Antimicrobial Activities of Six Types of Honey towards Selected Pathogenic Microorganisms

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Abstract

The emergence of strains of pathogenic microorganisms with resistance to commonly used antibiotics has necessitated a search for novel types of antimicrobial agents. It is of a great importance to understand the efficiency of honey against microorganisms. The main objective of this study was to investigate the biological activities of Black Seed (Nigella sativa) honey, Thymus honey, Myrrh honey, Tamarix honey, Spring honey and Zizphus honey before and after exorcism against four pathogenic bacteria and one pathogenic fungi. Agar diffusion, Minimum Inhibitory Concentration and Minimum Killing Concentration methods were used to evaluate the antimicrobial activity. All the Gram-positive and Gram-negative bacteria tested were found to be inhibited to some extent by the six different honeys, although the antimicrobial potency was highly dependent upon type of honey and test organism. The honey samples were especially active against methicillin-resistance Staphylococcus aureus (isolated from Tripoli Medical Center), S. aureus ATCC 43300), Pseudomonas. aeruginosa ATCC 27893, . Escherichia coli ATCC 35150 and the yeast Candida albicans that isolated from Al-jala hospital. S. aureus was found to be particularly sensitive to Thymus exorcism honey. However, C. albicans found to be sensitive to Spring honey. In addition, the results showed that Tamarix exorcism honey was significantly (P = 0.006) more active than the Tamarix honey, also N. sativa exorcism honey was significantly (P = 0.003) more active than the N. sativa honey. The Minimum Inhibitory Concentration (MIC) of Tamarix exorcism honey (determined by tube dilution) for MRSA is more effective than 32-fold lower at 3.125% (V/V) compared to the other five honeys. The Minimum killing concentration was 6.25% concentration against S. aureus. The honey samples studied proved to be a good source of antimicrobial agents that might serve to fight against several diseases. These data also demonstrate that antimicrobial potency of honeys is highly dependent upon both the target microbial species and the type of honey.

1. Introduction:

Honey is the natural sweet substance produced by honey bees, Apis mellifera, from the nectar of plants (blossoms) or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (Codex Alimentarius Commission (2001a, b)).

It has been possesses inherent antimicrobial properties, some of which are due to high osmotic pressure/low water activity, in which the low water activity of honey is inhibitory to the growth of the majority of bacteria, and to many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water absorbed from wounds, honeys would be likely to retain a water activity sufficiently low to inhibit growth of most bacteria. Honey is mildly acidic, with a pH between 3.2 and 4.5. Gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid, the low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect (Molan 1995).

Hydrogen peroxide, the end product of the glucose oxidase system and tetracycline derivatives has the antibacterial properties against pathogens. Low concentrations of this known antiseptic are effective against infectious bacteria and can play a role in the wound healing mechanism and in Stimulation and proliferation of peripheral blood lymphocytic and phagocytic activity. Other factors, such as low protein content, high carbon to nitrogen ratio, low redox potential due to the high content of reducing sugars, viscosity/anaerobic environment and other chemical agents/phytochemicals are also likely to play some role in defining antibacterial activity of honey.

Therapy with bee products as honey is an old tradition and honey has had many therapeutic uses from ancient times to the present. It has been suggested that pure honey is bactericidal for many pathogenic organisms, including various gram negative and gram positive bacteria. Other therapeutic effects of honey include its use in the treatment of fungal infections, burns, infantile gastroenteritis wounds and decubitus ulcers (Bergman et al. 1983).

It is known that honey strongly inhibits the growth of microorganisms. Already in 1892, the Dutch scientist Van Ketel demonstrated that honey has bactericidal effects. A great number of research reports have subsequently confirmed his findings. (Molan 1999) found that honey is becoming accepted as a reputable and effective therapeutic agent by practitioners of conventional medicine and by the general public. This is because of good clinical results that are being obtained. Honey has been reported to be effective in the healing of infected postoperative wounds. It has also been reported to inhibit the growth of many bacteria such as Bacillus cereus, Staphylococcus aureus, Salmonella dublin, and Sh. dysenteriae. It has also been reported to inhibit the growth of Bacteroides spp. (Elbugoury and Rasomy, 1993).

