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COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF SUDANESE HONEY SAMPLES COLLECTED FROM DIFFERENT REGIONS

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ABSTRACT

Objective: To assess the in vitro Antimicrobial activity of different samples of Sudanese natural honey collected from various regions against pathogenic microorganisms. **Methods:** Different samples of Sudanese honey were collected and studied in vitro using standard microorganisms. The antimicrobial activity of the samples was tested and compared against each type of the selected microorganisms. The antibacterial activity of these honey samples was determined by the cup plate method. **Results:** The mean MIC of different samples of honey for control and test microorganism was 6.7- 8.3 and 12.3- 18.6% (v/v) respectively, the highest diameter of inhibition zone 40 mm (*E.coli*) was from Honey H2(100mg/ml) and the lowest, 14 mm (*Bacillus Subtilis*) from Honey H2, while the diameter of inhibition zone for *C.albicans* 21 mm was from Honey H1(100mg/ml) . Honey collected from *Blue Nile State (Ad-Damazin).*has shown better antimicrobial activity than other sites. Over all the MIC of all microorganisms was between 6.25-50 and 12.5-100% (v/v) respectively. **Conclusions:** From the results obtained it can be concluded that all the samples tested possess antimicrobial activity, all collected honey samples showed varied antimicrobial activities, and none of the controlled microorganism were resistant to tested honeys, and can be developed into effective antimicrobial agents.

KEYWORD: Antimicrobial activity, inhibition zone, Sudanese natural honey.

INTRODUCTION

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections. It is also recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds.[1] This leads to the search for different types of honey with antibacterial activity.[2] The healing effect of honey could be due to various physical and chemical properties.[3] The floral source of honey plays an important role on its biological properties.[4] Honey is being used in a few hospitals, especially in the clinical treatment of ulcers, bedsores, burns, injuries and surgical wounds. The antibacterial properties of honey may be particularly useful against bacteria which have developed resistance to many antibiotics, e.g.*Staphylococcus aureus*, which is a major cause of wound sepsis in hospitals.[5] Honey is thus an ideal topical wound dressing agent in surgical infections, burns and wound infections.[6] The use of honey as a medicine has continued into the present-day medicine. It has been shown that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria.[7,8,9] The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low PH[10], the ability to produce hydrogen peroxide which plays a key role in the antimicrobial activity of honey and phytochemical factors.[11,12] Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of microorganisms, including multi-antibiotic resistant strains. Other studies demonstrated the antibacterial activity of honey against: *Escherichia coli*, *Campylobacter jejuni*, *Salmonella entercolitis*, *Shigelladysenteriae*[13,14], *Mycobacterium* [15], Methicillin-resistant *Staphylococcus aureus* and Vancomymin-resistant *Enterococci*[16,17],Common gastrointestinal pathogenic bacteria[18]and the development of *streptococcus pyogenes* biofilms.[19] The antifungal activity of the honey, especially anti-Candida activity[20,21,22]has also been reported.

The present study aimed to evaluate the antimicrobial activity of some honey samples collected from various regions of Sudan. The honey samples are classified as winter honey (collected in the months of January-February).

MATERIAL AND METHODS

Materials

Honey samples and the standard: Four natural honey samples(H1, H2, H3, H4) were collected between the months of January and February. The samples were collected from

different regions in Sudan; and stored in dark place at a room temperature(25-35^{o}C) until used. The antibiotic(Gentamycin) was used as standard.

Table No. (1): Common details about Sudanese Honey samples.

Samples	Area of Collection (State)	Time of Collection
H 1	*South of kordofan(kaduqli)*	January
H 2	*Blue Nile State (Ald-Damazin).*	January
H 3	*South of Dar fur(Nyala)*	February
H 4	*West of Dar fur(Geneina)*	January

Collected by: Experts in the field of honey.

Picture No (1): The areas from which the study samples were collected.

Microbial Strains

Strains of micro-organism namely: *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis*, *Staphylococcus aureus* and *C. albicans*, were used for both sensitivity tests and determination of minimum inhibitory concentration (MIC).

Source and dilution of standard drug

A concentration of 80mg/2ml gentamycin was obtained for use. Gentamycin 400 mg was emptied into 10 ml sterile distilled water to make up the stock solution and employed for standard organisms.

Preparation of media

To evaluate the antimicrobial efficacy of honey using Mullar Hinton Agar (Hi Media Laboratories, Mumbai, India), different types of honey were added to molten warm agar to achieve the resulting Petri dishes which were used for tested microorganism.

Determination of Antimicrobial activity: The antimicrobial activity was determined by an agar well diffusion method, Gentamycin being used as standard antibiotic. Activity was determined with and without addition of catalase to remove any hydrogen peroxide generated by the honey, so allowing the detection of non-peroxide antibacterial activity (i.e. that due to phytochemical components) as well as total antibacterial activity.

