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Antimicrobial Activity of Different Saudi Arabia Honeys

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Abstract: Honey is used as an old traditional medicine. In this study, the antimicrobial activity of twelve Saudi honey samples were investigated against 6 potential Gram positive and Gram negative bacterial pathogens. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* and *Corynebacterium pseudotuberculosis* and the Gram negative bacteria were *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218). Honey samples were obtained from 12 different floral sources. The collected honey samples were Sidr or birds or Sidr North honey, Tabah honey, Rok honey, spring Lena honey, Harbingers honey, Valley offense (Qtad) honey, Abu roses honey, spring hospitality honey, Agaa mount honey, spring honey pride, then Sair honey, Hegaz Spring honey and Shamar mount honey. The findings indicated that samples with different Saudi honeys were effective antibacterial agents against tested pathogenic bacteria as they inhibited their growth when honey was added to their growing cultures. It was clear that the different types of honey were less effective against *E. coli* than other bacteria.

Key words: Honey · KSA · Antimicrobial Activity

INTRODUCTION

Honey has been used since ancient times for the treatment of some respiratory diseases and for the healing of skin wounds [1, 2]. Also honey is recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds [3]. Renewed interest in honey for various therapeutic purposes, including treatment of infected wounds, has led to the search for different types of honey with antibacterial activity [4]. The healing effect of honey could be due to various physical and chemical properties [5]. The floral source of honey plays an important role on its biological properties [6]. The use of honey as therapeutic substance has been rediscovered by medical provincials in more recent times and has been accepted as antibacterial agent for treatment of ulcers, bed sore, surface wound infection and surface infections resulting from wounds [3, 7]. Also honey has been found to be effective in treating bacterial gastroenteritis in infants [8] and liver diseases [9].

The antimicrobial activity of honey could be attributed to several factors [10] including endogenous hydrogen peroxide content [3, 11], inhibin [12] which acts as antibacterial factor other than H_2O_2 [13], osmotic effect of honey and the low pH of honey being between 3.2 and 4.5 [14, 15], defensin-1, as well as the presence of phytochemical factors [16, 17]. The antibacterial activity of different honeys was studied by many authors [18-24].

A large number of honeys are available in the Saudi market. These honeys are either locally produced or imported from different countries. Some of these honeys are traditionally used as remedy for several ailments. Previous studies has been conducted on the antibacterial activity of 24 types of honeys available in the Saudi market which were compared with Manuka honey and several types of the tested honeys are recognized as potent antimicrobials [25]. A comparison between Egyptian and Saudi Arabia honeys was also conducted by Hegazi [24]. Thus the aim of the present study was to investigate the antibacterial activity of different Saudi Arabia honeys against six Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Bacterial Strains: Six Gram positive and Gram negative bacterial species were used. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923),

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Streptococcus pyogenes and *Corynebacterium pseudotuberculosis*. Where, the Gram negative bacteria were *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218).

Honey: Fresh twelve Saudi honey samples (1 kg each) were kindly provided by Alnahal aljwal Company, 2010 flowering season. The mono floral honeys were harvested from apiaries (From Authorized private apiary farm), these honeys are vended as "monofloral", meaning that the honey must be derived from at least 55 % of pollen from a single floral source according to Louveaux *et al*. [26]. The collected honey samples were Sidr or birds or Sidr North honey, Tabah honey, Rok honey, spring Lena honey, Harbingers honey, Valley offense (Qtad) honey, Abu roses honey, spring hospitality honey, Agaa mount honey, spring honey pride then Sair honey, Hegaz Spring honey and Shamar mount honey. Each honey sample was collected in a sterile universal glass container and kept at 2- 8°C until tested. Physiological saline (PBS, pH 7.2) was used for all dilution steps under aseptic condition according to the method described by Nzeako and Hamdi [27].

