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Evaluation of Pharmacological Applications of Honey

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1. Abstract

1.1 Background: Honey is a complex mixture of concentrated sugar solution with main ingredients of fructose and glucose and any honey depends largely on the source of the nectar.

1.2. Objectives: To assess physicochemical properties and biological activities of ten honey samples collected from different regions of Karnataka *in vitro*.

1.3. Methods: Physical, chemical and biological properties studied using standard protocols.

1.4. Results: The colour of the samples varied from white to dark amber. The pH of the samples varies from 3.2 to 5.59. The electric conductivity falls between 1.100mS/cm to 0.177mS/cm. The protein content of honey sample ranges between 0.73±0.041mg/g and 0.08±0.06mg/g. The total sugar content in the honey samples were 1.9-9.75mg/g. The total phenol content in honey from Rubber tree plantation showed least (11.45 mg/100g) and highest content was recorded from honey samples of Arecanut tree plantations (84.17mg/100g). The radical scavenging activity of the honey samples varied from 24.92±1.535% to 56.46±0.462%. The FRAP antioxidant activity varied from 23.64± 1.767mg/100g to 3.58±0.424mg/100g. All the honey samples have been shown the zone of inhibition against the bacterial strains. Numerous varieties of pollen grains were seen in honey samples and their species were identified.

1.5. Conclusion: The present study revealed different properties of honey samples which were varied as per their collection place and associated plants which can be used for their applications in pharmacology.

2. Introduction

Honey is a natural sweet substance produced by honey bees from the nectar of blossoms or from the secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which honey bees collect, transform, and combine with specific substances of their own, store, and leave in the honey comb to ripen and mature. It has been reported that honey contains carbohydrates, mainly fructose and glucose in addition to about 25 different oligosaccharides. Honey possesses powerful antimicrobial properties that can be utilized at low cost and at no risk. Various studies have reported the antimicrobial activity of honey [1].

Honey is characterised by its complex composition, which varies with the origin of the raw material as nectar or honeydew, the bee species, the edaphoclimatic conditions, the available floral source and the storage conditions. Honey mainly consists of glucose and fructose but also contains amino acids, phenolic compounds, organic acids, vitamins, minerals, lipids, enzymes and other phytochemicals. Hundreds of bioactive substances have already been found in honeys from the *Melipona* species in different countries. Among the compounds with biological activity that are present in honeys, the compounds that display antioxidant capacity, such as phenolic acids, flavonoids and the enzymes glucose oxidase and catalase, have received special attention from research groups, due to their role in the prevention of diseases associated with oxidative stress [2]. However, the phenolic compounds present in honey are directly related to botanical resources, such as pollens, nectars, resins and oils that are supplied to the bees, and consequently, honeys from different floral origins possess distinct bioactive properties. The botanical and geographical origin of honey may be evaluated through melissopalynology, which is used to assess the pollen types present in the honey and to suggest its floral source [3].

Honey has been widely accepted as food and medicine by all generations, traditions, and civilizations, both ancient and modern.

For at least 2700 years, honey has been used by humans to treat a variety of ailments through topical application, but only recently have the antiseptic and antimicrobial properties of honey been discovered. Honey has been reported to be effective in a number of human pathologies. Clinical studies have demonstrated that application of honey to severely infected cutaneous wounds rapidly clears infection from the wound and improves tissue healing. A large number of *in vitro* and limited clinical studies have confirmed the broad-spectrum antimicrobial (antibacterial, antifungal, antiviral and antimycobacterial) properties of honey, which may be attributed to the acidity (low pH), osmotic effect, high sugar concentration, presence of bacteriostatic and bactericidal factors (hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides), and increase in cytokine release, and to immune modulating and anti-inflammatory properties of honey; the antimicrobial action involves several mechanisms. Despite a large amount of data confirming the antimicrobial activity of honey, there are no studies that support the systemic use of honey as an antibacterial agent [4].

Today, about three hundred types of floral honey have been documented. This variety is linked to a wide diversity in the types of nectar collected by honeybees. Honey is composed of about 83% solids; the rest is water. The main portion of the solid fraction consists of 95 to 97% carbohydrates. Moreover, honey contains proteins (diastase, invertase, glucose oxidase, catalase and acid phosphatase), vitamins, amino acids (all of the nine essential amino acids and all of the nonessential amino acids, except asparagine and glutamine), minerals (phosphorus, sodium, calcium, potassium, sulfur, magnesium, chlorine), and other organic acids, such as flavonoids, polyphenols, alkaloids, glycosides, anthraquinone, and volatile compounds. Scientific researchers have identified more than six hundred volatile chemicals that may contribute to honey's health benefits. The fraction of volatile chemicals in honey is low, but consists of aldehydes, ketones, alcohols, acids, esters, hydrocarbons, derivatives of benzene, derivatives of terpene, norisoprenoids, and cyclic compounds. Some bioactive chemicals, such as quercetin, luteolin, galangin, isorhamnetin, and kaempferol, exist in almost all types of honey. Flavonoids and polyphenols, which act as antioxidants, are the two main bioactive groups of chemicals that present in honey. Recent studies have shown the existence in honey of approximately thirty kinds of polyphenols. Their levels can fluctuate significantly, depending on the source of the nectar and on the environmental conditions. They are the mostly responsible ingredients for the antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects of honey [5].

Currently, honey has been shown to have excellent antibacterial activity for many wound pathogens. Honey has excellent antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and various species of *Pseudomonas* commonly associated with wound and burn infections.Honey dressings are used commonly to manage skin and burn wounds infections.Honeys also possess antifungal activity. Honey has antiviral activity against Rubella virus, and honey is used topically to treat recurrent herpes simplex lesions [6]. The objective of the present investigation was to study the phytochemical, antimicrobial, and antioxidant activities of different honey samples as well as their palynological parameters.

