Evaluation of Antibacterial Activity of Sudanese Bee honey Against Four Species of Bacteria

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Abstract

Background: Different researches on therapeutic effects of honey have been conducted in different regions; however the study on the antibacterial activity of Sudan honey is still limited.

Objectives: this study was aimed to evaluate the antibacterial activity of four concentrations of Sudanese bee honey (100%, 75%, 50%, and 25 %v/v) and gentamicine against four species of bacteria; namely, *Bacillus cereus*, *Pseudomonas aeruginos*a, *Staphylococcus aureus* and *Proteus mirabilis*.

Materials and Methods: This study was carried out under the laboratory conditions at the Department of Microbiology, Faculty of Veterinary Medicine; University of Khartoum, Sudan during 2013, Using sterile cork borer method; four holes were made in each Petri dish. The four holes in each Petri dish of each group were filled with one of honey concentrations or gentamicine (80mg/2ml). All Petri dishes were then incubated at 37˚C for 24 hours. The inhibition zone around each hole was measured by a ruler every 24 hours for three days.

Results: The results showed that all treatments had inhibition zone for each bacterium. The honey, as compared to gentamicine, showed inhibition efficiency for bacterial growth of 79%, 67%, 57% and 43% for 100%, 75%, 50% and 25% honey concentrations respectively. The results indicated that the four species of bacteria responded differently to the honey concentrations: *Bacillus cereus* was the least affected by the honey (23.46mm), followed by *Pseudomonas aeruginos*a (29.28mm), *Proteus mirabilis* (32.41mm), and *Staphylococcus aureus* (33.55mm).

Conclusion: All honey concentrations inhibited the bacterial growth of each species included in the study and some bacteria showed strong resistance to honey than other e.g. *Bacillus cereus* followed by *Pseudomonas areugnosia, Proteus mirabilis* and finally *Staphylococcus aureus but* none of honey concentration was equaled as effective as Gentamicin. Honey concentration at 100% and 75% were recommended for treatment of some pathogenic bacteria: *Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginos*a.

Key Words: *Bacillus cereus; Pseudomonas aeruginos*a; *Staphylococcus aureus; Proteus mirabilis;* Sudan bee honey

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Introduction

Beekeeping is an important component of agriculture, rural employment, human nutrition, and economic development. Honey is the most important primary product of beekeeping both from a quantitative and economic point of view and has been used by mankind for many years as a source of food, medicine and for religious and cultural ceremonies Cart land, (1970), Mcinerney, (1990) and Molan, (1999). Pure honey has shown bactericidal activity for many pathogenic organism including various Gram-negative and Gram positive bacteria (Andulem, 2013). Honey as most natural products may have a large variance in therapeutic components depending on its origin. The antibacterial potency of honey has been attributed to its strong osmotic effect and naturally low pH (Kwakman and Zaat, 2012). Krell, (1996) reported that the normal honey antibacterial activity attributed to the high sugar concentration and acidity (pH range 3.5 to5.0), also diluted honey has shown antibacterial activity, the active ingredient was attributed to an elusive substance generically termed"inhibin" much of this activity was later attributed to hydrogen peroxide (H_2O_2) and the responsible enzyme glucose oxidase which is basically inactive in concentrated normal honey. However the resistant pathogens develop and spread the effectiveness of the antibiotics is diminished. Therefore alternative antimicrobial strategies are urgently

needed (Mandal and Mandal 2011). In the past decades honey has been subjected to various laboratory and clinical investigations. The antimicrobial properties of honey have been attributed to both the hydrogen peroxide as well as non-peroxide components (Raju and Goli, 2013). Non-peroxide factors may also contribute to antimicrobial properties of honey such as lysozyme, phenolic acids and flavonoids (Weston *et al*, 2000).In recent time new interest in honey has been witnessed mainly due to an increase concern on the side effect of chemical medicines (Sommeijer *et al*, 1995).This situation forced the researcher to search for new antimicrobial substance *(Bauer et.al*, 1996).

