

Study of Phytochemical and Antimicrobial Activity of Honey Against Pathogenic Bacteria *E. Coli* and *Pseudomonas*

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Abstract

Honey is known for its health promoting properties, nutritional value and unique taste. Honey is also known as an antimicrobial agent, it strongly inhibits the growth of microorganisms. In present study, we examine and compare the phytochemical and antimicrobial activity of three types of branded honey. In vitro moisture content, total solids, electrical conductivity was observed in all honey samples. To examine antimicrobial activity by honey sample in *E. coli* and *Pseudomonas* we used agar well diffusion method and diameter of the inhibition zone was measured.

Keywords: Honey, phytochemical, antimicrobial, Inhibition zone

1. Introduction

In India and all over the world honey is known for its unique taste and health promoting properties. It is used in daily routine diet for its great nutritional value and health promoting properties it has a great value in human diet. It is also used as a traditional medicine in India since ancient time. It mainly consist of glucose, fructose and also minor amounts of oligosaccharides. Some phytochemicals compounds, minerals, vitamins, enzymes contribute to the health promoting factors of the product. Honey consists of beneficial antioxidants and is rich in iron content. Honey is also known as an antimicrobial agent and it strongly inhibits the growth of microorganisms.

Honey contains certain microorganisms mostly molds and yeast, but high osmotic pressure and high acidity inhibit the growth of microorganisms. As a result only few species of microorganism exist in honey and maintain their stable population at the time of storage. Some studies also suggested that some bacteria present in honey produce antimicrobial agents they protect honey for growth of other microorganisms and benefits for the consumers health.

Escherichia coli (*E. coli*) bacteria is commonly found in human gut and warm blooded animals. *E. coli* transmit to human by contaminated food, uncooked meat products and water. Few strains of *E. coli* caused diarrhoea and vomiting and stomach cramps.

Pseudomonas is a genus of Gram-negative bacterium. It is commonly found in the environment and can spread by human through contaminated hands, equipment, or surfaces. *Pseudomonas* caused blood infection, pneumonia in lungs and other body parts of the body in human. Present studies showed the phytochemical and antimicrobial effect of honey against *E. coli* and *pseudomonas*.

2. Material and method

2.1 Study area and sample collection – For the present study, three branded samples of honey were purchased from the local grocery store in Jabalpur city. Jabalpur is a city in Madhya Pradesh which is situated on the banks of Narmada River. We used three branded honey sample -Honey A, Honey B and Honey C to examine the phytochemical and antimicrobial properties.

This study was performed at Department Of Zoology St Aloysius College Jabalpur.

2.2 Phytochemical properties of honey:

(a)Moisture Content : In the present study moisture content of each honey sample was determined by measuring 5 grams of the sample and placed into a pre weighed aluminium drying dish (foil). The sample was dried to constant weight in an oven of 105 degree Celsius for 4 hours under vacuum. The final moisture content of the honey samples was determined by -

$$M.C = m1 - m2 / m1 - m0$$

m0 –Aluminium dish weight

m1- Aluminium dish +weight of

fresh sample

m2- Aluminium dish + weight of dried sample

(b) Determination of Total Solids – Total solids Percentage of each sample honey sample was determined using the following formula:

$$\text{Total solids (\%)} = 100 - \text{Moisture content}$$

(c) Determination Of Electrical Conductivity-- Electrical Conductivity of honey sample was observed through conductivity meter at 25⁰C.

2.3 Determination Of Antimicrobial Activity -

(a) Collection of culture – For the present study, Culture of E.coli and pseudomonas was collected from the Department Of Biotechnology St. Aloysius College Jabalpur.

(b) Preparation of agar media – 4.2 grams of Hinton agar powder was diluted in 100 ml distilled water. All the components were dissolved by heating and continuous stirring. the mixture was autoclaved at 121 degrees Celsius for 15 minutes. once the dissolved agar has been autoclaved, allow it to cool but not solidify. The agar media was poured into the petri plates and the plates were left on the sterile surface until the agar has solidified. Put the lid tight on each dish. Store the plates in a refrigerator.

(c) Inoculation And Pouring : Under hygienic conditions the laminar air flow was cleaned by 70 % ethanol every time before and after the pouring of agar media. The agar media was then poured into the autoclaved petri plates one by one. after some time when the agar solidifies in the petri plates After the agar solidifies the E. coli culture or pseudomonas culture is poured in the plate and mixed throughout the plate. After proper mixing a well is made in the petri plate with the help of micro pipette. The antibiotic strip of streptomycin is kept opposite to the well. The well is loaded each time with different samples in different petri plates. The petri plates are covered after pouring and on the cover, it is divided into two partitions with the help of marker with one side the well containing the sample and other side streptomycin strip. The petri plates were then placed inside the incubator at 37 degrees Celsius for 72 hours. Readings

were noted down for every sample in every 24, 48, 72 hours. After 72 hours the final diameter was noted down of every sample.