3. Materials and Methods

Ten honey samples from different regions of Karnataka were collected, numbered and brought to the laboratory for experiment. They were properly labelled and details were entered during the collection stage itself.

1. Sample 1: Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada

2. Sample 2: Rared honey, Kumta, Uttara Kannada

3. Sample 3: Honey collected from Mango tree, Kaup, Udupi

4. Sample 4: Rared honey, Virajapete, Kodagu

5. Sample 5: Honey from coffee plantation, Mudigere, Chikamagalur

6. Sample 6: Rared honey, Kaniyur, Dakshina Kannada

7. Sample 7: Honey from arecanutplantations, Panja, Dakshina Kannada

8. Sample 8: Honey from Sacred fig tree, Thirthahalli, Shimoga

9. Sample 9: Rared honey, Sakaleshpur, Hassan

10. Sample 10: Honey from forest area, Mundoor, Dakshina Kannada

3.1. Colour measurement

Colour is one of the most important sensory traits of honey for the consumers. Honeys originating from different plant species are different in colour, but there could be variability within them as well, if originating from different geographical locations [7]. The actual colours of the 10 honey samples were compared.

3.2. pH measurement

The pH meter is used to measure the pH of honey 10:1 concentration (Water: Honey). Usually a pH meter must be calibrated to ensure its accuracy and this is typically done by using a standard buffer ammonia solution whose exact pH is known. The pH meter is used to measure the pH of honey 10:1 concentration (Water: Honey). The pH meter must be calibrated to ensure its accuracy is typically done by using a standard buffer solution whose exact pH is known. Initially the pH meter was calibrated using standard buffer of pH 4.0 and pH 9.0 maintained at laboratory temperature ($25^{o}C$).

3.3. Moisture content

The moisture content of the honey can be determined by weighing 10g of honey samples in pre-weighed crucible which was then dried at 105°C until a constant weight was obtained.

3.4. Ash content

The percentage of ash present in the honey is determined by igniting pre-weighed crucible with 10g of honey at 550°C in muffle furnace to constant mass.

3.5. Electrical conductivity

Electrical conductivity was determined by suspending 20% (w/v) of honey samples using distilled water.

3.6. Determination of total protein content (TPC)

The amount of protein present in the given 10 honey sample was estimated colorimetrically using Lowry's method [8], the blue colour developed by the reduction of phosphotungstate and phosphomolybdate present in Folins reagent by aromatic amino acids tyrosine and tryptophan present in the protein. The blue colour was developed and the absorbance was taken at 610nm using spectrophotometer. Bovine serum albumin (BSA) used as standard.

3.7. Estimation of total sugar content

The total sugar content of 10 honey samples were estimated by phenol-sulphuric acid method [9]. The basic principle of phenol sulphuric acid method is that carbohydrates, when dehydrated by reaction with concentrated sulphuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops red colour. The absorbance was read at 490nm using spectrophotometer.

3.8. Total phenol estimation

Phenol estimation of honey sample was done by using Folin Ciocalteau (FC) reagent. The principle of the Folin-Ciocalteau assay is the reduction of the FC reagent in the presence of phenolics resulting in the production of molybdenum-tungsten develops blue colour and the absorbance was measured at 650nm using spectrophotometer [10].

3.9. Total flavonoid estimation

Aluminium chloride colorimetric method was used to determine the flavonoid content in the honey samples. The basic principle of aluminium chloride colorimetric method is that aluminum chloride forms acid stable complex with C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols [11]. The absorbance of reaction is measured at 415nm. Quercetin used as standard.

3.10. Antioxidant activity of honey

3.10.1. DPPH radical scavenging activity (Teow *et al.,* 2007) [2]: The different concentration of samples used as test and ascorbic acid was the standard. The absorbance was measured at 517nm and free radical scavenging activity was measured and calculated using the formula:

Per cent scavenging = $\frac{(As - Ab)\times 100}{AC}$

where,

As =absorbance of sample solution, Ab =absorbance of blank and Ac = absorbance of control

3.10.2. FRAP assay (Vijayalakshmi and Kandasamy, 2016) [12]: The absorbance was measured spectrophotometrically at 700nm. Ascorbic acid was maintained as standard antioxidant.

3.11.1. Determination of antibacterial activity of honey: The antimicrobial activity of the honey is determined using well diffusion method.

Bacterial strains and inoculums standardization: *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* selected for the experiment. Prior to the experiment the bacterial stain were taken and inoculated in nutrient broth incubated for 24 h in incubator so that 24h culture was prepared. The 24h bacterial strains was inoculated over the surface of Mueller Hinton agar media, four wells with a diameter of 6 mm was punched aseptically with a sterile cork borer, and the honey samples of concentration 50% and 75% were pipetted into each well.. Streptomycin was used as positive control and distilled water used as negative control. All the cultures were incubated at 37°C for 24 h and antibacterial activity was assessed by measuring the diameter of inhibition zone formed around the well.

3.11.2. Determination of antifungal activity of honey by fungal analysis (Pakdaman and Mohammadi, 2018) [13]**:**

Fungal strains and inoculums standardization: *Fusarium oxysporum, Botrytis cinerea, Cladosporium sphaerospermum*. Here the single mycelial discs of each test strain were inoculated in 100mL Erlenmeyer flask containing 50mL of liquid potato dextrose medium and 2mL of honey samples. The flasks were incubated at 28°C±1°C for 4 days. The obtained mycelial biomasses were filtered through pre-weighed and dried Whatman No.1 filter paper. The mycelial biomass was dried in hot air oven at 60°C±2°C until the constant weight is obtained and weight of the mycelial biomass was measured.

3.12. Palynologicalstudy of honey by determination of sediment content of honey

Based on the method of Luveaux *et al*. (1978) [14], 2 grams of honey was dissolved in 4 mL of warm distilled water (40°C). The solution was centrifuged for 10min at 2500rpm. The solution was poured into the small tube and centrifuged again for 10min at 2500rpm. The entire sediment was placed on a slide and spread out over an area about 20x20mm, after drying by slight heating at 40°C. The sediment was mounted with glycerine-gelatine, liquefied by heating in water bath at 40°C and observed under microscope. The different shapes of pollen grains were observed and identified using Pollen Atlas.