The aim of this study was to determine, the antimicrobial of six types of honey which representative (Thymus honey, Tamarix honey, N. sativa honey, Spring honey, Zizphus honey and Myrrh honey) and also to compare the antimicrobial of honey before and after exorcism against four types of bacteria Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus as gram-positive, Escherichia coli, Pseudomonas aeruginosa as gram-negative and one fungi (Candida albicans).

2. Materials and method:

2.1- Honey samples:

The samples of Thymus floral and zizphus floral (originating from Albyda), Tamarix floral, and Spring (originating from Yafran), Myrrh floral (originating from Gebel Akhdar) and N. sativa floral honeys were purchased from local shops in Tripoli (Libya).

Table 1: Examined natural honey samples and their floral sources.

Honey sample	Scientific name of plant cover	Aceae	Season
Thymus	Thymus capitatus (L.)	Limaceae	summer
Zizphus	Zizphus lotus Desf.	Rhamnaceae	Summer
Tamarix	Tamarix aphylla (L.) Karst	Tamaricaceae	Spring
Nigella staiva	Nigella staiva L.	Ranunculaceae	Spring
Myrrh	Arbutus pavarii	Ericacees	Autmen
Spring	Acacia cyanophylla Citrus limon (L.) Burm	Fabaceae Rutaceae	Spring

Muller Hinton Broth and Muller Hinton Agar (Oxoid) were cultivation of bacteria. Sabouraud Dextrose Broth and Sabouraud Dextrose Agar (Oxoid) were used for cultivation of fungi. All these culture media were obtained from Sigma-Aldrich Company.

2.2- Honey preparation and In Vitro Antimicrobial Assay:

Four bacterial species obtained from faculty of Medicinal, University of Tripoli (Libya) were selected for testing. Representative strains of Gram-positive bacteria were S. aureus ATCC 43300 and MRSA (Isolated from Tripoli Medical Center. Representative strains of Gram-negative bacteria were E. coli ATCC 35150 and P. aeruginosa ATCC 27893.

Representative fungal (yeast) strain was C. albicans (Isolated from Al-jala Hospital). The honeys samples were stored in the dark at room temperature. For agar well diffusion tests, the honeys were initially diluted in de-ionized water to obtain concentrations of 10%, 25%, 50 and 75% Wt/Vol.

The plates were prepared using 50ml of sterile Muller Hinton Agar. The surface of the plates was aseptically inoculated using a 100µl of suspension of bacteria grown overnight at 37ºC in Muller Hinton Agar Broth and allowed to dry. Agar cylinders of, 7mm in diameter were cut from the culture media using a sterile glass Derhum tube; and then filled with the dilutions of honeys. The plates were incubated at 37ºC and observed after 24 hours; the clear, circular inhibition zones around the wells were measured. For MIC determination, the honey samples were initially diluted in Muller Hinton Broth 100% (Vol/Vol). Six serial two fold dilution of the preparation were made in Muller Hinton Broth for bacterial inhibition testing; the lowest concentration was applied is 3.125% (Vol/Vol). Control comprised broth only (negative) or test organism and broth (positive control) were prepared.

2.3 Antibiotic Susceptibility Test:

Antibiotic susceptibility on organisms were studied against the Ciprofloxacin 10 µg and ketoconazole 10ug compared of concentrations of honey by the disk diffusion technique on (MHA), using inhibition zone criteria recommended by the disk manufacturer and based on the method of (Barry 1976). The selection of antibiotic disks was performed according to the guidelines recommended by ATCC.

3.-Result:

The six types of honey used in this study were inhibited the growth of all the bacteria and fungi tested. The antimicrobial activities of all types of honey on the different bacteria and fungi strains are tested is shown in Tables 2, 3, 4, 5, 6 and 7. The average zone of inhibition of honey against the strains ranged from 0.0 ± 0.0 (all strains) to 21 ± 0.88 (P. aeruginosa).

