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EFFECT OF HEAT ON THE ANTIBACTERIAL ACTIVITY OF HONEY ON BACTERIAL ISOLATES

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ABSTRACT

In this study, the antibacterial activity of raw honey and commercially sold honey on *Serratia marcescens, Proteus mirabilis* and *Vibrio cholera* was evaluated. Raw honey which was gotten from farmers in Etche community and two processed honey (Rowse and Princenic global honey) were bought from a superstore. The different honey samples were divided into two set. The heated honey and the unheated honey. The heated honey samples were heated for three days in a water bath at 90 ºC. Their antibacterial activities were evaluated using the well in agar method. Different concentrations of the honey samples were transferred into 6mm holes in freshly prepared Mueller-Hinton agar plates which have been seeded with the test isolates. This was done in duplicates. The antibacterial activities of the different honey samples showed that the zones diameter increased with increased concentration with the 100% concentration having the highest zones of inhibition. The zone diameter (mm) of the heated raw honey at 100% concentration on *Proteus, Serratia* and *Vibrio* was 17.50±0.71, 21.50±0.71 and 19.50±0.71, respectively. Despite the difference in the zone diameter, there was no significant difference at $P \leq 0.05$ in the zone diameters at the 100% concentration. The zone diameter of the unheated Raw honey at 100% concentration on *Proteus, Serratia* and *Vibrio* was 18.00±2.83, 28.50±7.78 and 22.00±0.00mm, respectively. The zone diameter (mm) of Rowse and Princein heated honey at 100% concentration on *Proteus, Serratia* and *Vibrio* were (20.00±0.00, 25.00±0.00, and 20.00±0.00mm) and (19.00±0.00, 26.00±1.41, and 24.50±3.54mm), respectively. More so, there was no significant difference of the efficiency of the Rows honey on the different isolates at 100% concentration. This is also similar to the PG honey at $p = 0.005$. The unheated honey showed higher zones of inhibition than the heated honey. Thus, the type of honey, the heat, the microorganism and the concentrations of honey were factors that affected the antimicrobial efficiency of the honey.

KEYWORDS: *Honey, Serratia, Vibrio cholera, Proteus mirabilis, raw honey, processed honey.*

INTRODUCTION

Honey is the natural sweet substance from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which honey bees collect, transform, and combine with specific substances of their own to ripen and mature. It is also defined as the nectar and saccharine exudation of plants, gathered, modified and stored as honey in the honeycomb by honeybees (Khandal *et al.*, 2010). It was reported that honey is the only food sweetener which can be used industrially without further processing (Bogdanov *et al*., 2004). Honey is a concentrated aqueous solution composed of a mixture of glucose and fructose but also contains at least 22 other complex carbohydrates, various amino and organic acids, proteins, antibiotic rich inhibin, enzymes, phenol antioxidants, aroma compounds, vitamins, minerals, pigments, waxes and pollen grains (Bogdanov et al., 2007). In a study by Aggad and Guemour (2014), it was reported that the antibacterial activity of honey varies greatly with the origin and method of processing since honey is produced from different sources. The antibacterial activity of honey is well documented and its antibacterial activity on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas* spp have been reported (Al- Naama, 2009).

Hydrogen peroxide and gluconic acid which originates from the dissolution of sugar by honey's glucose oxidase are the two main factors which have been reported to be associated with the potency of honey against bacteria (Ruiz-Argüeso and Rodriguez-Navarro, 1973). The use of honey in the treatment of infected wounds resulting from post-operation has been documented [Al-Waili and Saloom, 1999], also, Radwan *et al*. [1984] in a study reported that honey inhibited the proliferation of *Salmonella* and *Escherichia coli* during an invitro analysis. Due to the antibacterial activity of honey, it is used in clearing infections in wounds and protecting them from becoming infected (Boukraa *et al.*, 2008). According to Molan (2001), honey is very much important in treating infected surgical wounds, burn wounds, and decubitus ulcers (bedsores); and that it

