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Original article

Antibacterial properties of *Apis dorsata* honey against some bacterial pathogens

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ABSTRACT

Now-a-days, different bioproducts are being used extensively for the welfare of mankind. However, for proper utility of any bioproduct, the exact biotechnological potential of that product should be explored. Honey is produced in almost every country on the planet. It has long been used as a medicinal agent in addition to its broader use as a popular food throughout the human history. It can be used to treat various diseases without causing any negative side effects. In the present study, the antibacterial potential of honey produced by *A. dorsata* was investigated at its variable concentrations (25, 50, 75 and 100 %) against four pathogenic bacterial species. The highest antimicrobial action was seen against *E. coli* at 100 % concentration of the honey while showing zone of inhibition of 37.5 ± 3.5 mm. However, the lowest antibacterial action was observed against *E. faecalis*. The overall order of growth inhibition by the honey at its 100 % concentration for the implicated bacterial species appeared as: *E. coli* > *P. aeruginosa* > *S. aureus* > *E. faecalis*. The honey couldn't show antibacterial action at its 25 % concentration. Our findings of the present study will be helpful for utility of the honey as an alternative medicine for curing different complications caused by microbial pathogens.

1. Introduction

Honey has long been valued as a medicinal agent in addition to its broader use as a popular food throughout the human history. Honey is a sweet substance made by honey bees from plant nectar (Jaganathan and Mandal, 2009). Now-a-days, honey is mainly known for its sweetening properties and as a desirable natural food product. In ancient times, it was considered as an important medical treatment for all kinds of health complications (Zaghloul et al., 2001).

Honey is produced in almost every country on the planet. It has been used as a crucial ingredient in Ayurvedic and Yunani medicine for thousands of years. Today's scientists recognize honey as

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Table 1
Composition of nutrient broth.

Chemical constituent	Quantity (g/L)
'Lab-Lemco' powder	1.0
Peptone	5.0
Sodium chloride	5.0
Yeast extract	2.0
pH: 7.4 ± 0.2 at room temperature	

Table 2
Composition of nutrient agar.

Chemical constituent	Quantity (g/L)
Agar	15.0
'Lab-Lemco' powder	1.0
Peptone	5.0
Sodium chloride	5.0
Yeast extract	2.0
pH: 7.4 ± 0.2 at room temperature	

a highly effective treatment for a variety of disorders. Honey can be used to treat various diseases without causing any negative side effects. It does not harm even diabetic patients in the same way that it does not harm non-diabetic people (Kumar et al., 2010).

A range of studies dealing with the antimicrobial potential of honey has been carried out till now (Yuksel, 2011; Oryan et al., 2016; Wasihun and Kasa, 2016). After the discovery of penicillin, the rise of antibiotic-resistant microbes has attracted the attention of healthcare and medical professionals. Several bacterial genera including *Staphylococcus*, *Enterococcus* and *Mycobacterium* have been observed to produce novel antibiotic-resistant forms. However, with the discovery of multidrug-resistant microbes, the situation became much worse (Arias and Murray, 2009; Bereket et al., 2012). Furthermore, due to the high expenses of medication development, the pharmaceutical sector was unable to produce new antimicrobial drugs to combat the emerging threat of antibiotic-resistant bacterial forms.

Production of honey is linked with multifarious plant sources thus antibacterial properties of honey varies greatly depending on its source (Molan, 1992). Physical characteristics such as acidity (Mato et al., 2003), osmolarity (Weston, 2000) and chemical constituents (Weston, 2000) have been linked to its antibacterial properties. Commercial honey has shown antibacterial properties against a variety of pathogenic microbial species including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nzeako and Hamdi, 2000). In a study reported earlier by Subrahmanyam et al. (2001), bacteria procured from burns and wounds couldn't grow at 30 % of honey. In addition, it has shown to work against a variety of multidrug-resistant bacteria (Cooper et al., 2002; George and Cutting, 2007). In addition to its antimicrobial action, honey offers a number of advantages including lack of reaction, toxicity and side effects as well as low maintenance cost and local availability (Mandal and Mandal, 2011).

Four well-known honey bee species belonging to the genus *Apis* are found in Pakistan. *A. mellifera* was brought to the country in 1977 for commercial beekeeping (Hussain et al., 2015). Although *A. dorsata* contributes little to the production of honey due to its genetic makeup yet its ecological function in pollination cannot be ignored. In the present study, honey produced by *A. dorsata* was selected since honey from this wild species is the most popular for local consumption. Antimicrobial properties of the honey from *A. dorsata* against *E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus* were assessed in this study.

