhi* showed more resistance in case of citric acid and sodium benzoate mixed in the ginger - garlic paste.

Conclusion:

The conclusion of this result could be explained in two ways. The first way, as we noted previously in

our results, garlic has antimicrobial effect against tested microbes because it is a rich source of the organosulphur compound including (allicin) the most important chemical compounds of garlic which was thought to be responsible for garlic medicinal properties (**Koch**,1996). Honey, also, has antimicrobial effect against tested microbes because it is known to contain phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose and has high osmotic potential and low pH. These elements (either in garlic or in honey) which can acting alone or synergistically may be contribute significantly to the antibacterial activity of the combination of honey and garlic which resulting higher growth reduction, enhancing the killing activity. The second way, the antibacterial activity of honey and garlic, both have excellent nutritional values and would be an additional enhancer of immunity in aid to the treatments of bacterial infections. Honey in combination with garlic may prolong or improves the shelf life of each other (**Al-Masaudi and Al-Bureikan,2012**). Apparently, with the increasing interest in the use of alternative therapies coupled with the development of antibiotic-resistant bacteria, honey may finally receive its due recognition (**Christy et al., 2011**).

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