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Research Article

NOT ALL HONEY SAMPLES CONTAINS SIGNIFICANT LEVELS OF ANTIBACTERIAL ACTIVITY

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ABSTRACT
The bee's honey is well documented in literature as a healing panacea against numerous ailments, including antimicrobial potential. However, honey as a raw natural product could lose part of its quality due to many direct or indirect effects. In this report, a raw pure honey (authenticated sample), claimed as meadow honey, collected from the central eastern part of Sudan, did not show any significant antibacterial activity against the tested bacteria (referenced and clinical bacterial isolates) at $p<0.05$. The handling processes, botanical origin, transportation or storage conditions could be among the reasons explaining absence of antibacterial effects of this raw honey sample. The results suggested that, honey, which is sold in the markets as a food supplement or as a medicine must be subjected to physicochemical and biological testing and it should be included under the supervision of the official local health organizations.

Keywords: *Bee Honey, Antibacterial Activity, Disc-Diffusion Test, Negative Results*

INTRODUCTION
Since ancient times, the bee's honey used to be considered as a best remedy for almost all diseases. It used to be prescribed by physicians from many ancient civilizations like ancient Egyptians, Assyrians, Greeks, Chinese and Arabs; they used honey against many ailments such as wound healing, burns, anti-diarrhea, anti-ulcers, anti-inflammatory and for eye diseases (Molan, 1999). Although, modern medicine has neglected honey as an effective drug and allow use it only as food supplement, an increasing scientific studies and publications confirmed that it has a significant curative properties against many diseases and disorders (Jeffrey and Echazaarreta, 1996). Recent studies reported that honey has immunomodulatory activities, antioxidant, estrogenic, anti-proliferative, anti-tumor and anti-cancer activities (Ahmed and Othman, 2013). The honey is the plant's nectar that is collected and processed by honey bees (*Apis mellifera*), it has wide a variability in its compositions and features, which is affected by geographical, botanical, seasonal and environmental factors as well as the handling and treatment of beekeepers (El Sohaimy *et al.*, 2015). Honey is a complicated natural product, it contains about 200 compounds, sugar represents between 95-99% of the honey dry content, most of them are fructose (32.56% to 38.20%), glucose (28.54 to 31.30%) and small percentages of sucrose, maltose, isomaltose turanose, maltotriose, meli-biose, melezitose, panose and nigerose (Eteraf-Oskouei and Najafi, 2013). It is also contains vitamins (vitamin C and E), amino acids, minerals and phenolic compounds (Tasleem and Naqvi, 2014). Therefore, the bee honey enjoys great popularity as a remedy. Unfortunately, because of the absence of market supervision in developing countries in particular, getting original bee's honey is a great challenge. This investigation is part of a study aimed to evaluate the antibacterial activity of different Sudanese honey samples, in this report the author presents his laboratory observations on a raw (original) honey sample lacking any antibacterial properties.

MATERIALS AND METHODS
Sample Collection
Natural honey sample was purchased from trusted herbal store in Khartoum, Sudan. The product has an official certificate of purity from Industrial Research and Consultancy Center, Khartoum, Sudan (Certificate Number 2016/C217/352), this raw honey is claimed that it is a meadow honey and collected

from Gedaref Region, which lies in the central eastern part of Sudan, the so-called Mechanized Farming Schemes. Another commercial bee's honey sample was randomly purchased from a regular market in Qassim Region, Saudi Arabia for comparison purpose.

Purity Test

The two samples (raw and processed honey) were subjected again to the purity test to investigate presence or absence of synthetic inverted sugar as described by Tasleem and Naqvi, (2014) with minor modifications in the quantities used. Briefly, 20 g of honey sample was dissolved in 100 ml distilled water and left for up to 10 minutes with frequent gentle shaking. 10 ml of the honey solution was mixed with 5 ml of ether and left for up to 5 minutes, shacked well and left until the ether layer separated out. This layer was pipette and loaded in a clean Petri-dish and left to evaporate. The resorcinol solution was dropped to the dry residues on the plate and stirred. The appearance of reddish to reddish brown or pinkish color indicates the presence of inverted sugars.

Preparation of Honey Samples

Serial two folds dilution from the honey samples were prepared to make three concentrations; 100%, 50% and 25%, diluted with distilled water with shaking for up to 10 minutes until became homogenous. Sterile disc papers (6 mm) were prepared from Whatman No.1, and dipped in the different concentrations, which are used in the antibacterial testing.

