ANTIMICROBIAL ACTIVITYAND PHYTOCHEMICAL ANALYSIS OF HONEY

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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OF

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This is to certify that **MR. NIRANJAN. K. PATIL** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled **"Antimicrobial Activity and Phytochemical Analysis of Honey"** during academic year 2024- 2025

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Place: Kolhapur
Date: 18/12/2024.

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Ms. ANKITA KESARE.

INDEX

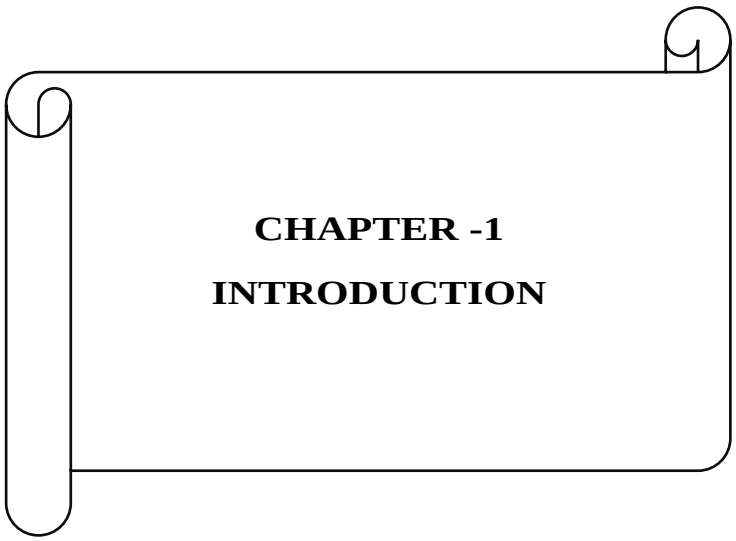
Sr. no	Title	Page no.
1.	Introduction	11-14
2.	Review of literature	16-17
3.	Aim and Objectives	18
4.	Materials and methodology	20- 26
5.	Results and Discussion	27- 38
6.	Conclusion	39-40
7.	Bibliography	41-44

LIST OF TABLES

- **Table No. 1:** Effect of honey on *Staphylococcus aureus*
- **Table No. 2:** Effect of honey on *Bacillus spp.*
- **Table no. 2:** Effect of honey on, *Escherichia coli*
- **Table no. 3:** Effect of honey on *Klebsiella spp.*
- **Table No. 4:** Effect of honey on *Salmonella typhi*

LIST OF PHOTOGRAPHS

- **Photograph no.1:** The Inhibition Zone of Honey *on Staphylococcus aureus*
- **Photograph no.2**: The Inhibition Zone of honey on *Escherichia coli*
- **Photograph no.3**: The Inhibition Zone of honey on *Klebsiella spp.*
- **Photograph no.4**: The Inhibition Zone of honey on *Salmonella typhi*
- **Photograph no.5**: Phytochemical analysis:



CHAPTER -1

INTRODUCTION

INTRODUCTION

Honey is valued not only for its sweetness but also for its medicinal properties. It has been used since ancient times to heal wounds and fight infections. Honey has anti-inflammatory, healing, and antioxidant effects and is a traditional remedy for bacterial infections, colds, coughs, and other diseases. The effectiveness of honey depends on factors like the type of flowers the bees visit, the bees' health, and how the honey is processed.

ANCIENT USE OF HONEY

In ancient text the *Atharvaveda*, one of the oldest Vedic texts, contains numerous references to honey, highlighting its use as a therapeutic agent and as an offering to the gods (Krishnamurthy, 2012). Honey was also prominently featured in Ayurvedic medicine, where it was described as a remedy for a wide range of ailments, including digestive issues, respiratory problems, and wounds (Sharma, 2007). In traditional Indian culture, honey has been considered a symbol of health and longevity. It is used in various religious rituals and offerings, often symbolizing purity and sweetness. The *Charaka Samhita*, an ancient Indian medical treatise, extols the medicinal properties of honey, emphasizing its role in balancing the body's doshas (humors) and improving vitality (Nadkarni, 2000).

The earliest evidence of honey harvesting dates back to prehistoric times. Rock art in Spain's *Cuevas de la Arana* (Cave of the Spider) in the *La Arana* cave shows images of early humans collecting honey from wild bees, estimated to be about 8,000 years old. This suggests that humans have been interacting with honeybees and collecting honey since the Paleolithic era (7000-6000 BCE) (Radovanovic, 2009).