Antibacterial Assay: Evaluations of the antibacterial activity of different honey dilutions were performed according to Moussa [28] and Hegazi [24]. Bacterial suspensions of the six studied strains were prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard ($5x10^7$ cells / ml) tubes. It was further diluted to obtain a final concentration of $5X10^6$ cells / ml. These bacterial strains were enriched on nutrient broth as well as on selective broth for bacterial propagation [29]. Each broth was inoculated by 0.2 µl of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium pseudotuberculosis*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli* and then 40 µl of 20.30% honey were added [20]. The tubes were incubated at 37°C for 24 h. The growth of control bacterial strains as well as inhibition of the bacterial growth due to honey was measured by turbidity at 420 nm wave length. The mean values of inhibition were calculated from triple reading in each test [24].

Statistical Analysis: Data were analyzed statistically using student "T" test showing mean±SD. Data were compared using one way Anova. Statistical significance was accepted at $p \leq 0.01$ according to Senedcor [30].

RESULTS

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

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

	Staphylococcus aureus	*Streptococcus pyogenes*	*Corynebacterium pseudotuberculosis*
Normal Bacterial growth	1.636±0.001	1.307±0.002	1.421±0.011
Tetracycline (50ug)	0.213±0.001*	0.321±0.001	0.326±0.002
Sidr or birds or Sidr North honey	0.581±0.015	0.322±0.001	0.333±0.012
Tabah honey	0.301±0.001	0.253±0.011	0.682±0.011
Rok honey	0.394±0.002	0.345±0.014	0.439±0.001
Spring Lena honey	0.425±0.002	0.311±0.001	0.381±0.002
Harbingers honey	0.597±0.003	0.351±0.001	0.859±0.003
Valley offense (Qtad) honey	0.521±0.001	0.401±0.001	0.701±0.001
Abu roses honey	0.594±0.002	0.544±0.002	0.294±0.002
Spring hospitality honey	0.325±0.002	0.625±0.002	0.425±0.002
Agaa mount honey	0.297±0.003	0.397±0.003	0.397±0.003
Spring honey pride, then Sair honey	0.339±0.013	0.511±0.011	0.233±0.021
Hegaz Spring honey	0.512±0.014	0.343±0.150	0.425±0.002
Shamar mount honey	0.454±0.011	0.632±0.011	0.379±0.023

* Growth Inhibition = Inhibition of the growth measured by turbidity at 420 nm analyzed by spectrophotometer

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

	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Escherichia coli*
Normal Bacterial growth	1.415±0.001	1.236±0.002	1.323±0.001
Tetracycline (50ug)	0.317±0.011*	0.509±0.032	0.443±0.001
Sidr or birds or Sidr North honey	0.462±0.002	0.398±0.001	0.298±0.001
Tabah honey	0.329±0.031	0.532±0.012	0.532±0.012
Rok honey	0.333±0.001	0.461±0.001	0.341±0.001
Spring Lena honey	0.561±0.014	0.449±0.002	0.249±0.002
Harbingers honey	0.401±0.001	0.311±0.011	0.311±0.011
Valley offense (Qtad) honey	0.319±0.031	0.422±0.012	0.499±0.001
Abu roses honey	0.433±0.001	0.561±0.001	0.501±0.002
Spring hospitality honey	0.361±0.014	0.649±0.002	0.634±0.014
Agaa mount honey	0.401±0.001	0.211±0.011	0.332±0.012
Spring honey pride, then Sair honey	0.539±0.016	0.282±0.003	0.558±0.001
Hegaz Spring honey	0.633±0.001	0.446±0.001	0.299±0.004
Shamar mount honey	0.387±0.003	0.502±0.021	0.738±0.002

* Growth Inhibition = Inhibition of the growth measured by turbidity at 420 nm analyzed by spectrophotometer

