Studies on the effects of the honey of two floral types (Ziziphus spp. and Acelia spp.) on organism associated with burn wound infections

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The honey of 2 Nigerian honey of 2 floral types (Ziziphus spp. and Acacia spp.) was studied. Standard physiological composition of honey quality, that is, ash, pH, acidity, color, refractive index, conductivity, moisture, mineral content, hydroxymethyl furfural (HMF) contents and diastase number (DN) of the species were determined. Compositional variation due to the floral types shows that Ziziphus honey has the highest ash and pH but lower acidity compared to Acacia honey statistically. Both Honey (show) indicates good quality with low level HMF and DN. Acacia honey was tested on burn wound healing potency as well as the sensitivity of wound infecting bacteria species within 6 weeks healing has take place, bacteria culture revealed that common wound pathogens such as Staphylococcus aurens, speuchomonas, methecilin resistant staphylococcus (MRSA), vancomycin-resistant enterocecci (VRE) and Acinctobacter tauari were rendered sterile in the honey treated wound. A wide antibaterual potency with minimum inhibitory concentration (MIC) range of 3.7 - 7.3% was obtained which is necessary to stop the growth of these strains.

Key words: Ziziphus spp. and Acelia spp., antibacterial potency, burn, wound, healing, culture of bacteria.

INTRODUCTION

Honey is a carbohydrate rich syrup produced by bees, primarily from floral nectars. It is prepared by bee from plant nectars, plant secretions and from excretion of plant sucking insects (Honey dew). The food standard code defines it as “the nectors and saccharine exudations of plants gathered, modified and stored by the honey bee.” Fructose which is approximately 38% w/w and glucose (-31%) are the major components present in honey, with less amount of sucrose (-1%). Other disaccharides and oligosaccharide. Glucose acids, other acids and small amounts of proteins enzymes (including glucose oxides), amino acids and mineral may also be present. Honey is midly acidic with a pH around 3.9. Moisture content is low (17%) as is water activity (0.562 - 0.620) (British Pharmacopia, 1993).

Chemical composition of honey varies depending on plant source, season and production methods. Storage conditions may also influence final composition, with the proportion of disaccharides increasing overtime (white et al., 1964).

Some aspects of the composition of honey have been identified to contribute to its antibacterial activity. The low water activity of honey is inhibitory to the growth of the majority of bacteria and many yeast and moulds. When applied topically to wounds, Osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissues and reduce bacterial growth.

The low pH alone is inhibitory to many pathogenic bacteria and in topical applications at least, could be sufficient to exert an inhibitory effect. Honey has an extensive history to traditional human medicine use, in a large number of societies. It may be used alone or in combination with other substance and has been administered both orally and tropically. A number of studies have been carried out on animal models of wound and burn healing in which honey is used as a treatment (Subrahmamaryam,

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Honey is a substance suitable for use as an active ingredient in list bale therapeutic goods in Nigeria. It is likely to find use in products such as ointments for treatment of minor burns, cuts and skin infections. While it may be suitable for use in oral rehydration products and to treat more severe burns and wounds, such treatment would require medical supervision and products for these uses would require registration. As a first step for this purpose, the chemical analysis and wound-healing phenomena involving honey will be required. Therefore, the major interest in this work is to evaluate the quality parameters from Nigerian honey form 2 floral types (Ziziphus spp. and Acelia spp.) that are relevant in wound healing properties and to study the potential of honey in treating infected burn wounds.

MATERIALS AND METHODS

MATERIALS

Treatment of honey samples

2 honey samples were brought from a traditional bee keeper in Ganye local government area of Adamawa state, Nigeria. The samples were harvested from Ziziphus and Acacia Flora plants in Ganye and stored in 5 liters plastic containers. Foreign matters such as wax sticks dead bees, particles of combs were removed by straining the samples through cheesecloth before use. The color of the samples was determined using a leribond comparator.

