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## Antimicrobial Activity of Different Egyptian Honeys as Comparison of Saudi Arabia Honey

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### ABSTRACT

Apitherapy or therapy with bee products as honey is an old tradition. In this study, the antimicrobial activity of 8 honey samples were investigated against 6 bacteria potential pathogens belonging to Gram positive and Gram negative bacteria. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* and *Corynebacteria pseudotuberculosis*. Where the Gram negative bacteria were *Klebsiella pneumonia* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218). Honey samples were obtained from 8 different floral sources. The honey samples were acacia honey, citrus honey, clover honey, coriander honey, cotton honey, palm honey and sesame honey. Beside one saudi honey sample (sider honey). The findings indicate that honey samples with different Egyptian honeys and Saudi honey sample (sider honey), were effective antibacterial against different pathogenic bacteria. Honey prevents growth of the isolates and inhibits their growth when honey was added to growing culture. The results also showed that most of honey samples at 20.30% (w/v) can completely inhibit the growth of all of the tested bacteria. It was clear that the different types of honey were less effective against *E. coli* than other bacteria.

**Key words:** Honey, Gram positive, Gram negative, growth inhibition, antimicrobial activity

### INTRODUCTION

Honey is recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds (Brudzynski, 2006; Jalali *et al.*, 2007a). Renewed interest in honey for various therapeutic purposes, including treatment of infected wounds (Jalali *et al.*, 2007b), has led to the search for different types of honey with antibacterial activity (Mullai and Menon, 2007). It has a valuable role in traditional medicine for centuries. It was described in many cultures since ancient times (Molan, 1992). The use of honey as therapeutic substance has been rediscovered by medical provincial in more recent times and has been accepted as antibacterial agent for treatment ulcers, bed sore and surface wound infection and surface infections resulting from wounds (Tossoun *et al.*, 1997; Brudzynski, 2006). Also honey has been found to be antioxidant activity (Atrooz *et al.*, 2008) and act as effective in treating bacterial gastroenteritis in infants (Brady *et al.*, 2004) and liver disease (Yoirish, 1977).

The antibacterial activity of honey referred to the endogenous hydrogen peroxide content (Brudzynski, 2006; Mercan *et al.*, 2007), inhibin (Nour, 1988) which acts as antibacterial factor other than H<sub>2</sub>O<sub>2</sub> (Molan and Russell, 1988). The antibacterial activity of different honey was studied by as Molan (1992), Elbagaury and Rasmy (1993), Al-Somal *et al.* (1994), Hegazi *et al.* (2002) and Brady *et al.* (2004). Thus the aim of the present study was to

investigate the antibacterial activity of different Egyptian honey and compared with Saudi Arabia honey against Gram positive and negative bacteria.

## MATERIAL AND METHODS

**Bacterial strains:** Six bacterial species included Gram positive and Gram negative were used. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* and *Corynebacteria pseudotuberculosis*. Where the Gram negative bacteria were *Klebsiella pneumonia* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218).

**Honey:** All reagents are of analytical purity grade. Distilled water was used for all dilution steps. Fresh seven Egyptian honey samples and one Saudi honey sample (suder honey, was kindly provided by El-Yahia Company 2004, flowering season) were mono floral honeys harvested from apiaries (From Authorized apiary farm). The collected honey samples from Egypt were acacia honey, citrus honey, clover honey, coriander honey, cotton honey, palm honey and sesame honey. Beside All of these honeys are vended as monofloral, meaning that the honey must derive from at least 55% of pollen from a single floral source according to Louveaux *et al.* (1978). All honey samples were stored in dark in tan containers at 4°C until being used. Under aseptic condition to different dilutions were prepared for each type of honey using sterile distilled water. Evaluations of the antibacterial activity of different honey dilutions were performed according to Nour (1988), Moussa (1997) and Hegazi and Abd-El-Hady (2002). The results of antibacterial activity against different examined bacteria were performed.

**Antibacterial assay:** Six bacterial strains were used: *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes*, *Corynebacteria pseudotuberculosis*, *Klebsiella pneumonia* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218). The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard ( $5 \times 10^7$  cells mL<sup>-1</sup>) tubes. It was further diluted to obtain a final of  $5 \times 10^6$  cells mL<sup>-1</sup>. These bacterial strains were enriched on nutrient broth as well as on selective broth for bacterial propagation (Cruickshank *et al.*, 1979). The broth was inoculated by the 0.20 µL/10 mL broth *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacteria pseudotuberculosis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* and then added 40 µL of 21.30% honey (Hegazi *et al.*, 2002). Honey was added to broth inoculated with isolates of different bacterial species after inoculation. The tubes were incubated at 37°C for 24 h. The growths of control bacterial strains as well as inhibitions of the bacterial growth due to honey were measured by turbidity at 420 nm wave length. The mean values of inhibition were calculated from triple reading in each test (Hegazi *et al.*, 2000).

