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RESEARCH ARTICLE

ANTIBACTERIAL POTENTIAL OF HONEY ON SOME SELECTED CLINICAL ISOLATES

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ABSTRACT

The antibacterial action of honey on five bacterial clinical isolates (Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Klebsiella sp and Proteus sp) was observed with 50% (v/v) as well as the neat concentration (100%) v/v, using agar- ditch diffusion technique. All the tested organisms were resistant to concentration of honey at 6.5, 12.5 and 25% (v/v).E.coli showed the highest zone of inhibition which increases with increased in concentration followed by Klebsiella sp, Proteus sp and Staphylococcus aureus. While Pseudomonas aruginosa showed the least. The minimum inhibitory concentration (MIC) of honey presented Escherichia coli as the most susceptible organism and Pseudomonas aeruginosa also, the least.

Key Words: Pseudomonas, Inhibitory, Concentration, Escherichia

INTRODUCTION

In developing countries all over the world, especially in Africa, a large number of people die daily of preventable and curable diseases because of lack of even simple health care (Sofowora, 1987). Honey has been used for the treatment of infected wounds hundreds of years ago, even before the discovery of bacteria as causes of infection (Gunther, 1967). Honey has been used to treat infections in a wide range of wound types, including leg ulcers, boils, pilonidal sinuses, and infected wounds from limb surgery (Betts and Molan, 2001).Furthermore, honey has been employed to shorten the duration of diarrhea in patient with bactericidal gastro-enteritis due to bacterial infection (Haffejee *et al.,*1985).Therefore the bactericidal action of pure honey on many pathogenic organisms including enteropathogens such as *Salmonella species, Shigella species, E.coli* and other gram negative organisms has also been reported (Jeddar, *et al.,* 1985). Stone Age painting in several location dating back to 6000BC or earlier depict honey hunting documenting human use of honey for at least 8000 years. References to honey as a medicine are found in ancient scrolls, estimated to be 6200 BC, Egyptian Papyri dated 1900-1250 BC, Veda (Hindu scripture) about 5000 years, the Holy Qur'an , the Talmud, both old and new testament of bible, the sacred book of India, china, Persia, Egypt, and Hippocrates 4560-357 (Molan, 1992). Honey is water soluble, may granulate at temperatures between 10^{o}C and 18^{o}C and is slightly acidic (pH 3.4-6.1).The sugars make honey hygroscopic (moisture absorbing) and viscous. Honey was almost the only source of sugar available to people in ancient times, and was valued for its medicinal benefits. It was used to make mead, a fermented beverages, and was mixed with wine and other alcoholic drinks.

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In Egypt, it was also employed as an embalming material. Honey is a powerful antiseptic and antimicrobial agent due to the high sugar concentration plus other factors including low pH, and the pressure of hydrogen peroxide flavonoids, phenolics and terpenes (Loveridge, 2001).

Aims and objective

1. To evaluate antimicrobial activity of honey using agar-ditch diffusion technique
2. To determine the minimum inhibitory concentrations of honey against test organisms.

MATERIALS AND METHODS

Source and dilution of honey

The honey used in this study was obtained from Danzaki town, Gezawa local government, kano state. It was diluted in sterile distilled water to different concentration of 6.5%, 12.5%, 25%, 50% and (v/v) 100%, 100% honey was referred to as neat.

Test organisms

Standard isolates and strains of micro organisms namely *E. Coli, staphylococcus aureus*, *Klebsiella sp, pseudomonas aeruginosa and proteus sp* were employed for both sensitivity test and determination of minimum inhibitory concentration (MIC).

Antibacterial sensitivity test

The method employed was described by (Bauer *et al.,* 1996) and (Barry *et al.,* 1985), it has been widely used for antimicrobial susceptibility testing.

Nutrient agar was prepared according to the manufacturer's instructions. Two to three colonies each of the clinical isolates were picked and inoculated into 10mlof nutrient broth in a test tube. These were then incubated for 4 hours at 37°C. The nutrient broth containing the incubated clinical isolates was then diluted with sterile normal saline to turbidity that corresponded 0.5 mcfarla standard. The suspension was then seeded evenly onto the surfaces of plates containing nutrient agar with a sterile swab. using a sterile capillary tube,5 wells were cut in the agar to which appropriate concentration of honey were added. The plates were incubated at 37^0c for 24 hours and were thereafter examined for zone of inhibition.

Determination of minimum inhibitory concentration

Determination of minimum inhibitory concentration (MIC) which is the lowest concentration of antimicrobial agent required to prevent visible growth was determined for honey. A tube dilution test (45, 40, 35, 30 (v/v) was carried out by adding dilution of honey to a nutrient broth in test tubes. A standardized inoculums of the test organisms was then added. The tubes containing the bacterial cultures were read macroscopically to determine the lowest concentration of the honey that did not permit any visible growth when compared with that of the control.

