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Nutritional Analysis and Antibacterial Effect of Honey on Bacterial Wound Pathogens

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Abstract: The nutritional analysis of honey sample purchased from Iyale, Dekina Local Government area in Kogi state, Nigeria was assessed using the recommended methods of the Association of Official Analytical Chemists. The results were as follow: total titratable acidity (32.6%), fat content (1.5%), protein content (0.88%), unhydrolysed and hydrolysed honey have glucose and fructose (63.0%) and (81%), vitamin C content (3.45%), moisture content (25.22%), ash content (1.67%), crude fibre (1.2%), soluble carbohydrate (69.53%). The antibacterial analysis of honey at 100% concentration revealed a significant activity against *Escherichia coli,* (25mm), *Pseudomonas aeruginosa* (23mm), *Streptococcus pyogenes* (22mm), *Staphylococcus aureus* (20mm), and *Proteus mirabilis* (17mm). At 75% concentration the bactericidal activity was slightly reduced but effective against *Escherichia coli,* (21mm), *Pseudomonas aeruginosa* (16mm), *Streptococcus pyogenes* (17mm), *Staphylococcus aureus* (14mm), and *Proteus mirabilis* (13mm). While at 50% concentration there was weak inhibition *Escherichia coli,* (11mm), *Streptococcus pyogenes* (9mm), *Staphylococcus aureus* (9mm), however some of the wound bacterial pathogens were resistance at these concentration such as *Pseudomonas aeruginosa* and *Proteus mirabilis.* The minimum bactericidal concentration and the minimum inhibitory concentration were 3.13mg/ml - 12.5mg/ml and 1.57mg/ml - 6.25mg/ml respectively. The result reveals that honey can be used in the treatment of wound infection associated with these pathogens.

Key words: Nutritional analysis, antibacterial, unhydrolysed honey, Minimum bactericidal concentration.

INTRODUCTION

Most tropical countries are blessed with a diversity of micronutrients substance which plays a basic role in nutrition and healthy body development (WHF, 2005). In order to have a health population that can promote development, the relation between nutrition and health should be reinforced. In developing countries, one of the ways of achieving this is through the exploitation of available local resources, in order to satisfy the needs of increasing population[2]. Among these micronutrients substance is honey and according to National Honey Board,[17] honey is secreted by bees as a food source, in cold weather or when food sources are scarce. Bees made use of their stored honey as their source of energy. Honey contains trace amounts of several vitamins and minerals[4,21,12,8] reported that honey contains tiny amounts of several compounds thought to function as antioxidants, which restores the damaged skin and give it a soft as well as young looks. Molan *et al.*,[14] observed that honey is hygroscopic in nature and hence speeds up growth of healing tissue and sometimes dries it.

Apart from possessing these nutritive qualities, honey had been found useful in management of wound which enables healing and prevents the spread of infection to other body tissues. Namias,[16] grouped wounds according to the cause, the environment in which they occur, their extent, and whether they are clean or contaminated. The clearing of infection seen when honey is applied to a wound may reflect more than just antibacterial properties.

Tonks *et al.*,[20] observed that honey at a concentration of 1% also stimulated monocytes in cell culture to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activated the immune response to infection. Dimitrova *et al.*,[7] discovered that phenolic compounds have antioxidant properties and are thought to inhibit microbial growth by disruption cell membrane function. In addition, Krishna,[11] confirmed that the antibacterial properties of honey may be due to its acidic nature and hydrogen peroxide produced enzymically. Although Molan,[3] discovered the existence of other antibacterial factors from honey that do not necessarily contain hydrogen peroxide, but also capable of inhibiting the growth of pathogens. Guardian Society,[9] also observed that the antibacterial and antiseptic properties of honey help in healing of sore throats and laryngitis.

However, Abuharfeil *et al.*,[1] showed that the proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at

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concentrations as low as 0.1%; and phagocytes are activated by honey at concentrations as low as 0.1%, which strengthens the white blood corpuscles to fight bacterial and viral diseases.

Despite these beneficial uses of honey, information is scanty on the antibacteric properties of honey on abrasion. So the study highlight proximate analysis of honey, isolation and identify pathogens associated with wound test on antibacteric property of honey, determine the MIC and MBC of honey against the tested pathogens.

