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Effect of Natural Honey (Produced by *African sculata in* Guyana) Against Bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) and Fungus (*Candida albicans*)

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Abstract: Honey is among many of the natural products that have been used to treat various aliments such as burns and ulcers since ancient times. Honey is also applied to treat burns based on research carried out earlier. In Guyana, honey is mainly used for coughs and colds. This research was carried out during the year 2006-2007 to investigate the anti-fungal and antibacterial action of honey produced by *African sculata* obtained in Guyana against *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* and *Candida albicans*, a fungus. Biochemical analysis of honey depends on the plant species on which the bees forage confirms to the earlier works (White, *et al.,* 1962). The present studies indicate strongly that the honey in pure form (with out dilution), was effective against bacteria: *Pseudomonas aeruginosa*, *Eschericha coli* and *Staphylococcus aureus* using the disk diffusion technique. However, the disk diffusion method had negative effect on the fungus *Candida albicans*. The results were conclusively substantiated by ANOVA analysis where the data was found to be highly significant at probability level of 0.05.

Key words: Honey · Antifungal · Antibacterial · Chemical properties · Bateriostatic · Bactericidal

INTRODUCTION

Honey is an organic substance that is produced from many different floral sources. Pollen from flowers are collected by honeybees and taken back to their nest. When the nectar enters the stomach of bee, it immediately becomes honey. Enzymes from the head gland called diastase-invertase and enzyme from the thoracic gland (glucose oxidase), mixed with the nectar in order to enhance the flavour of honey [1].

Honey has been used for its medicinal properties in many cultures, since in ancient times. Work carried out by Molan [2] reported that honey is becoming accepted as a reputable and effective therapeutic agent by practitioners of convectional medicine and by the general public. In today's society, it is still being used and is slowly replacing some of the artificial products used to treat human. According to Bangroo and co- workers, [3], despite the recent advances in antimicrobial activities of artificial products, none has eliminated the problem of infection. In some countries, honey is used as an antiseptic therapeutic agent for treatment of burns, ulcers and other wounds [3].

Raw and unprocessed honey is a type valued for its nutrient and medicinal properties. Honey contains as much as eight substances that are important to good nutrient in human. It is a sweet viscous liquid that has high enzyme content. Honey also contains proteins, carbohydrates, hormones, organic acids and antimicrobial and antibiotic properties.

The composition of honey varies according to the plant species on which the bee forages, but the main constituents are the same in all honeys [4]. Honey consists mainly of carbohydrates of which glucose and fructose are most abundant. These two sugars strongly attract water and as such honey when applied to wounds, absorb water, drying it out so that the growth of bacteria and fungi are inhibited (these micro-organisms thrive in moist environment). Secondly raw honey contains enzyme called glucose oxidase that, when combined with water, produces hydrogen peroxide [5], a mild antiseptic. In addition, honey also contains antioxidants and flavonoids that may function as antibacterial agents. The mineral and vitamin content of honey is very low (about 0.02% of its weight) and given the low consumption of honey, it thus

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have no significant nutritional benefit to man. The pH of honey is low and ranges from 3.2 to 4.5 with the most predominant one being gluconic acid.

These properties of honey are well established. Undiluted honey inhibits the growth of bacteria such as *Staphylococcus aureus*, certain gut pathogens and fungi such as *Candida albicans* [6]. At a concentration of 30-50%, honey has been shown to be superior to certain conventional antibiotics in treating urinary tract infections [7]. The exact mechanism of the anti-microbial effect of honey remains obscure. Low pH, osmotic disruption of pathogens and the presence of bactericidal substances, collectively called inhibine may all play a part.

Honey has been reported to be effective in the healing of infected postoperative wounds [8]. It has also been reported to inhibit the growth of a lot of bacteria such as *Bacillus cereus, Staphylococcus aureus, Salmonella dublin* and S*higella dysenteriae* [9, 10]. It has also been reported to inhibit the growth of anaerobic bacteriodes [11].

The evidence for the existence of other antibacterial factors is mainly that the peroxide-generating system does not account for all of the observed antibacterial activity, but there have also been some reports of isolation of antibacterial substances from honey that are not hydrogen peroxide. Furthermore, it has been found that heating honey, which inactivates the glucose oxidase, causes loss of activity against some species whilst it is retained against others [12]. Although the stability of the enzyme varies in different honey, 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 honeys treated with catalase to remove the hydrogen peroxide activity.

The purpose o f this study was to investigate the anti-fungal and antibacterial action of honey produced by *African sculata* obtained in Guyana, against *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungus). This research had stem from the rapid use of honey that was utilized for cough and colds. As such the researcher aim was to find out what in honey was responsible for its effective antimicrobial and anti fungal activities. The experiment was taken up using pure natural honey rather than commercially processed and strained honey which loses up to half its original vitamin content.

