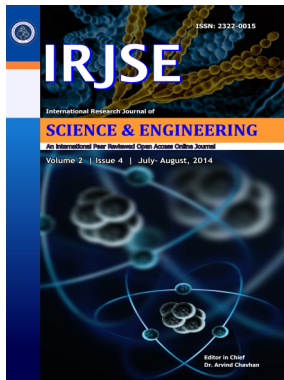


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RESEARCH ARTICLE

In Vitro Assessment of the Antimicrobial Potential of Honey against Enteric Pathogens

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ABSTRACT

Honey is well known for its health benefits and it has been used as traditional medicine for many years. Antimicrobial activity of honey samples was carried out against some bacterial pathogens by agar well diffusion method. The present study concluded that all of the tested honey samples such as Dabur honey, Crude honey-1 and Crude honey-2 exhibited inhibitory effects against different tested bacterial pathogens such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* except *Lactobacillus acidophilus*. The net honey i.e. 100% concentration of all the honey samples were most effective against the tested pathogens. The highest antimicrobial activity was found by Crude honey-2 (100%) against *E. coli* and *S. typhi* (50mm each) followed by Dabur honey against *E. coli* (48mm) and *S. typhi* (46mm). Honey samples were not antibacterial against *Lactobacillus acidophilus*. As the dilution of honey increased, the antimicrobial activity was found to be decreased.

Keywords: Honey, Antimicrobial Activity, Bacterial Pathogens

INTRODUCTION

Honey is a well known medicine from ancient time period. It is found to be more effective where conventional modern therapeutic agents are failing. Many researchers described the effectiveness of honey for the treatment of infected wounds. It acts as both antibacterial and antifungal agent with no adverse effects (Molan, 2001). In the medical sciences wound-healing activity and antimicrobial property has been documented. Honey contains cenic acid, antioxidant agent and some flavonoids which have been approved for antibacterial applications (Rahman et al., 2010). Wound healing property of honey is resulted from its antibacterial potential and the fact that it maintains a moist wound condition along with its high viscosity which helps to prevent infection. The antimicrobial activity of honey varies depending on its origin, type of flowers, the region, the nature of bees and the breeding techniques (Malika et al., 2004). However there is a need to identify and characterize its contents which may provide valuable information regarding therapeutic potential of honeys

and therefore the medicinal property of honey with emphasis on their antibacterial activities have been studied by many researchers (Levy and Marshall, 2004).

The global burden of infectious diseases tends to the use of antimicrobial agents. Because of the day to day emergence resistant pathogens, the effectiveness of the antibiotics is diminished. It can pose a great threat to the health of mankind (Mandal et al., 2009). For this, alternative antimicrobial strategies are urgently required which led to re-evaluation of the therapeutic use of ancient remedies (Mandal et al., 2010). Most of the researchers have reported the antimicrobial properties of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Lusby et al., 2005; Mundo et al., 2004). Therefore aim of the present project was to assess in vitro antimicrobial potential of honey against enteric pathogens.

MATERIALS AND METHODS

1. Collection of Microorganisms:

The cultures were obtained from National Chemical Laboratory (NCL) Pune which includes *Escherichia coli* NCIM 2064, *Salmonella typhi* NCIM 2257, *Pseudomonas aeruginosa* NCIM 2036, *Proteus vulgaris* NCIM 2027, *Lactobacillus acidophilus* NCIM 2285, etc. where, NCIM- National collection of industrial microorganisms.

2. Antimicrobial Activity of Honey by Agar Well Diffusion Method

A total of 3 honey samples were collected from different sources in sterile glass bottles. The antimicrobial activity of different honey samples against the five different organisms tested using agar well diffusion method (Bhakuni et al., 1974). Test materials were prepared by diluting each honey (Crude honey -1, Crude honey-2 and Dabur honey) in sterilized, double distilled water at different dilutions (concentrations) 50%, 25%, 12.5% and Net honey i.e. 100%. Nutrient agar plates and MRS agar plates were prepared. A 0.1ml of broth of the organism was inoculated by using sterile cotton swab on two solidified plates. After making lawn, wells were prepared using sterile cork borer having 6 mm diameter and 0.5 ml of the honey sample was added in it. The plates were incubated at 37°C for 24 hrs. The zones of inhibitions were observed on the plates (Sheikh et al., 1995).