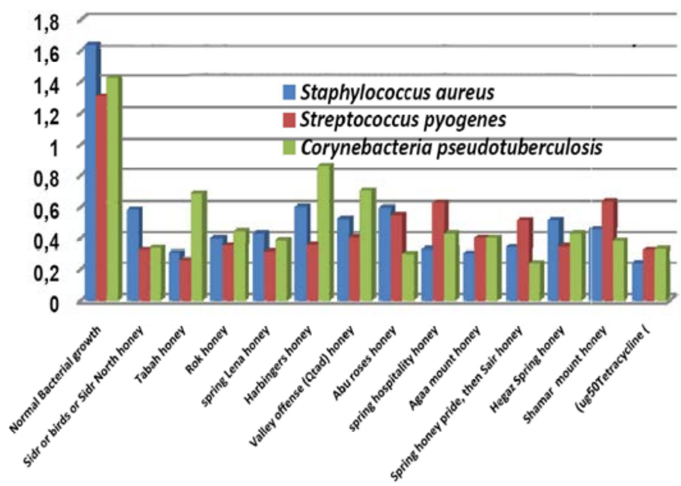

Fig. 1: Antibacterial activity of different honey types against gram positive bacteria

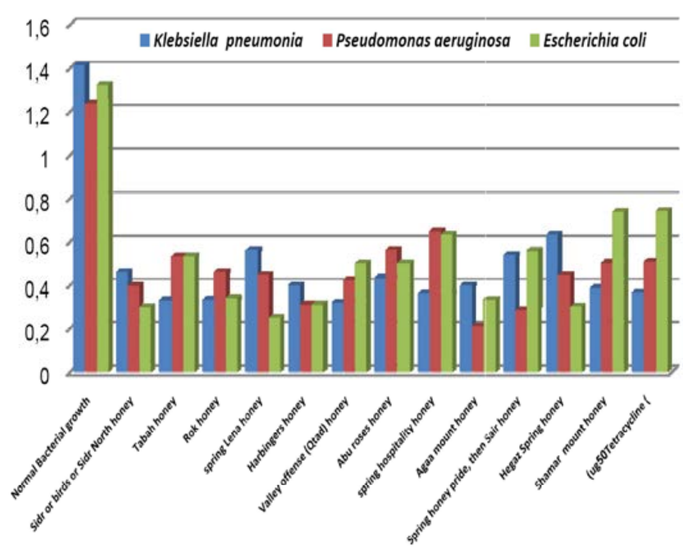

Fig. 2: Antibacterial activity of different honey types against gram negative bacteria

The result of antibacterial activity of different honey types against *Staphylococcus aureus* was illustrated in Table (1) and Figure (1). It was clear that all honey types at 20.30 % showed inhibition of bacterial growth. Tetracycline (50ug) inhibited *Staphylococcus aureus* (0.213±0.001). Agaa mount honey (0.297±0.003) gave highest activity against *Staphylococcus aureus* where the lowest inhibition was displayed by Harbingers honey (0.597±0.003).

The result of antibacterial activity of different honey types against *Streptococcus pyogenes* was presented in Table 1 and Figure 1. Tabah honey gave the highest antibacterial activity (0.253±0.011) where the lowest activity was demonstrated by Shamar mount honey (0.632±0.011). Tetracycline (50ug) inhibited *Streptococcus pyogenes* (0.321±0.001).

The antibacterial activity of different honey against *Corynebacterium pseudotuberculosis* was observed (Table 1 and Figure 1). It was obvious that the highest antibacterial activity was observed by spring honey pride, then Sair honey (0.233±0.021). While the lowest activity was observed in case of Harbingers honey (0.859±0.003). Tetracycline (50ug) inhibited *Corynebacterium pseudotuberculosis* (0.326±0.002).

The antibacterial activity of different honeys against *Klebsiella pneumoniae* was observed (Table 2 and Figure 2). It was obvious that the highest antibacterial activity was observed by Valley offense (Qtad) honey (0.319±0.031). The lowest activity was observed by Hegaz Spring honey (0.633±0.001). Tetracycline (50ug) inhibited *Klebsiella pneumoniae* (0.317±0.011).

The result of antibacterial activity of different honey types against *Pseudomonas aeruginosa* was tabulated in Table 2 and Figure 2. Agaa mount honey gave the highest antibacterial activity (0.211±0.011) while the lowest activity was observed by spring hospitality honey (0.649±0.002). Tetracycline (50ug) inhibited *Pseudomonas aeruginosa* (0.509±0.032).