Materials and Methods

Honey used in this study was obtained from the Kingdom Company of Honey and Bee Product. This honey was diluted in four different concentrations (25%, 50%, 75% and 100%v/v) prepared by thoroughly mixing honey with distill water.

Source of Bacteria

Four species of bacteria were obtained from the National Public Health Laboratory (stack laboratory). Those bacteria include *Bacillus ceruse* ATCC14529, *Pseudomonas aeruginos*a, ATCC27853, *Staphylococcus aureus* ATCC25923 and Proteus *mirabilis* ATCC 25659.The bacteria chosen for this study were both Gram-Positive *Bacillus cereus*, *Staphylococcus aureus* and Gram- Negative Bacteria, *Pseudomonas aeruginos*a, *Proteus mirabilis.*

Antimicrobial Susceptibility

Three to five bacterial colonies of 24-hour-old pure culture were suspended in 10 ml nutrient broth. The turbidity of the suspension was adjusted to achieve 0.5 McFarland (equivalent to that of 1.5 X 108 CFU/ml) with the absorbance range of 0.08 to 0.13 by spectrophotometer at wavelength of 625 nm .Then drops (200 micro litter) from the adapted bacterial suspension were taken by the micro pipette and spread it on surfaces of Muller and Hinton medium and left them to adapt for 30 minutes. Twenty Petri dishes for each species of bacteria were used and divided into five groups. Then four holes were made in each Petri dish using sterile cork borer (7mm).Then the four wells in each Petri of each group were filled with (1/ml) of one honey concentrations or Gentamicin .Then after that all plates were

incubated at 37˚C for 24hrs.The inhibition zone around each well was measured by a ruler every 24hours for three days. Then the means of all inhibition zones of all the five treatments were compared.

Statistical Analysis

The experiment was carried in complete randomized design with four replicates, following the method described by Gomez and Gomez (1984).The collected data were subjected to the analysis of variance & Duncan's multiple range tests was applied for means separation.

Results

The effect of different concentrations of honey and antibiotic on *Proteus mirabilis* after 72hrs: The analysis showed significant differences among all treatments. The mean zones of inhibition 38.25, 32.38, 26.63, 20mm for 100%, 75%, 50%, 25% concentrations of honey respectively. Zone of inhibition induced by gentemicin was 44.81 mm. (Table1 and plate 1)

The effect of different concentrations of honey and antibiotic on *Bacillus ceruse* after 72hrs: There were significant differences among all treatments. The results obtained from the study as indicated in Table1and plate 2 showed that means of inhibition zones induced by honey were 25.38, 19, 16.75, 13.94mm for the concentrations 100%, 75%, 50% and 25% respectively. While the antibiotic (Gentemicin) recorded 42.25 mm

The effect of different concentrations of honey and antibiotic on *Pseudomonas aeruginosa* after 72hrs: This study revealed that the mean of inhibition zone induced by honey 100% was 33.50 mm, 28.63 mm for 75%, 26.13mm for 50% and 25% 18mm. However the antibiotic (Gentemicin) recorded 40.13 mm (Table1plate3). Significant differences were recorded between all treatments.

The effect of different concentrations of honey and antibiotic on *Staphylococcus aureus* after 72hrs: The present study showed that the means of inhibition zones Induced by honey were 37.94 mm, 35.13 mm, 29.13mm and 22.69 mm for 100%, 75%, 50% and 25% of honey concentrations respectively. However the antibiotic (Gentemicin) recorded 42.88mm (Table1and plate 4).Significant differences were obtained between all treatments.