keeps up a damp injury condition that advances recuperating, and its high consistency gives a defensive obstruction to avert microbial contamination. Low concentrations of this recognized antiseptic are compelling against irresistible microscopic organisms and can play a role in the injury recuperating system (Molan, 2001) and in incitement and expansion of fringe blood lymphocytic and phagocytic action. Also, the gentle causticity and low-level hydrogen peroxide discharge helps both tissue repair and adds to the antibacterial action (Mullai and Menon, 2007). Furthermore, a wide range of honey have high sugar content yet a low water substance and acidity, which forestall microbial development. Most categories of honey produce hydrogen peroxide when weakened (diluted) as a result of the initiation of the glucose oxidase enzyme, which oxidizes glucose to gluconic acid and hydrogen peroxide (Schepartz and Subers, 1964). Hydrogen peroxide is the significant supporter of the antimicrobial action of honey, and the various convergences of this compound in various honey bring about their changing antimicrobial impacts (Molan, 1992). Other than its antimicrobial properties, honey can clear microbial contamination in various ways, including boosting the body natural defense framework, having anti-inflammatory and antioxidant activities, and by means of incitement of cell development [Al-Jabri, 2005]. There is no doubt that the activities of honey in respect to its antimicrobial action is well documented. Previous studies have evaluated this antimicrobial activity by either extracting the honey using different solvents or the direct use of the honey at different concentrations. Studies relating to the effect of heat on the antibacterial activity of honey is scanty. Reports of the presence of naturally occurring spore forming organisms which cause infant botulism to children between 0-1-year-old, who consume the substance have been documented (Prescott *et al.*, 2011). Thus, this study was aimed at investigating the antibacterial activities of different honey samples when subjected to heat treatment.

MATERIALS AND METHOD

Honey Samples

Freshly harvested raw honey was bought from farmers in Etche community, Rivers State, Nigeria. Rowse honey and Princenic honey were bought from a superstore in Port Harcourt. Honey samples were sent to the department of microbiology where they were preserved at 4˚C in a refrigerator for further analysis.

Bacterial Isolates

Proteus mirabilis and *Vibrio cholera* were isolated from oven dried crabs sold in Borikiri market in Port Harcourt, Rivers State. *Serratia marscence* was isolated from a well water sample in Ula-Ubie community, Ahoda, Ahoda-East local government, Rivers State, Nigeria.

Preparation of Honey Samples

The honey samples were divided into two batch; heated honey samples and unheated honey samples. The heated honey samples were heated in a water bath at 90˚C for fifteen minutes interval for 3 days until spore producing organisms were killed. The unheated honey samples were not subjected to any form of heat.

Concentrations of 100, 80, 60, 40 and 20%, of the honey samples (both heated and unheated) were prepared and were used to evaluate the antimicrobial activity of the honey on the three bacterial isolates. The concentrations were achieved by diluting the honey samples in 20mL, 40mL, 60mL and 80mL sterile distilled water for the 80%, 60%, 40% and 20% concentrations, respectively. The 100% concentration represent absolute honey which was not diluted.

Standardization of Inoculum

The bacterial inoculums were standardized using the method described by Cheesbrough (2005). In this method, colonies of the investigated bacterial isolates were suspended in 4mL sterile normal saline and the turbidity was compared with freshly prepared 0.5McFarland standard.

Antibacterial Activity of Honey Samples

The well in agar method was used in determining the antibacterial activity of the honey samples. Standardized bacterial isolates were seeded using sterile swab sticks on freshly prepared Mueller-Hinton agar plates. The seeded plates were allowed to dry (Wemedo and Robinson, 2018) for 3 minutes before 6mm wells were bored using a sterile cork borer. The wells were bored in such a way that it did not penetrate the bottom to expose the bottom of the Petri dish. Different concentrations of the honey samples were introduced into the wells and the wells were well crammed with the honey samples. The analysis was done in duplicates, after which the plates were incubated at 37˚C for 24 hours in the incubator. After incubation, clear zones around the wells were measured and recorded.

Statistical Analysis

The results obtained were expressed as mean ± standard deviations. The analysis was done using the SPSS version 22. The Duncan was used to separate the means and differences were considered significantly when $P \leq 0.05$.

RESULT

The result showing the antibacterial activity of the unheated and heated Rows honey is presented in Tables 1 & 2. The antibacterial activity of the unheated rows honey showed that at the 100% concentration, *Vibrio cholera* and *Serratia marcescens* had the highest zones of inhibition which was recorded as 27.50±0.71 mm while the zone of inhibition of *Proteus mirabilis* was 20.00±4.24 mm. The result also showed that there were very high zones of inhibition of the rows honey on the

three bacterial isolates at 80%, 60% and 40%. At 20% concentration, *Vibrio cholera* was more susceptible followed by *Serratia marcescens*, while the least susceptible isolate with zone diameter of 11.00±1.41 mm was *Proteus mirabilis* (Table 1). The result of the heated rows honey presented in Table 2 showed that at 100% concentration, *Serratia marcescens* was more susceptible and the zone diameter was 25.00±0.00, while *Vibrio cholera* and *Proteus mirabilis* were the second most susceptible with zone diameter of 20.00±0.00mm. The result also showed that at 80 % and 60 % concentration, *Serratia marcescens* was the most susceptible bacterial with zone diameter of 20.00±0.00mm and 18.00±0.00, respectively. While at 40% and 20% concentrations, *Vibrio cholera* had the highest zones of inhibition of 13.00±0.00mm and 10.00±0.00mm, respectively (Table 2).