Archaeological evidence suggests that honey was used by the ancient Egyptians, Greeks, and Romans, with honey being found in tombs and used in rituals, medicinal treatments, and as a sweetener (Eichhorn et al., 2009). In ancient medicine, honey was used to treat a variety of ailments, ranging from wounds to digestive issues (Molan, 1992). The renowned Greek physician Hippocrates is known to have prescribed honey for its healing properties, particularly for wounds and ulcers (Rasmussen, 2008). Throughout history, honey's value was not just as a food but also as a form of currency and a trade commodity (Lambertsen, 2003). The Roman Empire also valued honey, with Roman writings showing that the Romans used it in a variety of ways, including as an ingredient in foods, as a preservative, and in medicinal treatments. The Roman scholar Pliny the Elder (23-79 CE) even described how honey could be used to treat wounds, digestive problems, and as a component in beauty treatments (Pliny the Elder, *Natural History*)

MODERN USE

By the 19th century, beekeeping in Europe and North America had become more industrialized. The invention of the Langstroth hive, which allowed beekeepers to harvest honey without killing the bees. Medicinal importance of honey has been documented in the world's oldest medical literatures, and since the ancient times, it has been known to possess antimicrobial property as well as wound-healing activity. The healing property of honey is due to the fact that it offers antibacterial activity, maintains a moist wound condition, and its high viscosity helps to provide a protective barrier to prevent infection. Its immunomodulatory property is relevant to wound repair too. The honey, when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection. The application of honey can promote the healing in infected wounds that do not respond to the conventional therapy, *i.e.*, antibiotics and antiseptics including wounds infections.

The antimicrobial properties of honey have been widely recognized, with studies showing its effectiveness against a range of pathogens, including bacteria, fungi, and viruses (Molan, 2001; Gheldof et al., 2002). Honey is also said to be highly variable like must plant derived product and the chemical composition of honey also depends on the flower from which it is made. Antimicrobial effect may vary between different types of honey (Ovington, 2007). These antimicrobial effects are of particular interest in the context of the global rise in antimicrobial resistance (AMR), as honey may offer a viable alternative to conventional antibiotics in treating infections, especially those caused by multidrug-resistant pathogens (Ghanbari et al., 2016). The antimicrobial activity of honey arises from its low water content, acidity (low pH), production of hydrogen peroxide, and the presence of bioactive molecules such as phenolic acids and flavonoids. Honey is effective against a broad range of microorganisms, including *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

The composition of honey:

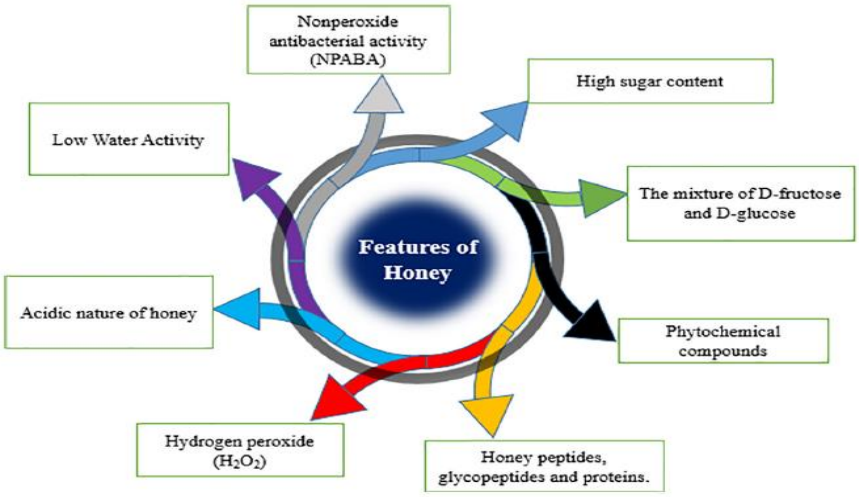


Fig. 1. Schematic diagram showing the parameters that contribute to the antimicrobial potential of honey.

Honey also contains helpful compounds like phytochemicals, peptides, and proteins that fight germs. Honey can be used as a traditional medicine because it does not cause harmful side effects and is widely used to cure various diseases. This is because of secondary metabolites found in honey, such as alkaloids, flavonoids, saponins, tannins, and essential oils. [2]. Besides containing secondary metabolites, honey contains many compounds, including carbohydrates (primarily fructose and glucose), vitamins, minerals, enzymes, organic compounds, free amino acids, and volatile compounds that contribute to color, aroma, and taste [3]. The content of honey

The content of honey consists of 80-85% carbohydrates (glucose and fructose), 15-17% water, 0.1-0.4% protein, 0.2% ash, small amounts of amino acids, enzymes, vitamins, and other substances [4]. The composition and quality of honey are very diverse and depends on the source of honey bee feed as a source of nectar, climate differences, honey maturity and processing and storage [3,5,6]. The honey content significantly affects the physicochemical properties of honey [1]. Honey has physicochemical and microbiological characteristics that can be used as honey quality parameters.

How Honey Fights Microorganisms:

1.Disrupting Biofilms: Honey can break down bacterial biofilms (protective layers), making it effective against bacteria like *Staphylococcus aureus*.