The result of antibacterial activity of different honey types against *Escherichia coli* was illustrated in Table (2) and Figure (2). It was clear that all honey types at 20.30 % showed inhibition of bacterial growth. Tetracycline (50ug) inhibited *Escherichia coli* (0.443±0.001). The spring Lena honey gave highest activity (0.249±0.002) while lowest activity was observed by Shamar mount honey (0.738±0.002).

DISCUSSION

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*, *Corynebacterium pseudotuberculosis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli* was dependent on the type of honey origin.

In the present study, it was clear that all honey types at 20.30 % showed inhibition of bacterial growth. Tetracycline (50ug) inhibited *Staphylococcus aureus* (0.213±0.001). The Agaa mount honey gave highest activity against *Staphylococcus aureus* (0.297±0.003) followed by Tabah honey (0.301±0.001), Spring hospitality honey (0.325±0.002), Spring honey pride, then Sair honey (0.339±0.013) and Rok honey (0.394±0.002). The moderate inhibition was found in spring Lena honey (0.425±0.002) and Shamar mount honey (0.454±0.011) where the lowest inhibition was found in Hegaz Spring honey (0.512±0.014) followed by Valley offense (Qtad) honey (0.521±0.001), Sidr or birds or Sidr North honey (0.581±0.015), Abu roses honey (0.594±0.002) and Harbingers honey (0.597±0.003).

Tabah honey gave the highest antibacterial activity (0.253±0.011) against *Streptococcus pyogenes* followed by spring Lena honey (0.311±0.001), Sidr or birds or Sidr North honey (0.322±0.001), Hegaz spring honey (0.343±0.15), Rok honey (0.345±0.014), Harbingers honey (0.351±0.001) and Agaa mount honey (0.397±0.003). The moderate activity was observed in Valley offense (Qtad) honey (0.401±0.001), spring honey pride, then Sair honey (0.511±0.011) and Abu roses honey (0.544±0.002), where the lowest activity was demonstrated in spring hospitality honey (0.625±0.002) and Shamar mount honey (0.632±0.011). Tetracycline (50ug) inhibited *Streptococcus pyogenes* (0.321±0.001).

The highest antibacterial activity against *Corynebacterium pseudotuberculosis* was observed in spring honey pride, then Sair honey (0.233±0.021) and Abu roses honey (0.294±0.002) followed by Sidr or birds or Sidr North honey (0.333±0.012), Shamar mount honey (0.379±0.023), spring Lena honey (0.381±0.002) and Agaa mount honey (0.397±0.003) and where the moderate activity was displayed by Hegaz spring honey (0.425±0.002), spring hospitality honey (0.425±0.002) and Rok honey (0.439±0.001). The lower activity was demonstrated by Tabah honey (0.682±0.011) and Valley offense (Qtad) honey (0.701±0.001) while the lowest activity was observed in case of Harbingers honey (0.859±0.003). Tetracycline (50ug) inhibited *Corynebacterium pseudotuberculosis* (0.326±0.002).

It was found that the highest antibacterial activity against *Klebsiella pneumoniae* was observed in Valley offense (Qtad) honey (0.319±0.031) followed by Tabah honey (0.329±0.031) and Rok honey (0.333±0.001) where spring hospitality honey (0.361±0.014), Shamar mount honey (0.387±0.003), Harbingers honey (0.401±0.001), Agaa mount honey (0.401±0.001), Abu roses honey (0.433±0.001) and Sidr or birds or Sidr North honey (0.462±0.002) showed moderate activity. Lower activity against *Klebsiella pneumoniae* was displayed by spring honey pride (0.539±0.016) and spring Lena honey (0.561±0.014) and the lowest activity was observed in Hegaz Spring honey (0.633±0.001). Tetracycline (50ug) inhibited *Klebsiella pneumonia* (0.317±0.011).