Table 1. Zone Diameter (mm) of Unheated Rows Honey on the bacterial isolates.

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	27.50±0.71[a]	25.00±0.0 [a]	23.0±2.83 [a]	20.50±2.12 [a]	18.50±2.12[a]
Serratia marcescens	27.50±0.71 [a]	24.00±0.00 [a]	23.50±0.71 [a]	18.50±0.71 [a]	14.50±2.12[ab]
Proteus mirabilis	20.00±4.24 [a]	19.50±0.71[b]	23.00±2.83 [a]	16.50±3.54 [a]	11.00±1.41[b]

Means with similar superscripts across the column have no significant difference at P ≤ 0.05

Table 2. Zone Diameter (mm) of Heated Rows Honey on the bacterial isolates.

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	20.00±0.00 [a]	17.00±0.00 [a]	15.00±0.00 [a]	13.00±0.00 [a]	10.00±0.00 [a]
Serratia marcescens	25.00±0.00 [a]	20.00±0.00 [a]	18.00±0.00 [a]	10.00±0.00 [a]	5.00±0.00 [a]
Proteus mirabilis	20.00±0.00 [a]	17.00±0.00 [a]	15.00±0.00 [a]	9.00±0.00 [a]	3.00±0.00 [a]

Means with similar superscripts across the column have no significant difference at P ≤ 0.05

The antibacterial activity of the unheated and heated Princenic global honey (PG) on the bacterial isolates is presented in Tables 3 and 4. The result in Table 3 showed that *Serratia marcescens* was the most sensitive bacterial isolates with zone diameters of 26.00±1.41, 25.00±1.41, 23.00±0.00, 18.50±0.71 and 11.00±2.83 for 100%, 80%, 60%, 40% and 20% concentrations, respectively. *Vibrio cholera* was the second most sensitive bacterial isolate at 80% concentration and was completely resistant at 20% concentration. In Table 4, *Serratia marcescens* and *Vibrio cholera* were the most sensitive bacterial isolates to the heated Princenic global honey (PG) and the zone diameters were very high. *Proteus mirabilis* had the least zone diameter of 4.00±01.41 at the 20% concentration (Table 4).

The effect of the unheated Raw honey on the bacterial isolates presented in Table 5 shows that the raw honey was very potent on the three bacterial isolates at the 100%, 80% and 60% concentrations. Though higher zones of inhibition were observed at the 40% and 20% concentrations on *Serratia marcescens* and *Proteus mirabilis*, *Vibrio cholera* was completely resistant at 20% concentration of the raw honey. Furthermore, the antibacterial activity of the heated raw honey on the bacterial isolates showed that *Serratia marcescens* was the most sensitive isolate with zones of 19.50±0.71mm at the 100% concentration and the second most sensitive bacterial isolates at 80% concentration (Table 6). The result also showed that *Proteus mirabilis* which was sensitive to the heated raw honey at the 100%, 80% and 60% concentrations exhibited high level of resistance (0.00±0.00mm) at 40% and 20% concentrations, respectively.

Table 3. Zone Diameter (mm) of Unheated Princenic global Honey on the bacterial isolates.

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	24.50±3.54 [a]	23.00±2.83[ab]	18.00±1.41 [a]	13.50±2.12 [a]	0.00±0.00 [a]
Serratia marcescens	26.00±1.41 [a]	25.00±1.41[b]	23.00±0.00 [b]	18.50±0.71 [a]	11.00±2.83 [b]
Proteus mirabilis	19.00±0.00 [a]	17.50±2.12[a]	19.50±0.71 [a]	15.00±2.83 [a]	11.50±0.71 [b]

Means with similar superscripts across the column have no significant difference at P ≤ 0.05

Table 4. Zone Diameter (mm) of Heated Princenic global Honey on the bacterial isolates.

