

Anti-microbial Efficacy of Honey Against Infectious Pathogens

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ABSTRACT

Honey has been known as an ancient traditional medicine, possessing numerous health benefits and recognized for its antimicrobial, anticancer, and antiulcer property. However, this study was undertaken to evaluate the anti-microbial efficacy of the honey against infectious pathogens. The antimicrobial activity was studied on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* by agar disc diffusion method. Honey was found to be effective against *Staphylococcus* (1.6cm), *Escherichia coli* (1.9cm), *Aspergillus niger* (1cm). However, the existing antibiotic gentamicin and antifungal Fluconazole had shown more inhibition zone than honey. Furthermore, honey was found to have 10 mM hydrogen peroxide per ml in the present study. In conclusion, honey may be used as a cheap and effective concomitant agent for treating infections caused by *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger*.

Keywords: Honey, anti-microbial activity, hydrogen peroxide, concomitant agent.

INTRODUCTION

Antimicrobial agents are pre-eminent in reducing the risk of infectious diseases. As the pathogens have developed the resistance due to their evolution, efficacy of antibiotics have been minimized that ultimately necessitates the need for an alternative antimicrobial strategies for which honey may be used as concomitant agent to enhance the effectiveness of the drug formulation¹. Honey has been coined the term “the remedy rediscovered” because of its therapeutic properties in the year 1989². The healing property of honey is due to anti-microbial activity and high osmolarity due to its high sugar content which maintains a moist condition and acts as a barrier for infection because of its viscosity nature¹. In ancient Ayurveda medicine honey was used as solvent for their medicinal formulation. Honey has been used along with extract of garlic, ginger and pepper to increase their activity efficiently in the treatment of human pathogen like cough, sore throat and common cold flu³. It also accommodates organic acids like lactic, formic, butyric, tartaric, pyruvic, acetic, citric, oxalic, succinic, malic, pyro glutamic and glycolic acid etc. Gluconic acid is produced by the action of glucose oxidase enzyme⁴. Major enzymes of honey are diastase, glucose oxidase, acid phosphatase, catalase and invertase that can be denatured by heating⁵. Glucose oxidase is a carbohydrate metabolizing enzyme that converts glucose into hydrogen peroxide and gluconic acid. The presence of hydrogen peroxide prevents the spoilage of unripe honey as the sugar concentration has not reached enough to prevent the microbial contamination⁶. Fungal disease are common and if left untreated can lead to harmful consequences, to make the existing antifungal drug more effective, it is concomitated with honey⁷. Honey has tremendous therapeutic benefits, used for the treatment of

bleeding disorders, leucoderma, urethritic discharges, sinusitis, upper respiratory infections, gluteofemoral fistulas, bed sores, inflammation, gingivitis, digestive disorders, colitis, dehydration, diabetes, osteoporosis, insomnia, chronic fatigue syndrome, multiple sclerosis, cardiovascular disease, hepatitis, tumors, cancer, and radiation/chemotherapy induced oral mucositis and Worm infestations^{8,9}. The aim of present study, was to analyze the efficacy of honey against infectious microorganisms and to estimate the amount of hydrogen peroxide present in it.

MATERIALS AND METHODS

Materials

Commercial honey was brought from a local store. It was then stored as such in room temperature.

Microbial strains

The microbial strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and a fungal strain *Aspergillus niger* were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial culture were maintained and subcultured regularly using nutrient agar media containing peptone, agar and beef extract. The subcultures were stored at 4°C. The fungal strain *Aspergillus niger* was subcultured in potato dextrose agar (PDA) medium.

Methods

All the antimicrobial activity discussed in this paper was done by agar disc diffusion method.

Medium preparation

500 ml of nutrient agar medium and 100ml of potato dextrose agar medium was prepared according to standard protocol with pH set to 7.0-7.2. Both the medium was plugged with cotton plug in a conical flask and sterilized upto 121°C for 15 minutes using autoclave. After

Table 1: Comparison of honey with antibacterial and antifungal agents towards inhibition of organism

Organism	Diameter of inhibition (cm)	
	Honey	Gentamicin Fluconazole
<i>Staphylococcus aureus</i>	1.6	3.7
<i>Escherichia coli</i>	1.9	3.9
<i>Pseudomonas aeruginosa</i>	1.3	3.5
<i>Bacillus subtilis</i>	0.8	3.2
<i>Aspergillus niger</i>	1.4	3.0

