

Determination of Antibacterial Efficacy of different varieties of Honey

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Abstract

Since time immemorial, honey has been pivotal in healing a variety of ailments. Apart from simple and complex sugars, honey contains potent antioxidants such as flavonoids, phenolic acids, and ascorbic acid. Hydrogen peroxide which is primarily responsible for the antimicrobial properties of honey, is produced naturally by the enzymatic activity of glucose oxidase. During the process of honey making, the honey bees secrete glucose oxidase. This study highlights the antibacterial efficacy of various types of honey. For the determination of antimicrobial properties agar diffusion assay has been used and the zone of inhibition formed by various types of honey samples have been recorded.

Keywords: Honey, glucose oxidase, antimicrobial properties, agar diffusion assay, zone of inhibition.

INTRODUCTION

Natural honey is a concentrated solution of sugars created by various species of genus *Apis*. In India, at least four species can be found, namely *Apis dorsata*, *Apis cerana*, *Apis florea* and *Apis andreniformis* [1]. Honey has been essential to human civilization, functioning as a source of nourishment and a healing substance. Ancient civilizations like the Egyptians, Greek and Chinese utilized honey in their cuisine and medical treatments.

In recent years, the global rise in antimicrobial resistance has become a serious public health concern, urging the scientific community to explore alternative solutions to conventional antibiotics. As pathogens evolve mechanisms to evade existing drugs, the search for natural antimicrobial agents has intensified. Among these, honey has emerged as a promising candidate due to its broad-spectrum antibacterial properties and historical use in traditional medicine.

Honey is a complex, supersaturated sugar solution produced by honeybees (*Apis spp.*) using floral nectar. Beyond its nutritional value, honey possesses a unique combination of antimicrobial factors, including high osmolarity, low pH, production of hydrogen peroxide, and the presence of bioactive compounds such as flavonoids and phenolic acids [2][3].

These properties not only inhibit bacterial growth but also reduce the risk of developing resistance—which is a striking feature of honey and a key advantage over many synthetic antibiotics[4][5].

The antimicrobial activity of honey is not uniform and it varies greatly depending on its botanical source, geographic origin, processing methods and storage conditions[6]. Different floral varieties contribute distinct phytochemicals, consequently, influencing the potency of honey[7]. In the Indian state of Jharkhand, beekeeping is a growing agro-based practice, and varieties such as Litchi, Karanj, and Multiflora honeys are widely produced by local farmers and promoted through institutions like Divyayan Krishi Vigyan Kendra, Ramkrishna Mission, Morabadi, Ranchi[13].

METHODOLOGY

The methodology used in this study was designed to evaluate the antibacterial properties of three different varieties of honey namely Litchi, Karanj, and Multiflora—collected from Divyayan Krishi Vigyan Kendra, Ranchi. These honey samples were tested against *Escherichia coli* using standard microbiological techniques such as Agar Well Diffusion assay.



Fig.1: Collection of different varieties of honey

First, the bacterial culture of *Escherichia coli* was prepared in nutrient broth and incubated at 37°C for 24 hours to obtain active bacterial cells. The honey samples were stored in sterile containers and were used without dilution for agar well diffusion, while serial dilutions were prepared for MIC analysis.

In the agar well diffusion method, sterile Mueller-Hinton agar plates were inoculated with the *E. coli* culture using a sterile cotton swab. Wells of uniform size (6 mm diameter) were then bored into the agar using a sterile borer, and each well was filled with 100 μ L of undiluted honey. Ampicillin was used as a positive control. The plates were incubated at 37°C for 24 hours, after which the diameters of the inhibition zones were measured in millimetres.

These methods allowed both qualitative and quantitative assessments of the antibacterial activity of the honey samples, ensuring accurate and reproducible results[12].

RESULTS

The antimicrobial activity of various samples, including Ampicillin and honey samples, were assessed using the zone of inhibition method at increasing concentrations. The results are summarized below:

- **At a concentration of 1000mg/ml:**

Karanj extract at a concentration of 1000mg/ml exhibited the largest zone of inhibition at 3.0 ± 0.08 cm, while lichi and multiflora at the same concentration showed a zone of inhibition measuring 2.6 ± 0.11 cm. Ampicillin, at a concentration of 50mg/ml, displayed the smallest zone of inhibition at 2.2cm, suggesting relatively lower activity at this dosage.

