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Compression Study of Anti-microbial Activity of *Honey-bees*

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Abstract: The antimicrobial activity of three types of Bees honey (Sidir, Sunflower and Sunut) were examined against (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerugenes*, *Pseudomonas aeruginosa* and *Candida albicans*) at concentration 100, 75, 50 and 25%. Sidir honey showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Candida albicans*, the zone of inhibition range between (9-50 mm), while the *E. coli* showed markedly resistance towards all concentrations used. Sunflower honey showed markedly sensitivity towards *E. coli*, *Staphylococcus aureus* and *Klebsiella aerogenes*, the inhibitions zone were between (15-50 mm), while both *Candida albicans* and *Pseudomonas aeruginosa* showed clearly resistance towards all concentrations used. Sunut honey showed antimicrobial activity toward *Staphylococcus aureus*, *E. coli*, *Klebsiella aerogenes* and *Candida albicans*, the inhibition zone range between (10-42 mm), while *Pseudomonas aeruginosa* showed resistance toward all concentrations used. The antibacterial activity was compared with Gentamicin and Tetracycline, while the antifungal activity was compared with Nystatin.

Key words: Antimicrobial activity, honey bees, gram-positive bacteria, gram-negative bacteria, *Candida albicans*

INTRODUCTION

Honey is widely used in flok -medicine throughout the world, however, it has a limited use in modern medicine due to lack of scientific support (Ali *et al*., 1991). Honey is used for any kind of weakness, cures digestive problems, improves growth of non-breast fed new borne infants, improves calcium fixation in bones and cures anemia. Honey also reduces and cures eye cataracts and conjunctivitis and applied honey directly to the eye cures various diseases of the cornea (Krell, 1996). There are many reports in the medical literature of honey being very effective as dressing of wounds, burn and skin ulcers, inflammation, the antibacterial properties of honey are not harmful to tissue and actually speed up the growth of new tissue to heal the wound (Lusby *et al*., 2002).

The antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics (Karayil *et al*., 1998; Patton *et al*., 2006). The bactericidal effect of honey was dependent on it's concentration and the nature of the bacteria, the bactericidal action was due neither to the normal acidity of honey, nor to it's high sugar content, enzymes, nitrogenous or other compounds, accumulation of hydrogen peroxide, which is produced by a natural glucose oxidize system in honey (Molan, 2000; Lusby *et al*., 2002; Namias, 2003).

The *in vitro* antimicrobial activity of honey was reported by Radwan *et al*. (1984) who observed that honey stopped the growth of *Salmonella* and *Escherichia coli*. Obaseik-Ebor and Afongo (1984)

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compared the antifungal activity of honey distillate with some antimycotic preparations against *Candida albicans* and found that all the strain resistant to conventional antimycotic agents where inhibited by the active fraction of honey distillate. Farouk *et al*. (1988) found that there were inhibitory effects on Gram positive and Gram-negative strains of both standard test organisms and clinical isolates from inflamed wounds.

Ali *et al*. (1991) reported that *Helicobacter pylori*, Gram positive and Gram negative bacteria growth were inhibited by 20% honey. Steinberq *et al*. (1996) found that honey at high concentration has an inhibitory effect against *Streptococcus mutans*. Hamdi and Zeako (2000) studied antimicrobial activity of commercial honeys using standard organisms, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The result showed that, six honey samples had differing levels of antimicrobial activity against standard organisms. Molan (2000) mention that honey have devoid from antifungal materials and prevents the development of fungi merely by it's high sugar concentration. Ceyhan and Alqur *et al*. (2001), investigated antimicrobial activity of honey samples against eight potential pathogenic bacteria and two fungi. The result of the survey showed that most honey samples at 50% concentration can completely inhibited the growth of all test bacteria. They added that fungi were less sensitive than bacteria to antimicrobial activity of honey. Miorin *et al*. (2003) evaluate the antimicrobial activity and the MICs of honey against *Staphylococcus aureus* while Asadi-Pooya *et al*. (2003) studied the antimycobacterial effect of honey. Postoienko (2004) mention that honey has high antimicrobial activity at different concentration against *Staphylococcus aureus*, *Bacillus subtilis*, *Sacchromyces cerevisiae*, *Micrococcus luteus* and *M. roseus*. Willinson and Cavanagh (2005) reported that *E. coli* was more sensitive than *Pseudomonas aeruginosa* toward honey.

