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*Full Length Research Paper*

# Total phenol, tocopherol and antibacterial quality of honey *Apis mellifera* sold in Owo community, Ondo State, Nigeria

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**The total phenol, the tocopherol and the antibacterial activity of different honey *Apis mellifera* sold in Owo Community, Ondo State, Nigeria were evaluated. The total phenol, expressed as garlic acid equivalents ranged from 2.85 mg/100 g 'Oja Oba' to 0.75 mg/100 g 'Hausa' honey, while the tocopherol ranged from 17.60/100 g 'Oja Oba' to 2.77/100 g 'Ilorin' honey. All the different honey sold in Owo Community were assayed for antibacterial activities against clinical isolates of *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli* and *Salmonella dysentriae* using the hole-plate diffusion method. 'Ikare' junction honey, 'Hausa' honey and 'Ilorin' honey showed the highest levels of antibacterial activity against all the bacterial used. The known safe use of honey without toxic effects suggest that honey could be used to treat diseases arises from these bacterial pathogens.**

**Key words:** Honey, total phenol, tocopherol, antibacterial activity, "Oja Oba.'

## INTRODUCTION

Honey is the natural substance produced by honey bees *Apis mellifera* in almost every country of the world. Raw honeys are usually produced by small farms and left in their natural state without undergoing processing (Blasa et al., 2006). Honey is essentially a concentrated aqueous solution of inverted sugar, but it also contains a very complex mixture of other saccharides, enzymes, amino acids, organic acids, polyphenols, and carotenoid-like substances maillard reaction products, vitamins and minerals (Gheldof et al., 2002). As collected from the honey comb, raw honey contains extraneous matter that is removed to make it marketable on a large scale (Wang et al., 2004). On the account of the nutritional value (303 kcal/100 g honey) and fast absorption of its carbohydrate, honey is a food suitable for humans of every age (Blasa et al., 2006). Honey is particularly recommended for children and sportsmen because it can help to improve the organism efficiency of the elderly and invalids (Blasa et al., 2006).

The beneficial role of honey was attributed to its anti-bacterial and anti-inflammatory properties with regards to its high osmolarity, acidity and content of hydrogen peroxide and non-peroxide components (Weston, 2000). The antimicrobial agents in honey is predominantly hydrogen peroxide of which the concentration is deter-mined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen (Weston, 2000). Current opinion suggests that oxidative and free radical-mediated reactions are implicated in degenerative processes related to aging (Ames et al., 1993) and various diseases such as cancer, athero-sclerosis and diabetes (Gutteridge and Halliwell, 1994). Almost all organisms possess antioxidant defense and repair systems that have evolved to protect them against oxidative damage but insufficient to protect them entirely (Oboh, 2005). Honey has been reported to be effective against enzymatic browning of fruits and vegetable, oxidative degeneration of some foods and in controlling the growth of or eliminating food borne pathogens (Chen et al., 2000; Taormina et al., 2001; Mckibben and Engeseth, 2002). Owo was the name of a Yoruba city-state in southwestern Nigeria that existed years 1400 and 1600.

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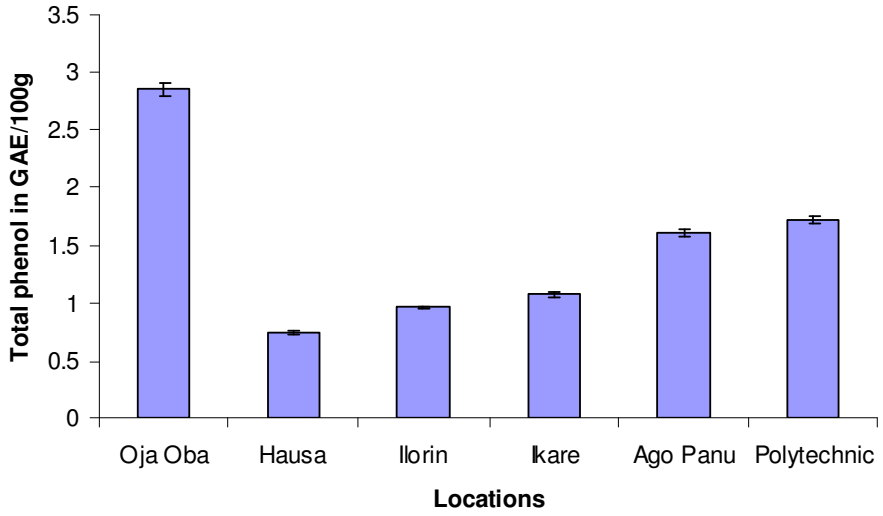