Determination of ash

2 g of a sample was ashes by Calcinations in a furnace at 600°C to a constant weight. Ash percentage was calculated as:

\[ \text{% ash} = \frac{\text{Weight of Ash}}{\text{eight of Sample}} \]

Elemental analysis

The sample were analyzed for elemental determination using the total reflection X-ray florescence (TXRF) spectrophotometer (model is 221520, Italy structures).

Determination of hydromethyl furfural; (HMF)

This is one of the indicators of quality parameters of honey and it was determined calorimetrically after dilution with distilled water and subsequent addition of P-thididine solution. Absorbance was determined at 55 nm using a 1 km cell in a buck model M500 sptrophotometer.

Determination of moisture content

2.50 g of each sample was put in a flat dish and dried in the oven at 105°C for 3 h, covered, cooled in a desiccators and weighed. The samples were dried again in an oven, cooled and re-weighed. This process was repeated at 1 h drying intervals until a constant weight was obtained.

Measurement of pH, conductivity, refractive index and acidity

The pH of honey was measure using a digital pH meter (Model HI, Hannah Instrument) while electrical conductivity measurement was done using a conductivity meter (model 20, Denver Instrument). An Abbe refractor was used in the measurement of refractive indices. To measure acidity, 10 g of the sample were accurately dissolved in 75 ml CO₂ free distilled and titrated with 0.1 M NaOH solutions.

Determination of diastase activity

To determine the diastase activity, 1 g of sample was mixed with 1% (w/v) and kept at 37°C. The diastase enzymes of the honey hydrolyzed the starch. The results were expressed as ml of 1% of starch hydrolyzed in 1 g honey per hour.

Wound healing process

Patient

A 26 years old female from a village near Demsa in Adamawa state who has wound on her left leg caused by hot water burns volunteered for this study. The wound had a small cavity with an offensive odor. Wound swabs were collected from the wound for culture and sensitivity test.

Wound dressing

Honey dressing was performed after the swabs were collected to see how honey stimulates wound healing. The wound cavity was washed with normal saline before the dressing process. A dressing consisting of absorbent pad with about 30 g of normal undiluted unprocessed honey samples added. This was inserted into the wound cavity and kept in place securely in position by a secondary dressing (a bandage). This method of soaking into an absorbent prevents the honey from running off. The wound was inspected every 2 days until healing.

Bacterial culture and sensitivity determination

The study concentrated on strains of antibiotic resistance bacteria which prevent wound healing (Dumfort et al., 2000). Isolates of methicillin resistance staphylococcus aureus (MRSA), Vancomycin resistance enterococci (VRE) and Pseudomonas from the infected wound were cultured in trypticase soy both overnight. They were then plated out using inoculators delivering all drops outs trypticase soy agar plates containing various concentrations of honey. The range of concentration of honey in the agar plates was in steps of 1% (v/v).

After incubation 24 h, the growth of the culture on the plates was examined. Plates containing no honey were used as controls to verify that the culture inoculated would grow. The minimum inhibitory concentration (MIC) of honey for each strains of bacteria was determined by finding the plate with the lowest concentration of honey on which the strain would not grow. All inoculation for each concentration of honey was carried out in triplicate.

RESULTS AND DISCUSSION

The quality parameters of honey are compositional requi-
The conductivity and ash content are within the range 9.49 - 172.9 µScm⁻¹ and 0.09 - 0.518% respectively found for other Nigerian honey (Adebiyi et al., 2004) and are in agreement with standard values (White, 1975). The results shows that the conductivity of the Ziziphus and Acacia honey are 19.23 ± 0.02 and 10.40 ± 0.11 µScm⁻¹ respectively. Ziziphus honey shows a higher ash content (0.56 ± 0.01%) than Acacia honey (0.08 ± 0.01%). Both samples indicated good quality with low level of hydroxymethyl furfural (MHF) 34.26 ± 1.35 and 36.42 ± 1.03 mg/kg. Hydromethyl furfural is a breakdown product of sugar. It is used as an index of heart treatment of honey. Also, both the honey samples are found to have low diastase (DN) values which is an indication of good diastase activity.