This study aimed to confirm the usage of honey as antimicrobial agent and evaluated this inhibitory action at different honey concentrations, it also aimed to compare between the antimicrobial activity of different types of honeys.

MATERIALS AND METHODS

This study was carried out throughout 2006 at Khartoum state, Sudan. Three types of commercial honey (sider, sunflower and sunut) at concentration 100, 75, 50 and 25%, were used in this study against some standard bacteria (*Staphylococcus aureus*, NCTC 25953, *Escherichia coli* ATCC 25922, *Klebsiella aerugenes*, NCTC 9633, *Pseudomonas aeruginosa*, *ATCC 27853* and *Candida albicans*, ATCC 7596). Honey was diluted by dimethyle sulphoxide.

Testing for Antimicrobial Activity

The cup-plate agar diffusion method was adopted according to Kavanagh *et al*. (1972) to assess the antimicrobial activity of the honey. 0.6 mL of standardized bacterial and *Candida albicans* stock suspensions (10^8-10^9) colony-forming units per mL was thoroughly mixed with 60 mL of sterile Muller and Hinton agar or nutrient agar which were distributed into three sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1 mL sample of each diluent's sample using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 18 h. Three replicates were carried out for each sample against each of the test organism. Simultaneously, controls involving the addition of the solvent instead of honey were carried out. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Gentamicin and tetracycline at concentrations of 100, 40, 20 and 10 mg mL^{-1} were tested against standard bacteria. Nystatin at concentration of 50, 15 and 12.5 mg mL^{-1} were used against *Candida albicans* using same procedure.

RESULTS

Antimicrobial Effect of the Sidir Honey Against Standard Organisms

Staphylococcus aureus showed marked inhibition of growth when Sidir honey used. These inhibition zones were gradually decreased with reduction of honey concentration. The maximum inhibition zone was shown at concentration of 100% as 50 mm, which reduced to 45 mm at 75%, 40 mm at 50% and 30 mm at 25% (Table 1).

Table 1 also showed that *Klebsiella aerogenes* was affected also with Sidir honey and the inhibition zone were 40 mm at 100% and 35 mm was shown in all 70, 50 and 25%.

Pseudomonas aeruginosa showed a little less inhibition zone with Sidir honey. These were 20 mm at 100%, 15 mm at 75% and 10 mm and 9 mm at 50 and 25%, respectively (Table 1), in this table it was clear that the *E. coli* has a marked resistant toward Sidir honey, all concentration revealed negative inhibition zone.

Sidir honey showed inhibition zones with *Candida albicans* at all used concentration; these were 25 mm at 100 and 50%, 35 mm at 75%. Unexpected the highest inhibition zone were 37 mm, was recorded at the lowest concentration 25% (Table 1).

Antimicrobial Effect of Sunflower Honey Against Standard Organisms

The Sunflower honey showed negative inhibition effect against both *Pseudomonas aeruginosa* and *Candida albicans*, while the highest inhibition zone was shown against *E. coli* as 50 mm at 100%, which gradually reduced to 40, 25 and 15 mm at concentration 75, 50 and 25% (Table 1).

Table 1 also showed that *Staphylococcus aureus* growth was inhibited by Sunflower honey and the inhibition zone was gradually decreased with reduction of honey concentration, these were 37, 30, 33 and 24 mm at concentration of 100, 75, 50and 25%, respectively.