3.13. Statistical analysis

All the quantifications and assays were performed in triplicates. The results were analysed and expressed as mean ± standard deviation.

4. Results and Discussion

The actual colour of the all 10 honey samples were compared visually according to the honey colouration chart. The samples 1,2,5, and 9 (Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada; Rared honey, Kumta, Uttara Kannada; Honey collected from coffee plantation, Mudigere, Chikamagalur and Rared honey, Sakleshpur, Hassan) are light amber in colour, the samples 3 and 10 (Honey collected from Mango tree, Kaup, Udupi and Honeycollected from forest area, Mundoor, Dakshina Kannada) are dark amber in colour, sample 4 and 8 (Rared honey Virajapete, Kodagu and Honey collected from Sacred fig tree, Thirthahalli, Shimoga) are amber in colour and sample number 7 (Honey collected from Rubber tree, Panja, Dakshina Kannada) which is only one sample white in colour (Table 1).

The average pH of honey is 3.9, but can range from 3.4-6.1. Honey contains many kinds of acids both organic and amino. However, different types and their amounts vary considerably, depending on the type of honey. These acids may be aromatic or aliphatic (nonaromatic). The sample 4 (Rared honey, Virajapete, Kodagu) has the lowest pH of 3.2 and sample 9 (Rared honey, Sakleshpur, Hassan) has high pH of 5.59 compared to the all the honey samples (Table 2). The pH values of the light-coloured honey sample (LNH1) and dark coloured honey sample (LNH2) were 5.2 and 5.0, respectively in the natural honey samples from Malda as reported earlier by Roy *et al*. (2016) [15].

The ash content of honey averages about 0.21% of its weight, but varies widely from 0.02 to over 1.0% (Codex Alimentarius Commission Standards (2001) for honey. The ash percentage for normal honey should not be more than 0.6%. Certain nitrogen compounds, minerals, vitamins, pigments and aromatic substances contribute to the ash content of honey. The sample 7 (Honey collected from Rubber tree, Panja, Dakshina Kannada) 0.1% has the highest percentage of ash and sample 1 (Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada) and 4 (Rared honey Virajapete, Kodagu) 0.01% has the lowest percentage of ash compared to the all the honey samples (Table 2).

The moisture content of the honey is related to its degree of fermentation. The control of the water content is an important requirement which sets an upper limit for moisture of 21% for honey in general. The moisture content of locally produced honey was in the range of 14.5 to 18.23%. All the samples examined contained moisture content within the standard limits.

According to the present results, compared to all the honey samples, sample 1 (Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada) has the high moisture content 14.78% and sample 10 (Honey collected from forest area, Mundoor, Dakshina Kannada) has lowest percentage of moisture content 10.45%. The electric conductivity of honey is limited by Codex Standard for honey to be less than 0.8mS/cm when 20g of honey solids is diluted with 100ml of water. It is influenced by the source of the honey, acidity, salt content, moisture and viscosity. Honey from honeydew has high conductivity than floral honeys. According to the following results the sample 9 (Rared honey, Sakleshpur, Hassan) and 6 (Honey collected from Arecanut tree, Kaniyur, Dakshina Kannada) has highest electric conductivity of 1.100mS/cm and 0.956mS/cm which is higher than the limit of Codex Standard of honey. The sample 2 (Rared honey, Kumta, Uttara Kannada) has the lowest electric conductivity of 0.177mS/cm, compared to the all the honey samples.

The protein content in the honey samples revealed that sample 8 (Honey collected from Sacred fig tree, Thirthahalli, Shimoga) contains high concentration of protein 0.73±0.041mg/g, while sample 5 (Honey collected from coffee plantation, Mudigere, Chikamagalur) contains least concentration 0.08±0.06mg/g. The rest of the honey samples contain the intermediate concentration of protein compared to sample 8 and 5. Honey is sweet because of its high concentration of the monosaccharides fructose and glucose. It is an excellent source of energy containing approximately 80g/100g carbohydrates. The total sugar content in the honey samples showed varied amount of sugar content in the honey samples. Sample 1 (Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada) contains high concentration of sugar content 9.75±0.026 whereas sample 10 (Honey collected from forest area, Mudoor, Dakshina Kannada) contains low concentration of sugar content 1.90±0.036.

The total phenolic content varied in a different types of honey, the lowest content was found in sample 7 (Honey collected from rubber tree, Panja, Dakshina Kannada) that is 11.45±0.02 and highest content of total phenol is found in sample 6 (Honey collected from Arecanut tree, Kaniyur, Dakshina Kannada) that was 84.17±0.035 whereas rest of the samples contains medium quantity of total phenol compared to sample 7 and 6. The TPC of the honey samples were ranged from 0.55 (sample 10) to 0.92 mg GAE /gr sample (samples 4 and 8). Honey sample No. 6 also had a high TPC. Manuka honey had a moderate TPC (0.71 mg GAE /g sample) compared to other honey types [16]. The TPC of honey samples was in the narrow range from 110.394 to 196.500 mg GAE/100 g honey from Malaysia. The TPC results were higher than commercial Indian honeys (47-98 mg GAE/100 g honey) and Argentinean northwest honeys (18.730 -107.213 mg GAE/100 g honey), as well as Burkina Fasan honeys (32.59-114.75 mg GAE/100 g honey) [17] (Chua *et al*., 2013). The Himalayan honey possess higher phenolic content ranged from 0.125 to 4.18 mg/100mg [18].