Table (1): shows antibacterial activities of different concentrations of honey against four bacteria species compared with Gentamicin

honey Treatments concentration	*proteus*	*Bacillus*	*pseudomonas*	*Staphylococcus*	Mean of inhibition zone (mm)	Percentage %
100%	38.25 dc	25.35 h	33.50 fe	37.94 d	33.77 B	79%
75%	32.38 f	19.00kg	28.63 g	35.13 e	28.78 C	67%
50%	26.63 h	16.75 L	26.13 h	29.13 g	24.66 D	57%
25%	20.00 j	13.94 m	18.00 Lk	22.69 i	18.66 E	43%
Gentamicin	44.81 a	42.25 b	40.13 c	42.88b a	42.52 A	100%
Mean spp	32.41B	23.46 D	29.28 C	33.55 A		

The Means followed with same letter(s) in the same colum or row are not significanly different at (p=0.05) according to Duncan

Comparison between different species subjected to the different concentrations of honey

Analysis of variance showed that there were highly significant differences among the species, and the highest inhibition zone was recorded in *Staphylococcus aureus* (33.55mm) followed by *Proteus mirabilis* (32.41mm), *Pseudomonas aeruginosa* (29.27mm) and *Bacillus cereus* (23.46 mm) (Table 1and Fig 1)

Comparison between different concentration of honey and gentamicin

Analysis of variance showed that there were significant differences between honey concentrations and antibiotic. The means of inhibition zones were 33.77mm, 28.78mm, 24.66mm and 25% 18.66mm for 100%, 75 %, 50 % ,25% respectively ,while antibiotic recorded 42.52mm/24hrs.On the other hand honey concentrations :100%,75% 50% and 25% recorded an inhibition zone by: 79%, 67%, 57%, and 43% as compared to the efficiency of gentamicin respectively (Table1and Fig 2).

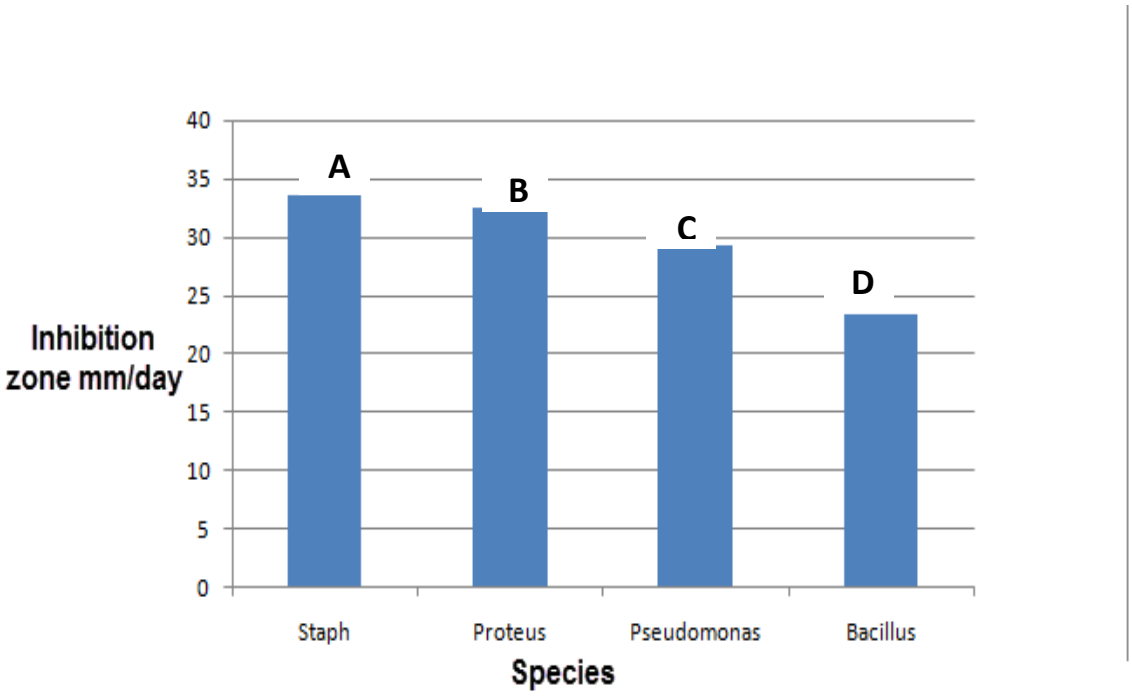

Fig.(1): Comparison between species subjected to different concentrations of honey.