2. Materials and methods

2.1. Sampling and preparation of honey concentrations

Honey was procured from the local beekeepers in District Kasur, Punjab, Pakistan. The honey was obtained conventionally from honeybee colonies of the local strain *A. dorsata* by uncapping the comb frame. No diluent or additive was added for the extraction of honey. The obtained honey was purified by filtration through sterile cotton gauze. The purified honey was then stored in a glass bottle at room temperature. Twenty-five percent (v/v) honey solution was prepared by adding 0.25 mL of honey in 0.75 mL of distilled water. Similarly, 50 % and 75 % dilutions of honey were

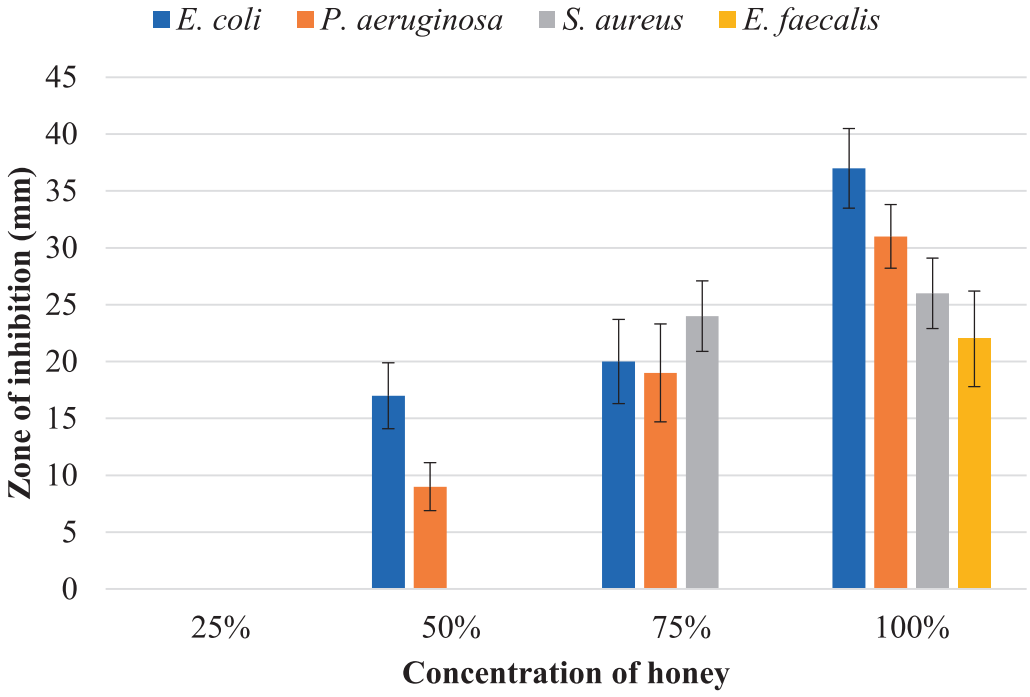

Fig. 1. Pathogenic bacterial growth inhibition at different concentrations of honey produced by *A. dorsata*.

made by the addition of 0.5 and 0.25 mL of distilled water in 0.5 and 0.75 mL of honey samples, respectively following Mama et al. (2019).

2.2. Nutritional profiling of honey

Carbohydrates, proteins, fats, dietary fiber, ash and water contents of the honey were estimated following standard protocols of Association of Official Analytical Chemists (AOAC, 2005). Different sugar types were analyzed through HPLC following the procedure as described by Mustafa et al. (2021).

2.3. Bacterial species and culture media

Four pathogenic bacterial species (*E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus*) were procured from MB Laboratory, Institute of Zoology, PU, Lahore, Pakistan and stored at low temperature (4 °C) in a refrigerator. The bacterial growth was revived in nutrient broth (CM0001B, Oxoid) prior to all further experiments of antibacterial susceptibility. The composition of nutrient broth is shown in Table 1.