MATERIALS AND METHODS

The honey was obtained from Linden High way in Georgetown (Guyana) and subjected to biochemical analysis. The analytical work was carried out at the Guyana Sugar Corporation Central Laboratory. Following parameters were analyzed using the standard techniques [13].

pH, moisture content, electrical conductivity, ash content, acidity, reducing sugars, sucrose and nutrients (potassium, iron, zinc, magnesium, copper and manganese).

Antimicrobial disc diffusion suspecting tests were carried out. The technique involves using disk diffusion susceptibility testing where disks from both the pure and diluted honey as well as the antibiotic disk erythromycin (control) were impregnated onto the surface of the Mueller Hinton agar. At areas where the concentration of both the diluted and undiluted honey were sufficient to prevent bacterial growth, a distinct margin known as the inhibition zone [14] can be seen. Similarly, if the organism tested has no resistance to the antibiotic disk impregnated on agar surface then as the antibiotic diffused from the disk an inhibition zone is formed around the disk. Treatment of each bacterium as well as the fungus *Candida albicans* was administered using concentrations of 50%, 25% and 1%. In addition, both the diluted and undiluted form of the honey was directly applied to the growth population of bacteria and the fungus. Candida BCG Agar culture media was used for *Candida albicans.*

All the Petri dishes (with replications) were inoculated with respective bacterium and fungus which were isolated from pure cultures. The various concentrations (pure, 50%, 25% and 1%) of honey disks were placed on the surface of agar. An antibiotic control disk, erythromycin was also inserted onto the surface of agar. The inoculated plates were incubated for twenty four hours at 37°C. Each plate was then observed accordingly for any inhibition zones. Inhibition zones present were measured in diameters using a ruler. The growth of colonies was also estimated for each plate.

In another set of experiments, 1 ml of pure honey was applied to the growing population of the organisms mentioned above. One of each Petri-dish with growing microorganism was left untreated and was used as a control. The treatment was done in triplicate. Before each treatment, the colonies of all of the tested organisms were counted using population estimation charts.

RESULTS AND DISCUSSION

According to research earlier carried out honey has the following average composition [4].

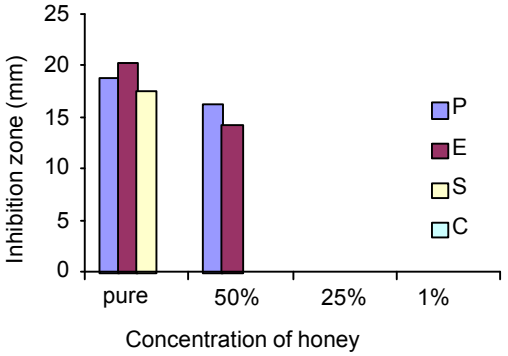
The biochemical properties of honey are listed above in table which confirms to the research earlier carried out honey [4]. Honey is produced from different floral sources and as such its composition depends on the substrates that are utilized from plants and bees [4]. The chemical parameters obtained showed that honey is acidic and because of its acidic environment there is no development of harmful microorganism that survive well in honey called sugar tolerant yeast, mostly belonging to the genera Saccharomyces and Zygosaccharomyces [15]. Most of the solutes (Bricks) found in honey are carbohydrates and the sensitive analytical separation techniques having revealed honey to be a highly complex mixture of sugars, most of which are in the immediately digestible form in the small intestine [4]. According to the research, many of these sugars are found in the nectar but are formed during the ripening and storage effects of bee enzymes and the acids of the honey [16].In addition, the most predominant substances in honey are gluconic acid and the reducing sugars (fructose and glucose) are the major sugars.The composition of honey also contains various elements with the exception of zinc. This metal is very toxic and once present in the nectar can be poisonous to the bees. However, iron was found to be most abundant among the various elements detected.

The bacteria used during experiment were both gram positive and gram negative organisms. Each organism tested was analyzed separately and the comparison was carried out to determine against which organism is honey most effective. After treatment with pure honey it was observed that the undiluted honey was most effective against *E. coli* whereas 50 % dilution was most effective against *P. aeruginosa.*

At concentrations of 50% solution of honey, it was found that *Pseudomonas aeruginosa* and *Escherichia coli* were inhibited. However, this concentration was not effective against *Staphylococcus aureus* and *Candida albicans.* However, all three of the bacteria were inhibited by the pure honey but the fungus growth population was only affected when the pure form of the honey was directly applied. This shows that honey can be used as an antimicrobial agent. The data obtained can be supported by earlier works of Wahdan and co-workers [17] where they did a comparative study on honey and syrup and found that honey because of its high sugar content was effective against twenty one bacteria and two fungi. Also, when the inhibition zone of pure honey was compared to that of erythromycin control, it was found to have a greater inhibition zone than the antibiotic zone of inhibition.