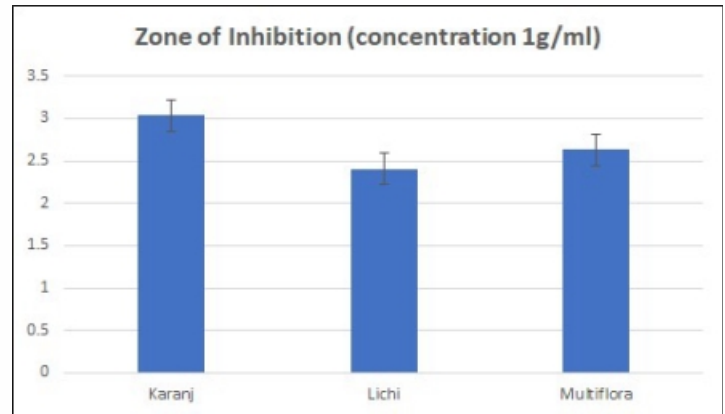


Fig.2: Zone of inhibition of different varieties of honey samples at initial concentrations (Ampicillin 50 mg/ml; honey samples 1000mg/ml)

- **At a concentration of 1500mg/ml:**

Karanj (1500 mg/ml) demonstrated a marginally better zone 3.1 ± 0.17 cm, retaining the highest effectiveness. Lichi (1500 mg/ml) and Ampicillin (100 mg/ml) displayed similar inhibition zones 2.9 ± 0.12 cm. Multiflora honey (1500 mg/ml) produced a zone of 2.8 ± 0.21 cm, which is slightly lower than the others.

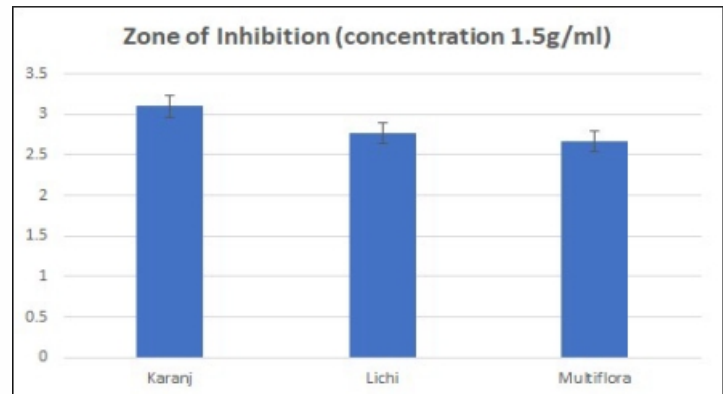


Fig.3: Zone of inhibition of different varieties of honey sample at increased concentration (Ampicillin 100mg/ml; honey samples 1500mg/ml)

- **At a concentration of 2000 mg/ml:**

Karanj exhibited the highest antibacterial effect with a 3.3 cm zone of inhibition. Multiflora improved to 3.1 cm, indicating increased effectiveness. Lichi presented 2.8 cm, reflecting a minor enhancement. Ampicillin surprisingly showed a reduced zone of 2.5 cm, possibly due to variability or saturation effects at higher concentrations.

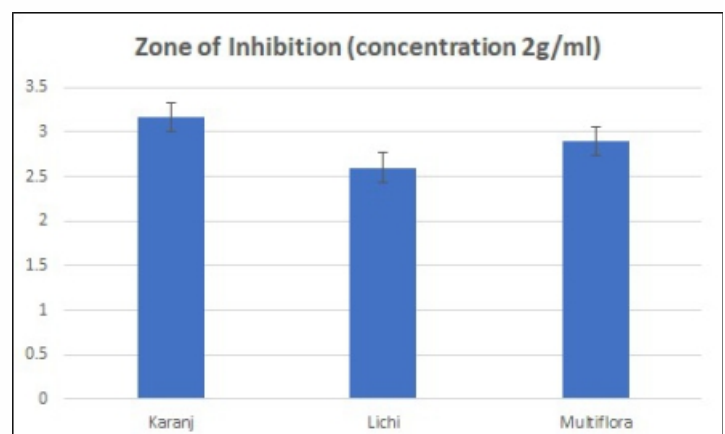


Fig.4: Zone of inhibition of different varieties of increased concentrations (Ampicillin 200mg/ml; honey solutions 2000mg/ml)

DISCUSSION

This study aimed to evaluate and compare the antimicrobial potential of three honey types—Litchi, Karanj, and Multiflora—collected from Divyayan Krishi Vigyan Kendra, Ranchi, Jharkhand. Using *Escherichia coli* as the test organism, which is a Gram-negative bacterium known to cause gastrointestinal and urinary infections. Ampicillin served as the standard reference antibiotic.

All honey samples showed clear zones of inhibition against *E. coli*, confirming their antibacterial activity. Karanj honey demonstrated the highest efficacy, followed by Litchi and then Multiflora. This variation likely stems from differences in botanical origin, affecting the concentration of bioactive compounds like polyphenols and flavonoids. The multiflora honey's slightly lower activity may result from the dilution of potent compounds present in monoflora sources [10].

Honey's antimicrobial action is multifaceted: its high sugar content creates an osmotic effect, its low pH inhibits microbial growth, and it enzymatically produces hydrogen peroxide [8]. Additionally, non-peroxide components such as phenolic acids and organic compounds (e.g., methylglyoxal) contribute to its antibacterial properties [9].

Although Ampicillin exhibited superior activity overall, the ability of natural honey to inhibit *E. coli* suggests its potential as a complementary therapy. It could be useful in wound care, topical applications, or in combination with antibiotics to reduce resistance.

In the Jharkhand context, where apiculture supports rural livelihoods and traditional knowledge favours honey for healing, validating its antimicrobial properties adds value. This can enhance local economic development, promote biodiversity, and support evidence-based use of natural products [13].

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