Honey is rich in phenolics and flavonoids, which exhibits a wide range of biological effects and act as natural antioxidants. The total flavonoid content was expressed as mg of quercetine equivalent per 100g of the sample. The highest content of flavonoid were recorded in sample 4 (Rared honey, Virajapete, Kodagu) 7.0±0.020mg/100mg and lowest content was record-

ed in sample 3 (Honey collected from Mango tree, Kaup, Udupi) 2.5±0.040mg/100mg. Rapeseed, buckwheat, and lavender monofloral honeys from the Republic of Moldova exhibited higher polyphenols and flavonoids [19]. 100 different honey samples collected from different regions of Kosovo and their flavonoid content is determined. According them results, in monofloral honey, the content of total flavonoids was lower (1.11±0.62 to 5.24±1.59mg/100g) compared to multifloral honey samples (3±1.52 to 7.51±3.57mg/100g) [20].

The radical scavenging activity of the honey samples varied from 24.92±1.535% to 56.46±0.462%. Sample 10 (Honey collected from forest area, Mundoor, Dakshina Kannada) showed the lowest radical scavenging activity 24.92±1.535% whereas sample 6 (Honey collected from Arecanut tree, Kaniyur, Dakshina Kannada) showed highest radical scavenging activity 56.46±0.462%. Rest of the samples ranges in between these two samples 10 and 6 (Table 3). The lowest inhibition caused by LNH1 and LNH2 honey samples were 43.46% and 36.09%, respectively, at concentrations 3 mg/ml, while the highest scavenging activities (75.18% and 77.23%, respectively) were found due to 15 mg/ml and 18 mg/ml concentrations of honey samples, respectively. The highest concentration of the honey samples used was 18 mg/ml, at which the scavenging activities were decreased to 69.61% for LNH1 and increased to 77.23% for LNH2. The IC_{50} values calculated were 3.83 mg/ml and 6.75 mg/ml for LNH1 and LNH2 honeys, respectively [21]. They also reported that the corn syrup treatment contained 0.21 (0.06 mg of phenolic antioxidants per gram, and the two buckwheat honey treatments contained 0.79 (0.02 and 1.71 (0.21 mg of phenolic antioxidants per gram. Following consumption of the two honey treatments, plasma total-phenolic content increased ($P<0.05$) as did plasma antioxidant and reducing capacities ($P<0.05$). The TPCs of the honey samples (LNH1 and LNH2) were calculated as 195.5 µg/mL and 333 µg/mL respectively.

A strong DPPH radical scavenging activity was exhibited by honey type 3, 4 and 1, with IC_{50} values of 10.0, 11.5 and 16.0 mg/ml, respectively [16]. In the ABTS•+ assay, the IC_{50} values ranged from 4.5 (sample 8) to 81.0 mg/ml (sample 11). Apart from honey type 8, in that assay, honey sample 4, 13 and 7 exhibited potent antioxidant activity, with IC_{50} values of 10.0, 17.5 and 19.0 mg/ml, respectively. Although some samples (e.g., 8, 4 and 13) exhibited similar antioxidant activity in both assays, the statistical comparison between the two assays revealed a moderate significant correlation ($r=0.574$, $P<0.05$). Manuka honey exhibited a weak antioxidant activity (IC_{50}, 68.0 mg/ml) in DPPH assay and a moderate activity in ABTS•+ assay (IC_{50}, 21.0 mg/mL) compared to the other honey types. Tiwari *et al*., (2017) [18] observed a significant correlation between the antioxidant activity and phenolic content which ranged from 1.68 to 8.70 mg/mL. The honey from the Garhwal Himalayas can be used as a source of potential antioxidants to dietary components to give it additional function or might be useful in reducing oxidative stress. Hulea *et al*. (2022) [22] recorded the maximum antioxidant activity and amounts of TPC and TFC were found in monofloral Manuka honey, followed by linden honey samples.

The FRAP antioxidant activity of the 10 honey samples varied from 23.64±1.767mg/100g to 3.58±0.424mg/100g. Sample 10 (Honey collected from forest area, Mundoor, Dakshina Kannada) has a significant amount of antioxidant activity 23.64±1.767mg/100g while sample 2 (Rared honey, Kumta, Uttara Kannada) has least amount of antioxidant activity 3.58±0.424mg/100g compared to other honey samples. The pine honey from Turkey and Greece the showed antiradical activity ranged from 42.43 to 79.33% and the total phenolic content was 451.38 120.38 mg GAE/kg, the FRAP values (1.87 to 9.43 mmol Fe^{+2}/kg) were generally greater than those noted in the literature [23].

Honey exhibits a broad-spectrum of antibacterial activity against both Gram positive bacteria and Gram-negative bacteria, including antibiotic-resistance (MRSA) ones. Honey has been shown to have strong activity against many bacteria in both media and in culture. The antimicrobial activity of 10 honey samples was examined against *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive) *in vitro* condition. All the samples have been shown the zone of inhibition against the bacterial strains.

Sample 3 (Honey collected from Mango tree, Kaup, Udupi) has shown the high zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* compared to all the samples and sample 2 (Rared honey, Kumta, Uttara Kannada) has the least inhibition zone against *Escherichia coli*compared to all the samples. Sample 9 (Rared honey, Sakleshpur, Hassan) also has been shown the least zone of inhibition against *Staphylococcus aureus*. Sample 7 (Honey collected from rubber tree, Panja, Dakshina Kannada) has the high zone of inhibition against the *Bacillus subtilis* and sample 6 (Honey collected from Arecanut tree, Kaniyur, Dakshina Kannada) has the least (Table 4).