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	20.50±0.71[a]	18.50±0.71 [a]	15.50±0.71 [a]	11.00±0.00 [a]	9.50±0.71 [a]
Serratia marcescens	22.00±1.41 [a]	18.50±0.71 [a]	15.50±0.71 [a]	12.50±0.71 [a]	10.00±1.41 [a]
Proteus mirabilis	19.50±0.71 [a]	14.00±0.00[b]	11.00±0.00[b]	8.50±0.71[b]	4.00±01.41 [b]

Table 5. Zone Diameter (mm) of Unheated Raw Honey on the bacterial isolates

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	22.00±0.00 [a]	20.00±0.00 [a]	17.00±11.31 [a]	13.50±2.12 [a]	0.00±0.00 [a]
Serratia marcescens	28.50±7.78 [a]	18.50±2.12 [a]	19.00±0.00 [a]	17.00±0.00 [a]	12.50±2.12 [b]
Proteus mirabilis	18.00±2.83 [a]	20.00±9.90 [a]	12.50±0.71 [a]	10.50±7.78 [a]	9.50±0.71 [b]

Table 6. Zone Diameter (mm) of Heated Raw Honey on the bacterial isolates.

Isolates	100%	80%	60%	40%	20%
Vibrio cholera	19.50±0.71 [ab]	15.50±0.71 [a]	12.50±0.71 [a]	10.50±0.71 [a]	9.00±0.00 [a]
Serratia marcescens	21.50±0.71 [b]	14.50±0.71 [a]	10.50±0.71 [ab]	6.50±0.71 [b]	4.50±0.71 [b]
Proteus mirabilis	17.50±0.71 [a]	11.50±0.71 [b]	9.50±0.71 [b]	0.00±0.00 [c]	0.00±0.00 [c]

DISCUSSION

The effect of heat treatment on the antibacterial activity of honey samples on *Proteus mirabilis, Serratia marcescens* and *Vibrio cholera* was investigated. The results showed that the antibacterial activity of the different honey samples varied across the type of honey and the concentrations of the honey. This agreed with Saad *et al.* (2017) who also reported that different types of honey possess different efficacies and mechanisms against the same type of bacteria. Higher concentration of the honey exhibited higher zones of inhibition on the tested bacterial isolate whereas at low concentrations, the zone diameter reduced. Some isolates were not affected at lower concentrations. Moist heat was used in treating the different honey samples as the presence of spore forming bacteria was detected in the three honey samples. Reports of the presence of spore formers in honey which makes honey not good for children below one year old is well documented (Prescott *et al.*, 2011). The effect of heat on the antibacterial activity of the different honey samples was compared with honey samples which were not treated with heat. The results showed that the zone diameters produced by the honey samples which were treated with heat on the different bacterial isolates were less than the zone diameters of honey samples not treated with heat. Thus, the none heat treated honey were more effective on the bacterial isolates than heat treated honey. Subjecting the honey samples to heat at 90℃ could have denatured vital components or nutrients which led to the reduced antibacterial effects. This agreed with findings of Sandra *et al.* (2016) who reported that different processing or storage conditions could affect the constituents of honey, thereby modifying or altering its antimicrobial activity. The use of honey in the treatment of infections caused by bacteria, viruses and fungi has been documented (National honey board, 2002; Oelschlaegel *et al.*, 2012). The findings in this study showed that the effect of natural honey was less than the effect of the processed honey (sold honey). Despite this disparity, the natural honey still possessed great antimicrobial activity against the bacterial isolates as seen in Table 5 & 6. The zone of inhibition of raw (not heated) honey at 100% concentration on *Vibrio, Serratia* and *Proteus* was 27.50±0.71, 27.50±0.71 and 20.00±4.24 mm, respectively. Abhishek *et al.* (2010). Reported that the maximum zone of inhibition produced by raw honey extracts against *P. aeruginosa* and *S. typhi, E. coli, B. cereus, S. aureus* and *B. subtilis* was 35.95, 34.39, 17.51, 11.11, 8.90 and 8.55mm. It has been reported that natural honey contains carbohydrate, water and minor components such as proteins, minerals, phytochemicals and antioxidants and these minor ingredients are responsible for the pharmacological activities of the honey (Saad *et al.*, 2017). The findings in this study agreed with findings of other studies which had previously reported that honey possess antimicrobial activities against wide varieties of microorganisms (Oelschlaegel *et al.*, 2012; Saad *et al.*, 2017; Abhishek *et al.*, 2010). Other studies acknowledged that the antimicrobial activities of honey are influenced by osmolarity, pH, production of hydrogen peroxide, flavonoids, phenolic compounds and the presence of other phytochemical components, such as methylglyoxal, leptosin, melanoidins, bee defensing, jelleins, and hydroxyl radicals (Lee *et al.*, 2008; Kato *et al.*, 2012).

CONCLUSION

In respect to the findings in this study, the antibacterial activity of honey depends on the type of honey and the concentration of the honey used for treatment of infections. Also, treating honey with moist heat at 90℃ for fifteen minutes in three consecutive days could kill microbial spores present in the honey without having much effect on the antibacterial activity of the honey.

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