Klebsiella aerogenes showed inhibition zone with Sunflower honey as 37 mm at 100%, 30 mm, 27 and 20 mm at concentration of 75, 50 and 25%, respectively (Table 1).

Antimicrobial Effect of Sunut Honey Against Standard Organisms

The highest inhibition effect was shown with *Staphylococcus aureus* at concentration of 25% as 45 mm, these were reduced to 37 mm at concentration of 100%, 32 and 29 mm at concentration of 75 and 50%, respectively (Table 1).

Table 1 also showed that the lowest concentration of 25% gave highest inhibition zone with *Klebsiella aerogenes* as 42 mm, these were followed by 75% as 37 mm, 33 mm at 50% and the lowest inhibition zone 29 mm was shown at concentration of 100%.

E. coli was inhibited by Sunut honey at all used concentration, these were shown as 27 mm at both concentration of 100 and 75%, which reduced to 22, 17 mm at 50 and 25%, respectively (Table 1). From Table 1 it was clear that *Pseudomonas aeruginosa* showed resistant toward Sunut honey at all concentration.

Table 1: Antimicrobial effect of different bees honey against standard organisms

	Inhibition zones (mm)											
	Sidir honey				Sunflower honey				Sunut honey			
Microorganisms	100%	75%	50%	25%	100%	75%	50%	25%	100%	75%	50%	25%
Staphylococcus aureus	50	45	40	30	37	30	33	24	37	32	29	25
Escherichia coli	0	0	0	0	50	40	25	15	27	27	22	17
Pseudomonas aeruginosa	20	15	10	9	0	0	0	0	0	0	0	0
Klebsiella aerogenes	40	35	35	35	37	30	27	20	29	37	33	42
Candida albicans	25	35	25	37	0	0	0	0	10	30	17	14

(0) = No inhibition zone appeared

Candida albicans was also inhibited by Sunut honey at all concentrations, lowest inhibition zone was shown at 100% as 10 mm, the highest inhibition zones was shown as 30 mm at 75%, which reduced gradually to 17 and 14 mm at 50 and 25%, respectively (Table 1).

Comparison Between Three Types of Honey Against Standard Organisms

Sidir Honey

The highest inhibition zone was shown with *Staphylococcus aureus* as 50 mm followed by *Klebsiella aerogenes* as 40 mm at concentration of 100%, while 37 mm was recorded as highest inhibition zone against *Candida albicans* at concentration of 25%, on the other hand *Pseudomonas aeruginosa* showed highest inhibition zone of 20 mm at 100%, while no antimicrobial effect was shown against *E. coli*.

Sunflower Honey

The highest inhibition zone was shown with *E. coli* as 50 mm followed by *Staphylococcus aureus* and *Klebsiella aerogenes* as 37 mm at concentration of 100%, while no antimicrobial effect was shown with *Pseudomonas aeruginosa* and *Candida albicans*.

Sunut Honey

Staphylococcus aureus was shown the highest inhibition zone as 45 mm at concentration of 25%, followed by *Klebsiella aerogenes* as 42 mm at concentration of 25%, while 30 mm was recorded as highest inhibition zone against *Candida albicans* at concentration of 75%, on the other hand *E. coli* showed highest inhibition zone as 27 mm at 100 and 75%, while no antimicrobial activity was shown with *Pseudomonas aeruginosa*.

Comparison of Sensitivity of the Microorganism Toward Three Types of Honey

Staphylococcus aureus and *Klebsiella aerogenes* showed sensitivity towards the three used honey (Sidir, Sunflower and Sunut), *E. coli* was sensitive toward Sunflower, Sunu and resistant toward Sidir honey, *Pseudomonas aeruginosa* was sensitive to Sidir honey but resistant to both Sunflower and Sunut honey and *Candida albicans* was sensitive toward Sidir and Sunut honey but resist Sunflower honey.

