

Assessment of the *in vitro* antibacterial activity of honey on some common human pathogens

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ABSTRACT:

The aim of the present study was to assess the *in vitro* antibacterial activity of bee honey on certain potentially pathogenic bacterial isolates. Different concentrations (10.0, 20.0, 40.0, 60.0, 80.0 and 100.0%) of honey sample were checked for their antibacterial activities, using disc diffusion assay, on some medically important bacteria including *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Pseudomonas aeruginosa*, *Enterobacter* sp., *Staphylococcus aureus*, and *Proteus* sp. The minimum inhibitory concentrations (MIC) of the honey sample were determined on the selected bacteria using agar dilution technique. The sample of honey showed pronounced bacterial inhibitory effect *in vitro* at honey concentrations of 40% and above on the various tested bacteria. No growth inhibition effect was observed at 10% concentration of honey. The MIC for the tested bacteria ranged between 12.5 and 25.0%. The MIC for *Klebsiella* sp. and *S. aureus* was 12.5% while for *Proteus* sp., the MIC was 25.0%. All the other tested bacterial isolates showed MIC value of 15%. The study shows that honey, like antibiotics, has certain organisms sensitive to it, and provides alternative therapy against certain bacteria and is also shown to have antibacterial action against a broad spectrum of bacteria, both gram- positive and gram-negative bacteria.

Keywords:

Honey, antibacteria, antibiotics, minimum inhibitory concentration.

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INTRODUCTION

Honey is a thick sweet liquid made by bees from the nectar of flowers. Honey is essentially a highly concentrated water solution of two sugars, dextrose and levulose, with small amounts of at least 22 other more complex sugars. Many other substances also occur in honey, but the sugars are by far the major components. The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents – such as flavouring materials, pigments, acids, and minerals – are largely responsible for the differences among individual honey types (Molan, 1992a).

Honey produced by honeybees (*Apis mellifera*) is one of the oldest traditional medicines considered to be important in the treatment of various diseases and ailments including gastrointestinal infection, respiratory ailment, wound infections and others. Two millennia before bacteria were identified as the cause of disease, physicians at the time were aware that certain types of honey are the best therapy for particular ailments and infections.

The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature, which include its content of tetracycline derivatives, peroxides, amylose, fatty acids, phenols, ascorbic acid, terpenes, benzyl alcohol and benzoic acid (Bogdanov, 1989; Molan, 1992a). However, large variations in the *in vitro* antibacterial activity of various types of honey have been reported and thus hampered its acceptance in modern medicine (Kwakmann, 2008). The production and type of honey produced by honeybees is dependent on the natural vegetative flowers blooming in different seasons. Thus the flowers from which bees gather nectar to produce honey may contribute to the difference in the antimicrobial activities. Molan (1992a) had reported that honey from certain species of *Leptospermum* flora native to Australia and New Zealand contains additional phytochemical components that further enhance its antibacterial activity. It has further been reported that physical property along with geographical distribution and different floral sources may play an important role in the antimicrobial activity of honey (Taormina *et al.*, 2001).

Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antimicrobial agent. Honey has been reported to have inhibitory effect on several bacteria including aerobes and anaerobes, Gram-positive and Gram-

negative and is effective against methicillin resistant *Staphylococcus aureus* (MRSA), β -hemolytic streptococci and vancomycin-resistant enterococci (VRE) as reported by Allen *et al.* (2000) and Kingsley (2001).

Clinical studies in several countries showed that there are differences in the spectrum of antimicrobial action of honey (Efem, 1988; Cooper and Molan, 1999; Obi *et al.*, 1994). It would be important to note the sensitivity in different places, and for differing samples of honey. This should serve as a guide to its clinical use.

The present study aims to evaluate the *in vitro* antimicrobial effect of bee honey collected in Nsukka, Nigeria on certain bacterial species involved in causing infections in humans compared with that of certain antibiotics that are commonly used in the treatment of infections.

MATERIALS AND METHODS

Collection of samples of honey

The honey sample used in this study was collected from Nsukka town in Southeast, Nigeria. Several samples were collected in sterile screw-cap containers and immediately transported to the laboratory for processing.

Processing of samples

Each sample was first filtered with a sterile mesh to remove debris. The samples were checked for purity by streaking on blood agar plates, and incubated overnight. Sample that showed uncontamination was stored at refrigeration temperature of about 4°C until used. The honey sample was diluted with sterile distilled water to 10, 20, 40, 60 and 80%, and the undiluted honey (100.0%) referred to as net.

Bacteria used

The bacterial species used in this study and known to be potentially pathogenic to human were obtained from the clinical laboratory of the Department of Microbiology, University of Nigeria, Nsukka, Nigeria. The bacterial species include *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* sp., *Enterobacter* sp. and *Proteus* sp. Two reference strains, *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used for comparison.