The result of these 2 parameters (HMF and DN) indicates that sugar levels in the honey samples were not affected during storage. The implication of these results in wound healing process than is that honey creates a film liquid between the tissues and the dressing that allows wound dressing to be lifted off painlessly and without tearing the newly grown cells. By reducing swelling in the surrounding inflamed tissues, honey also reduces primary cause of pain, hence maintaining high sugar level of honey eliminates the unpleasant odor associated with major burns and skin ulcer as well.

In the course of the study, Acacia honey was tested for its wound healing and antibacterial potency. The study shows that the wound treated with Acacia honey healed within 6 weeks of treatment. There was no significant different in the time taken for wound healing as a result of the application of honey. By week 5, significant healing was observed and wound odor as well as all the methicillin resistant s. aureus (MRSA) was eliminated. During the application of the honey, the patient observed a transient pain. This was probably due to the high osmolality or it is more likely that the wound is sensitivity to aci-

### Table 1. Elemental concentrations (µg/g) in honey samples.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Ziziphus honey</th>
<th>Acacia honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1115.17 ± 36.01</td>
<td>1065.25 ± 25.20</td>
</tr>
<tr>
<td>Ca</td>
<td>265.05 ± 0.10</td>
<td>285.08 ± 0.13</td>
</tr>
<tr>
<td>Mn</td>
<td>2.05 ± 0.01</td>
<td>3.01 ± 0.01</td>
</tr>
<tr>
<td>Fe</td>
<td>271.05 ± 1.25</td>
<td>265.02 ± 2.36</td>
</tr>
<tr>
<td>Cu</td>
<td>16.01 ± 0.02</td>
<td>18.00 ± 0.01</td>
</tr>
<tr>
<td>Zn</td>
<td>32.05 ± 0.06</td>
<td>35.08 ± 0.03</td>
</tr>
<tr>
<td>Se</td>
<td>2.15 ± 0.01</td>
<td>2.62 ± 0.02</td>
</tr>
<tr>
<td>Br</td>
<td>2.45 ± 0.021</td>
<td>2.78 ± 0.06</td>
</tr>
</tbody>
</table>

**Table 2. Physiochemical parameters of honey samples.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ziziphus honey</th>
<th>Acacia honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>16.35 ± 0.03</td>
<td>16.75 ± 0.01</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.56 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>6.05 ± 0.01</td>
<td>3.56 ± 0.02</td>
</tr>
<tr>
<td>Acidity (megk)</td>
<td>5.72 ± 0.01</td>
<td>14.96 ± 0.03</td>
</tr>
<tr>
<td>Color</td>
<td>60.21 ± 1.03</td>
<td>55.60 ± 2.05</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.486 ± 0.01</td>
<td>1.489 ± 0.02</td>
</tr>
<tr>
<td>Conductivity</td>
<td>19.23 ± 0.02</td>
<td>10.40 ± 0.11</td>
</tr>
<tr>
<td>HMF (megk)</td>
<td>34.26 ± 1.35</td>
<td>36.42 ± 1.03</td>
</tr>
<tr>
<td>Diastase number</td>
<td>14.63 ± 1.63</td>
<td>11.62 ± 1.10</td>
</tr>
</tbody>
</table>

...rements for use as food or other application such as in medicinal purpose. These quality parameters which include ash content, color, sugar content, moisture content, electrical conductivity, free acid, diastase activity and hydroxymethyl furfural content were determined in the 2 honey samples used for this study.

Table 1 shows the elemental compositions of the 2 honey samples used. Potassium was found to be the highest in concentration 1115.17 ± 36.01 for Ziziphus honey and 1065.25 ± 25.20 for Acacia honey as compared to other elements detected.

Generally all the elements detected from the honey samples were not significantly different. However, the results shows that these honey samples are very good sources of rich minerals. This will contribute to the nutrients supply for growth of new tissues which is usually limited because of damage from injury or infection to the underlying circulation. Worthy of note is that the honey samples used contain bromine, this may also contribute to the antibacterial activities of this honey samples.