Bacterial Strains

Five bacterial clinical isolates representing gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Ec= *Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*), were collected from the hospital, these bacterial strains were identified using microbiological methods by Dr. Mohamed Elgadi in Al-Rass General Hospital, Pathology and Laboratory medicine, Saudi Arabia. In addition to one referenced gram-positive strain (*Bacillus cereus* ATCC 10876) available in our laboratory, Qassim University, Saudi Arabia. These six bacterial strains were sub-cultured again and fresh bacterial solution adjusted to 0.5 McFarland's standard, using sterile normal saline.

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was performed by Kirby–Bauer disk diffusion method as mentioned in (Abdallah *et al.*, 2015). Briefly, standard antibiotic discs (MASTRING-S TM, UK) were aseptically loaded in to Mueller Hinton agar plates seeded with the tested bacteria. Then, plates were incubated for 18h at 37 ^{o} C. The diameter of inhibition zones around the antibiotic discs were measured and interpreted as sensitive/resistant according to CLSI guidelines.

Antibacterial Testing

The antibacterial activity of the honey samples were evaluated using the modified Kirby-Bauer disc diffusion method as cited in Abdallah, (2016), 20 ml of hot autoclaved Mueller Hinton agar (Watin-Biolife, KSA), was poured onto sterile plates and left at room temperature until solidified. 100 µl of the bacterial suspensions (previously prepared), were gently spread on the surface of the Mueller Hinton agar plates, using sterile cotton swabs. Sterile blank discs of 6 mm saturated with 100%, 50% and 25% of the honey (previously prepared) were loaded on the inoculated plates. Gentamicin disc (10 µg/disc) (Oxoid, UK), was used as positive antibiotic control, Plates were incubated at 37°C for 24 hrs. Mean zone of growth inhibition in millimeter (mm) was calculated from three replicates.

RESULTS AND DISCUSSION

The antibiotic susceptibility test for the pathogenic bacteria collected from the hospital are shown in Table 1, which revealed that all these bacterial strains are multi-drug resistant (MDR), except *Escherichia coli* strain which was sensitive to all tested antibiotics. In the last two decades, there was a dramatic increase in MDR infectious diseases, which returned many parts of the world to the pre-antibiotic era (Basak *et al.*, 2016). This fact justifies the growing interest in searching for new antibacterial alternatives as in the case of honey. As shown in Table 2 and Figure 1, the mean zone of inhibition at 100% concentration of *Bacillus cereus* ATCC 10876 was 7.6±0.5 mm for raw honey and 7.0±1.0 mm for processed honey, *Proteus vulgaris* was 7.5±0.8 mm for raw honey and 7.0±1.0 mm for processed honey,

the results of the other bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli*) were ranging from 6.6±0.2 mm to 6.0±0.0 for raw and processed honey. However, the inhibition zones did not show significant results ($P<0.05$). In general, since the blank disc diameter is 6 mm, the inhibition zones less than 10 mm indicates that this product has no potent antibacterial activity. Moreover, this small zone of inhibition could be attributed to the high content of sugar in the sample and not to the antibacterial compounds in honey (Figure 1).

The similarity in results between the raw honey and the processed honey support this claim. Numerous studies reported that the raw honey from different sources has significant antibacterial activities, Tasleem and Naquiv (2014) published that the honey samples from ten different floral sources exhibited good antibacterial activity against 17 different gram-negative and gram positive bacteria and these activities were comparable to antibiotics.

The manuka bees honey showed inhibitory effects against coagulase-positive *Staphylococcus aureus* isolated from wounds (Cooper *et al.,* 1999). It was also found that, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* except *Lactobacillus acidophilus* are susceptible to different honey samples collected from India and the 100% concentration of all the honey samples were most effective against the tested pathogens (Gomashe *et al.,* 2014). Another study stated that honey has excellent antibacterial activity against the tested clinical bacterial Isolates which were *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi (Mandal *et al.,* 2010). Negative results regarding the antibacterial potential of honey are scantly. However, Tumin *et al.,* (2005) claimed that 2 of 5 different honey samples from different location in Malaysia did not have any antibacterial effect against tested bacteria, but the remaining 3 samples showed good antibacterial activity. The results of Tumin *et al.,* (2005) supports the findings of this study.

In searching for an explanation about the vast variation in the antibacterial activity of honey, Molan (1992) summarize the reasons in 4 main reasons which are; osmotic effect, acidity, presence of hydrogen peroxide and plant-derived antibiotic substances.

However, the exact reasons behind absence of antibacterial effects in the studied honey sample are still unknown, but it is believed that it could be either related to the handling processes or this honey sample which sold as an original wild honey is from honey farms where nourishing bees with some food additive such as sugar is a major behavior of farmers. And since the curative properties of honey comes from substances in the collected plant's nectar, this sample succeeded in the purity test but failed some bioactivity tests like the antibacterial assessment. It is strongly recommended to find out all information regarding the origin, source and bioactivity of the honey samples as well as physical and chemical examinations before use honey as remedy.