2. High Sugar Content: Honey's high sugar concentration dehydrates and kills microbes.

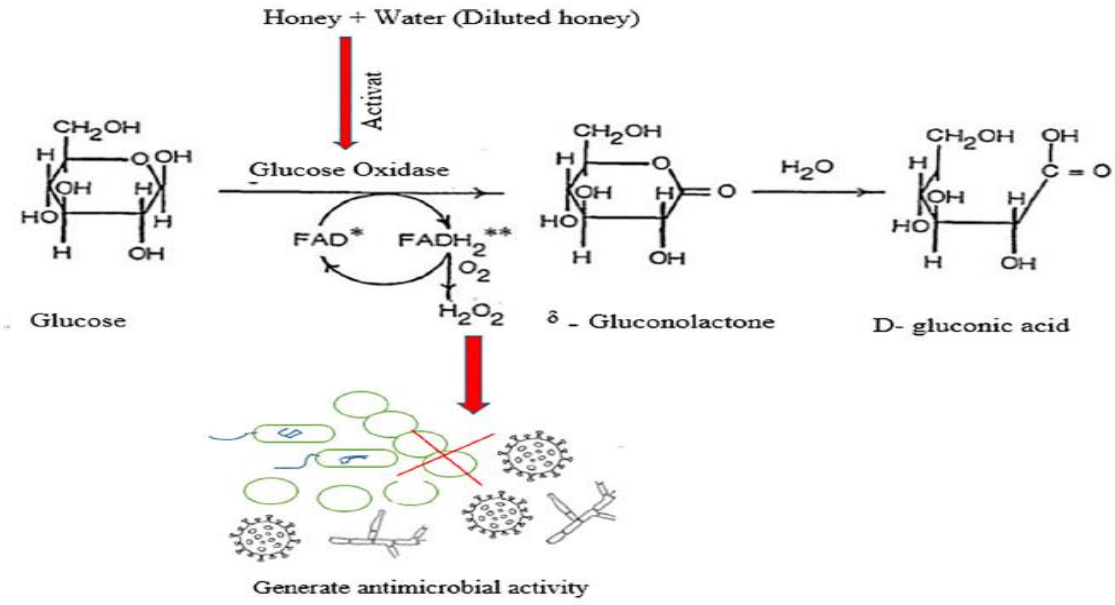
3. Low pH: Its acidic nature prevents many pathogens from surviving.

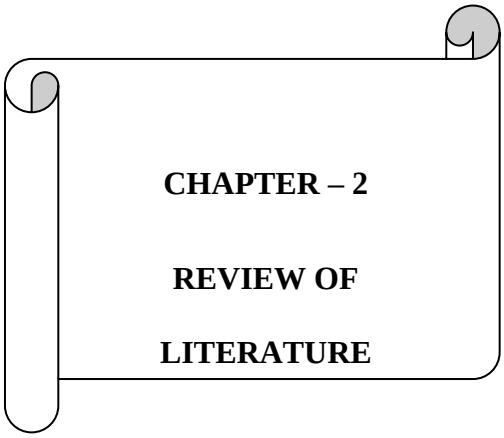
4. Hydrogen Peroxide: Honey produces hydrogen peroxide, which kills bacteria.

5. Non-peroxide Activity: Certain types of honey, like Manuka honey, have unique antimicrobial compounds such as methylglyoxal (MGO).

6. Bioactive Compounds: Honey contains polyphenols and flavonoids, which have antimicrobial, antioxidant, and anti-inflammatory effects

7. Defensins: Honey includes peptides like defensin-1, which directly kill microbes.





CHAPTER – 2

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

2.1 Antimicrobial activity of honey:

Antibiotic-resistant bacteria and their resistance genes emerge and spread globally among people, food, animals, plants, and the environment (soil, water, andair) (Berendonk et al. 2015). Erratic success in treating infectious diseases results in important societal and economic costs to human health and well-being (WHO 2020). Excessive and improper use of antibiotics in farm animals amplify and accelerate this development (Manyi-Loh et al. 2018). Introducing a novel antimicrobial requires lengthy efficacy and safety studies before introduction entry into the market. Increasing worldwide emergence of multidrug resistant pathogens emphasize the need to develop alternative or complementary treatment strategies, effective substances, formulas, or active ingredients. Honey is a natural sweet substance consisting of hundreds of compounds (Maddocks and Jenkins 2013; Nolan et al. 2019). Honey has been used both as food and as a traditional medicine for centuries (Zumla and Lulat 1989). Comprehensive reviews and several studies have been published on honey varieties with promising antibacterial and medicinal properties (Maddocks and Jenkins 2013, Zainol et al. 2013, Huttunen et al. 2013, Salonen et al. 2017, Mandal and Mandal 2011, Combarros-Fuertes et al. 2020), and especially in wound healing (Al-Waili et al. 2011). Recently, a meta-analysis confirmed the activity of honey against viral respiratory infections (Abuelgasim et al. 2020), and immunomodulatory effects of different sources of honeys have also been demonstrated (Ota et al. 2019). Honeys have not been reported to have toxic or other harmful side effects (Zainol et al. 2013). There are several reports showing activity of honey against antibiotic-resistant bacteria (Kwakman et al. 2008, Cooper et al. 2010, Maddocks and Jenkins 2013, Huttunen et al. 2013, Shah Pratibha and Williamson Manita 2015, Natarajan et al. 2001).