MATERIALS AND METHOD

Collection of Honey Samples: The sample of honey purchased from Iyale, Dekina Local Government area in Kogi state was put in sterile free sample bottles and then transported to the laboratory. The honey samples was tested for its purity, by keeping it in the refrigerator at 4°C for 24 hours to check the purity of honey, the honey did not freeze[18]

Sources of Bacterial Isolates: The bacteria isolate of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus mirabilis* were obtained from wound swab at General Hospital Minna, Niger State. The cultural characteristics and biochemical profile of these organisms were reconfirmed by biochemical test and the cultural characteristic as described by Oyeleke *et al.*,[18].

Biochemical Analysis and Cultural Characteristics: The biochemical activities were carried out according to the method described by Cheebrough,[6] and Oyeleke *et al.,*[19]. The isolates from the sample were identified using their morphology, gram staining, motility, catalase, indole production, oxidase, urease, citrate, and other biochemical tests.

Nutritional Analysis of Honey: The recommended methods of the Association of Official Analytical Chemists (AOAC, 2000) were adopted for the determination of the proximate composition while total titratable acidity and reducing sugar in unhydrolysed and hydrolysed honey were estimated using the method of Annon,[5].

Determination of Antibacterial Property of Honey: Using the cork borer method the antibacterial activity of honey was carried out using the method adopted by Oyeleke *et al.*,[19] in which a 4mm sterile cork borer was sterilized by flame and was used to bore four wells in the solidified nutrient agar plates aseptically. The nutrient agar plates were then inoculated with 3 hours incubated suspension of the bacteria isolate using a sterile swab stick which was seeded evenly on the surface of the agar plate. Each well was filled with the honey samples of different percentage i.e. 100%, 75% and 50% and 100mg/ml of Ampiclox was used as control and these was repeated for each test organism. The plates were then incubated at 37°C for 24 hours. The inhibition zone around each well was measured.

Determination of Minimum Inhibitory Concentration (MIC): Agar dilution method was used to determine the MIC of the honey using the method adopted by Mohammed *et al.*,[19] in which 500mg of honey was added to 20ml of nutrient broth, and 0.5ml of these honey (50mg/ml) was measured into sterile nutrient broth test tubes containing (0.5ml, 1.5ml, 4ml and 8ml each). 0.5ml of honey was measured into 8ml sterile nutrient broth, making the concentration of 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.13mg/ml, and 1.57mg/ml respectively. The test tubes were serially seeded with a loopful of test organisms and incubated for 24 hours at 37°C. Control (Ampiclox) containing the same honey concentration in sterile nutrient broth without test organism and were incubated at 37°C for 24 hours. After incubation period, the lowest concentration which shows no turbidity (no growth of organisms) is regarded as the minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration of the honey was determined by sub-culturing from those without visible growth and transferred to agar plates as described by Oyeleke *et al.*,[19]. The lowest concentration that yields no growth on the sub-culture agar plate after incubation for 24 hours at 37°C is regarded as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Discussion: The results obtained from the study as indicated in Table 1 shows that honey possesses some nutritive contents which include protein, carbohydrate, crude fibre, fat, vitamin C, glucose and fructose in various compositions. This is in agreement with the study of Habonimana *et al.,*[10], Molan *et al.*,[15] and Abuharfeil *et al.*,[1] who had independently reported that honey can be used as a nutrition supplement of human need.

The biochemical test results shown in Table 2 revealed that the honey is characteristically quite acidic, the pH value ranged between 3.2 and 4.5, which is low enough to inhibite the growth of pathogens. The optimum pH for growth of bacterial species normally falls between 7.2 and 7.4. The minimum pH values for

growth of some common wound-infecting species as reported by Martos,[12] and Gheldof,[8] are: Escherichia coli, 4.3 pH; Salmonella sp, 4.0 pH; Pseudomonas aeruginosa, 4.4 pH; Streptococcus pyogenes, 4.5 pH. This pH value signifies that undiluted honey has significant antibacterial factors. This further supports Krishna,[11] who had earlier confirmed that the antibacterial properties of honey may be due to its acidic nature and hydrogen peroxide produced enzymically.