The antibacterial activity of the honey sample LNH1 showed growth inhibitory action against Gram positive bacteria: *Staph. aureus* (ZDIs: 28-32 mm, for non-autoclaved honey and 27-28 mm, for autoclaved honey), as well as Gramnegative bacteria: *E. coli* ATCC 25922 (ZDIs: 30-35 mm, for non-autoclaved honey and 28-33 mm, for autoclaved honey), *Ps. aeruginosa* (ZDIs: 25-30 mm, for non-autoclaved honey and 24-28 mm, for autoclaved honey) and *S. enteric* serovar. Typhi (ZDIs: 26-28 mm, for both non-autoclaved and autoclaved honeys). The Gram-negative bacteria, *E. coli* ATCC 25922 and *S. enteric* serovar Typhi, were sensitive to the honey sample LNH2, having ZDIs 31-34 mm, for both non-autoclaved and autoclaved honeys, and 18-22 mm and 17-20 mm, respectively, for non-autoclaved and autoclaved honeys; for *Ps. aeruginosa*, ZDIs ranged 19-22 mm, for non-autoclaved honey

and 18-19 mm, for autoclaved honey (at 48 h incubation). Due to the action of honey sample LNH2, the ZDIs obtained against *Staph. aureus*, at 48 h incubation, ranged 15-21 mm [15].

All the tested 21 honey types from Mount Olympus, Greece exhibited antibacterial activity against *S. aureus* and *P. aeruginosa*. In any case, the antibacterial effects exerted by the tested honey types (including Manuka honey) were more potent against *S. aureus*, as demonstrated by larger inhibition zones, compared to the effects against *P. aeruginosa*. A Spearman's correlation analysis was performed using the data of the well diffusion assay for *S. aureus* and *P. aeruginosa*. This analysis revealed that there was no correlation between the antimicrobial activities of honey against these two bacterial species [16]. Brazilian SBH shows more effective antibacterial activity against Gram negative bacteria (*E. coli* and *S. typhimurium*) compared to Gram positive ones [24]. All of the clinical isolates were susceptible to the antibacterial properties of honey, with the exception of *B. cepacia* and *P. vulgaris*. With the exception of *P. aeruginosa*, monofloral manuka honey 10% was the most potent inhibitor of the ATCC strains. In conclusion, study's classification of efficacy was Manuka honey > brassica honey > acacia honey > linden honey [22]. Tsavea *et al*. (2022) [23] in their study on pine honey found statistically significant correlation between moisture, antioxidant, and antibacterial activity against *Salmonella* spp.*, S. typhimurium, Klebsiella pneumoniae* and both. Interestingly a statistically significant negative association has been observed between diastase activity and *Staphylococcus aureus* antibacterial activity.

Table 1: Colour measurement of the 10 different honey samples.

Honey samples	Colour
1	Light amber
2	Light amber
3	Dark amber
4	Amber
5	Light amber
6	Extra light amber
7	White
8	Amber
9	Light amber
10	Dark amber

Table 2: Physicochemical and phytoconstituents* of different honey samples collected from 10 different locations

Honey samples	pH	Ash (%)	MC (%)	EC (mS/cm)	Protein (mg/g)	Sugar (mg/g)	Phenolics(mg/100g)	Flavonoid (mg/100g)
1	4.0	0.01	14.78	0.679	0.41±0.005	9.75±0.026	13.4±0.045	4.6±0.01
2	3.59	0.04	11.54	0.177	0.58±0.041	5.60±0.02	25.76±0.16	3.9±0.02
3	3.56	0.02	13.32	0.809	0.65±0.017	2.51±0.020	35.77±0.045	2.5±0.040
4	3.2	0.01	13.21	0.467	0.46±0.251	3.82±0.037	46.48±0.023	7.0±0.020
5	4.0	0.03	13.89	0.769	0.08±0.06	4.76±0.017	46.33±0.02	6.6±0.01
6	4.0	0.05	14.37	0.956	0.20±0.151	3.38±0.020	84.17±0.035	5.4±0.026
7	3.75	0.1	13.49	0.823	0.55±0.055	4.51±0.036	11.45±0.02	4.4±0.036
8	3.9	0.03	13.6	0.209	0.73±0.041	7.65±0.020	28.67±0.045	3.1±0.03
9	5.54	0.09	12.56	1.1	0.38±0.011	8.78±0.05	33.16±10.16	5.3±0.026
10	4.67	0.06	10.45	0.435	0.24±0.05	1.90±0.036	56.71±09.10	4.6±0.01

*protein, sugar, phenolics and flavonoid contents mean± standard deviation, N=3

Table 3: DPPH radical scavenging activity of10 different honey samples

Samples	DPPH radical scavenging activity* (%)	FRAP* (mg/100g)
1	28.27±0.610	12.20±0.424
2	52.68±0.979	3.58±0.424
3	37.89±0.993	5.35±0.120
4	43.77±1.912	16.97±0.671
5	52.93±0.697	22.33±0.473
6	56.46±0.462	7.60±0.219
7	26.42±1.330	21.64±0.346
8	49.48±0.899	4.21±0.799
9	35.67±0.427	8.86±1.555
10	24.92±1.535	23.64±1.767

*mean value+ SD, N=3

Table 4: In vitro antimicrobial activity of the honey samples against bacterial strains