Comparison Between Three Types of Honey and Antibiotics

Gentamicin and Tetracycline at concentration range between 10-100 mg mL^{-1} gave inhibition zone with *Staphylococcus aureus* between 14-31 mm, while honey at concentration between 25-100% inhibited *Staphylococcus aureus* as 24-50 mm diameter zone. The used antibiotic showed antibacterial activity with *Escherichia coli* range between 20-25 mm, while used honey showed inhibition zone range between 15-50 mm. Gentamicin and Tetracycline showed inhibition zone with *Pseudomonas aeruginosa* range between 14-25 mm, while honey zone inhibition ranged between 9-20 mm. *Klebsiella aerogenes* showed markedly resistance toward both used antibiotics but gave an excellent sensitivity toward all types of honey with inhibition zones ranged between 20-42 mm (Table 1 and 2). On

Table 2: Antibacterial activity of gentamicin and tertracycline against the standard organisms

	Gentamicin (conc. mg mL^{-1}) Inhibition zones (mm)				Tetracycline (conc. mg mL^{-1}) Inhibition zones (mm)				Nystatin (conc. mg mL^{-1}) Inhibition zones (mm)		
Microorganism	100	40	20	10	100	40	20	10	50	15	12.5
Staphylococcus aureus	20	18	17	14	31	30	26	22	ND	ND	ND
Escherichia coli	25	24	23	20	24	25	23	20	ND	ND	ND
Pseudomonas aeruginosa	25	20	15	14	0	0	0	0	ND	ND	ND
Klebsiella aerogenes	0	0	0	0	0	0	0	0	ND	ND	ND
Candida albicans	ND	ND	ND	ND	ND	ND	ND	ND	28	26	23

0 = No inhibition zone appear, ND = Not Detected

the other hand *Candida albicans* showed inhibition zone with Nystatin at concentration of 12.5-50 mg mL^{-1} ranged between 23-28 mm, while the activity of honey against these microbe was ranged between 10-37 mm (Table 1 and 2).

DISCUSSION

In this study the three types of honey showed antimicrobial activity with standard organisms. The antimicrobial activity showed best results with *Staphylococcus aureus*, these might be due to the osomatic effect, the effect of pH and the sensitivity of these organism to hydrogen peroxide, *Staphylococcus aureus* is widely spread organism in the environment and relatively acquired resistance towards used antibiotics, these results supported by the finding of Miorin *et al.* (2003).

The mechanisms of honey's antimicrobial action are not yet fully understood, but the following seem to be involved higher sugar content, acidity and (on dilution) release of hydrogen peroxide and presence of certain organic compounds. The acidity of honey and it's content of sugars and hydrogen peroxide generation in honey is responsible for some of the other therapeutic effects seen in wound treatment as well as for the antibacterial activity. Also antioxidants in honey prevent the formation of free radicals, which are responsible for this inflammatory effect, the antioxidants in honey are also the likely explanation of the anti-inflammatory action of honey and these might support Molan (2000) and Lusby *et al.* (2002) findings.

The acidity of the honey also explained the relatively resistance of *Candida albicans* compared with other bacteria, it is well known that fungi can grow well and survive at acidic atmosphere.

In this study, *Pseudomonas aeruginosa* showed resistance towards Tetracycline, Sunut honey and Sunflower honey evenly the weak antibacterial activity of Sidir honey (9-20 mm) These might be due to the exopigmentation produced by these bacteria that play significant role in the persistence and resistant of *Pseudomonas aeruginosa* to certain environmental conditions, these results confirm the finding of Efem *et al.* (1992) who reported resistance of *Pseudomonas aeruginosa* to honey.

Our antimicrobial activity findings also supported by the finding of Farouk *et al.* (1988), Steinberq *et al.* (1996) Hamdi *et al.* (2000), Ceyhan and Alqur (2001), Miorin *et al.* (2003) and Postoienko *et al.* (2004).

The resistance of *E.coli* towards Sidir honey may need further investigation. This study concluded that the antimicrobial activity of honey would warrant further studies on the clinical application of honey against pathogenic microorganisms.

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