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ISOLATION AND IDENTIFICATION OF BACTERIA FROM RAW AND COMERCIALLY PROCESSED HONEY AND EVALUATION OF THEIR ANTIBACTERIAL PROPERTIES

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ABSTRACT

Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical profession. There have been reports that honey contains many microorganisms including bacteria and fungi. This study was carried out to investigate the bacterial quality of raw and commercially processed honey sold at Khulna city by local honey collectors and different super markets in Dnajpur city respectively. And also, to evaluate In vitro antibacterial activity of raw and commercially available honey against three-gram negative Bacteria (E. coli. Pseudomonas spp. and Shigella spp.) at different concentrations of 100%, 75%, 50% and 25%. The results of the isolation and identifications show that raw honey contained more bacterial load than the commercially processed honey. Bacillus spp. and Staphylococcus spp. were isolated from raw honey whereas only Staphylococcus spp. is found in commercially processed honey. However, both types of honey showed antibacterial activity against test organisms with the zone of inhibition ranging from 6.0 to 40.0mm. The potency of honey at 100% concentration was found to be higher than all other concentrations tested. However, no effect was observed at concentration of 25% v/v honey in the case of both samples.

Key words: *Raw honey, commercially processed honey, antibacterial activity.*

INTRODUCTION

Honey is the sugary substance produced from the nectar of flowers by the worker bees. As defined by the (Codex Alimentarius Commission, 2001), honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects living on parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. Bees use a variety of plants to create honey, consequently compositional differences that can influence the value of a specific honey for medicinal or health promoting purposes arise (Flodhazi, 2004). Bees that produce enough honey worth harvesting belong to two subfamilies of the family Apidae: Apinae (honeybees) and Meliponinae (stingless bees). Apinae has only one genus *Apis* of which the species *Apis mellifera* is of much greater economic importance than any other. The bases of the world's beekeeping industry are races and strains of the honeybee *Apis mellifera* (Crane, 1990).

Honey as a natural product has various constituents (Bertoncelj *et al.*, 2007). Honey is a supersaturated sugar solution of two sugars, glucose and fructose, with small amounts of other more complex sugars. Many other substances also occur in honey such as flavonoids, phenolic acids, water, vitamins, organic acids, proteins, phytochemicals and minerals which are largely responsible for the differences among individual honey types (White and Doner, 1980; Bertoncelj *et al.*, 2007). It has been used from ancient times as a method of accelerating wound healing (Van den Berg *et al.*, 2008; Mullai and Menon, 2007). Traditional importance and use of honey as therapeutics have been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago (Maryann, 2000; Patton *et al.*, 2006). It has been shown that natural unheated honey has some broad-spectrum antibacterial activities when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Mundo *et al.*, 2004). Natural honey exhibits bactericidal activity against many organisms including Salmonella, Shigella, Escherichia coli (Alvarez; *et al.*, 2010 and Echazarreta, J.A; 1996), *Helicobacter pylori* (Chowdhury M; 1999), etc. Honey in spite of its usefulness is known to contain certain microbes. It is in fact described as a reservoir for microbes. Some view the use of honey to treat infected wounds, with skeptic. For example, an editorial in the Archives of Internal Medicine in 1976 on medical folklore (Soffer A; 1976) ridiculed the use of honey, placing "honey from selected geographic areas" in the category of "worthless but harmless substances". Micro-organisms in honey may influence the stability of the products and its hygienic quality (Snowdon; 1996). Due to the natural properties of honey and control measures in the honey industry, honey is a product with minimal types and levels of microbes. Microbes of concern in post-harvest handling are those that are commonly found in honey (i.e., yeasts and spore-forming bacteria), those that indicate the sanitary or commercial quality of honey (i.e., coliforms and yeasts), and those that are under certain conditions could cause human illness. Primary sources of microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust, air, earth and nectar sources which are very difficult to control (Popa, M; 2009). The same secondary (after-harvest) sources that influence any food product are also sources of contamination for honey. These include air, food handlers, cross-contamination, equipment and buildings. Secondary sources of contamination are

controlled by hygienic processing practice (Snowdon; 1996).

Since both raw honey and commercially available honey is used extensively in Nigeria and globally, the present study is undertaken to isolate and identify the different bacterial specie present in raw and processed honey. And also to evaluate in vitro antibacterial activity of raw and commercially processed honey against three different bacterial species such as *Escherichia coli, Pseudomonas aeruginosa and Shigella spp.*

MATERIALS AND METHOD

Study area

Two kinds of honeys were used in this study. They are raw honey and commercially processed honey. Raw honey was collected from the Khulna city and commercially processed honey was bought from the Dnajpur market.

Collection of honey samples

Sample of raw honey was collected directly from local honey collectors by aseptic way in a sterile bottle. Different brands of commercially available processed honey were collected from the super market. The samples were kept in the dark at room temperature. Sterility of honey was checked by spreading a loopful quantity on nutrient agar medium and incubated at 37^0C for overnight. The samples, in which growth was observed, were used for isolation and identification of bacteria. Whereas those samples in which no growth was observed were used to test the antibacterial activity of the honey.

Preparation of sample

Different concentrations of the samples (100%, 75%, 50% and 25%) were made. The results are obtained in the form of reduce number of bacterial colonies in order to get pure colonies. Each of the colonies was seen for size, shape, pigment, diameter, form, margin and elevation.

Determination of antimicrobial activity of honey against test organisms

Antimicrobial activity of honey against test organisms was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (Barry and Thomsberry, 1985). For this assay following test organisms were used: E. coli, Psudomonas sp, Shigella sp.

