Comparison of Antimicrobial Activity of Honey from Various Floral Sources against Food Spoilage Bacteria

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Abstract

Honey is a natural product that has found to possess numerous beneficial properties. In this study, antimicrobial activity of honey produced by various floral sources: black cumin, litchi, mustard, wild honey from the forests of Sundarban (one was new and another was stored) and a commercial honey (Dabur honey) was tested against four food spoilage bacteria: *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Salmonella spp.* by disc diffusion assay. All honeys were checked at four concentrations (1%, 5%, 10% and 100% v/v) and the mean zones of inhibition were measured. The effect of temperature on the antibacterial activity was determined by treating the honey samples at three different temperatures: 50°C, 75°C and 100°C. Some physicochemical properties such as color, pH, moisture content and brix value of these honeys were also measured using standard methods. Research findings showed that, all honey samples had an inhibitory effect on the growth of bacteria. Among them, honey from black cumin flower was found to be the most active followed by mustard, litchi and Sundarban honeys. Of all honeys, commercial honey was the least active. None of the honeys were active at 1% (v/v) concentration, as the concentration increased activity also increased. Although, antibacterial activity found to be reduced with increasing temperature treatment, but it was remain effective even after heat treatment at 100°C for 30 minutes. The results of this study confirmed the possibility of using honey in preventing the growth of food spoilage bacteria to extend the shelf life of foods.

Key Words: Antibacterial activity, Food safety, Food spoilage bacteria, Zone of inhibition

Introduction

Human being need food for their survival, but this food could be the cause of disease or death if it contains deleterious physical, chemical and microbial components. For this reason from ancient time human being have tried to keep their food safe from all harmful agents. Today modern science has invented many sophisticated technology and approach to combat food borne diseases [1]. In spite of modern improvements in food production and processing techniques, food safety is an increasingly important public health issue [2].

It has been estimated that approximately 1.8 million children in developing countries (excluding China) died from diarrhoeal disease in 1998 [3]. According to WHO, in 2004 at least sixty nine thousand people died from diarrhoeal disease in Bangladesh caused by microbiological agents, mostly originating from food and water [4].

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So it is evident that, there is still a need for new methods of reducing or eliminating food borne pathogens, possibly in combination with existing method [5]. At the same time, consumer and health specialist are concerned about the safety of food containing synthetic chemicals as preservatives because of the side effects and carcinogenicity produced by the synthetic chemicals [2]. On the other hand, it was specified in a recent FAO report that one-third of food produced for human consumption is lost or wasted globally, which amounts to about 1.3 billion tons per year [6]. One of the cause of this wasting is spoilage and lack of preservation facilities in low income countries. It is a matter of great sorrow that everyday 870 million people go hungry where around 1.3 billion ton food is wasted each year.

Moreover, there is also alarming reports about increasing antibiotic resistance of bacterial food borne pathogens [3]. For these reasons, there is a growing interest in the use of natural antibacterial compounds present in food, because they have both characteristic flavor as well as a potential antimicrobial activity [7].

Thus, the antibacterial activity of honey has become subject of considerable interest. Honey is a natural product from honeybee *Apis mellifera*. It has many useful properties and is one of the most commonly used natural antimicrobial agents in food. It has been used traditionally for thousand of years by many cultures for preserving foods as well as food additives to enhance aroma and flavor. Recently honey has received attention in their useful physiological functions and antioxidant activity [8]. In spite of a wide body of research on the antibacterial property of honey in different parts of the world, to the best of our knowledge, systematic study on the possible antibacterial activity of Bangladeshi honey has not yet been done. Similarly, the large variation in the antimicrobial activity of honeys from different floral sources is still unknown. Moreover, the effect of heat treatment and storage on honey is also not realized. Hence, the present study was designed to identify the antibacterial activity of honey from various floral sources along with some physicochemical properties to give a general idea about the variation in their activity and also the effect of heat treatment on honey samples.

Methods and Materials

Materials

Honeys from various flora sources such as black cumin, litchi, mustard, wild honey from the forests of Sundarban (one new and one stored) and a commercial honey were used in this study. The honey samples were collected from local market and kept in a cool and dark place (at room temperature). Four bacterial species known to cause food spoilage such as *Staphylococcus aureus* (BTCC 43), *Bacillus cereus* (BTCC 19), *Eschericia coli* (ATCC 25922), and *Salmonella spp.* (BTCC 197) were used. These strains were obtained from the microbiology lab of Institution of

Nutrition and Food Science, University of Dhaka. Nutrient agar and nutrient broth used as culture media.

