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PHYTOCHEMICAL ANALYSIS OF DIFFERENT HONEY SAMPLES AND ITS ANTI-BACTERIAL ACTIVITY AGAINST CLINICAL PATHOGENIC BACTERIA

Ms. A. Deepa*[1], Ms. C. Muthu pritha[2], Ms. M. Abiyoga[3]

Pg Scholar*[1], Pg Scholar [2], Pg Scholar [3]

Department of Microbiology, Tiruppur Kumaran College for Women,

Mangalam road, Tirupur – 641687.

Corresponding author

A. Deepa

E Mail: deepashi2410@gmail.com

ABSTRACT

Honey is a natural product composed of numerous substance and microorganisms. Honey have antimicrobial characteristics, certain microorganisms are able to survive and multiply in honey. In this present study to evaluate the phytochemicals analysis for three different honey samples. The honey samples were collected from dalmia (near small village) at Trichy district. The honey samples were named as sample 1, sample 2 and sample 3. Honey samples were diluted by using the nutrient broth. The phytochemicals analysis of honey such as carbohydrate, amino acid, potassium, calcium, Iron, magnesium, flavonoids and alkaloids are Qualitative analysis of honey sample and some quantitative analysis were also done such as Total phenol test, Hydrogen peroxide test and Total Antioxidant test were performed by standard laboratory methods. The antimicrobial activity of Honey samples were studied against clinical pathogenic bacteria by well diffusion method.

Keywords: Clinical pathogenic bacteria, Honey Samples, Phytochemical analysis, Qualitative test, Quantitative test and Antimicrobial activity.

1. INTRODUCTION

Honey was a sweet, viscous food substance made by honey bees. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (honeydew) by enzymatic activity and water evaporation. Honey bees stored honey in wax structures called honey combs, where stingless bees store honey in pots made of wax and resin (Ngalimat*et al*., 2019).

The different variety of honey produced by honey bees was the best known, due to its worldwide commercial production and human consumption. Honey contain carbohydrates, fat, protein, vitamins and minerals and they are essential for nutritional requirements of humans. Honey got its sweetness from the monosaccharides fructose, glucose & sucrose and 15ml of honey provides around 190 Kilo Jules of food energy (Alvarez-Suarez *et al*., 2014).

Honey has been highly valued and plays a significant role in a novel branch of alternative medicine, termed 'Apitherapy', which emphases the medicinal use of honey as well as other bee and hive products (Majtan *et al*., 2009).

Honey has been extensively used as a therapeutic agent for the treatment of numerous diseases. Honey demonstrates beneficial effects in many physiological systems, for example, the cardiovascular, nervous, respiratory and gastrointestinal systems. Honey might exert antimicrobial and antioxidant activities due to its high osmolality, acidity, generation of H_2O_2 and NO on exposure to water, as well as the presence of methylglyoxal (MGO) (Mavric*et al*., 2008). Furthermore, phenolic compounds, organic acids, enzymes (e.g., diastase, glucose oxidase, and invertase), minerals (e.g., potassium, iron, zinc) and other minor constituents also have potential anti-parasitic, and antidiabetic activities (Cianciose*t al*., 2018).

Honey was a supersaturated sugary and flavorful natural product of great nutritional value. It was also used for its positive impacts on human health, in particular for its antioxidant, antimicrobial and anti-

inflammatory and antimicrobial properties. Honey has been found to provide beneficial effects to different stages of wound healing (i.e., hemostasis, inflammation, remodeling) and thus to influence positively the natural physiology of wound healing, particularly by reducing edema and wound exudate. The antibacterial and antioxidant activities of honey also contribute to its wound remedial effects (Alvarez-Suarez *et al*., 2013).

These benefits of honey to be used as a potential antibacterial and anti-inflammatory agent in a range of medicinal products (Saikaly*et al.,* 2017).Bacterial resistance is less likely to develop as a result of treatment of bacteria with honey. This is because of the composition of honey which contains a number of different components (Carnwath*et al*., 2014). Gram negative organisms such as *Pseudomonas aeruginosa* have been a major problem in hospital acquired infections and cause most severe wound and burn infections (Krondaet *al*., 2013).