**Statistical analysis:** Data were analyzed statistically using student t test showing Mean±SD. Statistical significance was accepted at p<0.01 according to Senedcor (1961).

## RESULTS

The results of antibacterial activity of different honey types against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacteria pseudotuberculosis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* were recorded in Table 1 and 2.

Table 1: Antibacterial activity of different honey types against Gram positive bacteria

Parameters	*Growth inhibition		
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Corynebacteria pseudotuberculosis</i>
Normal bacterial growth	1.236±0.001	1.397±0.002	1.321±0.011
Acacia honey	0.387±0.015	0.542±0.001	0.733±0.012
Citrus honey	0.204±0.001	0.253±0.011	0.582±0.011
Clover honey	0.594±0.002	0.321±0.014	0.339±0.001
Coriander honey	0.325±0.002	0.242±0.001	0.351±0.002
Cotton honey	0.497±0.003	0.451±0.001	0.459±0.003
Palm honey	0.399±0.013	0.301±0.011	0.433±0.021
Sesame honey	0.432±0.014	0.543±0.015	0.625±0.002
Sider honey	0.394±0.011	0.532±0.011	0.579±0.023
Tetracycline (50 µg)	0.234±0.001	0.421±0.001	0.326±0.002

\*Growth inhibition measured by turbidity at 420 nm analyzed by spectrophotometer

Table 2: Antibacterial activity of different honey types against Gram negative bacteria

Parameters	*Growth inhibition		
	<i>Klebsiella pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Normal bacterial growth	1.425±0.001	1.346±0.002	1.263±0.001
Acacia honey	0.562±0.002	0.498±0.001	0.682±0.003
Citrus honey	0.419±0.031	0.532±0.012	0.599±0.001
Clover honey	0.233±0.001	0.461±0.001	0.601±0.002
Coriander honey	0.461±0.014	0.349±0.002	0.534±0.014
Cotton honey	0.501±0.001	0.511±0.011	0.632±0.012
Palm honey	0.409±0.016	0.482±0.003	0.758±0.001
Sesame honey	0.733±0.001	0.646±0.001	0.499±0.004
Sider honey	0.487±0.003	0.302±0.021	0.638±0.002
Tetracycline (50 µg)	0.367±0.014	0.509±0.032	0.743±0.001

\*Growth inhibition measured by turbidity at 420 nm analyzed by spectrophotometer

The result of antibacterial activity of different honey types against *Staphylococcus aureus* was illustrated in Table 1. It was clear that all honey types at 20.30% showed inhibition of bacterial growth. Tetracycline (50 µg) was inhibited *Staphylococcus aureus* (0.234±0.001). The Egyptian honey gave higher activity than Saudi Arabia (0.394±0.011) honey (Sider honey). Citrus honey (0.204±0.001) gave the highest inhibition followed coriander honey (0.325±0.002), acacia honey (0.387±0.015), palm honey (0.399±0.013). The moderate inhibition was found in sesame honey (0.432±0.014) and cotton honey (0.497±0.003) where the lowest inhibition was found in clover honey (0.594±0.002). Sider honey (0.394±0.011) was more effective than Sesame honey (0.432±0.014), cotton honey (0.497±0.003) and clover honey (0.594±0.002) against *Staphylococcus aureus*.

The result of antibacterial activity of different honey types against *Streptococcus pyogenes* was tabulated in Table 1. Coriander honey gave the highest antibacterial activity (0.242±0.001) followed by citrus honey (0.253±0.011), palm honey (0.301±0.011) and clover honey (0.321±0.014). The moderate activity was observed in cotton honey (0.451±0.001), where the lower activity was demonstrated in sider honey (0.532±0.011) and acacia honey (0.542±0.001). The activity of sider honey was lower than all Egyptian honey except Acacia honey. Tetracycline (50 µg) was inhibited *Streptococcus pyogenes* (0.421±0.001).

The antibacterial activity of different honey against *Corynebacteria pseudotuberculosis* was observed (Table 1). It was obvious that the highest antibacterial activity was observed in Clover honey ( $0.339\pm 0.001$ ) and coriander honey ( $0.351\pm 0.002$ ) followed by palm honey ( $0.433\pm 0.021$ ), cotton honey ( $0.459\pm 0.003$ ) where the lowest activity was observed in case of acacia honey ( $0.733\pm 0.012$ ). In comparison between Sider honey and other Egyptian honeys it was clear that the sider honey ( $0.579\pm 0.023$ ) more effective than sesame honey ( $0.625\pm 0.002$ ) and acacia honey ( $0.733\pm 0.012$ ). Tetracycline (50  $\mu\text{g}$ ) was inhibited *Corynebacteria pseudotuberculosis* ( $0.326\pm 0.002$ ).