Honey samples	Conc. of samples (mg/mL)	*Escherichia coli*	*Staph. aureus*	*Bacillus subtilis*
		Diameter of zone of inhibition (cm)		
1	75	0.65±0.0701	0.7±0.1414	0.8±0.1414
	50	0.45±0.0701	0.55±0.0701	0.55±0.0701
	Streptomycin	0.85±0.0701	0.6±0.0701	0.550.0701
2	75	0.6±0.1414	0.55±0.0701	0.75±0.0701
	50	0.35±0.0701	0.5±0.0701	0.55±0.0701
	Streptomycin	0.8±0.1414	0.55±0.0701	0.5±0.1414
3	75	1.05±0.2121	0.75±0.0701	0.85±0.0701
	50	0.6±0.1414	0.4±0.1414	0.6±0.1414
	Streptomycin	0.9±0.1414	0.65±0.0701	0.65±0.0701
4	75	0.75±0.1414	0.65±0.0701	0.85±0.0701
	50	0.45±0.0701	0.45±0.0701	0.55±0.0701
	Streptomycin	1±0.2121	0.55±0.2121	0.55±0.0701
5	75	0.8±0.1414	0.6±0.1414	0.8±0.1414
	50	0.45±0.0701	0.45±0.0701	0.5±0.1414
	Streptomycin	0.9	0.5±0.1414	0.65±0.0701
6	75	0.6±0.1414	0.55±0.0701	0.45±0.0701
	50	0.3	0.6±0.1414	0.35±0.0701
	Streptomycin	0.8	0.25±0.0701	0.75±0.0701
7	75	0.85±0.0701	0.65±0.0701	1.15±0.2121
	50	0.6±0.1414	0.35±0.0701	0.65±0.0701
	Streptomycin	0.9±0.1414	0.9±0.1414	0.85±0.0701
8	75	0.9±0.1414	0.85±0.0701	0.85±0.0701
	50	0.750701	0.55±0.0701	0.65±0.0701
	Streptomycin	0.8±0.1414	0.4±0.1414	0.95±0.0701
9	75	0.8±0.1414	0.45±0.0701	0.8±0.1414
	50	0.6±0.1414	0.35±0.0701	0.35±0.0701
	Streptomycin	0.7±0.1414	0.45±0.0701	0.65±0.0701
10	75	0.7	0.65±0.0701	0.95±0.0701
	50	0.55±0.0701	0.35±0.0701	0.75±0.0701
	Streptomycin	0.95±0.0701	0.5±0.1414	0.9±0.1414
Negative control		0	0	0

4.1. Determination of Antifungal Activity of Honey Samples

Results of mycelial dry weight of *Fusarium oxysporum, Botrytis cinerea* and *Cladosporium sphaerospermum* are presented in Table 5. The sample 1 (Honey collected from Cluster fig tree, Bantwal, Dakshina Kannada) was highly effective against the *Fusarium oxysporum, Botrytis cineria* whereas sample 2 (Rared honey, Kumta, Uttara Kannada), sample 3 (Honey collected from Mango tree, Kaup, Udupi) and sample 10 (Honey collected from forest area, Mundoor, Dakshina Kannada) has similar results and more effective against the *C. sphaerospermum* fungal discs. On other hand sample 10 (Honey collected from forest area, Mundoor, Dakshina Kannada) was least effective against *Fusarium oxysporum*, sample 2 (Rared honey, Kumta, Uttara Kannada) was least effective against *Botrytis cinerea* and finally sample 6 (Honey collected from Arecanut tree, Kaniyur, Dakshina Kannada) was least effective against *C. sphaerospermum* compared to all the samples. The Portugese honeys showed antibacterial and antifungal activity with minimum inhibitory concentrations (MIC) between 6.25–25% (w/v) and minimum fungicidal concentrations (MFC) in the range 12.5–50% (w/v), respectively. The bioactivity and physicochemical parameters of honey samples were correlated and depended on the honey floral source. The darkest honey, i.e., heather honey, showed the highest antioxidant and antimicrobial activities, which can be attributed to its higher phenolic, flavonoid and protein content [25]. All examined 21 samples of honey from Pakistan shown resistance against *Candida albicans*. The minimum inhibitory concentration (MIC) against *Candida albicans* and *Rhodotorula* species for branded, unbranded, and natural comb honey, respectively, ranged from 53.33% to 88.12% and 1.76% to 90.22 and 61.3% to 93.8% and 9.90% to 95.5% [26].

Table 5: In vitro antifungal activities of 10 different honey samples

Samples	Mycelial dry weight* (g)		
	Fusarium oxysporum	***Botrytis cinerea***	***C. sphaerospermum***
1	0.06±0.014	0.08±0.007	0.11±0.014
2	0.12±0.042	0.34±0.007	0.09±0.007
3	0.19±0.014	0.23±0.035	0.09±0.002
4	0.10±0.021	0.08±0.021	0.13±0.021
5	0.32±0.042	0.14±0.021	0.18±0.028
6	0.24±0.042	0.17±0.028	0.28±0.014
7	0.21±0.035	0.26±0.042	0.24±0.035
8	0.08±0.007	0.2±0.014	0.19±0.014
9	0.21±0.035	0.11±0.035	0.15±0.042
10	0.32±0.056	0.22±0.035	0.09±0.007
Positive control	0.05±0.015	0.07±0.055	0.09±0.026
Negative control	0.82±0.035	1.15±0.030	0.64±0.040

*mean value± standard deviation, N=3

4.2. Palynological Study of Honey

Palynology is the study of plan pollen, spore and certain microscopic plankton organisms (collectively termed palynomorphs) in both living and fossil form. Numerous varieties of pollen grains were seen, and their species were determined. Sample 2 contains fewest number of pollen grains compared to all the samples (Rared honey, Kumta, Uttara Kannada). In sample number 3 (Honey collected from Mango tree, Kaup, Udupi), there were more pollen grains of various shapes observed (Table 6, Figure 1).

A total of 36 pollen types belonging to 18 families were identified. The pollen grains of the honey samples belonging to the family Apiaceae, Asteracae, Betulaceae, Boraginaceae, Brassicaceae, Campanulcaeeae, Caprifoliaceae, Caryophyllaceae, Cistaceae, Ericaceae, Fabaceae, Onagraceae, Plantaginaceae, Ranunculaceae, Rosaceae, Salicaceae were identified at different rates. As a result of the pollen analysis, 18 plants were identified at the family level, 18 of them are genus and 1of them is species. The families with the highest numbers of pollen types situated in the honey samples were Asteraceae, Cistaceae, Fabaceae, Lamiaceae, Moraceae, Rosaceae and Salicaceae families. All of the samples were classified as multifloral because they contain the pollen of many plant taxa [27]. Multifloral honeys with pollen from 57 plant species including *Prunus persica, Pyrus pashia, Citrus sinensis, Aesculus indica, Rhododendron arboreum*, and *Brassica campestris* were shown to have high phenolic content was reported by Tiwari *et al*. (2017) [18].