RESULTS

Table No. (2): Antibacterial activities of different types of honey compared with gentamycin.

Organism	Honey 1(100mg/ml)	Honey 2(100mg/ml)	Honey 3(100mg/ml)	Honey 4(100mg/ml)	St.(Gentamycin)
S.aureus	23 mm	20 mm	22 mm	21 mm	20 mm
B.subtilis	19 mm	14 mm	0	16 mm	18 mm
P.aeruginosa	30 mm	28 mm	33 mm	29 mm	21 mm
E.coli	34 mm	40 mm	35 mm	34 mm	20 mm
C.albicans	21 mm	16 mm	20 mm	10 mm	0

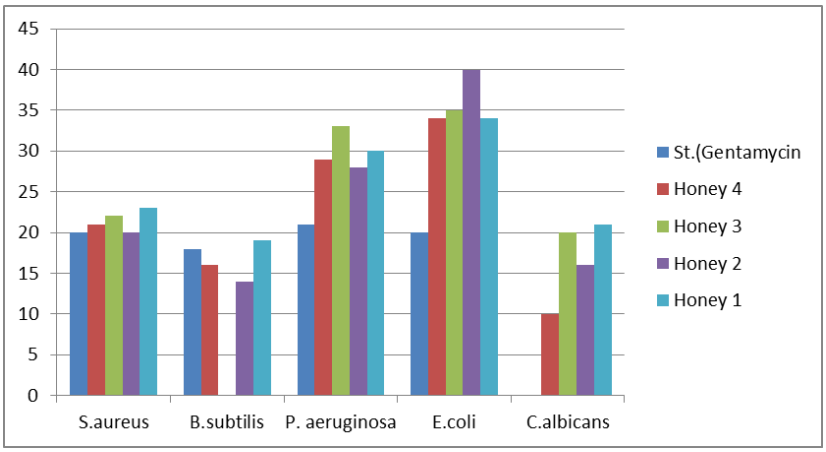

Chart No. (1): Antimicrobial activities of different concentrations of honey compared with gentamycin.

4. DISCUSSION

The honey samples collected from different origins were evaluated for their antibacterial activity against the Gram positive species such as *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative species as *E.coli* and *Pseudomonas aeruginosa*, and fungi (*C.albicans*). Among the four honey samples studied H2and H3 samples showed maximum antibcateial activity especially against *E.coli*. The average diameter of the inhibition zones produced by these samples was 37.5mm. The growth of bacteria was also inhibited by these honey samples, although to a lesser extent. Among the honey samples normally the honeyH2 shows

the maximum inhibition zone 40 mm, whereas the honey sample H4 shows minimum inhibition zone 10 mm. Among the bacterial strains tested *E.coli was* the most sensitive against all honey samples, whereas the *Bacillus subtilis* shows the less sensitive against all honey samples. Antibiotic resistance of bacteria is on increase, which makes the Search for alternative therapeutic agents is urgently needed. Honey possesses therapeutic potential, including wound healing properties and antimicrobial activity. The antimicrobial properties of honey can be attributed to several factors; the following mechanisms have been suggested to explain the antimicrobial actions of honey:1. Presence of an 'inhibine', factor in honey, which is hydrogen peroxide.[23,24] Hydrogen peroxide is a well-known antimicrobial agent and its harmful effects when added in isolation is not noticeable with honey since the latter sequesters and inactivates the free iron which catalase formation of oxygen free radicals produced by hydrogen peroxide. Its antioxidant components help to mop up free radicals.[25]

2. Osmotic property: Honey being a super- saturated sugar exerts an osmotic pressure which makes little or no water available for the micro-organisms to survive.[26]

3. Stimulation of lymphocytic and phagocytic activity.[27,28] Recent studies showed that the proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentration as low as 0.1% and phagocytes are also activated by honey at such low concentrations. Furthermore, honey stimulates monocytes in cell culture to release cytokines, tumor necrosis factor (TNF) – alpha, interleukins (IL) -1 and (IL) –6, which stimulate the immune response to infection. In addition, the glucose content of honey and the acidic pH (typically between 3 and 4) may assist in the bacterial destroying action of macrophages.

It could be said from the fore going that honey, when used in vivo might produce a greater effect than the in-vitro study, the antimicrobial profile might compare favorably with the present observation. However, the present study has clearly demonstrated that honey might adequately offer a total solution to the current problems facing bacterial chemotherapy.

5. CONCLUSION

The present study reveals that among the Sudanese honey samples against pathogenic micro-organism, the honey sample H2was more effective in inhibiting the pathogenic organism than other honey samples. All the honey samples were more effective against gram negative bacteria: *P.aeruginosa* and *E.coli*.

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