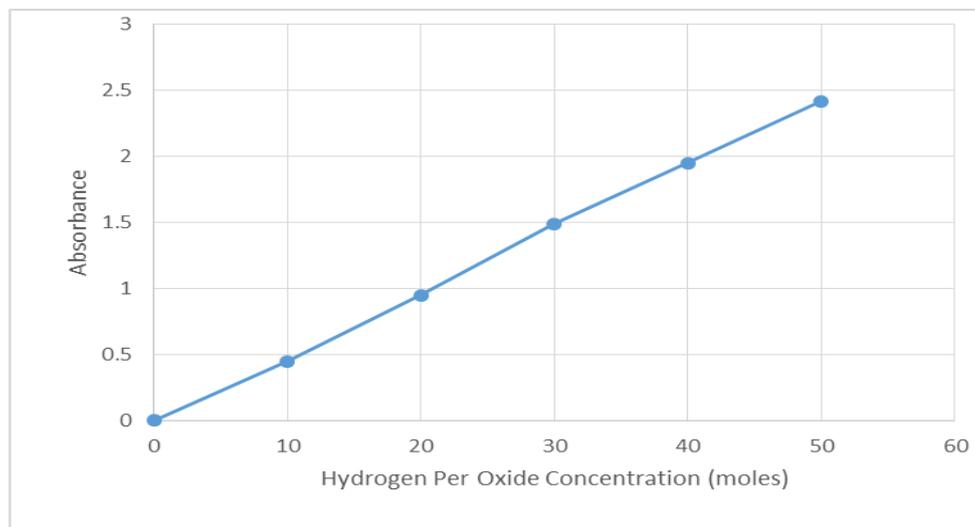


Figure: 1. Estimation of Hydrogen Peroxide

sterilization they was allowed to cool down to a handling temperature (around 35°C to 45°C) and poured into the petri dishes under sterile conditions inside the laminar airflow chamber and allowed to solidify at room temperature.

Agar disc diffusion assay

After the solidification of agar 1 ml of each strain was inoculated into each petri plate and spread evenly using L-rod. Sterile filter paper discs of 6.5 mm in diameter were dipped in honey using sterile forceps and placed on each inoculated petri dishes. Their position on the petri dishes was marked and noted. Similarly the same procedure was followed for the standard Gentamicin and Fluconazole. The petri dishes were incubated at 37°C and the diameter of inhibition zone was measured for each strain after 24 hours.

Estimation of hydrogen peroxide

50 µl of honey was added to 1.95 ml of 0.01M potassium phosphate buffer (pH 7.0) in a test tube. Then 2 ml of 5 per cent potassium dichromate and glacial acetic acid (1:3; v/v) was added to the mixture. The tube was kept in boiling water bath for 10 min and then cooled. After cooling reading for absorbance was taken at 570 nm.

RESULTS

In the present study, the effectiveness of honey was evaluated against microbial pathogen shown in the table 1. It shows the inhibition zone formed by honey and the standard Gentamycin on the bacteria and Fluconazole on *Aspergillus niger* respectively. The assessment of antimicrobial activity was based on inhibition zone formed

around the disc. Honey had antimicrobial activity against *Staphylococcus aureus* (1.6cm), *Escherichia coli* (1.9cm) and *Aspergillus Niger* (1cm), *Pseudomonas aeruginosa* (1.3cm) and *Bacillus subtilis* (0.8cm). Fig. 1 shows the gradual increase in absorbance with increase in concentration of hydrogen peroxide. This indicates that the Hydrogen peroxide present in honey is proportional to its concentration. From the graph it can be assessed that for every 1ml of honey, 10mM of hydrogen peroxide was present.

DISCUSSION

The microbial strain used in this study possess clinical importance in accordance to pathogenic infections. *Escherichia coli* were shown to exhibit Urinary tract infection, diarrhea, septicemia and wound infections^{10,11}. *Staphylococcus aureus* cause superficial skin lesions, urinary tract infection, and nosocomial infections^{12,13}. *Pseudomonas aeruginosa* cause wound infections, diabetic foot ulcer and Urinary infections^{10,11,14}. *Bacillus subtilis* cause severe pulmonary diseases¹⁵. *Aspergillus niger* cause Pulmonary aspergilloma, Allergic bronchopulmonary aspergillosis¹⁶. The pathogen being susceptible to inhibition by honey reveals the therapeutic efficacy of honey in several infectious diseases. Broad spectrum of antibiotics commonly used for both gram positive and gram negative bacteria was ceftriaxone¹⁷. As the pathogens evolve and develop, they tend to adapt resistance to antibiotics whose effectiveness is diminished. The existing antibiotic efficacy were increased by using

concomitant agent, honey may be fulfill the criteria as concomitant agent. Among these organisms, majority of the wound infection were caused by *staphylococcus aureus* and *pseudomonas aeruginosa* which shows that the property of the honey has not only restricted to the treatment of diseases, but also its effectiveness against wound care had shown interest to several clinicians and researchers. Honey has been considered as a good antimicrobial agent for both gram positive organism *staphylococcus aureus* which shows maximum zone of inhibition (1.6cm) and gram negative organism *Escherichia coli* also with having a considerable zone of inhibition (1.9cm). The antimicrobial and antifungal activity of honey was attributed due to the presence of hydrogen peroxide. Concentrations of hydrogen peroxide found was very low, hence cytotoxic damage by hydrogen peroxide is very minimum, making it safe for daily consumption. In conclusion, Honey has therapeutic potential with less side effects and can enhance immunity against pathogenic microbes, when taken as concomitant with other medicinal supplements.

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