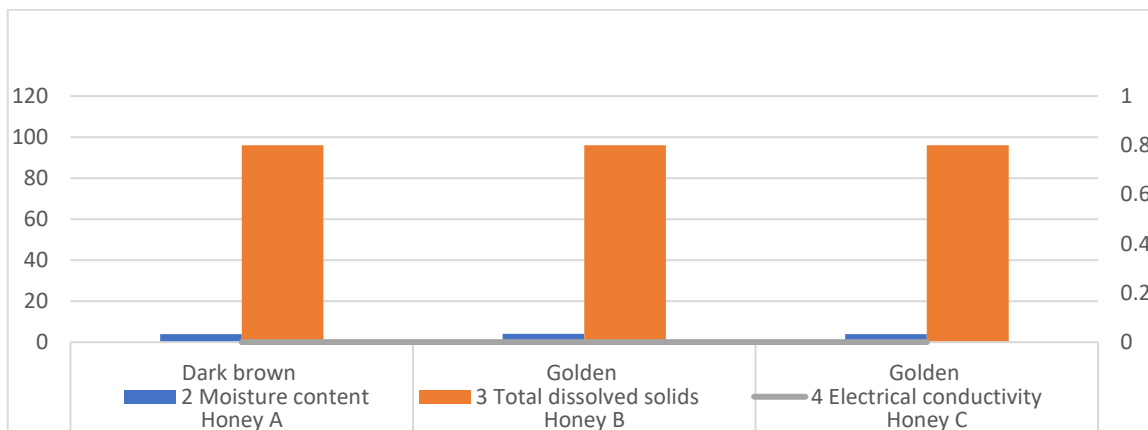
3. Result and Analysis –

3.1 Phytochemical analysis of honey samples-

All the three samples were analysed in the department of zoology St Aloysius college Jabalpur. The different parameters were observed including colour, moisture content , total solids, and electrical conductivity of all honey samples . The colour of all honey samples was reddish brown to golden in appearance and moisture content of all the samples was observed to be 3.98g ,4.03g, 3.99g respectively. Total solids determination was observed to be 96.01, 96.02, 95.97 respectively.

Table1 – Comparative Phytochemical Analysis Of Honey Samples

S.no	Physical properties	Honey A	Honey B	Honey C
1	Colour	Dark brown	Golden	Golden
2	Moisture content	3.98	4.03	3.99
3	Total dissolved solids	96.01	96.02	95.97
4	Electrical conductivity	0.0	0.0	0.0



3.2Antimicrobial assay of honey sample

Antibacterial activity was observed in all natural and commercial honey samples against E. Coli and Pseudomonas. Sample A, Sample B and Sample C was obtained from local market of Jabalpur city.

(a) Antimicrobial effect of Honey Sample On E.Coli.

The effect of E.coli on honey samples was recorded in every 24 hours time interval and final diameter was noted down after 72 hours of incubation .Sample A and B showed positive effect on E.coli . The diameter of the inhibition zone for the three Samples Honey A and Honey B was 19mm ,18mm and honey C shows negative effect on E.coli.

Table 2 -Antimicrobial effect of honey on E.coli

s.no	Sample On E.Coli	Diameter	Effect
1.	Sample Honey A	19 mm	Positive result
2.	Sample honey B	18 mm	Positive result
3.	Sample honey C	-----	No effect

(b) Antimicrobial activity of Honey Sample on Pseudomonas

In the case of Pseudomonas honey was more effective antimicrobial agent. The effect of honey on pseudomonas samples was also recorded in every 24 hours time interval and final diameter was noted down after 72 hours of incubation . The diameter of the inhibition zone for the all three samples A, Sample B and Sample C was 24mm , 26mm , 33mm respectively. Sample C was more effective as compare to others .

Table 3- Antimicrobial activity of honey on Pseudomonas

S.No	Sample On Pseudomonas	Diameter	Effect
1.	Sample Honey A	24 mm	Positive result
2.	Sample honey B	26 mm	Positive result
3.	Sample honey C	33 mm	Positive result



Discussion

Honey used as an effective antimicrobial agent since ancient time. Honey show great antimicrobial activity against both Gram-positive bacteria and Gram-negative bacteria. In present study we found that all three commercial honey inhibit growth of both bacteria *E.coli* and *pseudomonas*. According to Irish et al., 2011; Hydrogen peroxide is well known antimicrobial agent present in honey sample that cause DNA degradation in *E. coli*. Another study suggests (Vishnu Prasad Shenoy 2012) Inhibine is another antibacterial substance present in honey which cause Shrinkage and disruption of the bacteria cell wall.

Antibacterial property of honey on *E.coli*, We observed that among the three samples, commercial honey was more effective than local honey but in the case of *pseudomonas* all honey were effective with zone inhibition diameter of 30mm, 24mm diameter, 26 mm diameter.

Conclusion

In conclusion, the finding of this work is that all sample of honey showed different antimicrobial activity depending on geographical location and types of flower from which was made. Several earlier authors reported that many unidentified antimicrobial components and Hydrogen peroxide, sugar and protein compounds were present in honey sample.

In conclusion, the honey showed excellent antibacterial activity against *E. coli* and *pseudomonas*. It is suggested that honey is a good antimicrobial agent against some harmful pathogens and it can be used as a multidrug resistant and as an antiseptic ointment.

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