The present study was made considering consumer perspectives and bioactivity among randomly selected organic honeys available in supermarkets. Organic honeys from supermarkets in Kolhapur were investigated. They were tested for their antibacterial activity against six important bacterial pathogens isolated. The studied medically important bacteria ***Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus spp, and Staphylococcus aureus*** represent various disease conditions. ***E. coli*** is a versatile bacterial

species with the ability to cause intestinal or systemic diseases in humans and in animals (Leimbach et al. 2013). ***E. coli*** is associated with urinary tract infections, diarrhea, septicemia, wound, and other infections, such as neonatal meningitis. *E. coli* causes bacterial infections in humans and is

prominently associated with diarrhea in pets and farm animals. The therapeutic treatment is compromised by the emergence of antimicrobial resistance (Allocati et al. 2013). Human infections with typhoidal ***Salmonella typhi*** cause typhoid fever, the treatment of which is complicated by increasing drug resistance (Johnson et al. 2018). Typhoidal serovars cause a systemic infection (Raffatellu et al. 2008). ***P. aeruginosa*** causes infections and diseases in both plants and animals, including several human diseases, e.g., wound infections, diabetic foot ulcers, urinary infections, and many hospital-acquired infections especially in immune-compromised patients. P. aeruginosa is an opportunistic pathogen and the occurrence of antimicrobial resistance makes it difficult to treat and eradicate (Azam and Khan 2019). ***K. pneumoniae***, known as a major threat to public health, is the most common factor of hospital- and community- acquired infections. (Shah Pratibha and Williamson Manita 2015). ***Bacillus spp*** has been associated with food poisoning. The bacterium causes two types of gastrointestinal disease, the diarrheal and the emetic syndromes, which are caused by very different types of toxins (Stenfors Arnesen et al. 2008). ***S. aureus*** is an important commensal organism of the human skin and mucous membranes. ***S. aureus*** can cause opportunistic infections including biofilm-associated infections on indwelling medical devices and nosocomial sepsis (Nguyen et al. 2017). ***S. aureus*** can be found in food products, e.g., artisanal cheeses from raw whole cow milk, being a threat for humans with more virulence factors and antibiotic resistance through mobile genetic elements (Chajęcka-Wierzchowska et al. 2019). Great variabilities of honey samples regarding quality and bioactivity are available for customer use in the market. Organic honeys are produced using strict ecological and natural principles which are meant to enhance the good quality (Estevinho et al.2012). Antimicrobial activity of the organic honeys is less studied. The aim of the present study was to collect commercial organic honey samples from supermarkets in order to investigate their antimicrobial activity against important human pathogens.

2.2 Phytochemical analysis of honey:

Honey is a substance that contains high antioxidant, antibacterial, anti-inflammatory, analgesic, wound- healing, anti-cancer and free radical scavenging activity. The antioxidant properties are conferred by compound varies in honey, including flavonoids, phenolics, vitamin C and amino acids. Phytochemical screening is a method for identifying active compounds in honey which can be potential antibacterial and antioxidant. This study aims at determining the phytochemical content of honey by qualitative tests on alkaloids, steroids, flavonoids, tannins, saponins, triterpenoids, and quinones. The result of this study is expected to provide information in searching for compounds with pharmacological effect.

AIM AND OBJECTIVES

❖ **AIM:** To study the antimicrobial activity and phytochemical analysis of honey

❖ **OBJECTIVES:**

I. To verify the authenticity of honey**.**

I. To evaluate the antimicrobial activity of honey against various microorganism.

II. To identify the Phytochemical compound present in honey

CHAPTER- 3

METHODS AND MATERIALS

3. Methods and materials:

3.1 Collection of samples

Honey sample – *(Dabur and Royal bee Himalaya multifloral honey)*

The different variety of honey is available in the market. For study, Dabur and Royal bee Himalaya multifloral honey was purchased from Kolhapur market.

Purity of honey:

The purity was checked in the laboratory by the following process:

Water test

One tablespoon of honey was mixed with 10 ml water and stirred with glass rod. If honey get settled at bottom, it indicates that honey is pure.

Heat test

Heat a small amount of honey in a microwave-safe container for 30 seconds or in a saucepan on low heat. Pure honey should caramelize slightly and leave a caramel like aroma.

Paper test

Few drops of honey on a piece of paper, pure honey will remain intact and will not get absorbed into the paper. It might make the paper slightly sticky.

3.2. Materials Needed

- Microorganisms
- Agar plates
- Nutrient Broth media
- Well Diffusion

1. **Microorganisms Bacteria** (e.g., *Staphylococcus aureus, Escherichia coli salmonella typhi, klebsiella spp, Bacillus, Pseudomonas aeruginosa.)*
2. **Agar plates:** Nutrient agar
3. **Well diffusion plates**: Plates with wells for well diffusion.
4. **Incubators**: Temperature-controlled incubators for culturing microorganisms
5. **Cyclomixer**: Mixing of the honey dilutions.

