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Comparative Pharmacognostic and Pharmacological Profiling of Honey Obtained from Different Plant Sources

Barkatullah[a], Saira Faiz[a], Shah Khalid[a], Sami Ullah[b] and Muhammad Nafees[b]*

[a]Department of Botany, Islamia College, Khyber Pakhtunkhwa, Pakistan

[b]Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract. Honey is natural biological sweet drug formed from the combination of plant pollen, nectar and the secretion of honey bees' body. In the present study eleven samples of honey (*Robinia pseudoacacia, Eriobotrya japonica, Justacia adhatoda, Zizyphus jujuba, Calathea allouia, Punica granatum, Otostegia limbata, Accasia modesta, Thymus seralis, Citrus sinensis* and *Trifolium resupinatum*) from different plant sources were collected from Khyber Pakhtunkhwa, Pakistan. The honey samples were tested for physical and chemical parameter including hydroxy methyl furfuryl **(**HMF) test, pH, moisture content, acidity, electrical conductivity and pollen analysis. Various ranges including pH (3.43-6.00), moisture content (1.89-3.73%), EC (0.36-2.91 µS/cm) and acidity (0.345-2.42 meq/Kg) has been recorded. HMF result was tested for adulteration and the purest honey recorded was *Eriobotrya japonica*, *Justacia adhatoda*, *Trifolium resupinatum*. Samples of honey contained pollen from seventeen different species, most of which belonging to the Fabaceae family and included unifloral and polyfolral honey. Honey samples tested for antibacterial potential against *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeroginosa* showed significant potential against *P. granatum* and *E.coli*. Antifungal potency of honey against different fungal strains *Aspergillus niger, Aspergillus terreus, Fusarium oxysporum* and *Alternaria alternate* showed that the multifolral honey was highly effective against the afore mentioned fungal species. The honey obtained from *Accasia modesta* shown high effectiveness against the fungus *F. oxysporum*. In conclusion, the physio-chemical characteristics and pharmacognostic advantages of honey derived from various plant sources showed varied features. More recommendations citing the physio-chemical and antibacterial properties of various honeys for health advantages.

Keywords: honey, pharmacognostic profiling, physico-chemical properties, antimicrobial activities, pollen analysis

Introduction

Honey is natural drug as well as good source of food and recorded that about 190 Kilojoules of energy can be obtained from 15 mL honey. Due to its important nature and it can be adulterated for easy cash. So, various pharmacognostic characteristics of honey samples help in preservation of the honey and prevent these samples form adulteration. Pharmacognosy generally describe crude drugs having natural origin with health benefits other than pharmacy which means break down, appending causes, flavouring, appending causes etc. related closely to plant science and plant chemistry. The exact meaning about drugs which are natural has been portion of medical skill and science so human beings start drugs use for diseases (Nafees *et al*., 2019). Pharmacognosy provides very direct, conceivable, whole and accurate facts about natural crude drugs and is remarkably significant constituent in production, safety and helpfulness of the herbal substances (Badukale *et al*., 2021). Current study of pharmacognosy contain mainly the comprehensive study of natural yields from different sources including floras, micro-organisms, fungi and marine organisms (More *et al*., 2021). The World Health Organization (WHO) guesses that 4 billion individuals (80%) of the biosphere's people currently consume herbal remedy for one form of main fitness maintenance or extra. It is intimately entangled through that of modem drug but pharmacologist not use the whole plant but just use the important parts of plant which are important for health care (Khosravi and Kumar, 2021). Pharmacognostic study is medicinally important they involve phytochemical screening antimicrobial characters, physicochemical parameters and pharmacological evaluation and so on (Ajmire *et al*., 2021).