Agaa mount honey and spring honey pride, then Sair honey gave the highest antibacterial activity against *Pseudomonas aeruginosa* (0.211±0.011 and 0.282±0.003 respectively) followed by Harbingers honey (0.311±0.011) and Sidr or birds or Sidr North honey (0.398±0.001). The moderate activity was observed in Valley offense (Qtad) honey (0.422±0.012), Hegaz spring honey (0.446±0.001), spring Lena honey (0.449±0.002) and Rok honey (0.461±0.001) where the lower activity was demonstrated in Shamar mount honey (0.502±0.021), Tabah honey (0.532±0.012) and Abu roses honey (0.561±0.001). The lowest activity was observed by spring hospitality honey (0.649 ± 0.002). Tetracycline (50ug) inhibited *Pseudomonas aeruginosa* (0.509±0.032).

It was found that tetracycline (50ug) inhibited *Escherichia coli* (0.443 ± 0.001). The spring Lena honey, Sidr or birds or Sidr North honey and Hegaz Spring honey gave highest activity (0.249±0.002; 0.298±0.001 and 0.299±0.004 respectively) followed by Harbingers honey (0.311±0.011), Agaa mount honey (0.332±0.012) and Rok honey (0.341±0.001). The lowest activity was observed in Shamar mount honey (0.738±0.002).

Many authors studied the antibacterial activity of honey as Hodgeson [31] 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*. [32] 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 Molan *et al*. [18] 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*. [33] who found that 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 [4] 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 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 Muğla exhibited high anticandidal activity on *C. albicans* [10].

A total of ten honey samples collected from different floral areas around Riyadh were investigated by Ayaad *et al*. [1] for their antimicrobial activity against one yeast, *Candida albicans* (ATCC 10231) and four standard bacterial strains; *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *S. aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) using standard antimicrobial assays. The isolated polypeptide and the different honey samples revealed comparable marked variations in antimicrobial activities and their sensitivity might be depending on their floral source.

Halawani and Shohayeb [23] examined nine widely used honeys in Saudi Arabia (Yemeni Sidr, Taify Sidr, Kashmiri Sidr, Shaoka, Somra, Black Seed, Black Forest and Clover honeys) and Manuka honey against 5 pathogenic Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella Enterica* serovar *typhimurium, Shigella flexneri* and *Klebsiella pneumoniae*), 2 pathogenic Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), a food spoilage Gram-positive bacterium (*Bacillus subtilis*) and an acid fast bacterium (*Mycobacterium phlei*). They found that *P. aeruginosa* was the most sensitive Gram-negative bacterium and *St. pyogenes* was the most sensitive Gram-positive bacterium. The MIC of the tested bacteria ranged between 5 and 20% honey (w/v) and the MBC ranged between 5 and 30% (w/v). Shaoka had lowest MICs and MBCs against Gram negative bacteria and Yemeni Sidr had lowest MICs and MBCs against Gram-positive bacteria. Halawani and Shohayeb [25] concluded that several honeys available in the Saudi market especially the locally produced Shaoka and Taify Sidr, in addition to imported Yemeni Sidr, black seed, Clover and Orange blossom are as potent as Manuka honey. Therefore we recommend these honeys for use in the treatment of bacterial infections.

Estrada *et al*. [34] 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 recorded for the microbiological characterization of honey that 91% of samples had counts equal or lower than 1.0 x 10(1) CFU/g. No positive result was obtained for the isolation *of C. botulinum*. 24 of samples analyzed inhibited the growth of *S. aureus* even in a 25% v/v concentration; nevertheless, *A. niger* wasn't inhibited by any of the samples tested.

Iurlina and Fritz [35] 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 for all 70 samples. Fecal coliforms, *Escherichia coli, Salmonella spp., Shigella spp*. and Clostridium sulfite-reducers were not detected but *P. larvae* subspp. larvae, *Bacillus cereus, Bacillus pumilus* and *Bacillus laterosporus* were found among samples.

The variation of the activity of different honeys was attributed to the previously mentioned factors which influence the antibacterial activity [24] as osmotic properties of honey and honey pH [14, 15], or activity of glucose oxidase [36], hydrogen peroxide [3,11], non peroxide substances [37, 38] and presence of propolis which contains flavinoides [37, 19] and volatile antibacterial substances [39].

It can be concluded that all studied honey types showed inhibition of bacterial growth. Also it was obvious that the inhibition of the studied strains was dependent on the type of honey origin. Agaa mount honey gave the highest activity against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria.

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