RESULTS AND DISCUSSION

The present study has undertaken to study the efficacy of honey against the enteric pathogens. A total of three different types of honey samples were analyzed for antibacterial effect on the pathogens. The three different types included Dabur honey, Crude honey-1, Crude honey-2 which were collected from different sources. The four different types of dilutions of all three honey samples were prepared. The dilutions prepared were 100%, 50%, 25% and 12.5%. Every dilution of each honey sample was tested against five enteric pathogens such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Lactobacillus acidophilus*. All three different types of honey samples used in this study were effective against *E. coli*, *S. typhi*, *Ps. aeruginosa* and *P. vulgaris* and non-effective against *L. acidophilus* (Table1-3). These findings were correlated with several earlier reports by Somerfield (1991) on antimicrobial activity of different brands of honeys from other countries. In their study, total of three honey samples from different sources were evaluated for their antibacterial activity against selected bacteria species representing the Gram - positive species and the Gram negative species. However, their study does not reported significant inhibition zone against *L. acidophilus*.

The study revealed that 100% dilution of all the honey samples had more antibacterial activity (Fig. 3-6). Badawy et al. (2004) suggested that the concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent. Thus, it has been shown by French et al., (2005) that the antimicrobial activity of honey may range from concentrations < 3 % to 50 % and higher. The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria (Basualdo et al., 2007).

It was found that 100% dilutions of all the honey samples were found to be more effective against *Escherichia coli* as compared to the 50% and 25% dilution. However, 12.5% dilution was not effective against *E. coli*. Thus as the dilutions increases the zone of inhibition decreases and ultimately the antibacterial activity decreases. Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source. The antibacterial activity of 100% dilution showed Dabur honey (48mm), Crude honey-1 (46mm) and Crude honey-2 (50mm) zone of inhibition against *E.coli* (Fig. 3) (Figure 1).

Table 1: Antimicrobial Activity of Dabur Honey against Bacterial Pathogens

Organisms	100%	50%	25%	12.5%
<i>Escherichia coli</i>	48mm	32mm	25mm	NZ
<i>Pseudomonas aeruginosa</i>	40mm	36mm	NZ	NZ
<i>Proteus vulgaris</i>	36mm	27mm	NZ	NZ
<i>Salmonella typhi</i>	46mm	36mm	22mm	NZ
<i>Lactobacillus acidophilus</i>	NZ	NZ	NZ	NZ

Table 2: Antimicrobial Activity of Crude Honey-1 against Bacterial Pathogens

Organisms	100%	50%	25%	12.5%
<i>Escherichia coli</i>	46mm	31mm	24mm	NZ
<i>Pseudomonas aeruginosa</i>	27mm	24mm	NZ	NZ
<i>Proteus vulgaris</i>	30mm	23mm	NZ	NZ
<i>Salmonella typhi</i>	34mm	31mm	16mm	NZ
<i>Lactobacillus acidophilus</i>	NZ	NZ	NZ	NZ

Table 3: Antimicrobial Activity of Crude Honey-2 against Bacterial Pathogens

Organisms	100%	50%	25%	12.5%
<i>Escherichia coli</i>	50mm	30mm	23mm	NZ
<i>Pseudomonas aeruginosa</i>	40mm	28mm	NZ	NZ
<i>Proteus vulgaris</i>	32mm	25mm	NZ	NZ
<i>Salmonella typhi</i>	50mm	31mm	23mm	NZ
<i>Lactobacillus acidophilus</i>	NZ	NZ	NZ	NZ