Differences regarding inhibition were observed for the Thymus honey before and after exorcism (Table 2). Thymus exorcism honey was more active with the largest inhibition against S. aureus and MRSA organisms. Thymus exorcism honey showed marked inhibition of growth on S. aureus, the maximum inhibition was shown at concentration of 100% as 21 ± 0.66 mm, which reduced to 16 ± 0.57 mm at 75% mm and 15 ± 0.33 mm at 50% concentration. While, Thymus honey showed marked inhibition of growth on S. aureus, the inhibition was shown at concentration of 100% as 17 ± 0.88 mm (Table 2).

Table 2: Antimicrobial activities of the Thymus honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	Thymus honey				Thymus exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram- Positive								
S. aureus	0 ± 0	0 ± 0	0 ± 0	17 ± 0.88	0 ± 0	15 ± 0.33*	16 ± 0.57*	21 ± 0.66
S. aureus (MRSA)	0 ± 0	0 ± 0	0 ± 0	15 ± 0.88	0 ± 0	12 ± 1.0*	15 ± 0.88*	19 ± 0.57
Gram- Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	13 ± 0.88	0 ± 0	0 ± 0	0 ± 0	15 ± 1.0
P. aeruginosa	0 ± 0	0 ± 0	0 ± 0	19 ± 0.58*	0 ± 0	0 ± 0	0 ± 0	12 ± 0.88
Fungi								
C. albicans	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	12 ± 0.88*

*Significant of compared to two values in the same row.

Also the table showed that MRSA growth with inhibition zone at concentration of 100% as 19 ± 0.57 mm, which reduced to 15 ± 0.88 mm at 75% and 12 ± 1.0 mm at 50% concentration for Thymus exorcism honey and at concentration of 100% as 15 ± 0.88 mm for Thymus honey. The inhibition of growth on E. coli was shown at concentration of 100% as 15 ± 1.0 mm for Thymus exorcism honey and at the same concentration as 13 ± 0.88 mm for Thymus honey. Inhibition of growth on P. aeruginosa, the inhibition zone was at concentration of 100% as 19 ± 0.58 mm. However, Thymus exorcism honey only was inhibited the growth of C. albicans zone at concentration of 100% as 12 ± 0.88 mm in comparison with Thymus honey. However, no effect was observed at concentrations of 25%, 50%, 75% and 100% for Thymus honey.

Table 3 showed marked of inhibition on P. aeruginosa, the maximum zone was shown at concentration of 100% as 21 ± 0.66 mm, which reduced to 19 ± 0.57 mm at 75%, 18 ± 0.88 mm at 50% and 16 ± 1.20 at 25% concentration for the Tamarix exorcism honey which compared to the Tamarix honey at concentration of 100% at zone of 18 ± 0.58 mm. Also the table exhibited that MRSA grow with inhibition zone at concentration of 100% as 18 ± 1.76 mm, which reduce to 14 ± 0.88 mm at 75% and 11 ± 0.88 mm at 50% concentration for Tamarix exorcism honey and the Tamarix honey was inhibition at only concentration of 100% as 16 ± 0.88 mm. S. aureus shows inhibition zone with Tamarix honey as 19 ± 1.0 mm at 100% and with Tamarix exorcism honey was 17 ± 1.45 mm at the same concentration. E. coli shows inhibition zone with Tamarix exorcism honey as 12 ± 0.88 mm at 100% concentration and with Tamarix honey was 11 ± 0.66 mm. C. albicans showed inhibition zone at concentration of 100% as 13 ± 0.33 mm for Tamarix honey.

Table 3: Antimicrobial activities of the Tamarix honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	Tamarix honey				Tamarix exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram-Positive								
S. aureus	0 ± 0	12 ± 0.33*	15 ± 0.33*	19 ± 1.0	0 ± 0	0 ± 0	0 ± 00	17 ± 1.45
S. aureus (MRSA)	0 ± 0	0 ± 0	0 ± 0	16 ± 0.88	0 ± 0	11 ± 0.88*	14 ± 0.88*	18 ± 1.76
Gram-Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	11 ± 0.66	0 ± 0	0 ± 0	0 ± 0	12 ± 0.88
P.aeruginosa	0 ± 0	0 ± 0	0 ± 0	18 ± 0.58	16 ± 1.20*	18 ± 0.88*	19 ± 0.57*	21 ± 0.66
Fungi								
C. albicans	0 ± 0	0 ± 0	0 ± 0	13 ± 0.33*	0 ± 0	0 ± 0	0 ± 0	0 ± 0

*Significant of compared to two values in the same row.