**Figure 1.** Total phenol content of honey sold in Owo community in Nigeria. Value represent mean of triplicate ± standard deviation.

Owo is also the name of a present-day town in the Ondo state, situated halfway between the towns of Ile Ife and Benin City. The present day town is an agricultural center, it is the location of a state polytechnic, a museum, a university (Achievers university) and the best hospital in the state and beyond (Federal medical center) (en.wikipedia.org). The purpose of the present study was therefore to evaluate the antioxidant and antibacterial quality of various honey sold in Owo community, Ondo State, Nigeria.

## MATERIALS AND METHODS

### Sources of honey

The honey used for this studies were collected from different locations where they were been sold in Owo community ("Oja Oba', honey hawked by the hausas, honey hawked by the Ilorins, Ikare junction, 'Ago Panu' and Polytechnic community).

### Source of microorganisms

The microbes used for this study were clinical isolates of *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli* and *Salmonella dysentriae*. They are medically significant organisms capable of causing infections. They were collected from the Federal Medical Center, Owo.

### Antioxidant analysis

The total phenol was determined by mixing 0.2 ml of the phenolic extract of the honey with 0.8 ml Folin-Ciocalteu reagent and 2 ml of 7.5% $Na_2CO_3$. The mixture was diluted using 7 ml distilled water and the absorbance was measured after 2 h at 765 nm, the result was calculated as garlic acid equivalent (Iqbal et al., (2004). The tocopherol content was determined using the AOAC (1990) method.

### Antibacterial assay

The antibacterial activity of all honeys was assayed by the Hole-Plate Diffusion Method. Each organism was cultured to 250 ml nutrient agar (OXOID) to reach concentrations of $10^3$. The mixture was shacked well and poured into sterile Petri dishes to obtain the media. The plates were left at room temperature for solidification. The wells were made in plates by using sterile cork borer (6 mm in diameter) and 150 µl of the neat undiluted honey was placed into each well with sterile micropipette. The plates were left at room temperature prior to incubation till the honey diffused. The plates were incubated at 37°C for 18 h. After incubation the inhibition zones were measured in millimeters (mm).

### Statistical analysis

All values were expressed as the mean standard deviation. Standard deviations were calculated using spread sheet soft ware (Excel©)

## RESULTS AND DISCUSSION

Phenols have antioxidant capacities that are much stronger than those of vitamin C and E (Oboh, 2005). Raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants (Blassa et al., 2006). The total phenol (Figure 1) content of honey sold in Owo community ranges from 2.85 mg garlic acid equivalent (GAE)/100 g 'Oja oba' to 0.75 mg GAE/100 g hawked by Hausa. These values of total phenol were low when compared with the value reported for Millefori and Acacia honey 3 - 17.5 mg caffeic acid equivalent (CAE)/100 g (Blasa et al., 2006) but in the range of values reported for sunflower honey (2.13 mg/100 g) (Lihu et al., 2005). Gheldof et al. (2002) had also reported 4.6 mgGAE/100 g Acacia ho-