The physiochemical parameters of the honey samples are shown in Table 2. The results indicated, the color of all the honey samples is amber, which on Lovribound comparator scale, range from 55.6 - 60.21. The color range is due to the fact that the samples may contain high amount of minerals. There was no significant variations found in the moisture contents and refractive indices of Ziziphus honey and Acacia honey (16.35 ± 0.03 and 16.75 ± 0.01) and *1.486 v 0.01 and 1.489 ± 0.02* respectively. However, the moisture content was slightly lower than honeys from US (Whites, 1975). This different may be due to the time of extraction from the comb in relation to the ripening process by the bees (Kamal et al., 2002).

It was observed that Ziziphus honey has highest pH value (6.05 ± 0.01) as compared to Acacia honey (3.56 ± 0.02). This difference may be due to the variation of different acids and minerals present in honey (Kamal et al., 2002).

Ziziphus honey was found to have lower acidity (5.72 ± 0.01 megk⁻¹) than the value of 14.96 ± 0.03 megk⁻¹ found in Acacia honey. This variation seems to be due to the source of nectars. The acidity of honey, its sugar content and other nutrients are important in the healing process. Acidification of the wound prevent ammonia produced by bacteria metabolism from harming body tissues. It also promotes healing by increasing oxygen release from the blood's haemoglobin. Oxygenation of the tissue being essential for growth of tissue.

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Table 3. Minimum inhibitory concentration (MIC) of Acacia honey for each strain of bacteria.

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>3.5 ± 0.01</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>5.6 ± 0.01</td>
</tr>
<tr>
<td>MRSA</td>
<td>6.3 ± 0.02</td>
</tr>
<tr>
<td>VRE</td>
<td>7.3 ± 0.03</td>
</tr>
<tr>
<td>Acinetobacter baumari</td>
<td>5.0 ± 0.04</td>
</tr>
</tbody>
</table>

dity (low pH) of the honey.

Previous studies have shown that honey heals due to its high osmolarity (Molan, 1992) which inhibited the growth of bacteria and also accelerate the cleansing and desloughing of dirty wounds. Honey also contains a thermolabile factor, inhibine, related to the plant source of honey which helps in the sterilization of burn wounds (Dumfort et al., 2000).

Positive swab culture were obtained from the wound (treated and untreated) of the patient. The organism isolated from the swab culture was *S. aureus* and *Pseudomonas* which are common wound pathogens. Others include epidemic strains of MRSA, VRE and *Acinetobacter baumari*. These antibiotic-resistant strains were found to be sensitive to honey as the antibiotic sensitive strains of the same species.

Table 3 is a result showing the minimum inhibitory concentration (MIC) of Acacia honey used from the result, a wide range of (MIC) value (3.5 - 7.3) of Acacia honey necessary for complete eradication of bacterial growth was observed. This antibacterial potency is well in excess needed to stop the growth of the strains found in this study. These results are interesting because the common wound infecting species of bacteria have been tested as sensitive to the antibacterial activity in the honey. Clearing bacteria infection by honey as shown in this study is essential to allow for wound healing process to occur and may be beneficial in treating burn wounds with mix infections.

**Conclusion**

In this study, honey obtained from *Ziziphus* and *Acacia* floral species were found to be of good quality. Honey from *Acacia* floral was further investigated for it burn wound healing and antibacterial potency. The study revealed that, this honey healed the wound by making the wound sterile through its antibacterial effects. The common wound infecting pathogens were sensitive to honey, hence wound infection by these bacteria which cause serious clinical problems and which cannot be treated with systematic antibiotics will now be healed with honey.

This honey could therefore be used in clearing chronic infected wounds such as leg ulcers, cavity wound etc. However, further evidence and understanding of therapeautic and other chemical properties of the honey are needed to optimize its uses in the clinical management of wounds.

**REFERENCES**