3.3. Phytochemical analysis:

Chemicals:

3.3.1. Mayer's reagent:

Mercuric chloride (1.36 gm)

Potassium Iodide = 5 gm

Distilled water (P.W) = 100 ml

3.3.2. Dragendorff's reagent:

Bismuth sub nitrate (1.7 g)

Potassium Iodide = (40 gm)

Citric acid = 20 ml

Distilled water (P.W) = 100 ml

3.3.3. Wagner's reagent:

Iodine = 1.27 gm

Potassium Iodide = 2 gm

Distilled water (P.W) = 100 ml

3.3.4. Bacterial cultures

Following bacterial cultures available in our laboratory were used in the study

Gram positive organisms

- *Staphylococcus aureus*
- *Bacillus*

Gram negative organisms

- *Salmonella*
- *Klebsiella*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

The microorganisms were reconstituted by sub culturing on to freshly prepared nutrient agar slants. They were incubated at 37º Celsius for 24 hours.

3.3.5 Culture media

Nutrient agar medium was used for cultivation and checking the antibacterial activity of honey.

Peptone:	0.5 gm
Meat extract / yeast extract:	0.3gm
Sodium chloride:	0.5gm
Agar:	2gm
Distilled water:	100 ml
pH:	7 to7.2
Distilled water:	100ml

3.3.6. Sample Preparation

3.6.1. Honey-

The bottle of **Royal bee Himalaya** multifloral honey was opened aseptically and different dilutions of honey were done using sterile potable water as diluents.

Table -1

Dilutions of honey.

Sr.No.	Dilutions of honey (%)	Sterile Distilled Water (ml)	Honey (ml)
1	1	9.9	0.1
2	10	9	1
3	20	8	2
4	30	7	3
5	40	6	4
6	50	5	5
7	60	4	6
8	70	3	7
9	80	2	8
10	90	1	9
11	100	-	pure

3.6.2. Honey-

The bottle of Dabar multifloral honey was opened aseptically and different dilutions of honey were done using sterile potable water.

Dilutions of honey: Same dilution was prepared using Dabar honey

- ***Staphylococcus aureus:***

To study antibacterial activity of honey against *Staphylococcus aureus*

1. **Preparation of suspension of *Staphylococcus aureus***

A loop full of freshly grown *Staphylococcus aureus* was added in to sterile saline to prepare thick suspension of organism.

2. Preparation of seeded agar

A sterilized 20ml nutrient broth medium was prepared in 5 flasks.

3. The suspension of an organism is added into 20ml nutrient broth medium.

4. Pouring of plates

After addition of the organism into the nutrient broth medium, the plates were poured.

5. **Solidification of medium**

Plates were allowed to solidify.

6. **Preparation of wells.**

Wells of approximately 10 mm diameter were cut using the sterile cork borer in solidified seeded agar.

7. **Addition of honey dilutions**.

Each dilution of honey was added in a respective well. Each dilution of honey was respectively added in well-labelled petri plates, and proper labelling was done. In one well sterile pure honey was added.

8. All plates were kept in refrigerator at 4° c for 15 mints

9. After that plates were incubated at 37°c for 24 hrs.

10. After incubation plates were examined for inhibition zone around each well.

Similar procedure was followed for all other pathogens

- *Salmonella typhi*
- *Escherichia coli*
- *Klebsiella spp*

- *Bacillus*
- *Pseudomonas aeruginosa*

3.7. Phytochemical analysis:

Chemicals:

1. Mayer's reagent:

Mercuric chloride (1.36 gm)

Potassium Iodide = 5 gm

Distilled water (P.W) = 100 ml

2. Dragendorff's reagent:

Bismuth sub nitrate (1.7 g)

Potassium Iodide = (40 gm)

Citric acid = 20 ml

Distilled water (P.W) = 100 ml

3. Wagner's reagent:

Iodine = 1.27 gm

Potassium Iodide = 2 gm

Distilled water (P.W) = 100 ml

3.7.1. Analysis of Alkaloid compound:

1. 2ml honey + 6ml D/W (in test tube)
2. 3ml of solution were piped out.
3. Add 0.3 ml of 2N HCL.
4. Heat the solution in boiling water bath for 3 minutes and allow it to cool.
5. 1ml of solution is transferred in 3 test tube
6. 2 drops of Wagner's reagent, Drangendroff reagent, Mayer's reagent was added in each test tube.
7. Positive results: Drangendroffs reagent – Red deposit
   Wagner's reagent - Brownish red deposit
   Mayer's reagent – No result (Negative).

3.7.2. Analysis of saponin compound:

1. 2 ml honey is mixed with 6 ml D/W (in test tube)
2. Boiled for 2-3 min or shaken 10 min, cooled for 15 min
3. Shaken vigorously added 2 drops of HCL
4. If a stable form, then the sample will positively contain saponins.

3.7.3. Analysis of Tannin Compound:

1. 1 ml honey is mixed with 3ml D/W in test tube.
2. Then 3 drops of Solution transferred into a spot plate and titrated 2-3 drops of 1% $FeCl_3$ solution.
3. Positive result dark blue of greenish black colour.

