



ISOLATION OF LACTOBACILLUS SP FROM FERMENTED HONEY PEPPER AND ITS ANTI DIABETIC ACTIVITY

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ABSTRACT

Honey, a natural remedy for various health conditions for centuries. In addition to being a delicious food, honey produced by stingless bee has been shown in numerous studies to contain a specific quantity of antimicrobial components, including organic acids, proteins, flavonoids, phenolic acids, glucose oxidase, catalase, ascorbic acid and cinnamic acid. Black pepper, or *Piper nigrum* L., is a popular spice as the "King of spices" since it not only improves the flavor of other ingredients but also adds flavor to food. In-depth research on the biological characteristics and bioactive phytochemicals of *Piper nigrum*. Probiotics are known to be beneficial microorganisms that can have adjuvant or therapeutic effects on living things. The isolated probiotic bacterial sample from the fermented honey pepper has the pharmacological potential of Anti-diabetic activity

KEYWORDS : Honey, Pepper, Probiotic, Antidiabetic activity

INTRODUCTION

For generations, honey has been used as a natural treatment for a wide range of illnesses. It is an organic supply of carbs, which can provide you a rapid energy boost. Its vitamin and mineral richness may also contribute to bettering general health (Fratianne, F et.al). Most of Antimicrobial studies against pathogenic bacteria were investigated on honey produced by the honey bee. In addition to being a delicious food, honey produced by stingless bee has been shown in numerous studies to contain a specific quantity of antimicrobial components, including organic acids, proteins, flavonoids, phenolic acids, glucose oxidase, catalase, ascorbic acid and cinnamic acid. Black pepper, or *Piper nigrum* L., is a popular spice that is known as the "King of spices" since it not only improves the flavor of other ingredients but also adds flavor to food. In-depth research has also been done on the biological characteristics and bioactive phytochemicals of *Piper nigrum*. Probiotics are now known to be beneficial microorganisms that can have adjuvant or therapeutic effects on living things. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) classified probiotics as live microorganisms that, when given in sufficient proportions, have positive effects on the host. Experimental studies and clinical trials have documented that probiotics can modulate the GM, inducing beneficial effects and increasing overall wellness. Over the last few years, studies on probiotics have been growing sharply due to their beneficial health effects, which have been used as adjuvant therapy for metabolic disorders. A number of preclinical and clinical studies have investigated the effectiveness of probiotics by evaluating the intestinal microbiota after probiotics use, showing promising results in treating metabolic diseases

MATERIAL AND METHODS

Samples (*Apis mellifera*, *Piper nigrum*) were collected from Saravampatti in Coimbatore, Tamil Nadu, in the month of November 2023. The fresh and disease free pepper and honey were collected. The fermented honey and pepper were taken for isolation of probiotic bacteria. Honey and pepper were taken in the ratio of 1:1 and allowed to ferment for 24 days

Fermentation Process Of Honey And Pepper:

100ml of Honey and 100mg of Pepper in the ratio of 1:1. Mixed the pepper with honey into the sterile bottles and then left that for 28 days fermentation process at room temperature.

Isolation Of Probiotic Bacteria:

Take the sample from fermented honey pepper mixture and then serially diluted. The 10^{-4} , 10^{-5} , 10^{-6} is made spread plate on

MRS medium and incubated at 37°C for 24 hours. The bacterial culture was grown. Isolated bacterial culture were picked from the plate, purified by streaking techniques, and incubated at 37°C for 24 hours.

Bile Salt Tolerance Test:

Strains were grown in MRS broth at 37°C overnight. MRS media was prepared and different concentration of phenol (0.05%, 0.15%, 0.3%) was added and sterilized the broth cooled down. The culture was inoculated and incubated at 37°C for 24 hours then bacterial growth was monitored by measuring absorbance with a spectrophotometer at 600 nm.

Anti-diabetic Properties:

Take series of nine test tubes and add different concentration sample (25µg, 50µg, 75µg, 100µg, 250µg, 500µg, 750µg, 1000µg) 200µl of PBS was added to all the nine tubes and incubated at 37°C for 10 mins. 500µl of Starch was added and kept in incubate at 37°C for 20 mins. Add 1 ml of phenol, 1 ml of water was added to the solution, kept in room temperature for 20 mins and the absorbance was read at 415 nm using UV spectrometer.

RESULT AND DISCUSSION

Fermentation Process Of Honey And Pepper



Isolation Of Probiotic Bacteria



Lactobacillus sp on MRS medium
Bile Salt Tolerance Test

S.No	Bile Salt	OD at 600nm
1.	0.05%	1.4767
2.	0.15%	0.6344
3.	0.3%	1.1583

The OD at 600 nm falls as the bile salt concentration rises from 0.05% to 0.3%. This implies that probiotic bacterial growth or proliferation is adversely affected by increased bile salt concentrations.

Anti-diabetic Properties

S.No	Concentration(μ g/ml)	% of inhibition
1.	25 μ l	36.90%
2.	50 μ l	38.72%
3.	70 μ l	39.27%
4.	100 μ l	43.3%
5.	250 μ l	43.3%
6.	500 μ l	43.8%
7.	700 μ l	48.38%
8.	1000 μ l	52.05%

As the probiotic extract concentration rises, there is a corresponding increase in the inhibition of alpha-amylase activity. This indicates that the probiotic extract's bioactive ingredients may inhibit the alpha-amylase enzyme, which is involved in the breakdown of carbohydrates.

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