Effect of N. sativa exorcism honey against the strains: The honey showed pronounced activity against P. aeruginosa, the maximum inhibition zone was shown at concentration of 100% as 21 ± 0.88 mm and 15 ± 0.66 mm at 50% concentration (Table 4) and figure1.

Table 4: Antimicrobial activities of the N. sativa honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	N. sativa honey				N. sativa exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram-Positive								
S. aureus	0 ± 0	0 ± 0	0 ± 0	13 ± 0.88	0 ± 0	13 ± 0.88*	17 ± 0.88*	20 ± 0.88*
S. aureus (MRSA)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 0.57*	14 ± 2.40*	16 ± 2.40*	19 ± 2.08*
Gram-Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	12 ± 1.0	0 ± 0	0 ± 0	0 ± 0	18 ± 0.66*
P. aeruginosa	0 ± 0	0 ± 0	14 ± 0.58	18 ± 0.33	0 ± 0	15 ± 0.66*	18 ± 0.57	21 ± 0.88
Fungi								
C. albicans	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 0.33*	12 ± 0.88*	15 ± 1.45*

*Significant of compared to two values in the same row.

N. sativa honey showed marked inhibition of growth on P. aeruginosa at concentration of 100% as 18 ± 0.33 mm and no activity against MRSA figure 1. S. aureus showed inhibition zone with N. sativa exorcism honey as 20 ± 0.88 and 13 ± 0.88 mm at 100% concentration with N. sativa honey figure2. Also showed that MRSA grow with inhibition zone at concentration of 100% as 19 ± 2.08 mm. C. albicans showed a little less inhibition zone with N. sativa exorcism honey which 15 ± 1.45 mm at 100%, 12 ± 0.88 mm at 75% and 10 ± 0.33 mm at 50% concentration and no effect was observed for all concentrations of N. sativa honey, compared to the N. sativa exorcism honey (Table 4).

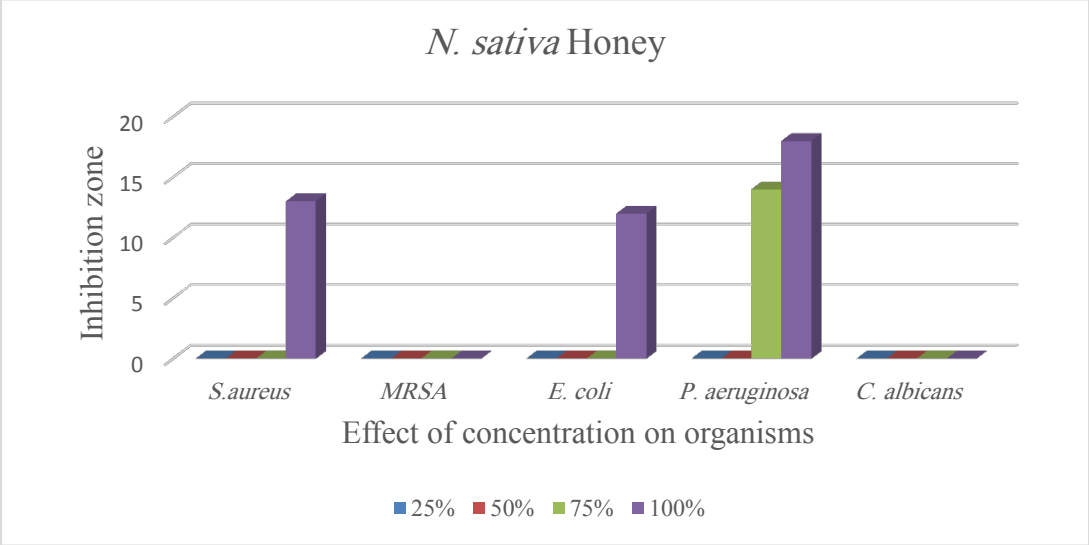

Figure1: The antibacterial activity of N. sativa honey compared with N. sativa.