Methods

Physicochemical properties like color, pH, moisture content and brix value of honey samples were determined using methods adopted by the International Honey Commission [9].

In vitro screening was carried out using disc diffusion method [10]. In this study, original honey sample were considered as 100% concentrated. From these samples 1%, 5%, 10% solutions were prepared using sterile distilled water.

In disc diffusion method, surface of freshly prepared nutrient agar plates were uniformly inoculated with overnight stock cultures that were previously prepared in nutrient broth. Discs made from Whattman filter paper n.1 (ADVANTEC; Toyo Roshi Kaisha, Ltd, Japan), 8 mm in diameter were used. Test materials (50 µl of each dilution or net honey) were soaked into the paper discs, dried at room temperature and carefully placed on the petri dishes seeded with inocula. Plates were kept at 4°C for four hours to provide sufficient time to diffuse the test material into the medium and finally incubated at 37°C for 8–12 hours. The diameter of the zone of inhibition produced around the discs was measured by vernier calipers as index of putative antibacterial activity of test materials. The size of the inhibition zone further represented a quantitative measure of antibacterial activity of the test material. All experiments were performed in triplicate and the zone of inhibition was measured twice for each honey dilution and net preparation. Effect of temperature on antibacterial activity of the sample was determined by the method as described by Leeching [11]. Different honey samples (10% v/v) were incubated at 50°C, 70°C and 100°C respectively in water bath for 30 minutes and cooled down. The antibacterial activity of this heat treated samples were carried out against the test organisms by the disc diffusion method as previously described. The inhibition zones were calculated as means ± sd. (n= 3 or 4)

Results

Several physical parameters such as color, pH, moisture content and brix value of these honeys were determined and showed in Table 1. The pH range of the five honeys was from 3.3 to 4.67. Moreover, among the five different floral sources of honey, "Black cumin honey" honey was the most acidic as compared to others. Moisture content of honeys was in between 13.3-25%. The brix value of "Black cumin honey" was the highest at 84.07°B and that of "Sundarban honey (new)" was the lowest at 75.50°B.

Table 1: Physicochemical properties of honey samples

Test Samples	Color	pH	Moisture (%)	°Brix
Black cumin honey	Dark Brown	3.3	13.3%	84.07
Mustard honey	Golden Yellow	3.35	13.7	81.75
Litchi honey	White	4.47	18.6	81.33
Sundarban honey (stored)	Brown	3.8	19	80.50
Sundarban honey (new)	Brown	3.69	25	75.50
Dabur honey	Light Brown	4.67	21.50	80.33

The antibacterial activity of honey against four different food spoilage bacteria was carried out and the inhibition zones are shown in Table 2. In the preliminary screening process it was observed that no honey was active at 1% concentration. The observed zones of inhibition ranged from 10.0 mm to 18.0 mm (for 5% v/v samples of different honey) and 12.0 mm to 27.0 mm (for 10% v/v samples of different honey). The zone of inhibition for original honey samples was showed in Figure 1, and it was ranged between 17 mm to 36 mm. Among the honeys, the black cumin honey showed maximum zones of inhibition whereas, the commercial honey (Dabur honey) showed minimum zone of inhibition. Mustard honey showed the second highest activity followed by litchi honey. Two honeys from Sundarban (one new and another stored) showed almost similar type of antibacterial activity. For all honey, the maximum zone of inhibition was observed with *Staphylococcus aureus* and the minimum zone of inhibition was observed with *Bacillus cereus*. Moreover, the antibacterial activity of all honey found to increase with increasing concentration.