The antimicrobial activity of 25% solution of honey had no effect on the various bacteria tested. According to literature, at a concentration of 30-50%, honey has been shown to be superior to certain convectional antibiotics. Conversely, this was not the same for *Candida albicans.* Research showed that pure honey suppressed fungi but when diluted to 50% and 30%, the honey lost efficacy against the fungi [18]. Similarly, 1% honey concentration had no effect on the organisms tested.

The effect of honey on the growth population of each tested microorganisms showed that honey can have both bacteriostatic and bactericidal activity [19]. When honey was applied directly to the growing populations of P. aeruginosa, E. coli, S. aureus and *C. albicans,* the population was inhibited. It was found that after separate treatment with 1ml of pure honey and 1ml of 50% diluted honey, the growth population of each plate was prevented and reduced. Analysis of this data corresponded with that of literature where it can be seen that both forms honey can affect bacteria but not *C. albicans* [18]. From the experiment, it was found that when honey is diluted there is an increase in the pH of the solution due to the high pH of water. Thus, the acidity of honey will be reduced and also its antimicrobial strength will decreased against pathogenic microorganisms. According to literature, it was found that in undiluted honey the acidity is a significant antibacterial factor but if the honey is diluted, the pH will not be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria [19].



Graph 1: Effect of diiferent concentration of honey on microbes

The graph above shows the different concentrations of honey on each microorganism and the zones of inhibition formed.

- *Pseudomonas aeruginosa (P)*
- *Escherichia coli (E)*
- *Staphylococcus aureus (S)*
- *Candida albicans (c)*
- The graph above indicates the effect of honey on each bacterium.
- Pure honey was more effective on bacteria but the difference is not significant.
- Diluted honey was more effective on *P. aerginosa*
- Diluted honey was only effective at 50 % concentration.
- *C. albicans* was not affected by honey disc diffusion method.

Table 1: Chemical properties of honey (Mean ± SD)

Parameters	Mean ± SD
pH	4.89±0.26
EC w (ms)	0.01±0.01
Brix	78.30±0.72
Water %	21.60±0.78
Sulphated Ash %	1.00±0.00
Acidity	3.17±1.53
Reducing sugar % (after inversion)	63.68±0.72
Reducing sugar % (before inversion)	67.9±1.390
Sucrose %	4.22±0.66
K (ppm)	23.0±6.68
Fe (ppm)	12.11±3.37
Zn (ppm)	0.00±0.00
Mg (ppm)	2.85±0.67
Cu (ppm)	0.32±0.24
Mn (ppm)	2.36±1.23

Table 2 Effect of honey concentrations on the tested microorganisms

	Honey Concentration (Inhibition Zone Mm)			
Species	Pure	50%	25%	1%
P. aeruginosa	18.74±5.30	16.44±1.67	0	0
E. coli	20.22±6.20	14.22±2.11	0	0
S. aureus	17.71±3.34	0	0	0
C. albicans	0	0	0	0

*Impact was measured in diameter of inhibition zones (mm) (mean ± SD)

Table 3: ANOVA analysis: two way factor analysis: both rows and columns

	F-calculated value	F-critical value
Rows (*P. aeruginosa, E. coli, S. aureus*)	1.29	5.14
Columns (concentration of honey)	13.80	4.76

Table 4: ANOVA analysis: Single factor analysis: column only

	F-calculated value	F- critical value
P. aeruginosa	484.55	2.9
E. coli	283.60	2.9
S. aureus	435.00	2.9

Analysis according to rows in table 3 shows that the effect of honey on the tested organisms was similar and thus insignificant. This was based on statistical data obtained which showed that at 0.05 probability level, the F-critical value was greater than the F-calculated value. Further, ANOVA analysis of the column showed that the F-calculated value was greater than the F-critical value at probability level 0.05. As a result, this indicates that the effect of honey was highly significant against bacteria.

There were significant differences of the antimicrobial activity of honey against each bacterium. In each case the statistical data obtained showed that the F-calculated value was always greater than the F-critical value at 0.05 probability level. Hence, the different dilutions of honey had significantly different effect on the size of inhibition zone.

Further, honey was cultured for any form of pathogenic microorganism but none was found. In addition, ghee was combined with honey to determine if its antimicrobial activity would increase but results obtained were negative since no inhibition zones were formed. The disadvantage of such a combination was due to the fact the honey is a viscous liquid and ghee is fat soluble. This may require further investigation for better refined techniques to overcome such difficulties.

CONCLUSION

Based on research conducted, this study showed that honey in its most concentrated form is very effective against pathogenic organisms. In some cases diluted honey up to 50% produces the same effect. Further, since honey is a cheap, easily available and also a non-toxic antimicrobial agent due to its properties, it can be very effectively used for medical purposes.

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