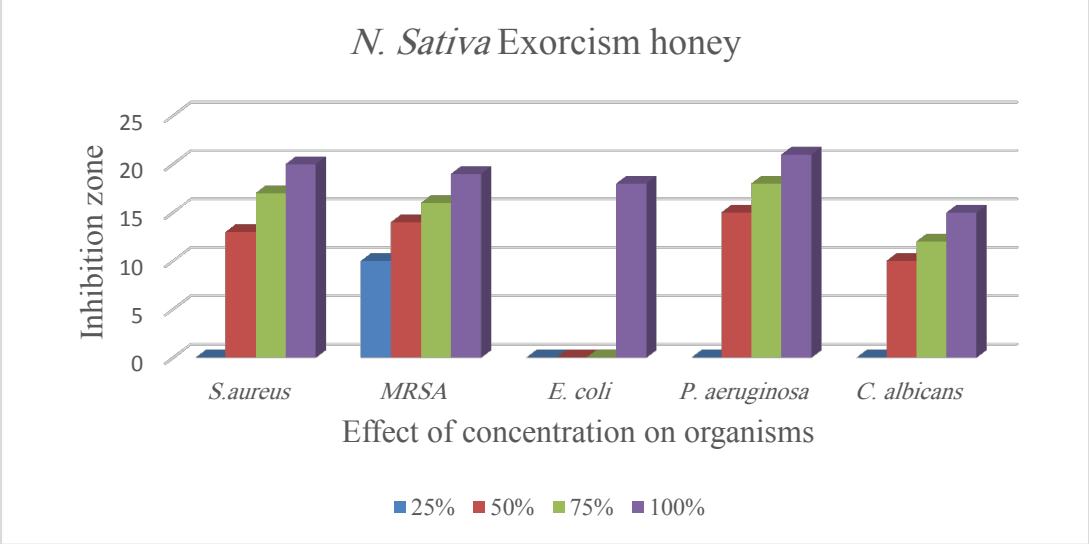

Figure2: The antibacterial activity of N. sativa exorcism honey .

Table 5 shows that only Spring exorcism honey has broad spectrum antifungal activity even in very low concentration 25% on C. albicans and the maximum inhibition zone was shown as 11 ± 0.58 mm.

In the case of E. coli there was no evidence of growth inhibition at concentration up to or including 75%, at concentration of 100% there was progressive increase in inhibition as honey concentration increased.

Table 5: Antimicrobial activities of the Spring honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	Spring honey				Spring exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram-Positive								
S. aureus	0 ± 0	0 ± 0	15 ± 0.58	18 ± 0.58	0 ± 0	0 ± 0	17 ± 1.0	20 ± 0.57
S. aureus (MRSA)	0 ± 0	0 ± 0	0 ± 0	19 ± 0.88	0 ± 0	10 ± 0.57*	13 ± 0.33*	16 ± 0.66
Gram-Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	15 ± 0.88
P. aeruginosa	0 ± 0	0 ± 0	10 ± 0.58*	14 ± 1.0	0 ± 0	0 ± 0	0 ± 0	12 ± 0.88
Fungi								
C. albicans	0 ± 0	10 ± 0.33	12 ± 0.33	15 ± 1.0	11 ± 0.58*	15 ± 0.58*	17 ± 0.88*	20 ± 0.88*

*Significant of compared to two values in the same row.

Zizphus honey before and after showed highly inhibition of growth and none of the organisms tested were resistance to the honey samples at concentration of 75% except E. coli (Table 6).

Upon comparison among the antimicrobial activities of the Zizphus honey samples before and after exorcism, highly statistical significant difference was found between their antimicrobial activity against MRSA and C. albicans. However, the Zizphus honey (before and after exorcism) have equipotent antibacterial against E. coli which was 14 ± 0.88 mm.