Honey is natural drug and food for human beings, about 190 Kilojoules of energy can be obtained from 15 mL. Honey is a sugary, sticky food constituent made by

*Author for correspondence;
E-mail: nafeesbotanist1990@gmail.com

honey bees and some related bugs. Bees collect nectar and pollen from flowers. Bees form waxy constructions called honey combs (Khan *et al*., 2018). Different types of honey produced by honey bees, the genus Apis, is the well-known for commercial production and human consumption (Al-Ghamdi and Ansari, 2021). Pollen are source of proteins for bees and nectar is the source of carbohydrates. When pollen are found in honey that is the surety of plant source. Pollen presence in honey from different plant flowers show that honey bee collect nectar due to which honey quality also change. Bees are responsible for pollination, about 16% pollination are carried out by bees (Didaras *et al*., 2020).

Honey is a balanced diet and equally popular for male and female in all ages as famous food (Khan *et al*. 2018). The only natural drug that doesn't require refrigeration and may be stored even at room temperature is honey (Hermanns *et al*., 2020). Characteristics and compositions of honey changes with geographical, climate changes, pollen and nectar utilized by bees for honey (Abu *et al*., 2020). Apitherapy developed in recent years, uses honey and other honey products against many diseases. The qualities of honey help us its medicinal uses antimicrobial as well antioxidant properties. The physical as well as chemical properties of honey concern mainly with its contamination. Therefore, physical and chemical properties of honey are responsible for its purity (Nair *et al*., 2020).

The characteristic and quality of honey generally change with the area and climatic conditions of the place along with the flower sources collected by the bees for honey. Honey bees combine complex saccharides and aqueous solution of inverted sugar, protein amino acids, organic acids, polyphenols, enzymes and carotenoids like ingredients, minerals vitamins mailers reaction. Different types of phenolic acid and flavonoids which are present in honey are responsible for removing potentially free radicals that's why they act for this purposes as antioxidant for antibacterial activities, infections, hypoallergenic, apoplexy and vasodilatory activities (Al-Ghamdi and Ansari, 2021). Honey is described to contain around 181 ingredients and is believed as part of customary medicine. Honey contain 15-17% water, 0.1-0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other constituents like phenolic antioxidants. Certain enzymes (glucose oxidase, catalase) the quantity and kind of polyphenolic substances depend on the flower source of honey and are major factors responsible for producing numerous nutritional (Al-Musawi *et al*., 2020).

Classification and sources of honey. Honey is categorized by its floral basis and divisions are made according to the packaging and processing used. Honey is graded on different environmental condition chemical as well as physical properties and plants source from which honey collected.

Flower source. Usually honey is classified by using nectar of different flower. Honey vary from definite types of nectar to the mix combination of nectar from flower the pollen of honey definite to specific flower and also du its climatic region. Pollen study and rheological characteristic can be used to distinguish source of plant nectar (McMinn-Sauder *et al*., 2020).

Poly-floral honey (multi-floral honey). Poly-floral honey, also known as non-cultivated flower honey is derived from the nectar of many types of flowers. The flavour may be different from time to time and the smell and the flavour can be more or less intense, dependent on which flowers are flourishing (Zhao *et al*., 2020).

Uni-floral honey (mono-floral honey). Mono-floral honey is consist of principally nectar of one type of flower. Mono-floral honeys have unique tastes and colors because of differences between their principal nectar sources. To produce mono-floral honey, the bees have access in those areas where honey bee can collect only one type of nectar. Typical examples are citrus, clover, orange blossom and blueberry (Zhao *et al*., 2020).

Honeydew honey. Honey bees can take honeydew, the sugary excretions of sap-sucking insects. The colour of honey dew is dark brown, with a rich smell of stewed fruit or fig jam, the taste is not sweet as that of nectar honey. In southwest Germany black forest is a famous source of honeydew contain honeys, as well as some regions in Bulgaria, Tara (mountain) in Serbia and northern California in the United State. 60-65% production of honey from Greece pine honey. The honeydew honey has some non-digestible things which give harms bees and destroy the colony (Karabagias *et al*., 2020).