Pseudomonas aeruginosa has become multidrug resistant due to its potential to acquire new antimicrobial resistance (Roberts *et al*., 2012). *Pseudomonas aeruginosa* is a bacterial pathogen that causes a substantial portion of hospital infections. It is frequently contributes to the high morbidity and mortality of patients in intensive care units, burns units and surgical wards(Abd-ElAal*et al*., 2007).

The antibacterial activity of honey is attributed and influenced by various properties such as low water content, high viscosity, acidity, hydrogen peroxide content, non-peroxide components, particularly and the presence of MGO (Weston *et al*., 2000) peptides, non-peroxidase glycol peptides and proteins. These are all, to varying degrees, prominent aspects of honey's antimicrobial action (Mavric*et al*., 2008).

The extracted honey can be helpful for humans in cooking, baking, desserts as a sweeteners in some commercial beverages and employed in medicinal treatment for wound, burns, cough, side effects of radiation therapy and some allergies (Oryan*et al*., 2016). With this basic information the study was planned to find out the "Phytochemical Analysis of Different Honey Samples and its Anti-Bacterial Activity against Clinical Pathogenic Bacteria".

2. MATERIALS AND METHODS

2.1 COLLECTION OF HONEY SAMPLES (Tsadila*et al.,* 2021)

Three different types of honey samples were collected from Dalmia region, Trichy district from beekeepers during September 2022.

2.2 PROCESSING OF HONEY SAMPLES(Tsadila*et al.,* 2021)

About 500 µl of the honey samples were diluted into 1.5ml of sterile Nutrient broth and mixed well and kept in rotatory shaker for 6 hours.

2.3 QUALITATIVE ANALYSIS OF HONEY SAMPLES(Olusolade*et al*., 2014)

2.3.1. Qualitative analysis of carbohydrates

2.3.1.1. Fehling's Test

About 2ml of diluted honey samples were taken in test tube and add 1ml of both fehling's Solution A and fehling's Solution B were mixed well and water bath for 10minutes. Then observe the precipitate found in the test tube were noted.

2.3.1.2. Barfoed's Test

About 1ml of diluted honey samples were taken in test tube and add 0.5ml of barfoed's Reagent were mixed well and kept the test tube at boiling water bath for 2minutes. Then the result were observed.

2.3.2. Qualitative analysis of Aminoacid (Ninhydrin test)

About 2 ml of diluted honey samples were taken in a test tube and add 2 drops of ninhydrin solution, then the result were observed.

2.3.3. Qualitative analysis of Potassium

About 2ml of diluted honey samples were taken in a test tube and add small amount of picric acid solution and mixed well. Then observe the precipitate found in the test tube were noted.

2.3.4.Qualitative analysis of Calcium

About 2ml of diluted honey samples were taken in a test tube and add a small pinch of ammonium chloride and ammonium hydroxide solution were mixed well and were filtered the solution. Add 2ml of ammonium oxalate solution in the filtrate. Then observe the precipitate found in the test tube were noted.

2.3.5. Qualitative analysis of Iron

About 2ml of diluted honey samples were taken in a test tube and add a few drops of concentrated Hydrochloric acid and placed on the water bath for 2to 3 minutes and cooled it. Then add 3drops of potassium sulphocyanide solution and observe the colour changes found in the test tube were noted.

2.3.6.Qualitative analysis of Magnesium

About 2ml of diluted honey samples were taken in a test tube and add a few drops of ammonium chloride solution and add small amount of ammonium phosphate solution. Then observe the side of the test tube for precipitation were noted.

2.3.7. Qualitative analysis of Flavonoids

About 1ml of diluted honey samples were taken in test tube and add 1ml of ferric chloride were mixed well. Then the result were observed.

2.3.8. Qualitative analysis of Alkanoids

About 1ml of diluted honey samples were taken in test tube and add 3 drops of wagner's reagent were mixed well. Then the result were observed.

2.4. QUANTITATIVE ANALYSIS OF HONEY SAMPLES (Morroni*et al.,* 2018)

2.4.1. Total Phenol Test

About 1g of the diluted honey samples were taken in a test tube and diluted with 10ml of distilled water. Then add 0.2ml of Folins phenol or Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 minutes. One ml of 20% sodium carbonates (Na_2CO_3) were added and incubate at 45°C for 45 minutes in water bath. Following incubation the absorbance were measured and total phenolic content was quantified by using OD at 765nm in UV visible Spectrophotometry.(Labtronics – Model LT- 291, ranges as from 200 to 800 nm).