Table 6: Antimicrobial activities of the Zizphus honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	Zizphus honey				Zizphus exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram-Positive								
S. aureus	0 ± 0	12 ± 0.58	15 ± 0.88	20 ± 1.15	11 ± 0.66*	14 ± 1.45	17 ± 1.0	20 ± 0.66
S. aureus (MRSA)	10 ± 0.33*	12 ± 0.58*	16 ± 1.76*	19 ± 1.86*	0 ± 0	0 ± 0	10 ± 0.88	14 ± 0.66
Gram-Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	14 ± 0.88	0 ± 0	0 ± 0	0 ± 0	14 ± 0.88
P. aeruginosa	0 ± 0	0 ± 0	11 ± 0.66*	15 ± 0.66	0 ± 0	0 ± 0	0 ± 0	13 ± 1.53
Fungi								
C. albicans	0 ± 0	0 ± 0	10 ± 0.33	14 ± 0.58	11 ± 0.33*	14 ± 0.33*	16 ± 0.66*	16 ± 0.58

*Significant of compared to two values in the same row.

In (Table 7) Myrrh exorcism honey was more susceptible against MRSA, the maximum inhibition zone was shown at concentration of 100% as 19 ± 0.88 mm. At concentration of 25%. In comparison to the all types of honey, only Myrrh and N. sativa exorcism honey were showed significant antibacterial activity on MRSA.

Table 7: Antimicrobial activities of the Myrrh honey before and after exorcism. **For each row,** Different letters after SE values indicate statistical significant differences between means ($P < 0.05$).

Organisms	Mean diameter of Inhibition Zone (mm) ± SE							
Honey	Myrrh honey				Myrrh exorcism honey			
	25%	50%	75%	100%	25%	50%	75%	100%
Gram-Positive								
S. aureus	0 ± 0	0 ± 0	10 ± 0.33*	14 ± 0.33	0 ± 0	0 ± 0	17 ± 0	15 ± 1.0
S. aureus (MRSA)	0 ± 0	0 ± 0	0 ± 0	15 ± 0.88	10 ± 0.33*	13 ± 0.33*	16 ± 0.66*	19 ± 0.88
Gram-Negative								
E. coli	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13 ± 0.88*
P. aeruginosa	0 ± 0	0 ± 0	0 ± 0	12 ± 1.0	0 ± 0	0 ± 0	0 ± 0	11 ± 0.66
Fungi								
C. albicans	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	15 ± 0.58*

*Significant of compared to two values in the same row.

The minimum inhibitory concentration of the six types of honey (before and after exorcism) against gram-positive bacteria are selected among the bacteria tested and results are shown in (Table 8). A lower MIC was observed for Tamarix exorcism honey 3.125% after 24 h incubation on MRSA, while the MBC was affected at concentration of 100% for the same honey. Tamarix honey was 50% against MRSA in comparison to Tamarix exorcism honey figure 2. In the case of S. aureus the lower MBC significantly was 6.25% by Zizphus exorcism honey. However, the MBC was 100% against MRSA. Generally, S. aureus was more susceptible to the six types of honey than the MRSA. Thymus honey exhibited no MBC values before and after exorcism toward S. aureus and MRSA (Table 8).

Finally, N. sativa honey (before and after exorcism) showed the same MIC and MBC values against S. aureus. While, significant difference were observed against MRSA at 100% concentration ($P < 0.05$).

Table 8: Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration of all honey samples necessary to inhibit 100% of the microbial growth in vitro expressed in % V/V solution.

Strain / Honey samples	S. aureus		S. aureus (MRSA)	
	MIC	MBC	MIC	MBC
Thymus honey	-	-	100	-
Thymus exorcism honey	-	-	-	-
Tamarix honey	25	100	50	-
Tamarix exorcism honey	6.25	25	3.125	100
N. sativa honey	50	100	50	-
N. sativa exorcism honey	50	100	50	100
Spring honey	-	50	50	100
Spring exorcism honey	-	25	50	100
Zizphus honey	-	50	50	100
Zizphus exorcism honey	-	6.25	50	100
Myrrh honey	25	100	50	-
Myrrh exorcism honey	6.25	25	-	50