Table 2: Comparison of antimicrobial activities of honey samples

Test samples	Zone of Inhibition (in mm)				
	Concentration (v/v)	*Staphylococcus aureus*	*E. coli*	*Salmonella spp.*	*Bacillus cereus*
Black cumin honey	5%	18.0±1.0	16.5±0.5	15.5±0.5	10.5±0.75
	10%	27.0±1.0	24.5±0.5	23.5±0.5	15±0.0
Mustard honey	5%	15.0±0.5	13.5±1.0	14.5±0.5	Not evident
	10%	20.0±0.75	18.0±1.5	19.5±0.5	12.0±0.5
Litchi honey	5%	14.0±0,75	12.0±0.5	13.0±0.75	Not evident
	10%	18.5±1.0	16.5±0.5	16.0±1.0	Not evident
Sundarban honey (stored)	5%	13.0±0.5	11.0±0.5	12.0±0.5	Not evident
	10%	18.0±0.5	14.5±1.0	16.0±0.75	Not evident
Sundarban honey (new)	5%	13.0±1.0	11.0±0.0	12.5±0.0	Not evident
	10%	18.0±0.0	14.5±0.75	15.5±1.0	Not evident
Dabur honey	5%	12.0±1.5	10.0±0.0	11.0±1.0	Not evident
	10%	15.0±1.0	13.0±1.5	12.5±0.75	Not evident

Foot note: The zones of inhibition were measured as the diameter (in millimeters) including the 8 mm diameter disc. Data are mean±standard deviation.

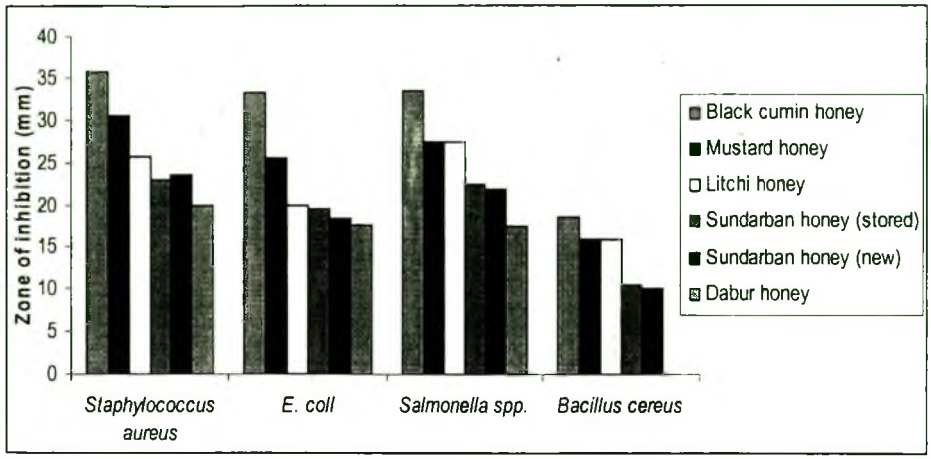

Figure 1: The antibacterial activity of 100% (v/v) honey samples on food spoilage bacteria

The effect of temperature on the antimicrobial activity of all types of honey (10% v/v) samples against all the test bacteria was determind and showed in Table 3. The sample's antimicrobial activity was found to be active at all the temperatures employed (50°C, 75°C and 100°C) suggested that, the honeys activity was not destroyed at high temperature treatment. Although, the antibacterial activity was observed to reduce as the temperature increased. The antibacterial activity of honey on *Staphylococcus aureus* at different temperature are showed in Figure 2. Similar to the previous findings, this figure also showed that, black cumin honey was the most effective after heat treatment followed by mustard, litchi, Sundarban and commercial honey.

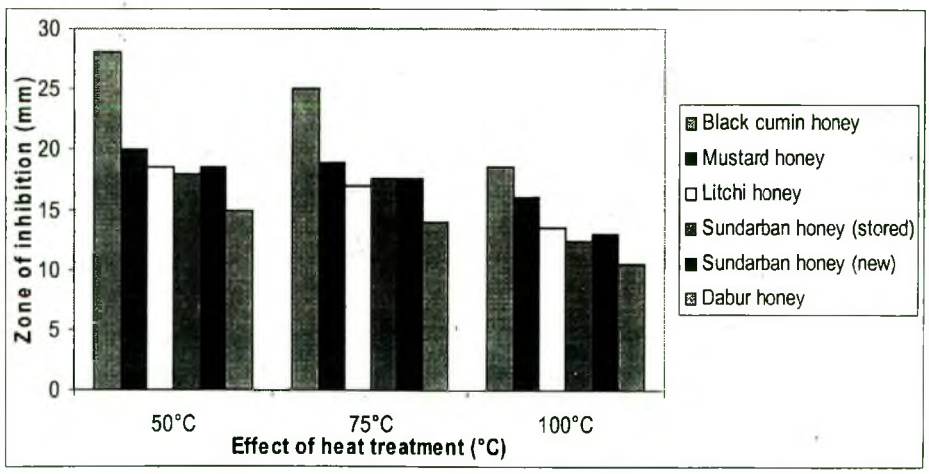

Figure 2: The antibacterial activity of honey samples after heat treatment on *Staphylococcus aureus* bacteria