2.4.2. Hydrogen Peroxide Test

About 0.5g of the diluted honey samples were taken in a test tube and diluted with 10ml of distilled water. Then add 2ml of 20mm hydrogen peroxide andmix well. And add 0.9ml of ethanol. Incubate the tubes at room temperature for 10 – 15 minutes. Following incubation the tubes were examined for hydrogen peroxide content present in honey was quantified using UV visible spectrophotometry for OD at 230 nm. (Labtronics – Model LT-291, ranges as from 200 to 800 nm).

2.4.3. Total Antioxidant Activity

The antioxidant activity of the honey samples were carried out by thphosphomolybdenum method. A 0.5ml of honey sample were properly mixed with 0.5ml of total antioxidant reaction mixture and incubated in water bath at 50°C for 90minutes and cooled at room temperature. The OD values

were calculated for honey samples were measured at 695nm by using a UV-Visible spectrophotometer. (Labtronics – Model LT-291, ranges as from 200 to 800 nm).

2.5. COLLECTION OF PATHOGENS(Obey *et al.,* 2022)

Collection of clinical pathogenic bacteria obtained from Sneha Clinical Laboratory, Madukarai, Coimbatore. The pathogenic bacteria were D1 and D2 as named as *Staphylococcus* sp. and *Pseudomonas*sp. these samples were identified and maintained as a pure culture by Microbiology Laboratory of Centre for Bioscience and Nanoscience Research, Coimbatore for research purpose.

2.6. ASSAY FOR ANTIBACTERIAL ACTIVITY(Kacaniova*et al.,* 2022)

The honey were tested for antagonistic activity against the clinical pathogens. Antibacterial activity of honey sample was tested against the target bacterial pathogens of health significance by *invitro* techniques using Muller Hinton agar plates at 37°C for 24 hours.

2.6.1. Well Diffusion Method

Antibacterialactivityof the honey sample was identified by usingagar well diffusion method against the clinical pathogenic bacteria. Antibacterial activity of honey as given in different concentration were filled on the respective well such as 10 µl, 20 µl, 30 µl, disc (Ampicillin) and control are active against the clinical pathogenic bacteria *Staphylococcus* sp. and *Pseudomonas*sp. were swabbed on MHA Agar plate.After incubation the plates were observed for the presence of zone of incubation around the wells. The antibacterial activity of the sample have extent of inhibitionwas estimated by measuring the zone of diameters in centimetre.

3. RESULT

3.1 COLLECTION OF HONEY SAMPLES

Three different types of honey samples were collected from various locations within the Dalmia region, Trichy district, as shown in Fig. 1

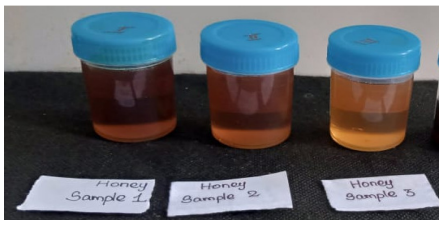

Figure 1. Collection of honey samples

3.3. QUALITATIVE ANALYSIS OF HONEY SAMPLES

The confirmatory test for carbohydrates were Fehling's test and Barfoed's test were produce the red colour it indicate the presence of reducing sugar. The presence of purple colour were indicates the amino acid compound present in the honey samples. Blood red colour were obtained in the test tube it indicates the presence of iron component in honey samples. White colour precipitate observed in the test tube it indicate the presence of calcium and magnesium content in honey sample. Brown colour were obtained it indicate the presence of flavonoids. Reddish brown colour indicate the presence of alkaloids content in honey samples.Yellow colour precipitate were observed it indicates the presence of potassium in honey samples(Akinatayo*et al*., 2014) had

showed that the presence of bioactive compounds were differed between the honey samples. Table 1 show the quantitative result for honey samples.

3.4. QUANTITATIVE ANALYSIS OF HONEY SAMPLES

Quantitative analysis of some compounds in honey sample is a very essential, it determine the antimicrobial activity of honey. The total phenol test, hydrogen peroxide test and total antioxidant test were performed and the honey sample 1 give high OD values, it have high antimicrobial activity.(Morroni*et al.,* 2018)had showed that the amount of bioactive compounds were differed between the honey samples. Figure 2 indicate the quantitative analysis of honey samples.