Medicinal uses of honey. Honey have diverse medicinal importance because of its use as anti-diabetes, injury (wound), bioceutical diseases (anxiety, ant nociceptive, epileptic seizures, improve oxidative agent of central nervous system also act against depression), digestive

tract diseases, anticancer, heart diseases (cardiovascular), cough, bronchospasm (asthma), eye diseases, antimicrobial activity, necrobiosis (apoptotic activity), antioxidant activity, enhance defence system and act as anti-inflammatory agent (Ghramh *et al*., 2021).

Considering the importance of honey as a natural remedy, the current study sought to examine the pharmacognostic and phytochemical analyses of honey taken from eleven sources in Khyber Pukhtunkhwa, Pakistan.

Material and Methods

Collection of honey samples. Eleven honey samples randomly were collected from different resources from different regions in Khyber Pakhtunkhwa, Pakistan and labelled as mentioned in (Table 1).

Physiochemical properties of honey. ***Hydroxy methyl furfuryl (HMF) detection in honey*****.** The presence of HMF in eleven samples of honey was determined by Fiehe's test following the methodology of (Anusha *et al*., 2020). Honey sample (5 g) was mixed with ethoxyethane (5 mL) and stirred with glass rod in beaker. The mixture of ethoxyethane and honey was poured in ceramic dish for evapouration of ethoxyethane. Left the solution till all the ethoxyethane was evapourated, then powder of benzene-1,3-diol was added in HCL (concentrated), the change in the colour occur which was sherry red colour, showed the presence of HMF in samples of honey and faint pink colour or any other colour show the range of HMF level.

Moisture content analysis**.** Moisture content in honey samples was determined by the following the methodology of (Pascual-Mate *et al*., 2018) with slight modification. Eleven ceramic dishes were taken, washed and dried. Each ceramic dish was labelled for each honey sample. 5 g of honey in each ceramic dish was taken and put in the oven for about overnight to desiccation at 110 °C. The dishes were weighted again. The % moisture quantity in honey was calculated with the following formula;

Moisture content measurement moisture content (%) = {(W1-W2)/W1}*100

whereas;
W1=weight of ceramic dish + weight of honey before placing in oven; W2=weight of ceramic dish + dry weight of honey.

pH analysis**.** pH of honey samples were determined by following the method of (Mateo and Bosch-Reig, 1998) with some modification. 10 g of honey and 75 mL carbonated water was mixed and stirred well with glass rod. The pH was recorded with pH meter.

Acidity determination**.** Acidity was determined following the standard procedure of (Chirsanova *et al*., 2021). 1 g of honey was dissolved in 7.5 mL carbonated water. 2 g caustic soda was dissolved in 1000 mL of carbonated water. The burets were filled with caustic soda solution, below the burets the honey solution in volumetric glass was titrated against the caustic soda solution. Then titration was carried out and 1 to 3 drops of phenolphthalein was added. Titration was continued till Pink colour appeared. The acidity of honey was then determined with the following formula;

Acidity % = (0.23 x volume of caustic soda required for titration)/mass in grams of the honey sample taken for the test (1 g)

Electrical conductivity of honey**.** The procedure of (Sharin *et al*., 2021) was followed with some modification. The honey samples of 5 g was dissolved in the carbonated water. Then the conductivity meter was used to determine all the honey electrical conductivity in µS/cm unit.

Pollen analysis. Different types of pollen in honey sample were determined following the standard procedure of (Pospiech *et al*., 2021) with slight modification. 10 g of honey was dissolved in 20 mL hot distilled water. The solution of honey samples were centrifuged for 10 min at the rate of 2500 r/min. The upper solution from the tubes was removed with dropper slowly, then acetic anhydrides and sulphuric acid in the ratio of 9:1 (v/v) were added to the remaining solution

