Antimicrobial Activity of Onion Juice (*Allium cepa*), Honey, And Onion-Honey Mixture on Some Sensitive and Multi-Resistant Microorganisms

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Abstract: The study conducted here to analyze the antimicrobial activity of onion juice alone which extracted from red Egyptian onion, honey alone (Langaneza honey, Black Forest) and honey-onion mixture (v/v: 1/1, 1/4, 4/1) with different concentrations 100 , 50 , 20 and 10% respectively, 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 investigated by broth dilution method. The results showed that onion juice at 100%, 50%, 20% and 10% concentration have a very strong effect on the growth of all tested species of microbes comparing with control and *Staphylococcus aureus* was the most sensitive microbe. 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 onion juice. When studying the effects of the onion- honey mixture with different concentrations, it became clear that the mixture (1/1) had a very noticeable effect on all species of examined microbes.. Results also showed that the honey-onion mixture was significantly more effective comparing with onion or honey alone.
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Key word: Onion, Honey, Onion- Honey mixture, Antimicrobial activity, MRSA, MSSA, VSE, VRE.

1. Introduction

The onion is one of the oldest cultivated vegetables in history. It is thought that bulbs from the onion family have been utilized as a food source for Millennia **(Azu and Onyeagba , 2007).** A number of studies have proven that onion having antibacterial and antifungal properties, and the potential use of onion against human pathogenic organisms. **(Hughes and Lawson, 1991; Augusti, 1996; Adeleye and Opiah, 2003; Amin and Kapadnis, 2005; Sohail *et al.,* 2011).** It is a rich source of flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are mainly responsible for its medicinal activities **(Sohail *et al.,* 2011).**In addition, the inhibitory effect of onion oil was demonstrated against the growth of various isolates of bacteria representing Gram-positive (four isolates), Gram-negative (four isolates) species and nine different species of dermatophytic fungi. The results showed that onion oil was highly active against all Gram-positive bacteria tested and only one isolate (*Klebsiella pneumoniae*) of Gram-negative bacteria, while all fungi inhibited at different concentrations **(Zohri *et al.,* 1995).** The antibacterial activity of onion extracts was studied on *Streptococcus mutans* and *Streptococcus sobrinus*, the results showed that the onion extracts possess an effect on all test bacterial strains and the effects were bactericidal. **(Kim, 1997). Mboto *et al*., 2009** 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.

2.Materials and Methods

Honey source and type

One brand of commercial honey, called Black Forest honey (Langaneza), Germany, is available in Saudi Arabia (Jeddah), and was used in current study.

Onion source and type

The onion which used in this study was Egyptian red onion (Allium cepa).

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 were collected from the Microbiology lab at King Khaled National Guard Hospital and King Abdulaziz University hospital in Jeddah, Saudi Arabia.

Preparation of *Allium cepa* extract

Fresh *Allium cepa* bulbs were rinsed thoroughly in distilled water and air dried; 200 grams were then blended. The resulting paste was allowed to

stand for 24 hours. Juice was then filtrated and squeezed out of it. The extract was stored bellow 4°C. **(Nelson** *et al*., 2007; Ige *et al***., 2009).**

Media used

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

3-Assay of antibacterial activity

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

The broth Dilution Method

A - Five ml of different concentrations (100, 50,20 and 10%) of (onion or honey) were prepared in Nutrient broth in test tubes. All the tubes were inoculated with 0.1 ml of Over Night culture of the tested organisms 1.5×10^6 cfu/ml. The tubes were incubated at 37^o C for 24 hrs and serial dilutions were made using sterile Nutrient broth and counts were determined as cfu/ml using Nutrient agar plate count (**Steve & Dennis, 2001**).

B - Onion- honey mixture was prepared in different volumes (1:1), (1:4) and (4:1). Then, every volume prepared with different concentrations in Nutrient broth (100, 50, 20 and 10%) in test tubes.