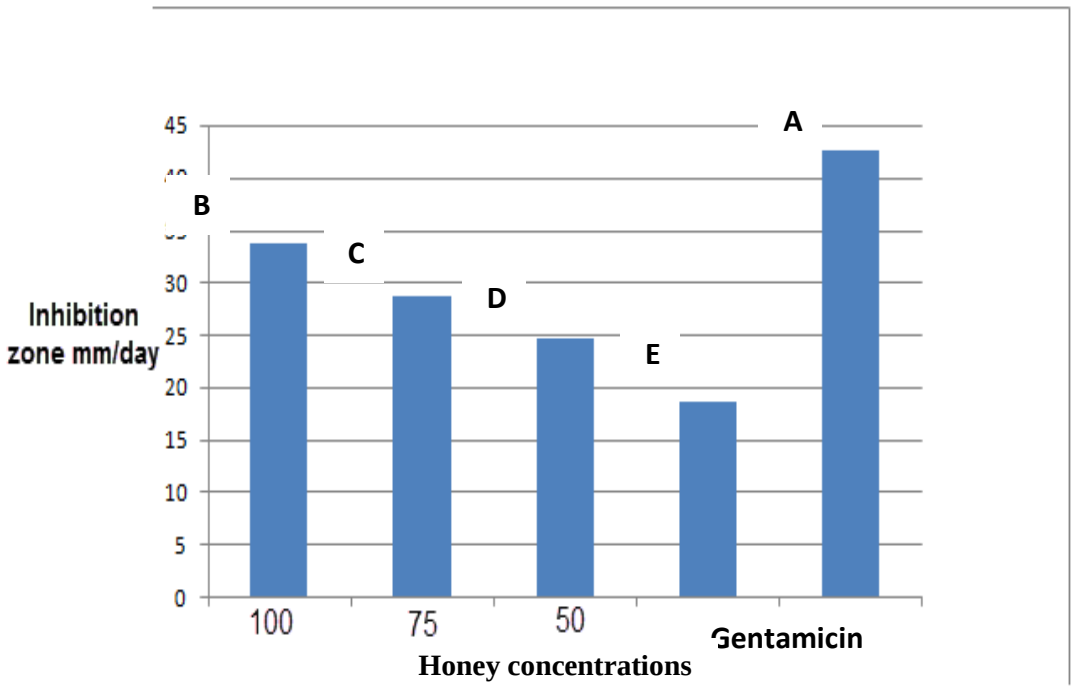

Fig. (2) Comparison between different concentrations of honey and gentamicin.