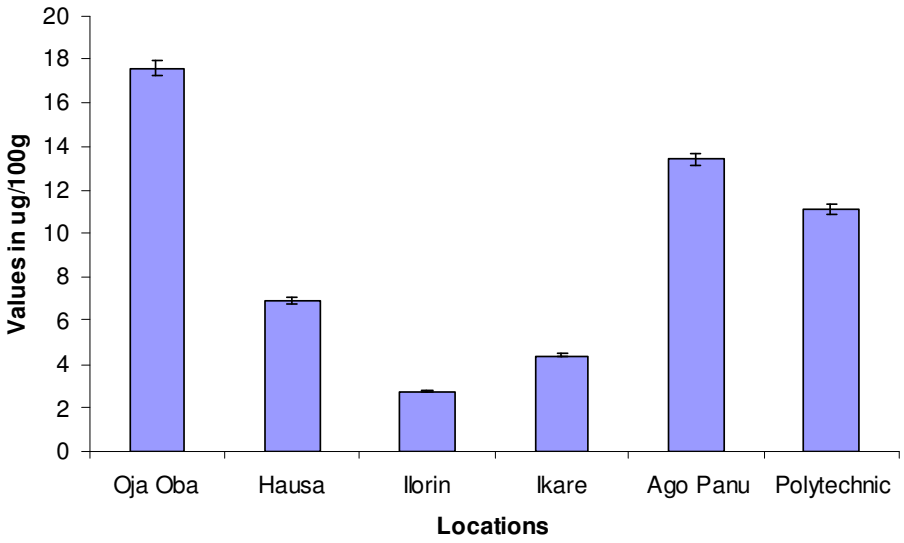


**Figure 2.** Tocopherol content of honey sold in Owo community in Nigeria. Value represent mean of triplicate ± standard deviation.

ney. It showed from the result that the honey from 'Oja Oba' had a high content of total phenol 2.85 mgGAE/100 g which was 3.0 fold higher than that of honey hawked by Hausa and it was significantly higher than honey from other sources in Owo community. There was no statistical significant difference in the total phenol of honey hawked by Hausa, 'Ilorin' people and that of 'Ikare' junction in Owo.

Tocopherol is the most important natural occurring compound with vitamin E activity (Esterbauer and Hayn, 1997). The tocopherol content of these honeys in Owo is reported in Figure 2. All the samples showed to contain tocopherol which varies from 17.60 µg/100 g 'Oja Oba' to 2.77 µg /100 g hawked by 'Ilorin' people. Honey sold in 'Oja Oba' showed a better tocopherol level (17.60 µg/100 g); however, they were not significantly different from the values obtained from honey sold in the polytechnic community and 'Ago Panu'. The values reported in this study are almost comparable to the tocopherol level of broccoli (Schlich et al., 2001). The variation in the antioxidant content of the honey sold in Owo could be due to the processing of honey which often removes many of the phytonutrients found in raw honey as it exists in the hive (Wang et al., 2004).

The antibacterial action of honey was first reported in 1892 by Van Ketel (Dustman, 1979). Honey is produced from many different floras, sources and its antimicrobial activity varies from origin and processing. The current prevalence of antibiotic resistance microbial species has led to a re-evaluation of the therapeutic use of ancient remedies of honey (SCST, 1998). Table 1 showed the antibacterial activity of honey sold at different locations within Owo Community in Nigeria. Honey sold at Ikare junction showed the best antibacterial activity against *S. aureus* with an inhibition zone of 10.2 ± 0.02 mm closely followed by honey sold by Hausas and Ilorins with inhibition zones of 9.3 ± 0.41 and 9.0 ± 0.06 mm respectively. This corroborates the works of Nzeako and Hamdi (2000), Bugadnov (1997) that honey has great antibacterial activity against *S. aureus*. Oja Oba honey had the least antibacterial activity as revealed by the inhibition zone of 6.3 ± 0.17 mm. *S. dysentriae* and *E. coli* were most recalcitrant of the test isolates to these different honeys. Honey sold by the Ilorins had the least zone of inhibition of 4.8 ± 0.31 mm on *S. dysentriae*, followed by Ikare junction honey (4.6 ± 0.14 mm) while Oja Oba and Rufus Giwa polytechnic honey showed least antibacterial activity against the isolate with inhibition zones of 3.5 ± 0.66 mm and 3.20 ± 0.18 mm respectively. Ikare junction honey showed the best antibacterial action on *E. coli* with inhibition zone of 7.00 ± 0.2 mm.