Where, NZ= No Zone

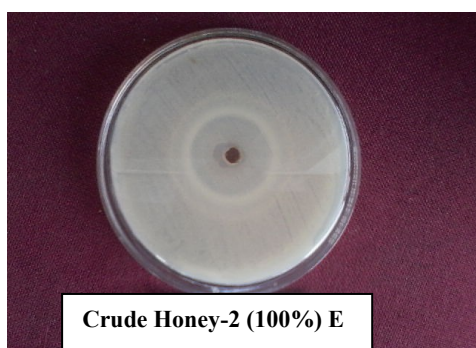


Figure 1: Antimicrobial Activity against *E. coli*

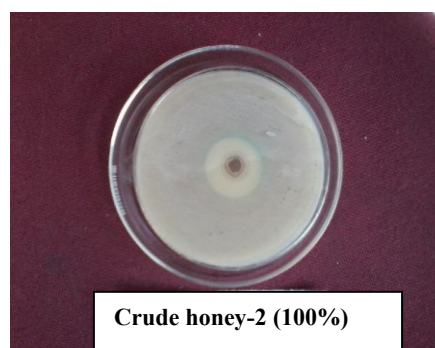


Figure 2: Antimicrobial activity against *Pseudomonas aeruginosa*

It was found that 100% dilutions of all the honey samples showed more zone of inhibition against *Pseudomonas aeruginosa* as compared to the 50% dilution. However 25% and 12.5% dilution was not effective against *Ps. aeruginosa*. At 100% dilution Dabur honey (40mm), Crude honey-1 (27mm) and Crude honey-2 (40mm) inhibition zone against *Ps. aeruginosa* (Fig. 4) (Figure 2). All the honey samples showed highest antibacterial activity at 100% dilution against *Proteus vulgaris* as compared to the 50% dilution. However 25% and 12.5% dilution was not effective against *P. vulgaris*. Dabur honey (36mm), Crude honey-1 (30mm) Crude honey-2 (32mm) showed zone of inhibition against *P. vulgaris* (Fig. 5) (Figure 3). It was

found that 100% dilutions of all the honey samples were found to be more effective against *Salmonella typhi* as compared to the 50% and 25% dilution. However, 12.5% dilution was not effective against *S. typhi*. Dilutions of Dabur honey showed 46mm zone of inhibition at 100% dilution where as Crude honey-1 (34mm) and Crude honey-2 (50mm) against *S. typhi* (Fig. 6) (Figure 4). Taormina et al. (2001) reported that the concentration of honey needed for complete inhibition of *S. typhimurium* growth is <25%. According to Basualdo et al., (2007), both raw and processed honey showed the inhibitory effects which were inherent mostly in all selected test organisms. Their study revealed the same results in which *S. typhi*, *Ps.*

aeruginosa and *E. coli* showed significant antibacterial activity with the zone of inhibition range between 37mm and 13mm. Al-Namma (2009) also observed that honey has a greater inhibitory effect on Gram negative bacteria. *S. typhi*, *Ps. aeruginosa*, and *E. coli* are more susceptible than other test organisms and honey may have potential as therapeutic honeys.

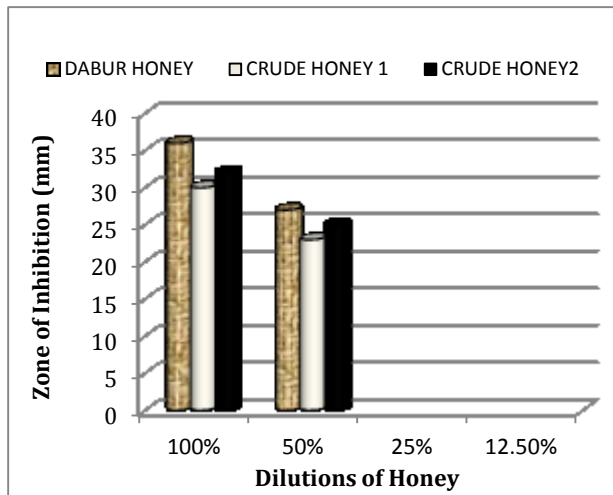


Fig. 6: Antimicrobial Activity of Honey Samples against *Salmonella typhi*.

It was found that the antimicrobial activity of all the honey samples showed varying zone of inhibition against tested pathogens. According to Badawy et al., (2004) the zone diameter of inhibition of different honey samples (5%-20%) has been determined against *E. coli*, *S. typhimurium*, *S. aureus*, *Ps. aeruginosa* and *Proteus mirabilis*. Another reason suggested that the antibacterial property of honey was also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties of gluconic acid and the antiseptic properties of its H₂O₂ (Lusby et al., 2002). Zumla and Lulat (1989) reported that honey is very good inhibitor to *Escherichia coli*, *Salmonella* and *Shigella*.

It was found that all the dilutions of all the honey samples were not effective against *Lactobacillus acidophilus*. No zone of inhibition was observed in any of the dilution of honey samples tested (Table 1-3). It was reported that the gut microflora plays an important role in maintaining gastrointestinal health. It is thought that by maintaining the beneficial microorganisms, humans may decrease the chance of suffering from gastroenteritis. According to El-Arab et al., (2006); Shamala et al., (2000) *L. acidophilus* had higher viable counts in a medium with a diluted honey. An in vivo study conducted by the same authors also showed that viable counts of lactic acid bacteria from both small and large intestines of rats fed with honey

were markedly higher than those from rats fed with sucrose. According to Angela (2012) *L. acidophilus* has been isolated from honey samples and contributed in antibacterial activity.