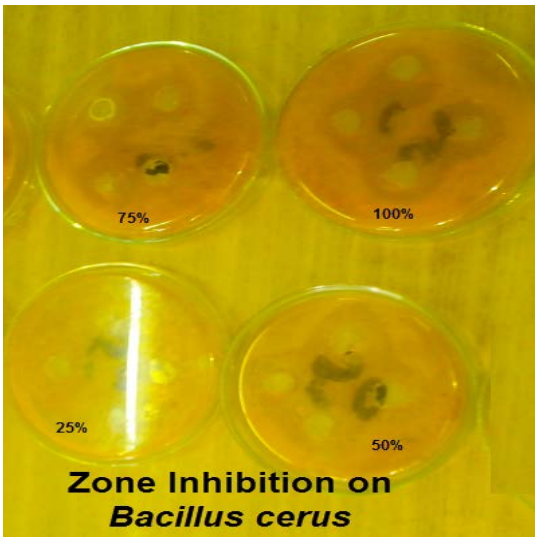

Plate. (2): Sensitivity of *Bacillus ceruse*

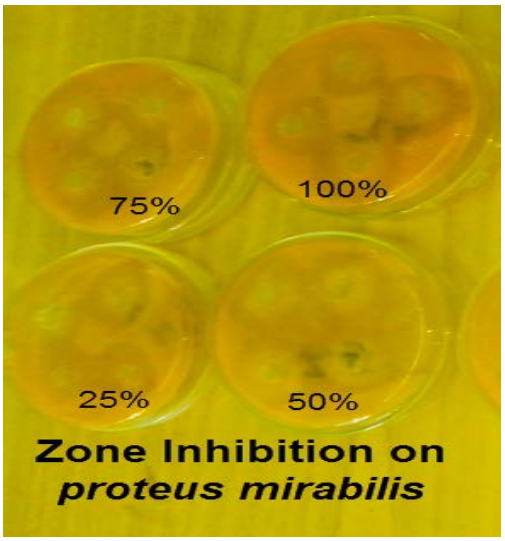

Plate. (1): Sensitivity of *Proteus mirabilis*

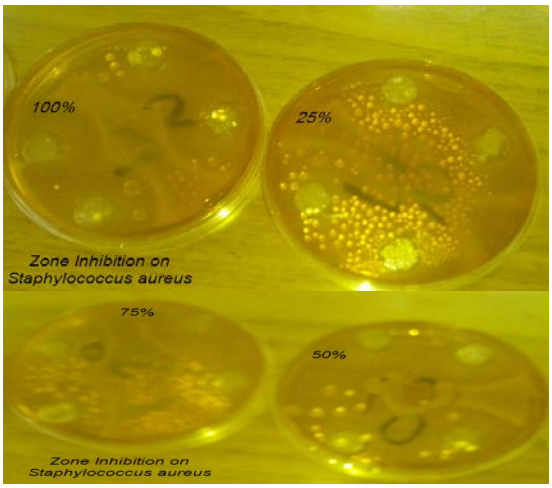

Plate. (4): Sensitivity *Staphylococcus aureus*

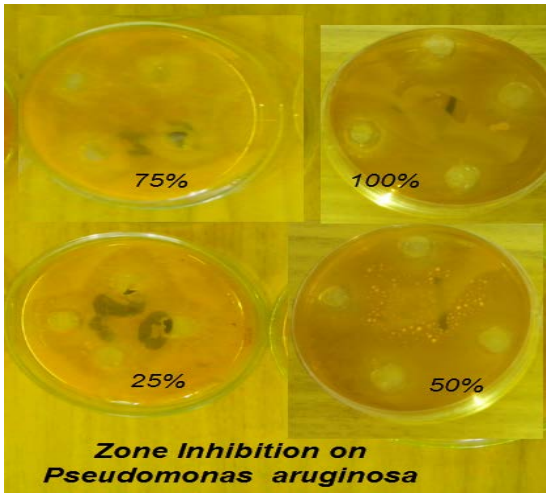

Plate.(3):Sensitivity of *Pseudomonas aruginosa*

Discussion

Natural products have recently been demonstrated as an alternative to synthetic substance. Today the scientists accepted honey as a very effective medicine for all kinds of diseases without any side effects. In the same line this current study was carried out in laboratory to evaluate antibacterial activity of honey against *Bacillus ceruse* , *Pseudomonas aeruginos*a , *staphylococcus aureus* and *Proteus mirabilis* .The results of the current study revealed that fresh honey in 100% concentration has