3.7.4. Analysis of Flavonoid Compound

1. 2 ml honey is dissolved in 6 ml D/W
2. 1 ml of solution was piped out, added 1 ml ethanol
3. Heat five min in test tube
4. 5 drops of HCl added (0.025 g of mg)
5. Positive result: Dark red colour in 3 min

CHAPTER – 4

RESULT AND DICUSSION

4.1. Antimicrobial activity of honey (Royal bee Himalaya honey)

The respective dilutions of honey were prepared according to the dilution table. The suspension of test organism is suspended in nutrient media. The nutrient media was spread on sterile Petri plates and plates were allowed to solidify.

After solidification plates was kept for diffusion in the refrigerator for 15 min and then incubated at 37°C. After incubation plates were observed for clear zone of inhibition.

The honey sample showed inhibition zones against:

Gram positive organisms

- *Staphylococcus aureus*

Gram negative organisms

- *Salmonella*
- *Escherichia coli*

The honey sample that does not showed inhibition zones against honey

- *Bacillus spp.*
- *Klebsiella.*
- *Pseudomonas.*

4.1.1 Staphylococcus aureus

Table 1. Effect of honey on *Staphylococcus aureus.*

Dilutions of honey	1	10	20	30	40	50
Zone of inhibition	-	2cm	2.5cm	2.6cm	2.3cm	2.9cm

Dilutions of honey	60	70	80	90	100
Zone of inhibition	3cm	3.2cm	3.5 cm	3.6 cm	3.8 cm

From table 1 it is clear that as concentration of Honey increases zone of inhibition also increases. The higher zone diameter was observed at 90% and 100% and lower zone diameter was observed at 10% concentration of honey.

Photo : Zone of inhibition of honey on *Staphylococcus aureus*

4.1.2. *Escherichia coli*

Table 2 Effect of honey on, *Escherichia coli,*

Dilutions of honey	1	10	20	30	40	50
Zone of inhibition	_	1.8 cm	2 cm	2.5.cm	2.6cm	3 cm

Dilutions of honey	60	70	80	90	100
Zone of inhibition	3.2 cm	3.5cm	3.6 cm	3.8 cm	3.9 cm

From table 2 it is clear that as concentration of Honey increases zone of inhibition also increases. The higher zone diameter was observed at 90% and 100% and lower zone diameter was observed at 10% concentration of honey.

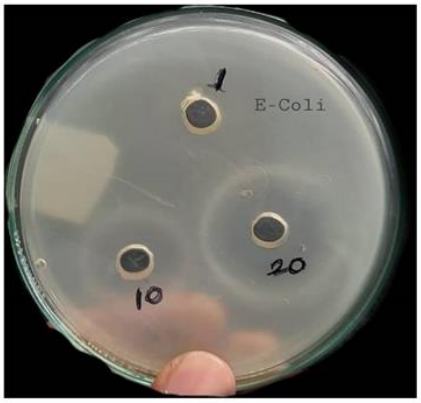


Photo : Zone of inhibition of honey on *Escherichia coli*

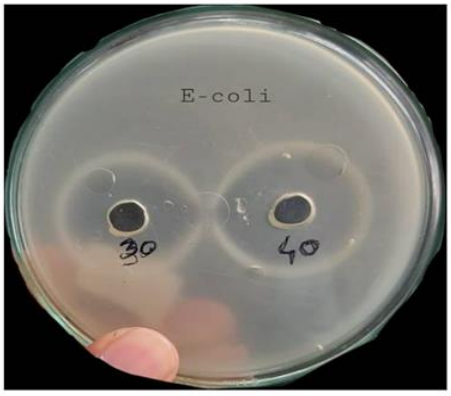
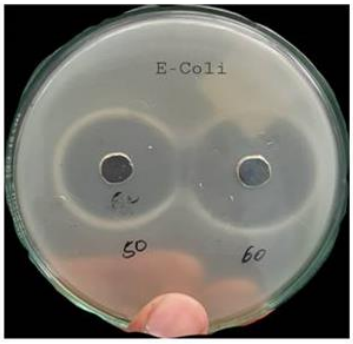


Photo : Zone of inhibition of honey on *Escherichia coli*



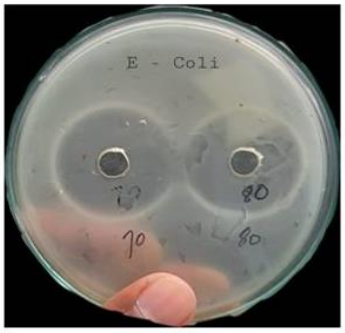


Photo : Zone of inhibition of honey on *Escherichia coli*

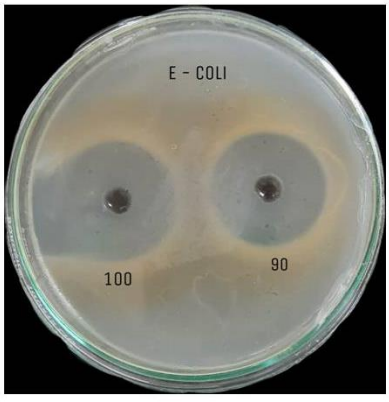


Photo : Zone of inhibition of honey on *Escherichia coli*

4.1.3 *Salmonella typhi*

Table 3 Effect of honey on *Salmonella typhi*

Dilutions of honey	20	40	60	80	100
Zone of inhibition	1.2	1.6	1.8	2.2	2.9

From table 4 it is clear that as concentration of Honey increases zone of inhibition also increases. The higher zone diameter was observed at 90% and 100% and lower zone diameter was observed at 10% concentration of honey.