Ago Pannu honey and honey sold by the Hausas has inhibition zone of 6.5 ± 0.32 mm and 6.3 ± 0.22 mm respectively. The effectiveness of the honey sample on both *E. coli* and *S. dysentriae* agreed with the report of Postmes et al. (1993) Obi et al. (1994) and Cooper et al. (2002) that honey may be used for the treatment of skin wounds, various gastrointestinal diseases and antibiotic resistant strains of bacterial. The overall antibacterial activity of those honey samples showed that *P. mirabilis* was the least susceptible of the test isolates, Ikare junction had 9.5 ± 0.23 mm inhibition zone, Hausa honey had 9.3 ± 0.42 mm, Ilorin honey has 9.1 ± 0.51 mm while Oja Oba and Ago panu honey had 8.6 ± 0.23 mm and 8.5 ± 0.14 mm inhibition zones respectively. The fact that these honey samples inhibit the growth of the clinical isolate further confirms the antibacterial activity of honey as describe by Jedder et al. (1985). Honey was found to shorten the duration of diarrhea in patient with bacterial gastroenteritis caused by organism such as *Salmonella*

**Table 1.** Antibacterial activity of honey sold in Owo community in Nigeria on selected pathogenic bacteria.

| Honey samples | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***P. mirabilis*** | ***E. coli*** | ***S. Dysentnae*** |
| Oja Oba | 6.3 ± 0.17 | 8.6 ± 0.23 | 4.3 ± 0.14 | 3.5 ± 0.66 |
| Hausa | 9.3 ± 0.14 | 9.3 ± 0.42 | 6.2 ± 0.18 | 4.5 ± 0.24 |
| Ilorin | 9.0 ± 0.06 | 9.1 ± 0.51 | 6.5 ± 0.32 | 4.8 ± 0.31 |
| Ikare junction | 10.2 ± 0.02 | 9.5 ± 0.23 | 7.0 ± 0.20 | 4.6 ± 0.14 |
| Ago Panu | 8.7 ± 0.21 | 8.5 ± 0.14 | 6.3 ± 0.22 | 4.2 ± 0.17 |
| Rufus Giwa Poly | 6.5 ± 0.16 | 7.6 ± 0.18 | 2.3 ± 0.25 | 3.2 ± 0.21 |

Value represent mean of triplicate ± standard deviation.

sp, *Shigella* sp, *E. coli*, *Vibrio cholerae*, *Enterobacter* sp and other gram negative and gram positive organisms (Ibrahim, 1985; Obi et al., 1994).

Literature sources indicated that antibacterial activity of honey considerably depends on the flora sources (Allen et al., 1991) and they could be distinguished by their predominant plant composition. The present study showed that 'Oja Oba' and Polytechnic honey which has the highest total phenol and tocopherol content has low antibacterial activity while Ikare junction and Hausa honey with low antioxidant content has the highest antibacterial activity against the tested isolates, though the color of these honey samples were different. It could be that the honey with high antibacterial activity has a better hydrogen peroxide constituent. It was well established that the hydrogen peroxide activity in honey correlates with floral sources and antibacterial activity of the honey is mainly due to the enzymatic formation of hydrogen peroxide (Mollan, 1992; Baltrusaityte et al., 2007) of which the concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase, originating from flower pollen (Weston, 2000). The variations in the antibacterial activity of these honey samples could also be due to the various processing and storage method for the honey during transportation to Owo. It is likely that processing and/or storage condition had negative influence on the antimicrobial properties of honey samples (Baltrusaityte et al., 2007).

## Conclusion

This study also shows that total phenol as non-peroxide component of honey does not play active role in the antibacterial activity of honey. The known safe use of honey without toxic effects suggest that honey could be used to treat diseases arises from these bacterial pathogens. Honey is shown to be a potential and suitable therapeutic agent against these bacterial pathogens in the search for a solution to the problem of antibiotic resistant strains of bacteria.

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