The antibacterial activity of different honey against *Klebsiella pneumonia* was observed (Table 2). It was obvious that the highest antibacterial activity was observed in clover honey ( $0.233\pm 0.001$ ) where palm honey ( $0.409\pm 0.016$ ), citrus honey ( $0.419\pm 0.031$ ), coriander honey ( $0.461\pm 0.014$ ) and sider honey ( $0.487\pm 0.003$ ) were showed moderate activity. Sider honey ( $0.487\pm 0.003$ ) was showed higher activity than cotton honey ( $0.501\pm 0.001$ ), acacia honey ( $0.562\pm 0.002$ ) and sesame honey ( $0.733\pm 0.001$ ). The lowest activity was observed in sesame honey ( $0.733\pm 0.001$ ) against *Klebsiella pneumonia*. Tetracycline (50  $\mu\text{g}$ ) was inhibited *Klebsiella pneumonia* ( $0.367\pm 0.014$ ).

The result of antibacterial activity of different honey types against *Pseudomonas aeruginosa* was tabulated in Table 2. Sider honey gave the highest antibacterial activity ( $0.302\pm 0.021$ ) followed by coriander honey ( $0.349\pm 0.002$ ), palm honey ( $0.482\pm 0.003$ ) and acacia honey ( $0.498\pm 0.001$ ). The moderate activity was observed in cotton honey ( $0.511\pm 0.011$ ) and citrus honey ( $0.532\pm 0.012$ ), where the lower activity was demonstrated in sesame honey ( $0.646\pm 0.001$ ). The activity of sider honey was higher than all Egyptian honey. Tetracycline (50  $\mu\text{g}$ ) was inhibited *Pseudomonas aeruginosa* ( $0.509\pm 0.032$ ).

The result of antibacterial activity of different honey types against *Escherichia coli* was illustrated in Table 2. It was clear that all honey types at 20.30% showed inhibition of bacterial growth. Tetracycline (50  $\mu\text{g}$ ) was inhibited *Escherichia coli* ( $0.743\pm 0.001$ ). The Egyptian honey gave higher activity than Saudi Arabia ( $0.638\pm 0.002$ ) honey (Sider honey) except acacia honey ( $0.682\pm 0.003$ ). Sesame honey ( $0.499\pm 0.004$ ) gave the highest inhibition followed coriander honey ( $0.534\pm 0.014$ ), palm honey ( $0.558\pm 0.001$ ), citrus honey ( $0.599\pm 0.001$ ). Sider honey ( $0.638\pm 0.002$ ) was more effective than acacia honey ( $0.682\pm 0.003$ ) against *Escherichia coli*.

## DISCUSSION

Regarding to the results of antibacterial activity of different honey types against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacteria pseudotuberculosis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*. It was clear that all honey types at 20.30% showed inhibition of bacterial growth. Also it was obvious that the inhibition of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacteria pseudotuberculosis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* depend on the type of honey origin. The Egyptian honey gave higher activity against *Staphylococcus aureus* than Saudi Arabia ( $0.394\pm 0.011$ ) honey (Sider honey). Citrus honey ( $0.204\pm 0.001$ ) gave the highest inhibition followed coriander honey ( $0.325\pm 0.002$ ), acacia honey ( $0.387\pm 0.015$ ), palm honey ( $0.399\pm 0.013$ ). Sider honey ( $0.394\pm 0.011$ ) was more effective than Sesame honey ( $0.432\pm 0.014$ ), cotton honey ( $0.497\pm 0.003$ ) and clover honey ( $0.594\pm 0.002$ ) against *Staphylococcus aureus*. Coriander honey gave the highest antibacterial activity ( $0.242\pm 0.001$ ) against *Streptococcus pyogenes* followed by citrus honey ( $0.253\pm 0.011$ ), palm honey ( $0.301\pm 0.011$ ) and clover honey ( $0.321\pm 0.014$ ). It was obvious that the highest antibacterial

activity against *Corynebacteria pseudotuberculosis* was observed in Clover honey (0.339±0.001) and coriander honey (0.351±0.002) followed by palm honey (0.433±0.021), cotton honey (0.459±0.003). The highest antibacterial activity against *Klebsiella pneumonia* was observed in clover honey (0.233±0.001) where palm honey (0.409±0.016), citrus honey (0.419±0.031), coriander honey (0.461±0.014) and sider honey (0.487±0.003). Sider honey gave the highest antibacterial activity (0.302±0.021) *Pseudomonas aeruginosa* followed by coriander honey (0.349±0.002), palm honey (0.482±0.003) and acacia honey (0.498±0.001). While Egyptian honey gave higher activity against *Escherichia coli* than Saudi Arabia (0.638±0.002) honey (Sider honey) except acacia honey (0.682±0.003). Sesame honey (0.499±0.004) gave the highest inhibition followed coriander honey (0.534±0.014), palm honey (0.558±0.001), citrus honey (0.599±0.001). The inhibition of bacterial growth may be due to many factors as the osmotic effect of honey as described by Listner (1975), Chirife *et al.* (1982), Molan (1992) and Omafuvbe and Akanbi (2009): The presence of hydrogen peroxide (Dustmann, 1987; Efem, 1988), non-peroxide substances (Bogdanov, 1984; Radwan *et al.*, 1984), propolis which contain flavonoid (Bogdanov, 1984; Hegazi *et al.*, 1996) and volatile antibacterial substances (Christov, 1961).