Figure - 4 The Inhibition Zone of honey on *Salmonella typhi*

4.1.4. Klebsiella spp.

Table 4 Effect of honey on *Klebsiella spp*.

Dilutions of honey	20	40	60	80	100
Zone of inhibition	0	0	0	0	0

From table 3. Zone of inhibition was not observed for any dilution concentration of honey

4.1.5 Bacillus spp.

Table 5 Effect of honey on *Bacillus spp.*

Dilutions of honey	20	40	60	80	100
Zone of inhibition	0	0	0	0	0

From table 4. Zone of inhibition was not observed for any dilution concentration of honey

4.1.6 Pseudomonas aeruginosa.

Table 6. Effect of honey on *Pseudomonas aeruginosa.*

Dilutions of honey	20	40	60	80	100
Zone of inhibition	0	0	0	0	0

From table 6. Zone of inhibition was not observed for any dilution concentration of honey

4.2. Antimicrobial activity of honey (Dabar honey)

The respective dilutions of honey were prepared according to the dilution table. The suspension of test organism is suspended in nutrient media. The nutrient media was spread on sterile Petri plates and plates were allowed to solidify.

After solidification plates was kept for diffusion in the refrigerator for 15 min and then incubated at 37°C. After incubation plates were observed for clear zone of inhibition.

The Dabar honey sample that do not show inhibition zones against honey

- *Staphylococcus aureus*
- *Bacillus spp.*
- *Klebsiella.*
- *Pseudomonas.*
- *Salmonella*
- *Escherichia coli*

4.2. Phytochemical analysis:

The phytochemical analysis is used to identify the secondary metabolites in a qualitative way is indicated by the colour intensities produced by the reagents.

The phytochemical analysis results for Royal bee Himalaya multifloral honey are as follows:

4.2.1 Analysis of Alkaloid compound:

The results of alkaloid compounds analysis in Royal bee Himalaya multifloral honey with Dragendorff's reagent, Wagner's reagent show the formation of deposits. The analysis results in red deposits with Dragendorff reagents, no white deposits with Mayer reagents and brownish red deposits with Wagner reagents. Therefore, there was presence of alkaloids in Royal bee Himalaya multifloral honey.

The principle of this method relies on depositional reactions. Mayer's reagent contains mercury chloride and potassium iodide. In these reactions, the nitrogen atoms in alkaloids have free electron pairs. These electron pairs can replace iodo ions in the reagent and form stable bonds (coordinate covalent bonds) with metal ions.

4.2.2 Analysis of Saponin compound:

The results of saponin compound analysis showed that Royal bee Himalaya multifloral honey contains saponins which are identified by stable form. Saponins are secondary metabolites that have antimicrobial activity. The ability of honey as antimicrobial substances is because of its low pH. The Royal bee Himalaya multifloral honey sample examined have the potential as antimicrobial is they contain active saponin compound. Saponins are natural surfactants that reduce surface tension, leading to the formation of stable form. If the stable form remains for a prolonged period, it suggests the presence of saponins in the honey.

4.2.3 Analysis of Tannin compound:

The analysis of tannin compound in Royal bee Himalaya multifloral honey showed that it did not contain tannins. The principle of this method is that, 1 ml honey is mixed with 3ml D/W in test tube. 3 drops of Solution transferred into a spot plate titrated with 2-3 drops of 1% $FeCl_3$ solution. The results of this reaction are seen in formation of dark blue or black colour.

Results: Not found (Negative)

4.7.4 Analysis of Flavonoid compound:

The analysis of Flavonoid compound in Royal bee Himalaya multifloral honey showed that it did not contain flavonoids . The principle of that method id that 2 ml honey is dissolved in 6 ml D/W. 1 ml of solution was piped out, added 1 ml ethanol. Heat five min in test tube 5 drops of HCl added (0.025 g of mg) . The positive result of this reaction shows dark red colour in 3 min.

Result: Not found (Negative)

4.8 Standard results for phytochemical analysis:

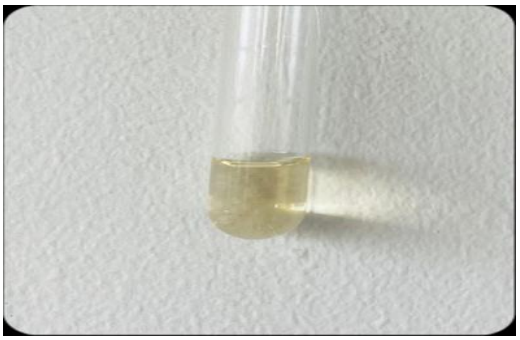
Sr.No.	Constituents	Method	Result
1	Alkaloid	Drangendroff reagent	+
		Wagner's reagent	+
		Mayer's reagent	-
2	Saponin	2 ml honey is mixed with 6 ml D/W (in test tube) Boiled for 2-3 min or shaken 10 min, cooled for 15 min Shaken vigorously added 2 drops of HCL	+
3	Tannin	1 ml honey is mixed with 3ml D/W in test tube 3 drops of Solution transferred into a spot plate titrated with 2-3 drops of 1% $FeCl_3$ solution.	–
4	Flavonoid	2 ml honey is dissolved in 6 ml D/W 1 ml of solution was piped out, added 1 ml ethanol Heat five min in test tube 5 drops of HCl added (0.025 g of mg)	–