2.4. Determining antimicrobial potential of honey

Susceptibility testing of honey sample was carried out using nutrient agar (CM0003B, Oxoid) plates through Kirby–Bauer disc diffusion technique following Kassim et al. (2016). The composition of nutrient agar is given in Table 2. Each plate was properly inoculated with each test organism by streak-plate method. Wells were made using a sterile cork borer and each well was filled with prepared concentrations (25, 50, 75 and 100 %) of the honey. A safer distance was maintained from the edges of the plates to pre-

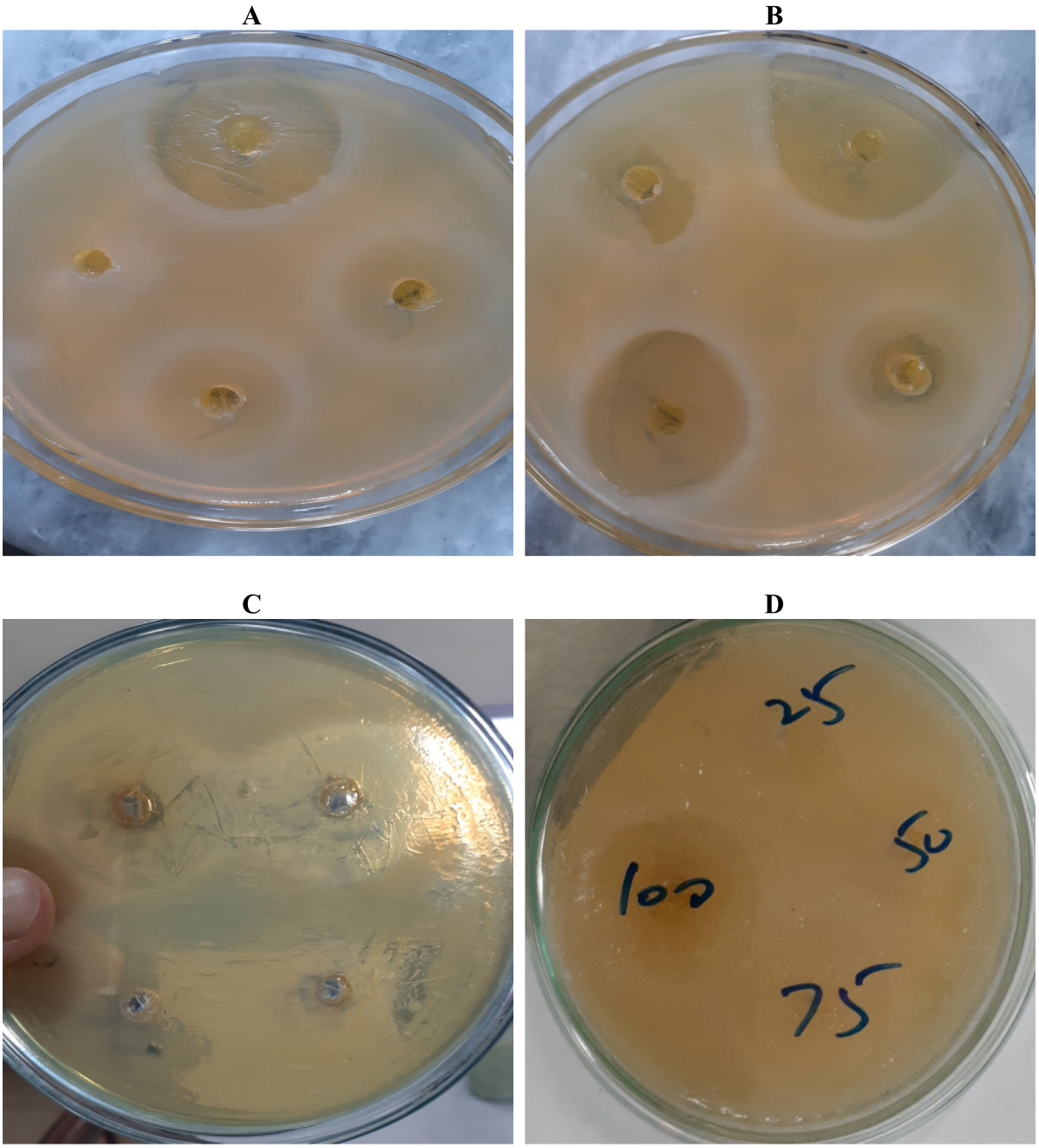

Fig. 2. Visible zones of inhibition produced by different honey concentrations against (A) *E. coli*, (B) *P. aeruginosa*, (C) *S. aureus* and (D) *E. faecalis*, respectively.