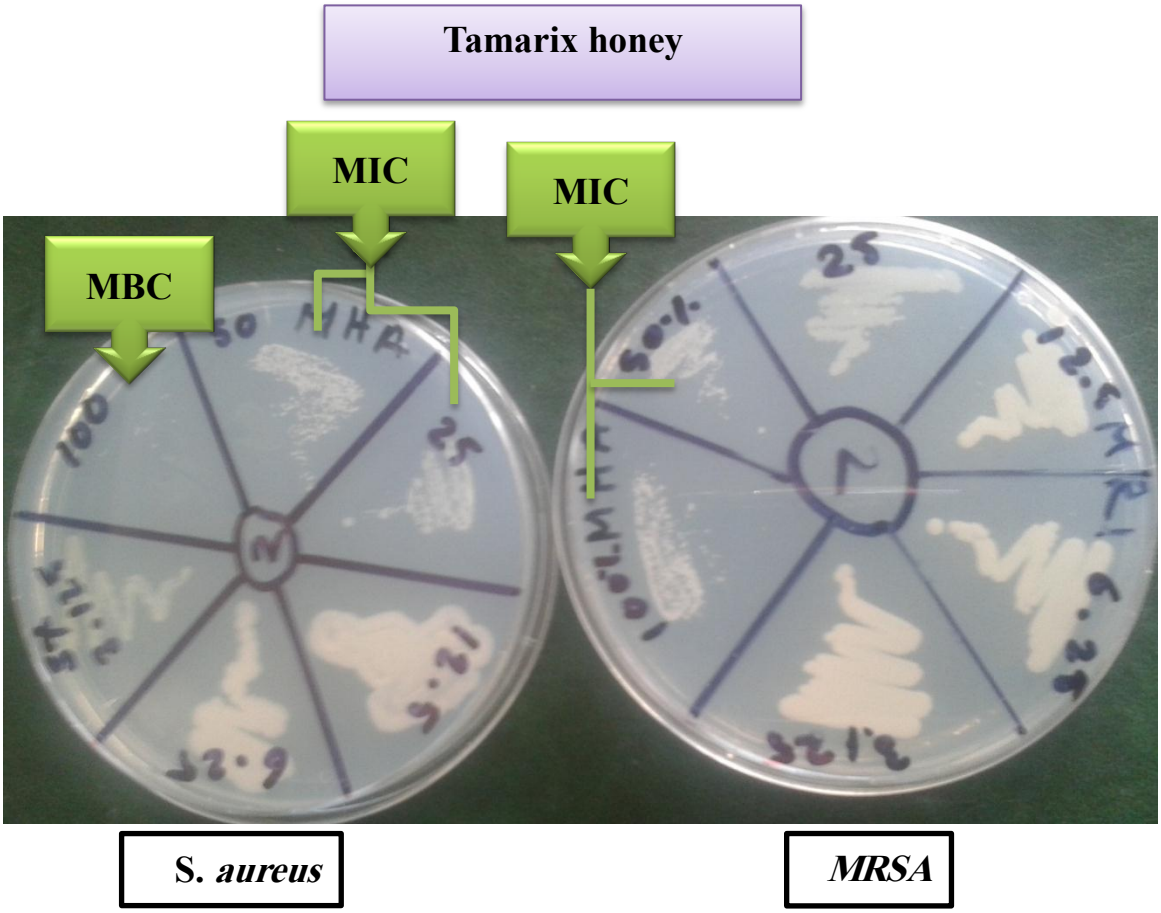

Figure2: The MIC and MBC activities of Tamarix honey against S. aureus and MRSA strains.

4. Discussion:

A total of six honey samples from different origins were evaluated for their antibacterial activity against selected bacteria and fungi species representing the Gram-positive species, S. aureus and MRSA, and the Gram negative species, P. aeruginosa and E. coli. And the fungi C. albicans. In general, as shown in results part. All tested honeys showed a measurable antibacterial activity against all of the tested bacteria with different values. Four of the tested bacteria were most sensitive to N. sativa exorcism honey comparable to other tested honeys showed a significant inhibition zone against Gram-positive bacteria, N. sativa, Thymus and Myrrh honey either showed inhibition to the tested fungi, especially. Spring honey displayed most a potent activity against C. albicans at concentration of 50 %. E. coli displayed the highest resistance for tested honeys. These data do not agree with the results reported by (Mohapatra et al. 2011) who showed that the Gram-negative bacteria are more susceptible to the inhibitory action of honey than are Gram-positive bacteria and agree with (Obeseiki-Ebor and Afonya 1984) and (Nzeako and Hamdi 2000) those found that ten honey samples investigation revealed that C. albicans sensitivity were less than other bacterial organisms tested and these are consistent with the data proved by our study. Also, In this study, honey sample showed the antimicrobial activity, and our result were in agreement with (Wi11ix et al. 1992) who found that

honey inhibited the growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas sp., and also in agreement with (Bilal et al., 1998) who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and Gram-positive bacteria, and a remarkable activity was observed with Pseudomonas aeruginosa and Staphylococcus aureus.