Antibacterial evaluation

The *in vitro* antibacterial evaluation of the honey was carried out using the disc diffusion method of Bauer *et al.* (1966). In the disc diffusion approach, surface of nutrient agar (Lab M) plate was uniformly inoculated in individual Petri dishes



with overnight stock culture of each of the bacterial species prepared in nutrient broth. The used bacteria were adjusted to 10^5 cfu/ml with sterile saline and inoculated onto the agar medium. Sterile Whatman No 1 filter paper discs (Whatman International Ltd., UK), 6 mm in diameter were used. The filter paper discs were soaked in the different honey dilutions or net honey, dried at room temperature, carefully and aseptically placed into the Petri dishes seeded with inocula. Standard antibiotic discs of chloramphenicol and tetracycline were used on the inoculated plates for comparison. The antibiotic discs used were commercially available discs. A disc without honey impregnation was used as control. Plates were kept at 4°C for four hours to provide sufficient time for the test material to diffuse into the medium and finally incubated at 37°C for 24 hours.

The diameter of the zone of inhibition produced around the discs was measured with transparent ruler as index of antibacterial activity of honey or standard antibiotic. The size of the inhibition zone further represented a quantitative measure of antibacterial activity of the test material. All experiments were performed in duplicate and the zone of inhibition was measured twice for each honey dilution and net preparation.

Determination of minimum inhibitory concentration (MIC) of honey

The MIC of the honey was determined using the agar dilution technique (Gaill and Washington, 1995) which was done by mixing molten nutrient agar with honey. Known volumes (ml) of honey,

namely, 2.0, 2.5, 3.0, 4.0 and 5.0 per 20 ml of the mixture were used. These were equivalent to honey concentrations (% v/v) of 10.0, 12.5, 15.0, 20.0 and 25.0, respectively. The MIC values were determined for the honey against the different bacterial isolates, namely, *Enterobacter* sp., *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* sp., *Klebsiella* sp. and the control strains.

Overnight broth culture of each bacterial strain in nutrient broth was prepared. The turbidity, which was visually compared with McFarland 0.5 standard which corresponds approximately to a homogeneous cell suspension of 1.5×10^8 cfu/ml, was adjusted to 10^4 cfu/ml. The test plates which contained media mixed with honey were inoculated with the adjusted culture broth and incubated at 37°C for 24 hrs. Agar plates without honey were similarly inoculated to serve as control. The lowest concentration of honey that completely inhibited visible bacterial growth was taken as the minimum inhibitory concentration of the honey.

RESULTS

The results of the *in vitro* antibacterial effects of honey against the different bacteria tested are shown in Table I. The results showed that honey exhibited a fairly good antibacterial activity against both Gram-negative and Gram-positive bacteria. The honey showed greater antibacterial effect against *E. coli*, *Pseudomonas aeruginosa* and *Salmonella* sp., than the other tested bacteria. *Proteus* sp. was the most resistant isolate tested as

Table I. Antibacterial activity of different concentrations of honey

Bacterial Strain	Honey dilution (% v/v)						Std antibiotics	
	10	20	40	60	80	100	Chl. 30µg	Tet. 30µg
	Diametre of zone of inhibition (mm)							
<i>Enterobacter</i> sp.	0	8	12	15	20	22	18	18
<i>E.coli</i>	0	10	15	18	21	24	20	20
<i>Klebsiella</i> sp.	0	8	10	14	20	22	18	19
<i>Proteus</i> sp.	0	0	8	13	18	20	18	18
<i>P. aeruginosa</i>	0	10	12	16	21	22	21	20
<i>Salmonella</i> sp.	0	10	14	16	20	24	21	20
<i>S. aureus</i>	0	8	10	12	19	21	20	20
<i>E. coli</i> (ATCC 25922)	0	10	16	18	22	23	21	20
<i>P. aeruginosa</i> (ATCC 27853)	0	12	14	18	21	22	20	19

Chl – chloramphenicol; Tet - tetracycline

shown in this study as it showed the least zone of inhibition of 18 mm at the highest concentration of honey, i.e., 100%. Clear zones of inhibition were produced by the net honey preparation on all the bacteria tested with the largest zones of inhibition observed for *E. coli*, and *Salmonella* sp. The maximum inhibition zone of 24 mm was shown against *E. coli* and *Salmonella* sp. at net honey concentration of 100%. The 10% concentration of honey did not show any inhibition of growth on any of the bacteria tested while 20% honey concentration did not show any inhibition on *Proteus* sp. Meanwhile, honey concentration of 40% and above showed different levels of growth inhibition on all the bacteria tested, including the reference strains. However, the diameter of zone of inhibition increases as the concentration of honey increases. Table 1 also shows that the net honey (100%) produced greater diameters of zones of inhibition against all the tested bacteria than the standard antibiotics, while the honey at 80% showed zones of inhibition that are comparable to the standard antibiotics.