RESULTS

Result of morphological, staining, cultural, and biochemical characteristics of the isolated organisms and antibacterial activity of the honey against test organisms are presented in table 1, 2, 3 and 4 below.

Table 1: Cultural, morphological and biochemical properties of isolated *Staphylococcus spp.*

Cultural characteristics		Biochemical Characteristics		Staining and morphological Characteristics
Nutrient Agar	MSA Agar.	Tests	Results	Staining properties
Circular, small, smooth, convex and gray-white or yellowish colonies were produced were prod were		Dextrose	Acid	Gram positive cocci arranged' in grape like cluster
		Maltose	Acid	
		Lactose	Acid	
		Sucrose	Acid	
		Mannitol	Acid	
		Catalase test	+	
		MIU	+	
		VP	+	
		TSI	+	
		MR	+	
		Indole test	–	

Table 2: Cultural, morphological and biochemical properties of isolated *Bacillus spp.*

Cultural characteristics				Biochemical Characteristics		Staining and morphological Characteristics
NA	EMB	SS	Mac conkey	Tests	results	Staining properties
Thick, grayish white or cream-colored colonies were produced.	No growth	No growth	No growth	Dextrose Maltose Lactose Sucrose Mannitole Catalase test VP MR Indole test	Acid Acid Acid Acid Acid + - - +	Gram-positive large rod-shaped organisms arranged in chain.

Legends:
AG = Acid and Gas, MR = Methyl-Red test, VP = Voges-Proskauer test,
+ = Positive reaction, - = Negative reaction, EMB = Eosin methylene Blue, MC = Mac Conkey

Table 3: Determination of antimicrobial activity of commercially processed honey against test organisms.

Test organisms	Concentrations of honey sample (v\v)				Inhibition zone (diameter in mm)			
Escherichia coli	100%	75%	50%	25%	27.0 mm	17.0 mm	8.0 mm	-
Pseudomonas spp.	100%	75%	50%	25%	38.0 mm	15.0 mm	9.0 mm	-
Shigella spp.	100%	75%	50%	25%	19.0 mm	12.0 mm	6.0 mm	-

Table 4: **Determination of antimicrobial activity of raw honey against test organisms.**

Test organisms	Concentrations of honey sample (v\v)				Inhibition zone (diameter in mm)			
Escherichia coli	100%	75%	50%	25%	29.0 mm	18.0 mm	10.0 mm	-
Pseudomonas spp.	100%	75%	50%	25%	40.0 mm	17.0 mm	9.0 mm	-
Shigella spp.	100%	75%	50%	25%	19.0 mm	15.0 mm	8.0 mm	-

DISCUSSION

A series of tests were conducted for isolation and identification of various types of bacterial species suspected to be present in the honey. Moreover, antibacterial effects of honey against three different species of bacteria were also conducted in this study.

However, the result of isolation and identification of the two types of honey revealed that both commercially processed and raw honey was found to contain some microbes, and the organisms identified in raw honey are *staphylococcus spp.* and *Bacillus spp.* whereas only *staphylococcus spp.* was identified in the commercially processed honey sample (Table 1 and 2). This finding is somewhat similar to the finding of Sacklet WG (1919) who observed that *Bacillus, Micrococcus* and *Saccharomyces* species could be readily isolated from honeycombs and adult bees. Due to the natural properties of honey and control measures in honey industry; processed honey is a product with minimal types and levels of microbes. While honey easily gets contaminated during the process of its production by bees and microorganisms also get introduced in to honey by activities of man including equipment, containers, wind, dust, the status of microorganisms found in honey is dormant. It has been observed that if honey is diluted with water, it supports the growth of non-pathogenic bacterial strains and killing of dangerous strain (White P.B 1996). The presence of *Bacillus spp.* may cause food poisoning. But it was present in marginal level and unable to produce disease.

The inhibitory actions of extracts of commercially processed and raw honey were evaluated against three bacterial strains, Gram negative bacteria such as *E. coli, Shigella spp.* and *Pseudomonas spp.* Both the commercially processed and raw honey sample showed the zone of inhibition ranged from 0 – 40 mm. The results of the antibacterial activity assays indicated that the honey have inhibitory activity against all the test organisms. On the other hand, *P. aeruginosa* have shown to be more susceptible to honey at 100% concentration than *E. coli* and *Shigella spp.* The potency of honey at 100% concentration was found to be higher than all other concentrations tested. However, no inhibitory effect was observed against any of the test organisms at 25 %(v/v) concentration (Table 3 and 4). The findings in this research nearly resembles that of others (El-Amari and Ben-Gweirif, 2010; Mulu et al., 2004; Cooper et al., 1999, 2002; French et al., 2005; Nzeako and Hamdi, 2000; Agbaje et al., 2006;

Basson and Grobler, 2008) who found that honey inhibited the growth of *S. aureus, E. coli, Shigella spp.* and Pseudomonas sp. and 100% concentrated honey is more effective than other concentrations (El-Amari and Ben- Gweirif, 2010). In the case of Shigella spp., our results differ from the result of other researchers (Mulu et al., 2004). This finding was also near similar the earlier reports that were made by Eman A. khairy; *et al.* (2013) in which they reported that *Pseudomonas spp.* was highly sensitive to flower and mountain honey.

CONCLUSIONS

From the present study, we can simply have concluded that both raw as well as processed honey can be the potential antimicrobial substance for control of different types of bacterial pathogens, it exhibits both bacteriostatic and bactericidal properties on both Gram-positive as well as on Gram-negative bacteria and also as a natural product has various constituents that help and promote human health. However, in spite of it's important to human health, it was also found to contain some microorganisms that may influence its nutritional and medicinal properties.

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