The secondary groups of pollens were the following taxa in three honey samples; *Helianthemum* sp., *Salix* sp. for H2 sample, *Onobrychis* sp., *Helianthemum* sp. for H1sample and *Centaurea* sp., *Onobrychis* sp. for H3 sample. Şık *et al*. (2017) [28] examined pollen content of honey samples from Ardahan, (Northeast Anatolia) and found 23 different taxa from 13 families. *Astragalus* spp., Apiacaee, Brassicaceae, Fabaceae pollen grains were the most abundant in honey samples. All of the samples were multifloral.

Principal component analysis (PCA) grouped the Brazilian honey into three categories with predominant pollen from Verbenaceae, Asteraceae and Sapindaceae families, confirming that SBH belonging to the same floral origin present similar characteristics [24].

Table 6: Palynological details of honey samples

Honey sample	Shape of pollen grain	Plant species/family
1	Isopolar, prolate spheroidal	*Ficus racemosa,* Anacardiaceae
2	Tricolpate	*Arecaceae,* Asphodelaceae
3	Radial, tricolporate	Apocynaceae ,*Mangifera indica*
4	Bilateral, elliptical amb, oval, round	*Tecoma stans, Coffea arabica*
5	Tricopolate, round	*Coffea arabica, Musa*
6	Colpus long	*Areca catechu, Musa*
7	Radial, apolar, circular amb, tripseudocolpate	*Hevea brasiliensis*, *Bougainvillea*
8	Reticulate, irregular	*Ficus religiosa, Rosa*
9	Colopus long, pore elongate,	*Trifolium repens*
10	Bilateral, bean, radial, subcircular	Jungle *Geranium, Acacia*

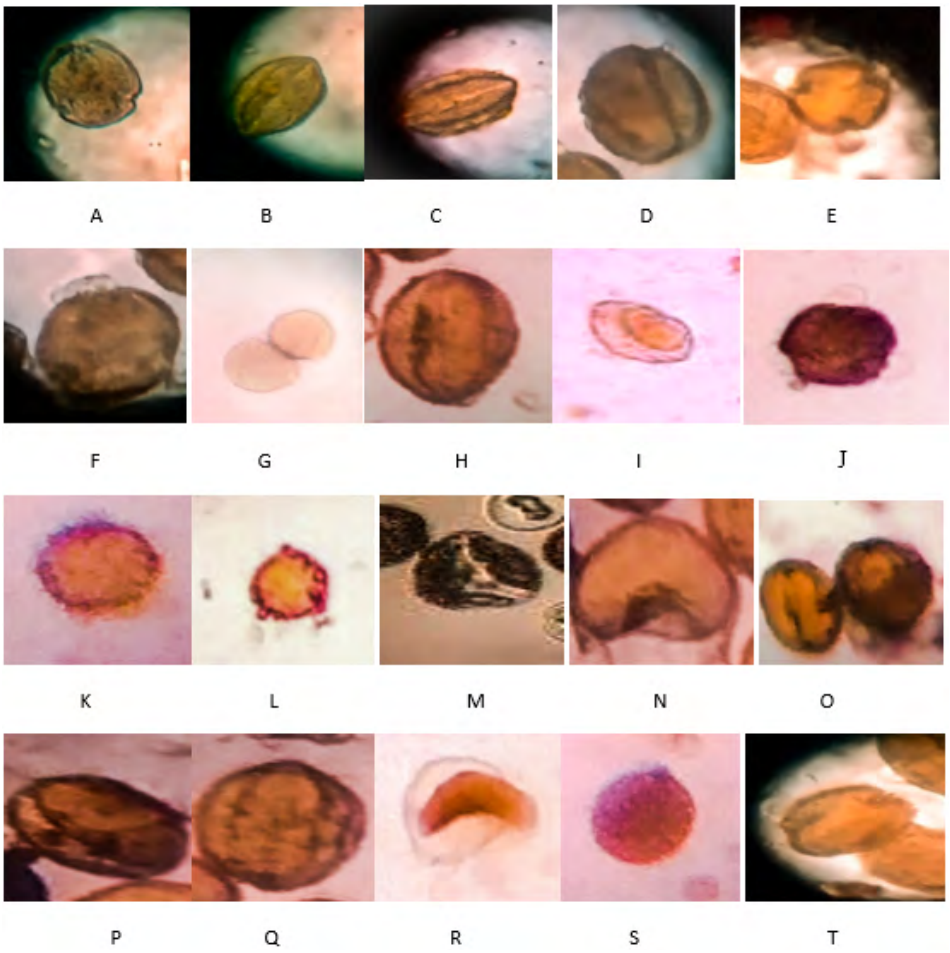

Figure 1: Pollen grain of different honey samples identified based on shape and structure

A. *Ficus racemose* (Isopolar shape), B. Anacardiaceae (prolate spheroidal shape) C. Arecaceae (icopolate), D. *Tecoma stans* (elliptical) E. *Mangifera indica* (tricolporate), F. *Coffea arabica* (tricolporate), G. *Musa* (round), H and T. Asphodelaceae (*Bilateral*), I. *Areca catechu* (Colpus long), J. *Hevea brasiliensis* (tripseudocolpate) K. *Bougainvillea* (Apolar), L. *Ficus religiosa* (irregular), M. *Rosa* (Reticulate), N. O and P. *Acacia* (Bilateral), Q and R. *Trifolium repens* (Colopus long, pore elongate), S. *Jungle Geranium* (radical, subcircular).

5. Conclusion

The 10 different honey samples from different places of Karnataka collected for their phytochemical, antioxidant, antimicrobial, antifungal and palynological studies. All of the tested honey samples showed presence of varied levels of phenols and flavonoids as well as DPPH radical scavenging activity and FRAP antioxidant activity. The results demonstrated that all the 10 honey samples meet the quality parameters established by international regulations and contains bioactive compounds. This study reported that, all the honey samples possessed the high concentration of protein and sugar content.