Results:

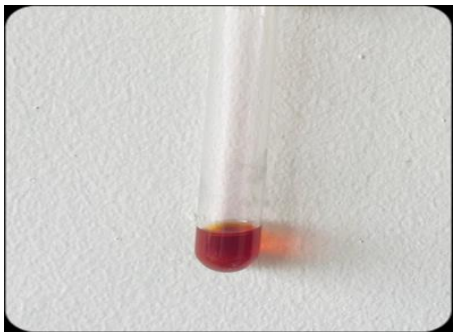
Positive = +

Negative = -

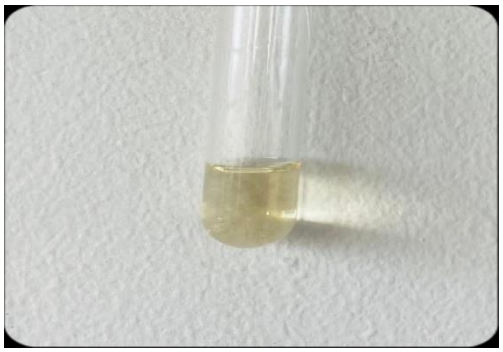
Control

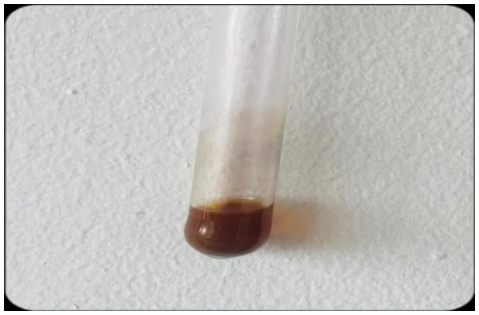


Drangendroffs reagent



Wagner's reagent





Saponins

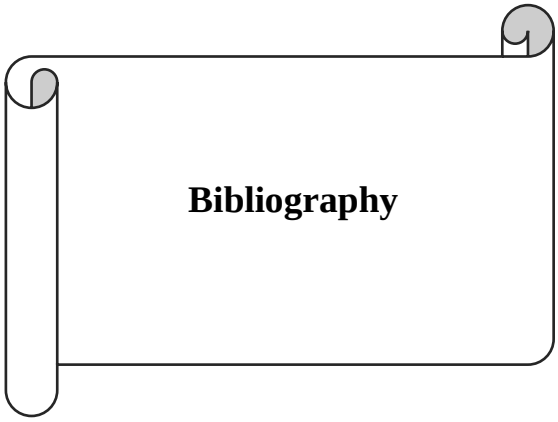
CHAPTER – 5

CONCLUSION

5.1 Conclusion:

The Royal bee Himalaya multifloral honey has antimicrobial activity against different pathogenic organism from this result we can conclude that honey has potential to use in against various pathogenic organisms. Another honey sample which is Dabur honey has not showed any positive results on the test organisms.

From the phytochemical analysis of honey, it is clear that honey is rich in alkaloids, saponins. Therefore, we can use this honey as antimicrobial agent against various pathogenic organisms. The Royal bee honey is active against pathogenic organisms. It is effective than any other commercially available honey in the market.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11. Comparison of methods to determine antibacterial activity of honeys against Staphylococcus aureus. Sandra M. Osés ∗, Ana Pascual-Maté, Daniel de la Fuente, Ana de Pablo, Miguel A. Fernández-Muino, ˜ M Teresa Sancho∗Department of Biotechnology and Food Science, University of Burgos, Plaza Misael-Ba˜nuelos s/n, Burgos, 09001 Spain

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20. Diego Romário-Silva 1,2, Severino Matias Alencar 3, Bruno Bueno-Silva 4, Janaína de Cássia Orlandi Sardi 2,4, Marcelo Franchin 5,, Rafaela Durrer Parolina de Carvalho 1, Thayná Ellen de Sousa Alves Ferreira 1,2, Pedro Luiz Rosalen 1,6,

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Jackie K. Obey. Moses M. Ngeiywa Marjatta Lehesvaara Jussi KauhanenAtte von Wright Carina Tikkanen-Kaukanen

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23. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece

Dimitrios Stagos Nikolaos Soulitsiotis Christina Tsadila Stamatina Papaeconomou Charalampos Arvanitis Alexandros Ntontos Fani Karkanta Soultana Adamou-Androulaki Konstantinos Petrotos Demetrios A. Spandidos Demetrios Kouretas Dimitris Mossialos

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