3.Results and Discussion

Table (1) illustrates the antimicrobial effect of different concentrations of onion on the tested microorganisms. At 100% and 50% concentration of onion, the results showed no growth, while when we use 20% concentration of onion the result showed growth of some microbial strains. However, at 10% concentration of onion all microorganisms were grown. Despite the appearance of growth at a concentrations of 20% and 10% of onion extract the growth was significantly less than the control sample.The results clearly showed that Onion had an antimicrobial activity against all tested microorganisms at different concentrations, and it reduced the growth significantly comparing with control which is agree with many previous studies **(Hughes & Lawson, 1991; Augusti, 1996; Adeleye & Opiah, 2003 ;Amin & Kapadnis, 2005; N. C. Azu, *et al*., 2007; Nelson and Onyeagba, 2007;** Nelson *et al*., 2007**; Watson, 2008; Hannan *et al*., 2010; Sohail *et al*., 2011).** Onion has clear effect on Gram-positive, Gram-negative and pathogenic yeast this result was not in agreement with **Adeleye & Opiah, 2003; Azu *et al*., 2007b** who proved that gram negative bacteria affected by Onion more than gram positive. The Welsh onion ethanol extracts were tested for their inhibitory activity against the growth and aflatoxins production of *Aspergillus flavus* and *A. parasiticus* where the results showed that the extracts have inhibitory effect toward aflatoxins production than the preservatives sorbate and propionate at pH values near 6.**5 (Fan and Chen, 1999). Azu *et al*., 2007** estimated the antibacterial activity of raw and aqueous extracts of onions against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, (from high vaginal swab) that are common cause of nosocomial (hospital-acquired) and urinary tract infections investigated using the cup-plate diffusion method, the result showed that *Pseudomonas aeruginosa* was more sensitive to the extract of onion bulbs compared to *Staphylococcus aureus*.). Moreover, onion has antimicrobial effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from High Vaginal Swab **(**Nelson *et al*., 2007)**.** Also, in other study onion (*Allium cepa*) showed antibacterial effect against *B. subtilis*, *Salmonella* sp. and *E. coli* **(Winston, 2008). Hannan *et al*., 2010** investigated the antimicrobial potential of onion against thirty-three clinical isolates of *Vibrio cholera;* the results indicated that onion (*Allium cepa*) has an inhibitory effect on *V. cholerae*

Table 1: Effect of onion juice on the microbial count (cfu/mℓ) of different pathogenic organisms by dilution method.

Total plate count at different concentrations of onion.					
Organism	Con.	Different concentrations of Onion (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	4.5×10^4	7.4×10^7
MSSA	7.8×10^8	0	0	0	3.2×10^6
MRSA	7.7×10^8	0	0	0	2.9×10^6
VSE	4.3×10^8	0	0	7.5×10^4	8.1×10^7
VRE	3.4×10^8	0	0	4.3×10^4	5.8×10^7
E.coli	1.5×10^9	0	0	0	6.8×10^8
P. aeruginosa	4.8×10^8	0	0	6.8×10^4	8.8×10^7
C. albicans	7.5×10^6	0	0	2.7×10^5	7.5×10^6