The results shown by honey samples in relation to S. aureus may be important, given that in recent decades there has been a marked increase in difficult to treat skin and underlying tissue infections associated with S. aureus (Halcon and Milkus, 2004). It has been informed that S. aureus has developed resistance against several antibiotics and that it is the principal contaminant agent in many clinical infections (Moreno et al., 2005).

Thus, new strategies to treat wounds infected with S. aureus are needed, and the possibility to use honey appears as a convenient and less costly treatment option. Poor activity of the honeys against S. aureus was unexpected as previous reports by (Cooper et al. 1999). Part of the explanation for the difference in results from other studies may be due to methodological differences between studies because the agar dilution method used by these authors different from an agar well diffusion method that is used in this study.

However it is also likely to be due to variation in the natural floral origin of the honey being produced and variability in the performance of Mueller- Hinton agars from different manufacturers has been shown to be statistically significant, especially when testing E. coli (Barry AL and Effinger LJ 1974). The size of inoculums used, depth of medium in the plates, inoculation technique and, time period between inoculation and application of discs, incubation temperature and time of incubation will also cause differences in the results obtained (Acar JF and Goldstein FW 1996).

Our honey samples also exerted antimicrobial activities on P. aeruginosa, which were resistant to some antibiotics.

The MIC of honey which tested in this study had antimicrobial activity in the range between 3.125% - 100% against the tested microorganisms. The antibacterial potency differences among different studied honey samples could be attributed to the natural variations in floral sources of nectar and the different geographical locations since honey micro components possess physicochemical and phytochemical characteristics resulting in its potency that differs associated with botanical and geographical origins (Alzahrani, Alsabehi, Boukra, Abdellah, Bellik Y and et al 2012). Different honey samples of different botanical or geographical origins; Egyptian honey had MIC and MBC values as 12.5 and 50% v/v (Ali MWN; Abdel-Rahman M and Abdel-Hafeez MM 2005), Malysian honey as 5% and 6.25% w/v (Zainol M, Yusoff K and Yusof M 2013), UK Manuka honey had MIC as 6% w/v (Jenkis R and Cooper R 2012) and Ethiopian honey as 6.25% w/v (Ewnetu Y, Lemma W and Birhane N 2013). Honey antimicrobial action involves several mechanisms but mainly the presence of bacteriostatic and bactericidal action is due to production of hydrogen peroxide (FeÃ¡s X, Iglesias A, Rodrigues S and Estevinho L 2013). H_2O_2 alone may not be sufficient to the full activity (Chen C, Campbell LT, Blair SE and Carter DA 2012), since it is in conjunction with other unknown honey components produce bacterial cytotoxic effects and DNA degradation. Fortunately, S. aureus (either MRSA or methicillin sensitive) which is the most predominant and virulent pathogen was the most sensitive Staph. to honey antimicrobial action with highly significant. It is documented and proved that S. aureus was the most sensitive species to the antimicrobial activity of honey among all tested bacterial species studied (Andualem B 2013).

5. Conclusion:

The majority of the tested honeys exhibited inhibitory effects against different microorganisms. These results suggest that they might be used in treating a wide range of pathogenic Gram-positive, Gram-negative bacteria and fungi.

Abbreviations

ZOI:	Zone of inhibition
MIC:	Minimum Inhibitory Concentration
MBC:	Minimum Bactericidal Concentration
MHA:	Mueller Hinton Agar
MHB:	Mueller Hinton Broth
SDA:	Sabouraud Dextrose Agar
SDB:	Sabouraud Dextrose Broth
CIP:	Ciprofloxacin
KCA:	Ketoconazole
ATCC:	American Type Culture Collection

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