3.5. COLLECTION OF PATHOGENS

The clinical pathogenic bacteriawere identified and maintained as a pure culture for research purpose by Centre for Bioscience and Nanoscience Research. The samples were sub-cultured in Luria bertani broth.

3.6. ASSAY FOR ANTIBACTERIAL ACTIVITY

3.6.1. Well Diffusion Method

Highest zone of inhibition were obtained at 30µl concentration against *Pseudomonas*sp. Lowest zone of inhibition were obtained at 10µl concentration against *Staphylococcus* sp.Obtained results were correlated with Kenfack*et al*., (2018) and Deglovic*et a*l., (2022) reported the zone of inhibition occur in well diffusion method.

S.No	Qualitative test	Sample 1	Sample 2	Sample 3	Result observed
1	Fehling's Test	+	+	+	Red colour
2	Barfoed's Test	+	+	+	Red colour
3	Ninhydrin test	+	+	+	Purple colour
4	Potassium test	+	+	+	Yellow colour
5	Calcium test	+	-	-	White colour percipitate
6	Iron test	+	+	+	Blood Red colour
7	Magnesium test	-	-	-	White colour percipitate
8	Flavonoids test	+	-	+	Brown colour
9	Alkaloids test	-	-	+	Reddish brown colour

Table 1. Qualitative analysis of honey

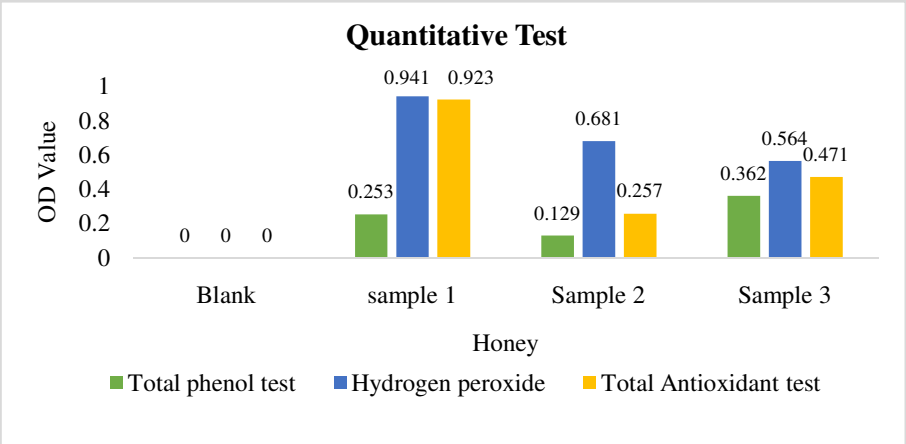

Figure 2. Quantitative analysis of honey

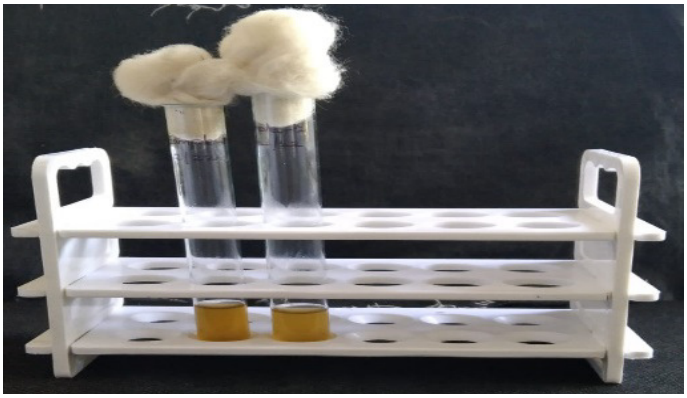

Figure 3. Pathogenic bacteria sub culture

Clinical pathogens	Conc in µl			Disc (Ampicilin)	Control (DMSO)
	10	20	30	10	50
Staphylococcus sp.	8mm	14mm	17mm	5mm	Nil
*Pseudomonas*sp.	12mm	15mm	18.7mm	7mm	Nil

Table 2. Zone of inhibition

Conclusion

In the conclusion, the finding of the present research provided an alternative way for the antibiotic treatment of the clinical pathogens. The future research will be designed as this honey were used in the pharma industry with some supplementary compounds to eliminate the effect of clinical pathogens.

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