Table 1: Antibiotics Sensitivity Profile of the Clinical Pathogenic Strains and the Referenced Bacterial Strain

Bacterial Strain	Origin	Source	AK	GM	CPM	TC	PRL	IMI
Proteus vulgaris	Clinical	Pus	R	R	R	R	R	S
Pseudomonas aeruginosa	Clinical	Sputum	S	S	R	R	R	W
Klebsiella pneumonia	Clinical	Sputum	S	W	R	W	S	S
Escherichia coli	Clinical	Urine	S	S	S	S	S	S
Staphylococcus aureus	Clinical	Pus	R	R	R	S	S	S
Bacillus cereus	ATCC 10876	-	S	S	R	R	S	S

AK: Amikacin 30 µg, GM: Gentamicin 10 µg, CPM: Cefepime 30 µg, TC: Ticarcillin 75 µg, PRL: Piperacillin 100 µg, IMI: Imipenem 10 µg, R: Resistant, S: Sensitive, W: Weak sensitivity or hazy zone. (bacterial inhibition zone ≤ 10 mm considered sensitive).

Table 2: Antibacterial Effect of Honey Samples against Different Pathogens

Tested	Concentration	Mean Zone of Growth Inhibition (mm)*					
		Gram-Positive		Gram-Negative			
		Sa	Bc	Pv	Pa	Kp	Ec
Raw honey	100%	6.6±0.2	7.6±0.5	7.5±0.8	6.5±0.0	6.5±0.0	6.6±0.2
(meadow honey)	50%	6.0±0.0	7.0±0.8	6.6±0.2	6.5±0.5	6.1±0.2	6.1±0.1
	25%	6.0±0.0	6.5±0.5	6.1±0.1	6.1±0.2	6.1±0.2	6.0±0.0
Processed honey	100%	6.0±0.0	7.0±1.0	7.0±1.0	6.0±0.0	6.0±0.0	6.1±0.2
(Farms honey)	50%	6.0±0.0	6.0±0.0	6.1±0.2	6.0±0.0	6.0±0.0	6.1±0.2
	25%	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Gentamicin	10 µg/disc	6.6±0.2	22.0±1.7	18.5±0.8	16.5±0.5	6.5±0.0	21.0±1.0

*Disc diameter=6.0 mm, 6 mm =no inhibition. zone of inhibition is the mean of three replicates ±standard deviation. Sa= *Staphylococcus Aureus*, Bc= *Bacillus Cereus* ATCC 10876, Pv= *Proteus Vulgaris*, Pa= *Pseudomonas Aeruginosa*, Kp= *Klebsiella Pneumoniae*, Ec= *Escherichia Coli*.