antibacterial activity and induced inhibition zone against tested pathogenic bacteria *Proteus mirabilis* (*38.25mm*), *Bacillus cereus* (25.38mm) *Pseudomonas aeruginosa* (33.50mm) and *Staphylococcus aureus* (37.94mm). These findings agreed with the results reported by Allen *et al*., (1991), Dimitrova *et al, (*2007) and Oyeleke *et al*, (2010). They showed that undiluted honey was also able to inhibit the growth of *Proteus mirabilis, P. aerussginosa, E.coli, Streptococcus faecalis, Clostridium perfingens* and *S. aureus*. The honey samples used in this study showed antimicrobial activity and this coincide with Wi11ix *et al*, (1992) who found that honey inhibited the growth of *S. aureus, E. coli* and *Pseudomonas* sp. and agreed with Bilal *et al*, (1998) who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and -positive bacteria and a remarkable activity was observed with *P. aeruginosa* and *S. aureus.* This might be due to the osmotic effect, the effect of pH and the sensitivity of these organisms to hydrogen peroxide. The results showed that honey at 75% concentration reduced the growth of these bacteria, *Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus*, but *Bacillua cereus* express slight resistant. This result agreed with Devarajan and Venugopal, (2011) who reported that honey at this concentration inhibited growth of *Staphylococcus aureus***.** On the other hand this result coincided with Iurlina and Fritz, (2005) who found that honey diluted from 75% to 1% concentrations had full-strength antibacterial activity. At the 50% concentration the results showed that honey inhibited the growth of *Proteus mirabilis* followed by *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* This results agreed with Nagaraj *et al*,(2012)**,** Basualdo *et al*, (2007) and Jeddar *et al*, (1985).They reported that honey is inhibitory to the growth of microorganisms at 50% dilution. On the other hand this finding were revealed that honey had antibacterial activity at 25% against tested bacteria, *Proteus mirabilis* followed by *Bacillua cereus*, *Pseudomonas aeruginosa* ,*Staphylococcus aureus*. These results were similar to Nagaraj *et al***, (**2012) who reported that diluted honey (25%) had an antibacterial effect on the tested bacteria.(*Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp*) and Hegazi and Fyrouz,(2012) said that honey concentration 20 to 30 % showed inhibition of bacterial (*Staphylococcus aureus*, *Streptococcus pyogenes, Corynebacterium pseudotuberculosis, Klebsiella pneumoniae, Pseudomonas aeruginosa Escherichia coli*) and that depends on the type of honey and origin. However this result disagreed with the findings reported by Tonk*s et al*, (2001) and Namias,(2003) who reported that if honey is diluted especially by body fluids which were well buffered the pH will be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria. This difference in the level of sensitivity may be due to the dilution water used in this experiment (distilled water) or may be due to the variation in

the antibacterial potential of honey used in the present study and the source of honey samples. The difference in sensitivity can also be due to the different of growth rate of microorganism's nutritional requirements inoculums size temperature and the test methods. This result also disagreed with Al-Naama**, (**2009) who said that there is no effect was observed at 25% concentration against *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa***.** Also disagreed with Ansari and Alexander, (2009) who reported that the antimicrobial activity of 25% solution of honey had no effect on the various bacteria tested**.** The antibacterial effect of honey at 25% may be attributable to the presence of glucose oxidase which is activated by dilution in water resulting in the production of hydrogen peroxide which is toxic to bacteria**.** At 25 %, 50%, 75%, 100% percentage of honey concentrations were 79%, 67%, 57%, 43% respectively they indicated that near activity to the gentamicin percentage 100%.

Based on this research, the findings showed that honey in its most concentrated forms, were very effective against pathogenic organisms. But the poisoning food bacteria such as *Bacillus ceruse* was resistance to honey in difference concentration this maybe due to the spores forming bacilli and also *Pseudomonas* showed slight resistance.

Conclusion

All honey concentrations inhibited the bacterial growth of each species included and some bacteria showed strong resistance to honey than other e.g. *Bacillus cereus* followed by *Pseudomonas areugnosia, Proteus mirabilis* and finally *Staphylococcus aureus but* none of honey concentration was equaled as effective as Gentamicin. Honey concentration at 100% and 75% were recommended for treatment of some pathogenic bacteria: *Staphylococcus aureus, Proteus mirabilis* and *Pseudomona*s *aeruginos*a.

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