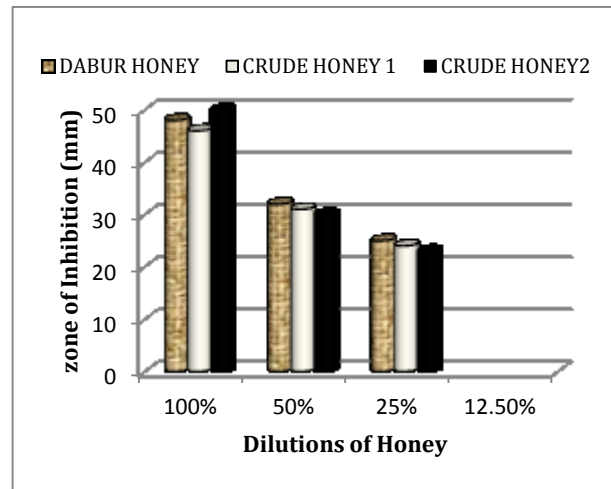


Fig. 2: Antimicrobial Activity of Honey Samples against *Escherichia coli*.

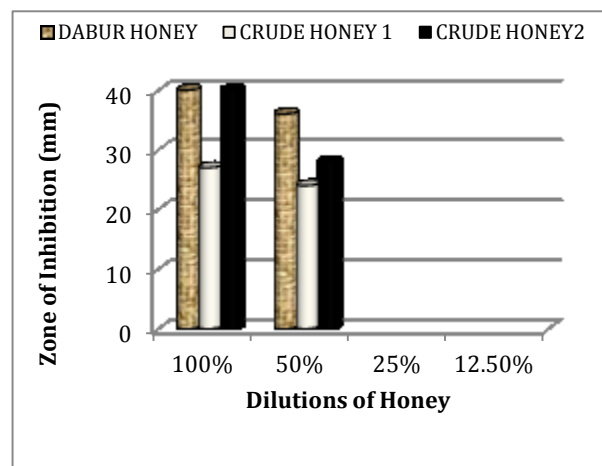


Fig. 3: Antimicrobial Activity of Honey Samples against *Pseudomonas aeruginosa*.

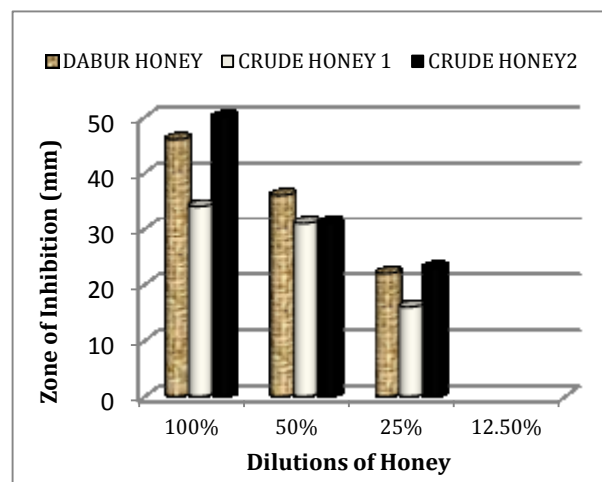


Fig. 5: Antimicrobial Activity of Honey Samples against *Proteus vulgaris*.

Molan and Cooper (2000) reported that the difference in antimicrobial potency among the different honeys depends on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are H₂O₂, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey.

CONCLUSION

The present study concluded that all of the tested honey samples such as Dabur honey, Crude honey-1 and Crude honey-2 exhibited inhibitory effects against different tested bacterial pathogens such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* except *Lactobacillus acidophilus*. The net honey i.e. 100% concentration of all the honey samples were most effective against the tested pathogens. The highest antimicrobial activity was found by Crude honey-2 against *Escherichia coli* and *Salmonella typhi*. Honey samples were not antibacterial against *Lactobacillus acidophilus*. As the dilution of honey increased, the antimicrobial activity was found to be decreased. The antibacterial activity against enteric bacterial pathogens was variable depending on the source of honey. The study showed that honey, a kin to antibiotics, possesses certain organisms sensitive to it and provides alternative therapy against certain bacteria. Therefore, there is need to characterize the active components of honey extracts and encourage to investigate possible benefits of the use of honey among therapies in the treatment of bacterial infections.

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