The result of the antibacterial activity of undiluted honey was effective against wound pathogenic bacteria which are *Escherichia coli,* (25mm), *Pseudomonas aeruginosa* (23mm), *Streptococcus pyogenes* (22mm), *Staphylococcus aureus* (20mm), and *Proteus mirabilis* (17mm), this agrees with the result of Allen *et al.*,[3] and Dimitrova *et al.,*[7] who 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*, that were cultured from wounds but were also found lethal to them.

75% concentration of honey has a bactericidal effect against *Escherichia coli,* (21mm), *Pseudomonas aeruginosa* (16mm), *Streptococcus pyogenes* (17mm), *Staphylococcus aureus* (14mm), and *Proteus mirabilis* (13mm) and at 50% concentration the activity was weak against the pathogens tested (*Escherichia coli,* (11mm), *Pseudomonas aeruginosa* (-), *Streptococcus pyogenes* (9mm), *Staphylococcus aureus* (9mm), and *Proteus mirabilis* (-). This agrees with the findings of Tonks *et al.*,[20] and Namias,[16] that if honey is diluted, especially by body fluids which are well buffered, the pH will be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria, hence this may probably be responsible for the resistant of *Pseudomonas aeruginosa* (-) and *Proteus mirabilis* (-).

The minimum bactericidal concentration and the minimum inhibitory concentration of honey were 3.13mg/ml - 12.5mg/ml and 1.57mg/ml - 6.25mg/ml respectively which corroborated with the findings of Oyeleke *et al.*,[18]. This result reveals that honey can be used as a therapeutic drug against tested bacterial pathogens.

Table 1: Proximate analysis of honey with their percentage values

Nutritional analysis of honey	values of honey analysis in %
Total titratable acidity content	32.6
Reduing sugar (%g+f sugar) for unhydrolysing honey	63.0
Reduing sugar (%g+f sugar) for hydrolysing honey	81
Vitamin C content	3.45
Fat content	1.5
Crude fibre	1.2
Protein content	0.88
Moisture content	25.22
Ash content	1.67
Soluble carbohydrate content	69.53

The organisms isolated and identified from wound are *Escherichia coli, Staphylococcus aureus, Streptococcus pyrogens, Pseudomonas aerugenosa* and *Proteus mirabilis*. These were later used to know antibacterial properties of honey.

Table 2: Antibacterial Activity of honey using 4mm cork borer.

Organisms	100% conc	75% conc	50% conc	Ampiclox(Control)
Pseudomonas aeruginosa	23mm	16mm	-	23mm
Staphylococcus aureus	20mm	14mm	9mm	21mm
Streptococcus pyogenes	22mm	17mm	9mm	18mm
Escherichia coli	25mm	21mm	11mm	22mm
Proteus mirabilis	17mm	13mm	-	20mm

The table shows that the reaction of organisms to different concentration of honey. *Escherichia coli* had the highest susceptibility to honey and the least was *Proteus mirabilis.*

Table 3: Minimum Inhibitory Concentration (MIC) of honey against the pathogenic bacteria tested

Organisms	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.57mg/ml
Pseudomonas aeruginosa	_	_	_	_	1.57
Staphylococcus aureus	_	_	_	3.13	0
Streptococcus pyogenes	_	_	_	0	0
Escherichia coli	_	_	_	_	_
Proteus mirabilis	_	_	_	0	0

Keys: + = Turbid, - = Not turbid.
The MIC ranged between 25mg/ml – 6.25 mg/ml, at any concentration below the least concentration will render the compound ineffective.

Table 4: Minimum Bactericidal Concentration (MBC) of honey against the pathogenic bacteria tested.

Organisms	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.57mg/ml
Pseudomonas aeruginosa	_	_	_	3.13	1.57
Staphylococcus aureus	_	_	_	0	0
Streptococcus pyogenes	_	_	_	0	0
Escherichia coli	_	_	_	_	0
Proteus mirabilis	_	_	6.25	0	0

Keys: + = Growth when subcultured, - = No growth when subculture.
The MBC values ranged between 25mg/ml to 6.25 mg/ml for most of the organisms.

Conclusion: This study reveals that honey possesses some nutritional quality that can be used as supplement for the need of human, and the antibacterial activities against these tested wound pathogens indicate that undiluted honey can be used in the treatment of wound infection associated with these pathogens.

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