Table 3: The antibacterial activity of honey samples after heat treatment

Test samples	Zone of Inhibition (in mm)			
	Temperature (°C)	*E. coli*	*Salmonella spp.*	*Bacillus cereus*
Black cumin honey	50°C	23.5±0.5	24.5±0.75	15.5±0.5
	75°C	21±1.0	22.0±0.5	13.5±1.5
	100°C	13.0±1.0	14.5±0.5	10.5±1.5
Mustard honey	50°C	18.5±0.75	19.0±0.5	12.5±0.75
	75°C	16.0±0.75	18.0±1.0	10.5±0.5
	100°C	12.0±0.5	13.0±1.0	No activity
Litchi honey	50°C	16.5±0.5	16.0±0.75	No activity
	75°C	14.5±0.5	15.5±0.5	No activity
	100°C	11.0±0.5	11.5±1.0	No activity
Sundarban honey (stored)	50°C	14.5±0.75	16.0±1.0	No activity
	75°C	13.0±1.0	15.0±0.5	No activity
	100°C	15.0±1.0	10.0±1.0	No activity
Sundarban honey (new)	50°C	11.0±0.0	15.75±0.75	No activity
	75°C	12.5±0.75	14.5±1.0	No activity
	100°C	10.0±1.0	11.5±0.5	No activity
Dabur honey	50°C	13.5±0.75	13.5±0.5	No activity
	75°C	11.0±0.75	11.5±1.0	No activity
	100°C	10.5±0.5	11.0±0.75	No activity

Foot note: The zones of inhibition were measured as the diameter (in millimeters) including the 8 mm diameter disc. Data are mean±standard deviation. Concentration of honey- 10%

Discussion

In the present study, physical characterization and antibacterial analysis were performed on different floral sources of honey. Physicochemical study (Table 1) revealed that all the honey were different in terms of their physicochemical properties except that all were acidic. Low pH or acidity of honey is believed to have an important contribution in its antibacterial activity. Indeed, previous studies claimed that the antibacterial activity of honey is credited to its acidity along with osmolarity and other factors [12]. Moreover, other physicochemical parameters, in particular the coloration of honey due to the presence of any particular chemical constituent(s) might also be contributed to its antimicrobial activity as reported by Taormina et al. [13].

The antimicrobial activity of different types of honey was determined against four food spoilage bacteria by disc diffusion method [10]. The result presented in Table-2, 3 showed that, the honey under investigation exhibited mark antibacterial activity as evidenced by their zone of inhibition. This finding correlated with other similar studies [12, 14] and it suggests that honey possess inhibitory effect on bacterial growth.

Honey has long been known as an antimicrobial agent and its activity was first recognized by Van Ketel in 1892 [15]. The pure honey contains alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids & reducing compounds. The antibacterial properties of honey includes the release of low levels of hydrogen peroxide and some honey have an additional phytochemical antibacterial compounds. The antibacterial property of honey is also due to osmotic effect of its high sugar content as it has an osmolarity sufficient to inhibit the microbial growth [12].

The concentration of honey had an impact on antibacterial activity as presented in table 2. In the disc diffusion test 1% concentration of six samples of honey produced no antibacterial effect on the test organism when compared to the honey concentration of 5% and 10%. At 5% concentration all the honey samples had produced an inhibitory effect against *Staphylococcus aureus, Escherichia coli, Salmonella spp.* but *Bacillus cereus* was found to be resistant to five honey samples. Only black cumin honey had an inhibitory effect against *Bacillus cereus* at 5% concentration. At 10% concentration, mustard honey also had an inhibitory effect against *Bacillus cereus* but other honeys had no effect. When the original samples (100%) were applied against the test organisms all honey showed an inhibitory effect on the growth of bacteria except the commercial honey (Dabur honey). According to studies, in undiluted honey, glucose oxidase responsible for hydrogen peroxide level in honey is inactive [17]. Therefore, with assistance from various antioxidant constituents hydrogen peroxide level in undiluted honey is said to be minimized. Very high osmotic pressures coupled with high acidity are the two main factors contributing to the antibacterial properties of honey at this stage [12, 18]. When honey is diluted to certain extents, glucose oxidase will be activated and start to utilize glucose to produce peroxide. At this point, the antibacterial activity of honey will gradually shift from osmotic- and pH-dependent to peroxide dependent.