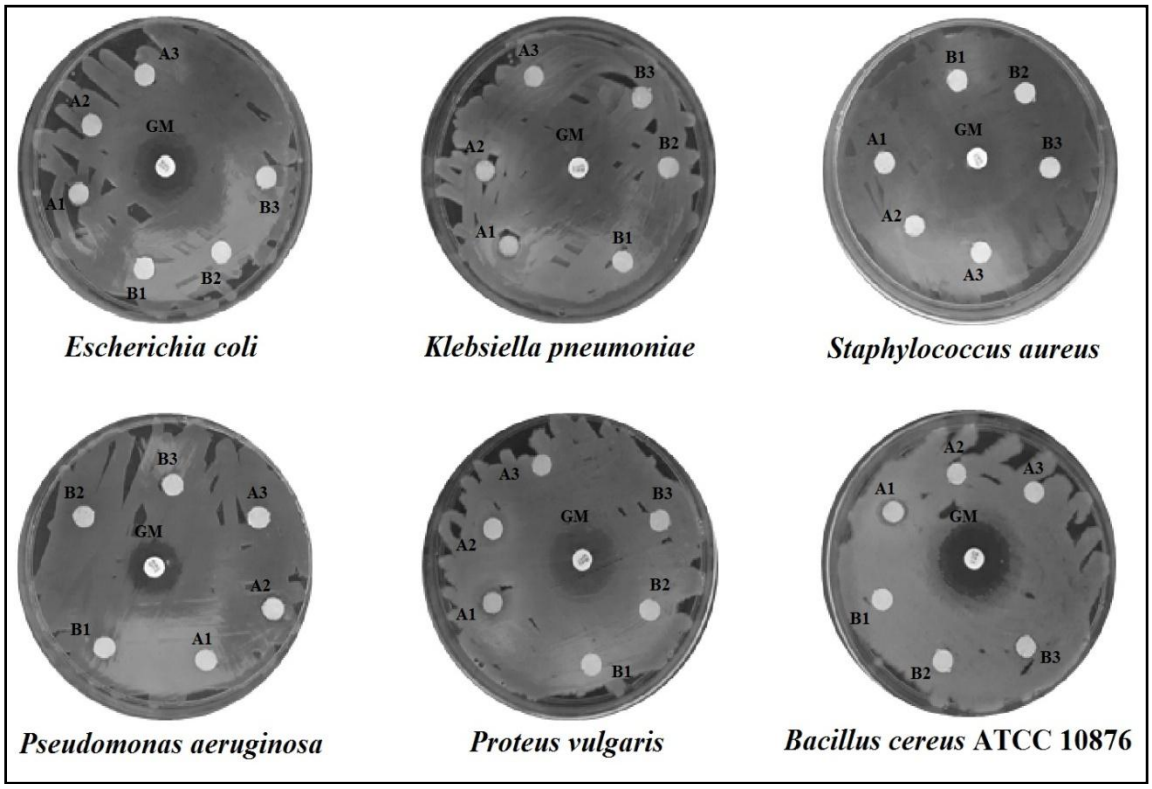

Figure 1: Susceptibility of Bacteria towards the Antibiotic (GM) Compared to the Raw Honey (A1, A2, A3) and the Processed Honey (B1, B2, B3)*

*A1, A2 and A3: The raw (meadow) honey at the concentrations 100, 50 and 25%, respectively. B1, B2 and B3: The processed (commercial) honey at the concentrations 100, 50 and 25%, respectively. GM: Gentamicin 10 µg/disc.

Conclusion

In view of results obtained, It can be concluded that the, honey as other natural products depends greatly on the botanical source as well as other indirect factors such as environmental conditions, storage, sugar-fed bees and handling. Besides, the immense diversity honey sources make it difficult to determine the medical benefits of each type accurately. Accordingly, not all honey samples contains significant levels of antibacterial activity. Although, the honey sample tested in this investigation could have another medical

benefits. Though, more biomedical and biochemical studies are required to understand the curative properties of the bees honey.

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REFERENCES

Abdallah EM (2016). Antibacterial Efficacy of *Acacia nilotica* (L.) Pods Growing in Sudan against Some Bacterial Pathogens. *International Journal of Current Research in Biosciences and Plant Biology* **3**(3) 6-11.

Abdallah EM, Ahamed F and Al-Omari AS (2015). Antibiotic susceptibility patterns of some clinical isolates from Al-Rass general hospital. *International Journal of Biosciences* **6**(9) 47-54.

Ahmed S and Othman NH (2013). Honey as a Potential Natural Anticancer Agent: A Review of Its Mechanisms. *Evidence-Based Complementary and Alternative Medicine* **2013** Article ID 829070, http://dx.doi.org/10.1155/2013/829070.

Basak S, Singh P and Rajurkar M (2016). Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. *Journal of Pathogens* **2016** Article ID 4065603, doi:10.1155/2016/4065603

Cooper RA, Molan PC and Harding KG (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine* **92**(6) 283–285.

El Sohaimy SA, Masry SHD and Shehata MG (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Science* **60**(2) 279–287.

Eteraf-Oskouei T and Najafi M (2013). Traditional and Modern Uses of Natural Honey in Human Diseases: A Review. *Iranian Journal of Basic Medical Sciences* **16**(6) 731–742.

Gomashe AV, Narad MV and Culhane PA (2014). In Vitro Assessment of the Antimicrobial Potential of Honey against Enteric Pathogens. *International Research Journal of Science and Engineering* **2**(4) 153-157.

Jeffrey AE and Echazarreta (1996). Medical uses of honey. *Revista Biomédica* **7**(1) 43-49.

Mandal S, DebMandal M, Pal NK and Saha K (2010). Antibacterial activity of honey against clinical isolates of Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica serovar Typhi. *Asian Pacific Journal of Tropical Medicine* **3**(12) 961-964.

Molan P (1999). Why honey is effective as a medicine. 1 Its use in modern medicine. *Bee World* **80**(2) 80-92.

Molan PC (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World* **73**(1) 5-28.

Tasleem S and Naqvi SBS (2014). Comparison of in vitro antimicrobial activity of different crude Pakistani honey samples and commercial antibiotics against clinical pathogens. *World Journal of Pharmaceutical Research* **3**(5) 88-104.

Tumin N, Halim NAA, Halim NAA, Shahjahan M, Noor INJ, Sattar MA, Khan A and Mohsin SSJ (2005). Antibacterial activity of local Malaysian honey. *Malaysian Journal of Pharmaceutical Sciences* **3**(2) 1-10.