The results of the studies on the minimum inhibitory concentration (MIC) of the honey on the test organisms are shown in Table 2. Results showed that the MIC values for the honey ranged between 12.5 and 25.0% (v/v) with the least MIC value of 12.5% demonstrated against *Klebsiella* sp. and *S. aureus*, respectively, while the highest value of 25.0% (MIC) was against *Proteus* sp. Other bacterial isolates had MIC of 15.0%.

DISCUSSION

The in vitro antibacterial activity of honey in this study showed that the honey was effective against several bacterial strains including *E. coli*, *Salmonella* sp., *Klebsiella* sp. and *Staphylococcus aureus* at all concentrations tested except the least

concentration of 10%. The honey showed the strongest activity against *E. coli* and *Salmonella* sp. This shows that the tested honey has antibacterial activity.

Significant inhibition of growth of all the tested bacteria was observed at 40% concentration and above. Nzeako and Hamdi (2000) in their study of six commercial honeys found that inhibition in agar diffusion of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur at honey concentrations less than 40%. Al-Waili (2004) had shown that growth of all the isolates used in a study was completely inhibited by 30–100% honey concentrations with the most sensitive microbes being *E. coli*, *P. aeruginosa*, and *H. influenzae*. It has been suggested that the inhibition of bacterial growth by honey probably resulted mainly from intrinsic antimicrobial properties other than its high osmolarity and acidity (Molan, 1992a, 1992b).

The antimicrobial activity of honey affects both Gram-positive and Gram-negative bacteria as obtained in the present study. These results are in agreement with Wilix et al. (1992) and Bilal et al. (1998) who found that honey inhibited the growth of *S. aureus*, *E. coli* and *Pseudomonas* sp., thus showing that honey exhibited a fairly good antimicrobial activity against both Gram-negative and Gram-positive bacteria. Molan (1992a) reported that the mode of action of honey has not yet been fully elucidated, but osmolarity, acidity, hydrogen peroxide generation and phytochemical components are all considered as contributory factors.

Variations in the antibacterial activities of different honey varieties have been suggested by Molan (1992a, 1992b) to be due to the amount of hydrogen peroxide and other additional antibacterial components derived from the nectar source. Wahdan (1998) had shown that the kinds of antimicrobial substances (inhibines) in honey include not only hydrogen peroxide but many other substances. Two important classes of these inhibines are the flavonoids and the phenolic acids (caffeic acid and ferulic acid). The antimicrobial activity of bee honey has been attributed to several properties of honey, including its osmotic effect, its naturally low pH, and the production of hydrogen peroxide, as also the presence of phenolic acids, lysozyme, and flavanoids (Abd-El Aal et al., 2007).

The honey used in this study was effective against *E. coli*, *Salmonella* sp., *Klebsiella* sp. and *S. aureus* and these findings agree with several earlier reports on antibacterial activity of honey from other countries (Taormina et al. 2001; Melissa et al.

Table 2. Minimum inhibitory concentration (MIC) of honey on the test bacteria.

Bacteria	Minimum inhibitory concentration (MIC) (% v/v)
<i>Enterobacter</i> sp.	15.0
<i>E.coli</i>	15.0
<i>Klebsiella</i> sp.	12.5
<i>Proteus</i> sp.	25.0
<i>P. aeruginosa</i>	15.0
<i>Salmonella</i> sp.	15.0
<i>S. aureus</i>	12.5
<i>E. coli</i> (ATCC 25922)	15.0
<i>P. aeruginosa</i> (ATCC 27853)	15.0



2004). Furthermore, this study also showed that some organisms were more sensitive to honey while others were less sensitive. Similar phenomenon was observed in terms of MIC values of the honey used as these values varied according to the bacterial strains. It was revealed in this study that *E. coli* and *Salmonella* sp were the most vulnerable organisms to the honey used as they both had the same highest diameter of zone of inhibition of 24 mm, while *Proteus* sp. and *S. aureus* appeared the least sensitive with MIC value of 12.5%. Different MIC values have been reported for similar organisms by different authors. For example, Mulu et al. (2004) showed the MIC of honey for 90% of test organisms to be 6.25% while *Pseudomonas aeruginosa* showed the highest MIC of 7.5%. On the other hand, Basson and Grobler (2008) reported the MIC values of different South African honeys to range between 12.5% and 50%. These results showed that honey has variable antimicrobial activity against different organisms studied.

The discrepancy in the observed bacterial sensitivity of several bacteria to honey can be due to several reasons. One possibility might be related to the differences in susceptibility of each species of microorganism to the antibacterial activity of honey used. Similar observations have been reported by other authors (Nzeako and Hamdi 2000; Ceyhan and Ugur 2001; Taormina *et al.* 2001).

In conclusion, the honey used in the present study has been shown to exhibit antibacterial activity when tested *in vitro*. The honey has been shown to prevent the growth of a wide range of potential human pathogens and therefore has a broad-spectrum of antibacterial activity.

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