The study found that honey inhibited both gram positive and gram negative bacteria. In general, and in most cases, Gram-negative bacteria are less sensitive to antimicrobials as compared to Gram positive bacteria due to its low permeability of the outer membrane which reduces the drug diffusion across the cell envelope [19]. However, once the drugs have entered have entered the cell membrane, then the cells defense mechanisms cannot prevent the drugs from exerting their toxic action. Probably, the tested honey samples possess some mechanism to evade the outer membrane so it inhibits both types of bacteria.

It was found that, honey from different floral sources exhibited differential antimicrobial activity. Among the honeys the black cumin honey showed maximum zone of inhibition, after that mustard honey, then litchi and wild honey from the forest of Sundarban and lastly commercial honey (Dabur honey), that showed minimum zones of inhibition. So, the antibacterial activity of honey varies with origin & processes. These differences are related to the amount of hydrogen peroxidases that is enzymatically present in different types of honey or other

antimicrobial constituents such as phenolic compounds with origin of nectar with which the bee is nourished [18].

Among all the honeys, honey from black cumin flower showed maximum zones of inhibition possibly because black cumin contains hydroxyl group (-OH) which is highly antimicrobial. Thymoquinone, p-cymene and carvacol are some major component of black cumin that had antimicrobial effect [20]. Hence, the antibacterial effects of black cumin honey may be closely related to their high percentage of these compounds. Honey from black cumin flower was darker in color and lower in pH than other honeys. There have been several studies in which dark honey have been found to have particularly high antimicrobial activity [21].

Mustard contain numerous chemical constituents, phytoalexins (sinalexin, sinalbins A and B), sterols and steryl esters (primarily sitosterol and campesterol), and flavonoids (eg, apigenin, chalcone). All of these compounds had antibacterial effect [22]. Mustard honey was less active than black cumin honey but more active than litchi honey, wild Sundarban honey and commercial honey.

Litchi flower contain significant amount of polyphenolic compounds which exhibit antimicrobial activity [23] Litchi honey and wild honey from the forest of sundarban showed almost similar zones of inhibition against the tested organisms. So, the antimicrobial activities of these honeys are almost same.

There were two types of Sundarban's honey, one was stored honey and other was new honey. But there was no significant difference between their antimicrobial activities. So the storage of honey does not affect its antibacterial activity.

The commercial honey (Dabur honey) was less active among all the six samples of honey. The commercial honey was pasteurized at a temperature of 70-75°C, to destroy yeast. This high temperature treatment hampers the antimicrobial activity of honey [21]. For this reason Dabur honey showed minimum zones of inhibition against tested organisms.

The antibacterial activity of all six honey samples were found to be active at all temperatures (50°C, 75°C, 100°C) as showed in Table-2, suggesting that components of honey were not destroyed at high temperature. A decrease in activity of honey was observed at 75°C and 100°C for all the tested organisms (Figure-2). Effective antibacterial activity of honey at 100°C indicates that these compounds may be used as potential preservatives for the food processed at high temperature. But, it was cleat that, high temperature treatment of honey was undesirable because it lowers the activity.

So, in light of the enormous potentials for the application of honey to use as a natural preservative, a continuing need exists to undertake more studies on honey. Identification and characterization of the active compounds may provide valuable information on the quality and possible therapeutic and preservative potential of these

Bangladeshi honeys. Similar studies are ongoing by the authors to identify other potent natural antimicrobials present in fruits, vegetables, herbs and spices.

Conclusion

The result of the study showed that honey possesses antibacterial property and it could be used as a natural preservative in Bangladesh. It is also evident that honeys from different floral sources have difference in their antibacterial activity. It was found that temperature decreased the antimicrobial activity of honey but significant activity still remains after heat treatment. So in the light of this study it can be specified that honey could be used as a safe alternative of chemical preservatives. More study is required on Bangladeshi honey to isolate and identify the compound responsible for honey's antibacterial activity and to use it in a feasible way.

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