RESULTS

Table 1. Zones diameter of inhibition at different concentration of honey (sensitivity test)

Test organism	Concentration of honey (%) v/v				
	6.5	12.5	25	50	100
	Zone of inhibition(mm)				
Staphylococcus aureus	0	0	0	9	11
E.coli	0	0	0	11	15
Pseudomonas aeruginosa	0	0	0	8	10
Proteus sp.	0	0	0	9	12
Klebsiella sp	0	0	0	9	14

Table 2.Minimum Inhibitory Concentration (MIC)

Test organisms	Concentration of honey(%) v/v			
	45	40	35	30
Staphylococcus aureus	+	+	-	-
E.coli	+	+	+	-
Pseudomonas aeruginosa	+	_	_	_
Klebsiella sp	+	+	+	_
Proteus sp.	+	+	-	_

DISCUSSION

The result for the study showed that all bacteria employed shows resistance to concentration of honey at 6.5, 12.5 and 25%, 50 and 100% honey inhibited growth of all the employed bacteria. E.coli was the most susceptible to honey as indicated by the clear zone of inhibition obtained at 100% (v/v) as well as the MIC value, followed by Klebsiella sp, proteus sp and staphylococcus aureus, while pseudomonas aeruginosa was the least susceptible to honey compared with others organisms employed. This showed that honeys antibacterial effect is a factor of its concentration. This is corresponded with the work of james *et al.,* (1972), who found that bacterial sensitivity to honey was more in high concentration.

The results for the MIC showed that E. coli and Klebsiella sp were the most susceptible with MIC at 35% (v/v), followed by proteus sp and staphylococcus aureus with MIC value at 40% (v/v) while pseudomonas was the least with MIC value of 45% (v/v) while pseudomonas was the least with MIC value of 45% (v/v).

The antibacterial action of honey have suggested to be attributed to the presence of:

1. **Osmotic property:** honey being a super-saturated sugar exert an osmotic pressure which makes little or no water available for the micro-organisms to survive (,molan, 1992).The carbohydrates present are monosaccharide-fructose (38.2%) and glucose (31 %) and disaccharide (about 29 %) sucrose, maltose, isomaltose, maltulose,maltulose,turanose,and kojibiose.There are also some oligosaccharides present (42 %) including erlose, theanderose and panose, formed from incomplete breakdown of higher saccharides present in nectar and honey dew (Loverbridge, 2001).

2. Presence of an" inhibine" factor in honey which is hydrogen peroxide (white *et al.,* 1963). 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 catalyses formation of oxygen free radicals produced by hydrogen peroxide (Molan, 1991). Honey contains a number of enzymes including invertase, which converts sucrose to glucose and fructose, amylase which breaks starch down into smaller units, glucose oxidase, which convert glucose to gluconolactone, which in turn yields gluconic acid and hydrogen peroxide, catalase, which breaks down the peroxide formed by glucose oxidase to water and oxygen and acid phosphorylase; which removes inorganic phosphate from organic phosphates. Honey also contains eighteen free amino acids of which the most abundant is proline (Loveridge, 2001).

3. **Non-peroxide component:** Among these are complex phenols and organic acids often referred to as flavonoids (Molan, 1988). The evidence for the existence of other antibacterial factors is mainly that the peroxide generating system does not account for all the observed antibacterial activity, but there have also been some report of isolation antibacterial substances from honey that are not hydrogen peroxide (Molan and Russell, 1988). Furthermore, it has been found that heating honey, which inactivate the glucose oxidase, causes loss of activity against some species whilst it is retained against others (Molan and Russel, 1988). Although the stability of the enzyme varies in different honeys, there have been reports of honey with stability well in excess of this variation, showing that there must be an additional antibacterial factor involved. The most direct evidence for the existence of non-peroxide antibacterial factors in honey is seen in the reports of activity persisting in honey treated with catalase to remove the H_2O_2 activity (Molan and Russell, 1988). Several chemicals with antibacterial activity have been identified in honey by various researchers pinocembrin terpenes, benzyl alcohol, 3,5-dimethoxy-4-hydroxybenzoic acid (synngic acid),methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl

syringate), 3,5,5-trimethoxybenzoic acid, 2-hydroxyl -3-phynylpropionic acid, 2 –hydroxybenzoic acid, and 1,4 –dihydroxy benzene. However,the quantities of these present were far too low to account for any significant amount of activity.

4. Stimulation of lymphocytic and phagocytic activity (Abuharfeil *et al.,* 1999).Studies showed that the proliferation of peripheral blood B –lymphocytes and T –lymphocytes in cell culture is stimulated by honey a concentration as low as 0.18 and phagocytes are also activated by honey at such low concentration. Furthermore, honey stimulates monocytes in cell culture to release cytokines,tumour necrosis factor (TNF) –alpha, Interleukines (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 of macrophages (Tonks *et al.,* 2001). Honey contains trace amount of several vitamins and minerals, the vitamins include riboflavin, niacin, folic acid, panthothenic acid and vitamin B6. It also contains ascorbic acid, the vitamins include B6. It also contains ascorbic acid (Vitamin C) and the minerals, calcium iron, zinc, potassium, phosphorus, magnesium, selenium, chromium and Manganese. The main group of antioxidants in honey is that of the flavonoids, of which one, pinocembrin is unique to honey and bee propolis. Ascorbic acid catalase and selenium are also antioxidants. Generally speaking the darker the honey, the greater the antioxidizing properties (Leveridge, 2001).

5. **Acidity:** Honey is characteristically quite acidic, its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens. The optimum pH for growth of these species normally falls between 7.2 and 7.4. The minimum pH values for growth of some common wound-infecting species is: *Escherichia coli* 4.3, *Salmonella SP 4.0,Pseudomonas aeruginosa 4.4*, *Streptococcus pyogenes 4.5*

Thus in undiluted honey the acidity is a significant antibacterial factor. But if honey is diluted, especially by body fluids which are well buffered, the pH will not be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria. Honey also contains organic acid such as acetic, butanoic, formic, citric, succinic, lactic, malic, pyroglutamic and gluconic acid, formed by the breakdown of glucose by glucose oxidase. Honey also contains hydroxymethylfurfural, a natural product of the breakdown of simple sugar below pH5 (Loveridge, 2001).

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