Many authors studied the antibacterial activity of honey as Hodgeson (1989) who compared the antibacterial effect of Manuka honey with ling heather honey. He found that whereas *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inhibited by both honeys, inhibition of *E. coli*, *Proteus mirabilis* and *Streptococcus fecalis* was not seen with ling heather honey, yet Manuka honey inhibited all these species. Also Jeddar *et al.* (1985) evaluated the growth of various gram positive and gram negative bacteria in media containing various concentrations of honey and they found that most pathogenic bacteria failed to grow in honey at a concentration of 40% or above. Where, Al-Somal *et al.* (1994) examined the sensitivity of helicobacter pylori to honey using five isolates from biopsies of gastric ulcers and found all five isolates were sensitive to 5% solution of Manuka honey incorporated in the agar media.

Dilution of honey was observed by Basualdo *et al.* (2007) who found honey inhibited the growth of *S. aureus* even at 50% dilution. Undiluted honey samples also inhibited the growth of *Staphylococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*, although to a lesser extent. Also Mullai and Menon (2007) assessed the antibacterial activity of different types of honey (manuka honey from Australia, heather honey from the United Kingdom, and locally marketed Indian honey). They found that locally available (khadikraft) honey produced the best activity against *Pseudomonas aeruginosa* and was found also it was better than all of the imported varieties of therapeutic honey. The honey samples which were obtained from Izmir proved more effective as inhibitors against *P. aeruginosa*, *E. coli* and *S. aureus*. The honey which was obtained from Mugla exhibited high anticandidal activity on *C. albicans* (Merican *et al.*, 2007). Khalil *et al.* (2001) found that the tested unifloral honeys available at the Northern Region of Bangladesh showed a significant antibacterial activity against the wound infecting and enteric pathogens. Among them honey from Mustard flower was more active.. Also Selcuk and Nevin (2002) found that honey collected from Rize-Anzer region, Turkey was found to be the most effective honey on clinically isolated bacteria.

Estrada *et al.* (2005) evaluated the antimicrobial activity of different honey concentrations (100, 75, 50, 25, 12.5 and 6.25% v/v) against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (UCR 2902), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC25922), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 19116) and *Aspergillus niger*.

They obtained for the microbiological characterization of honey show that 91% of samples had counts equal or lower than  $1,0 \times 10^1$  (1) CfU g<sup>-1</sup>. No positive result was obtained for the isolation of *C. botulinum*. 24 of the samples analyzed inhibited the growth of *S. aureus* even in a 25% v/v concentration; nevertheless, *A. niger* was not inhibited by any of the samples tested.

Iurlina and Fritz (2005) found that honey diluted to concentrations from 75 to 1% (w/v) of full-strength honey showed total antibacterial activity. The numbers of aerobic mesophilic bacteria, moulds and yeasts were less than 10 (3) cfu g<sup>-1</sup> for all 70 samples. Faecal coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and Clostridium sulfite-reducers were not detected but *P. larvae* subsp. *larvae*, *Bacillus cereus*, *Bacillus pumilus* and *Bacillus laterosporus* were found among samples. Honey diluted to concentrations from 75 to 1% (w/v) of full-strength honey showed total antibacterial activity. The numbers of aerobic mesophilic bacteria, moulds and yeasts were less than 10 (3) cfu g<sup>-1</sup> for all 70 samples. Faecal coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Clostridium sulfite-reducers* were not detected but *P. larvae* ssp. *larvae*, *Bacillus cereus*, *Bacillus pumilus* and *Bacillus laterosporus* were found among samples.

The variations of the activity of different honey was attributed to the previously mentioned factors which influenced the antibacterial activity as osmotic properties of honey (Listner, 1975; Chirife *et al.*, 1982; Molan, 1992); honey pH (Mairaj *et al.*, 2008) or activity of glucose oxidase; hydrogen peroxide (Dustmann, 1987; Efem, 1988), non peroxide substances (Bogdanov, 1984; Radwan *et al.*, 1984), Presence of propolis which contain flavonoid (Bogdanov, 1984; Hegazi *et al.*, 1996) and volatile antibacterial substances (Christov, 1961).

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