Table 3
Nutritional profile of honey produced by *A. dorsata*.

Parameter	Quantity (%)
Moister	7.79
Ash	1.21
Dietary fiber	2.12
Total carbohydrates	57.37
Total proteins	5.49
Total fats	0.36
Glucose	26
Fructose	41
Sucrose	2.7
Maltose	11

vent overlapping of the inhibition zones. The inoculated plates were incubated overnight at 35 °C. After successful incubation, the plates were examined and the diameter (mm) of the inhibition zones was measured in triplicate for each isolate.

2.5. Statistical analysis

The data collected for the measurement of inhibition zones were presented using descriptive statistics. Values represent the means of three replicates with standard error. A Student's *t* test was performed using MINITAB 16 to compare the size of inhibition zones formed against different concentrations of honey.

3. Results and discussion

The present study was carried out to check the antimicrobial potential of honey produced by *A. dorsata* against four pathogenic bacterial species. The results depicted the highest inhibition of *E. coli* at 100 % concentration of the honey while showing zone of inhibition of 37.5 ± 3.5 mm (Fig. 1). In all the experiments, growth of any of the bacterial species was not inhibited at 25 % concentration of the honey. The overall order of growth inhibition by the honey at its 100 % concentration for the implicated bacterial species appeared as: *E. coli* > *P. aeruginosa* > *S. aureus* > *E. faecalis*. This bacterial growth inhibition trend was also same at 50 and 75 % concentration of the honey. Ghramh et al. (2019) reported high efficacy of Saudi Arabian honey against *E. coli* among other tested pathogenic bacterial species. Similar findings were also reported by Hegazi and Allah (2012) while studying the antimicrobial potential of 12 honey samples from Saudi Arabia. Lesser resistance, mutation and low cellular permeability of *E. coli* might be attributed as possible reasons of low resistivity of *E. coli* to honey as reported previously by Wasihun and Kasa (2016).

The antimicrobial property of honey increased with increasing concentration of honey (Fig. 2). Similar dose-dependent antibacterial manner of honey was also observed by Deng et al. (2018) and Ghramh et al. (2019) while studying the antibacterial potential of honey sourced from different nectars. It is evident from different studies that variation in antibacterial potential of honey could depend on its botanical and geographical source, storage conditions and metabolism of honey bees (Molan and Cooper, 2000; Al-Waili et al., 2011; Almasaudi et al., 2017; Mohammed et al., 2017; Ghramh et al., 2019).

The nutritional profile of honey depicted the presence of significantly higher concentration of fructose (41 %). However, glucose concentration was not too much higher (26 %) as shown in Table 3. Higher concentration of fructose may contribute in the higher antibacterial property of the honey (Yuksel, 2011; Oryan et al., 2016; Cilia et al., 2020). Production of hydrogen peroxide by glucose oxidase from glucose may be another reason of the antibacterial potential of the honey (Sagona et al., 2015; Cilia et al., 2020). In our study, reasonable proportions of fructose and glucose may thus cumulatively contribute towards the antibacterial efficacy of honey produced by *A. dorsata*.

In the current study, the highest sensitivity was shown by *E. coli*, while the lowest was shown by *E. faecalis*. Different authors have shown variable antibacterial action of honey against different bacterial species (Yuksel, 2011; Oryan et al., 2016; Wasihun and Kasa, 2016; Almasaudi et al., 2017; Ghramh et al., 2019; Cilia et al., 2020). However, the antimicrobial potential of honey primarily depends on the source of honey as well as source of the microbial strain (Cilia et al., 2020). In some cases, monofloral, while in some other cases multifloral honey samples were appeared as efficient antimicrobial agents (Sakihama et al., 2002; Fratini et al., 2017; Felicioli et al., 2019).

4. Conclusions

Antibacterial potential of honey produced by *A. dorsata* was investigated at its different concentrations (25, 50, 75 and 100 %) against four pathogenic bacterial species (*E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus*). The highest antimicrobial action was seen against *E. coli*, however, the lowest antibacterial action was observed against *E. faecalis*. The honey couldn't show antibacterial action at its 25 % concentration. Our findings of the present study will be helpful for utility of the honey as an alternative medicine for curing different complications caused by microbial pathogens.

Ethical statement

Ethical statement is not applicable as this study does not involve any animal that require approval from the ethical committee.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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