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

Table (2) demonstrates the activity of different concentrations of Langaneza Black Forest honey against eight microorganisms. *Staphylococcus aureus* was the most affected microbe, while *Enterococcus faecalis* was the least affected organism. The results showed that honey has antimicrobial effect on all tested microorganisms at different concentrations which was in full agreement with the study performed by **Al-Masaud & Al-Bureikan, 2010; Manyi-Loh *et al.*, 2010b; Olawuyi *et al.*, 2010; Al-Waili *et al.*, 2011; Halawani & Shohayeb, 2011; Aurongzeb & Azim, 2011.** Moreover, results showed that honey significantly has similar antibacterial effect on sensitive and resistance microbes either MSSA or MRSA and VSA or VRE and this result confirms the results obtained by **Bilal & Alfalki, 1998; Cooper *et al.*, 2002; Al-Masaud & Al-Bureikan, 2010.** It could be also noted that *Candida albicans* was highly affected by honey and this is in accordance of **Zaghlooul *et al.*, 2001,** but not agree with **Lusby *et al.*,2005** who measured the antimicrobial effect of three kinds of honey on different organisms and found that *Candida albicans* did not affected with all kinds of honey. This may be attributed to use different method and different sources of honey. The effect of honey could be antibacterial or antifungal effect **(Nzeako and Hamdi, 2000;Taormina *et al.*, 2001; Al-Jabri *et al.*, 2003; Iurlina and Fritz, 2005; Lusby *et al.*, 2005; Manyi-Loh-*et al.*, 2011; Aurongzeb and Azim, 2011).** 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 **(Al-jabri, 2005).** Moreover, honey has similar antibacterial effect on sensitive and resistance microbes either MSSA or MRSA and VSA or VRE **(Bilal and Alfalki, 1998; Cooper *et al.*, 2002enkins *et al.*, 2011).** In addition, **Zaghlooul *et al.*, 2001** proved that *Candida albicans* affected by honey. Honey has anti-*Helicobacter pylori* activity (**Manyi-Loh *et al* 2010b**). **AL-Masaudi and Al-Bureikan, 2010** proved that honey has antimicrobial effect against *Streptococcus pyogenes*, *Staphylococcus aureus;*results of the dilution method showed that all kinds of honey had a very clear effect on all types of microbes with different concentrations. *Candida albicans* was the least microbe affected by the different kinds of honey with different concentrations even at 100% concentration. On the other hand, *Staphylococcus aureus* and *Streptococcus pyogenes* were the most sensitive microbes. When studying the antimicrobial effect of honey against microbes at concentration of 50%, it was found that some kinds of honey had static effect or cidal effect on different species. Comparing the effects of honey on resistance and sensitive *Staphylococcus aureus* **(MSSA and MRSA), results showed that there was no** significant difference in the effect of honey on both MSSA and MRSA tested strains. 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, 2010).**

Table 2: Effect of honey on the microbial count (cfu/mℓ) of different pathogenic organisms by dilution method.

Total plate count at different concentrations of honey.					
Organism	Con.	L.B.F Langaneza Black Forest honey (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	1.04×10^4	2.3×10^5	2.4×10^7
MSSA	7.8×10^8	0	0	0	2.4×10^3
MRSA	7.7×10^8	0	0	0	4.8×10^4
VSE	4.3×10^8	0	8.2×10^5	1.83×10^6	1.17×10^8
VRE	3.4×10^8	0	2.3×10^5	1.29×10^6	2.4×10^7
E.coli	1.5×10^9	0	0	0	2.35×10^8
P.aeruginosa	4.8×10^8	0	0	1.6×10^3	4.1×10^7
C . albicans	7.5×10^6	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*)

Tables (3- 5) illustrate that onion - honey mixture (v/v: 1:1, 4:1, 1:4) have antimicrobial activity on all tested microorganisms with different concentrations 100, 50, 20, and 10%. The results showed that onion - honey mixture (1:1) at concentrations of 100, 50 and 20% showed significantly the best antimicrobial effect on all tested organisms. Moreover, at a concentration of 10% the onion - honey mixture (1:1) exhibited significantly stronger antimicrobial activity on all tested organisms than onion alone and honey alone.

VRE and VSE were significantly affected by onion - honey mixture (1:1) more than onion alone and honey alone. However, the onion - honey mixture (1:1) significantly has the pest effect than onion - honey mixture (4:1, 1:4) on all organisms.