The results also shows that all the honey samples are effective against the selected Gram negative and Gram-positive bacteria. The antifungal effect of different honey samples was evaluated in culture media containing different concentration of honey. the data suggest that the components in the honey samples are responsible for the observed *in vitro* antifungal properties. All the honey samples have the potential to prevent the growth of a wide range of *Fusarium oxysporum, Botrytis cinerea and Cladosporium sphaerospermum*. The palynological study determined the different types and shapes of the pollen grains based on their botonical origin. This is the first report of this kind from the location.

References

1. Anyanwu, CU. Investigation of in vitro antifungal activity of honey. J Medicinal Plants Res. 2012; 6(18): 3512-3516.
2. Teow CC, Trung VD, MerFecters, Thompson RL. Antioxidant activities, phenolic and beta carotene contents of sweet potato genotypes with varying flesh colors. Food Chem. 2007; 103(3): 829-838.
3. Silva DIAA, Silva DTMS, Camara CA, Queiroz N, Magnani M, De Novais JS, et al. Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. Food Chem. 2013; 141(4): 3552-3558.
4. Israili ZH. Antimicrobial properties of honey. Am J Therap. 2014; 21: 304-323.
5. Iris A, Linayic AC, Bakar MFA. Effect of higher quality and quantity on the accumulation of flavonoids in plant species. J Sci Technol. 2018; 10(3).
6. Shahzas A, Cohrs RJ. In vitro antiviral activity of honey against Varicella zoster virus (VZV): A translational medicines study for potential remedy for shingles. Trans Biomed. 2012; 3(2): 2.
7. Szabo RT, Mezes, M, Szelai T, Zajacz E, Weber M. Color identification of honey and methodical development of its instrumental measuring. Columella J Agri Environ Sci. 2016; 3(1): 29-36.
8. Lowry OH, Rosbrough NJ, Farr AL, Randall RJ. Protein assay. J Biol Chem. 1951; 193: 265.
9. Sadasivam S, Manickam A. Biochemical Methods. New Age International (P.) Limited, Publishers, New Delhi. 2008; 51.
10. Swain T, Hillis WE. The phenolic constituents of Prunus domestica L. – the quantitative analysis of phenolic constituents. J Sci Food Agri. 1959; 10: 63-68.
11. Iris A, Linatic AC, Bakar MFA. Effect of high quality on the accumulation of flavonoids in plant species. J. Sci. Technol. 2018; 10(3).
12. Vijayalakshmi M, Ruckmani K. Ferric reducing antioxidant power assay in plant extract. Bangladesh J Pharmacol. 2016; 11: 570-572.
13. Pakdaman SB, Mohammadi GE. Effect of agricultural chemicals and organic amendments on biological control fungi. Sust Agri Rev. 2018; 31: 217-359.
14. Luveaux J, Maurizio A, Vorwohl G. Methods of Melissopalynology. Bee World. 1978; 59: 139-157.
15. Roy S, Mandal M, Pal NK, Das MK, Halder D, Sircar B, et al. Exploration of antibacterial and antioxidative property of two natural honey samples from Malda District, India. Trans Med. 2016; 2161-1025.6: 187.
16. Stagos D, Soulitsiotis N, Tsadila C. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. Int J Mol Med. 2018; 42: 726-734.
17. Chua LS, Rahaman NA, Adnan NA, Tan TTE. Antioxidant activity of three honey samples in relation with their biochemical components. J Anal Methods Chem. 2013; 8.
18. Tiwari P, Naithani P, Saklani S, Rawat DS. Phenolic content, antioxidant activity and palynological analysis of some multifloral honeys from Garhwal Himalaya. Int J Pharmacy Biol Sci. 2017; 7(3): 84-92.
19. Chirsanova A, Capcanari T, Boistean A. Palynological, physicochemical and biologically active substances profile in some types of honey in the Republic of Moldova, Technical University of Moldova. 2021.
20. Ibrahimi H, Hajdari A. Phenolic and flavonoid content, and antioxidant activity of honey from Kosovo. J Api Res. 2020; 59: 452- 457.
21. Gheldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agri Food Chem. 2002; 50: 3050-3055.
22. Hulea A, Obistioiu D, CocanI. Diversity of Monofloral Honey Based on the Antimicrobial and Antioxidant Potential. Antibiotics. 2022; 11(5): 595.
23. Tsavea E, Vardaka FP, Savvidaki E, et al. Physicochemical characterization and biological properties of pine honey produced across Greece. Foods. 2022; 11 (7): 943.
24. Avila S, Hornung PS, Teixeira GL, Malunga LN, Apea-Bah FB, Beux MR, et al. Bioactive compounds and biological properties of Brazilian stingless bee honey have a strong relationship with the pollen floral origin. Food Res Int. 2019; 123: 1-10.
25. Goncalves J, Ribeiro I, Marcalo J, Rijo P, Faustino C, Pinheiro L. Physicochemical, antioxidant and antimicrobial properties of selected Portuguese commercial monofloral honeys. J Food Nutri Res. 2018; 6 (10): 645-654.

26. Rahman KU, Ullah I, Ullah K, Husain A, Ullah S. Antifungal Activity of Honey Samples from Khyber Pakhtunkhwa (Pakistan) as affected by Botanical Origin. J Pure Appl Microbiol. 2022; 16 (2): 1147-1153.

27. Cakir Y, Cobanolu DN, Dervisoglu G, Kocyigit S, Karahan D, Yelkovan S, et al. Determination antimicrobial activity, palynological characteristics and chemical composition of some honey samples from Turkey. Mellifera. 2020; 20(1): 41-60.

28. Sik L, Guvensen A, Durmuskahya C, Erol O. Pollen analysis of honeys from Ardahan/Turkey. Biol Divers Conserv. 2017; 10: 2.