Table 3: Effect of onion - honey mixture (1:1) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (1:1).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	2.9×10^3	2.7×10^6
MSSA	7.8×10^8	0	0	0	2.6×10^3
MRSA	7.7×10^8	0	0	0	2.2×10^3
VSE	4.3×10^8	0	0	0	4.1×10^4
VRE	3.4×10^8	0	0	0	2.8×10^4
E.coli	1.5×10^9	0	0	0	4.2×10^7
P. aeruginosa	4.8×10^8	0	0	2.1×10^3	3.8×10^7
C. albicans	7.5×10^6	0	0	2.4×10^5	5.4×10^6

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

Table 4: Effect of onion - honey mixture (4:1) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (4:1).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	0	5.5×10^4	8.7×10^7
MSSA	7.8×10^8	0	0	0	4.1×10^6
MRSA	7.7×10^8	0	0	0	3.4×10^6
VSE	4.3×10^8	0	0	6.2×10^3	8.9×10^7
VRE	3.4×10^8	0	0	5.4×10^3	6.8×10^7
E.coli	1.5×10^9	0	0	0	6.1×10^8
P. aeruginosa	4.8×10^8	0	0	4.2×10^4	6.3×10^7
C. albicans	7.5×10^6	0	0	3.7×10^5	8.2×10^6

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

Table 5: Effect of onion - honey mixture (1:4) on the microbial count (cfu/mℓ) of different laboratory organisms by dilution method

Total plate count at different concentrations of onion - honey mixture (1:4).					
Organism	Con.	Different concentrations onion - honey mixture (%)			
		100	50	20	10
S.pyogenes	2.3×10^8	0	2.5×10^3	6.7×10^4	3.1×10^7
MSSA	7.8×10^8	0	0	0	3.9×10^3
MRSA	7.7×10^8	0	0	0	4.3×10^3
VSE	4.3×10^8	0	0	2.8×10^4	1.05×10^5
VRE	3.4×10^8	0	0	2.2×10^4	4.7×10^5
E.coli	1.5×10^9	0	0	0	8.9×10^7
P. aeruginosa	4.8×10^8	0	0	5.5×10^4	9.1×10^7
C. albicans	7.5×10^6	0	0	5.3×10^5	9.8×10^6

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

Our results showed that the antimicrobial activity of onion was significantly stronger on tested microbes than honey, but when we use the onion and honey mixtures especially (v/v: 1:1) it becomes clear that the

onion and honey mixtures have stronger effect on most microbes than onion alone or honey alone. This results agree with (Osman *et al,* 2003) who used combination of honey plus some natural additives and they found superior results with honey compound in its antibacterial, antifungal, and wound-healing promotion properties compared with pure bee honey and some other topical wound agents. Also, our results agree with **Al-Jabri *et al*. (2005a)** who found that the combination of honey and bovine milk had stronger antimicrobial effect than honey alone or bovine milk alone. The obtained results are in agreement with **Al-Jabri *et al*. (2005b)** who found that combination of honey and gentamicin had stronger antimicrobial effect than honey alone or gentamicin alone. **Mboto *et al*., 2009** also 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.

From our experience, it became clear that the combination of onion- honey (1v/1v) has stronger effect on microorganisms than onion alone or honey alone.The conclusion of this result could be explained in two ways. The first way, as we noted previously in our results, onion has antimicrobial effect against tested microbes because it is a rich source of flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are mainly responsible for its medicinal activities. 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 onion or in honey) which can acting alone or synergistically may be contribute significantly to the antibacterial activity of the combination of honey and onion which resulting higher growth reduction, enhancing the killing activity. The second way, the antibacterial activity of honey and onion, 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 onion may prolong or improves the shelf life of each other. 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).**

The combination of two or more antibacterial agents has been long accepted in the treatment of some microorganisms **(Al-jabri, 2005).** The combination of honey plus some natural additives has superior results in its antibacterial, antifungal, and wound-healing promotion properties compared with pure bee honey and some other topical wound agents alone (**Osman *et al.,* 2003**). The combination of medicinal plants like *G. kola* and *V. amygdalina extracts* suspended in honey inhibits the growth of some microbes stronger than honey alone or medicinal plants alone **(Mboto *et al*., 2009).** When honey mixed with an antibiotic it had best killing effect within half an hour of exposure to bacteria than either an antibiotic or honey used alone **(Al-Jabri *et al*., 2005a).** Synergy is known to exist between penicillin and streptomycin and between sulphamethoxazole and trimethoprim, between honey and gentamicin and between honey and milk **(Al-jabri, 2005).**

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