Table 1. Honey samples labelled obtained from different sources

Sample code	Common name	Botanical names
S1	Robinia	*Robinia pseudoacacia*
S2	Lokat	*Eriyobotrya japonica*
S3	Baker	*Justacia adhatoda*
S4	Beera	*Zizyphus jujuba*
S5	Marosa	*Calatheae allouia*
S6	Anar	*Punica Granatum*
S7	Spin azghkay	*Otostegia limbata*
S8	Palosa	*Accasia modesta*
S9	Spirky	*Thymus linearis*
S10	Malta	*Citrus sinensis*
S11	Shaftal	*Trifolium resupinatum*

of honey samples. The solution was left for about 30 min at the room temperature. Then the distilled water was added to the residues. The solution again was centrifuged for about 10 min. From the lower residue a drop was put on the glass slide with cover slip on the slides. It was examined under microscope with the power of objective 20X and 50X, compared with the reference pollen slide and identified.

Antimicrobial analysis of honey. ***Requisition of bacterial strains.*** Four strain of bacteria (*Salmonella typhi, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus*) were obtained from the laboratory of Khyber Teaching Hospital, Peshawar.

Requisition of fungal strains. Four fungal strains *(Alternaria alternate, Fusarium oxysporum, Aspergillus niger* and *Aspergillus terreus)* were obtained from Agriculture University, Peshawar.

Sterilization of apparatus. Apparatus comprises beaker, test tubes, cotton swab, petri dishes, cork borer, micropipette nozzles, Eppendorf tubes, smear loop and the prepared medium were autoclaved for 45 min at 121 °C. Ethanol solution spray bottle was used for decontamination of laminar hood cell unit for carrying out microbial activities using the methodology of Yadav and Mohite (2020).

Growth media preparation. Liquid media of nutrient broth was prepared for culturing bacterial and fungal strains. The media was then autoclaved for 45 min at temperature 121 ºC.

Growth of bacteria. The test tubes having 10 mL of growth media were labelled. Bacterial strains was taken in the labelled tubes with smear loop. The tubes were enclosed with aluminium foil and kept in the incubator for 24 h.

Formation of stock solution. For preparation of stock solution 1000 µL or 1000 µg/mL and working dilution 500 µL or 500 µg/mL, 20 mg of honey extract were dissolved in 2 mL DMSO (dimethylsulfoxide) with the help of sterilized syringe in the test tubes and shake well by vortex mixture, shifted to Eppendorf tube.

Standard control. 0.05% concentrated ciprofloxacin (25 mL/cc ciprofloxacin and 75 mL/cc was positive control in case of antibacterial bioassay (Yadav and Mohite, 2020), while 0.05% Nystatin (25 mL/cc and 75 mL/cc DMSO) was used as positive control in antifungal activities, while 500 µL DMSO was used as negative control in both cases (Othman *et al*., 2020).

Antibacterial activity. Nutrient agar media was prepared and autoclaved at 121 °C for 45 min. 44 petri dishes were labelled and media was poured in petri dishes and were left till the solidification. The bacterial strain of each sample was inoculated with sterilized cotton swab to labelled petri dishes. Sterilized cork borer was used and holed four wells of about 8 mm in each petri dish. The positive, negative, as well as 5%, 10% concentration of honey solution with micropipette was poured in the wells. All the petri dishes were paced in incubator for 24 h at 37 °C (Zainol *et al*., 2013).

Antifungal activity. 39 g of PDA (potato dextrose agar) was dissolved in 1000 mL of the carbonated water in bottle. The bottle containing media was placed on the thermo magnetic stirrer. All the materials used in the process with media in the bottle was autoclaved at 121 °C for 45 min. The laminar hood was sterilized with spray bottle containing ethanol. Media was added to sterilized petri dishes. Fungus of each strain was inoculated from tubes with cotton swab then wells were pored with sterilized cork borer. Control, 5% and 10% concentration of solution of all honey samples were poured in the wells with micropipette. All petri dishes were put carefully in incubator for 2 days at 28 °C (Anyanwu, 2012).

Statistical analysis. All the obtained data was analysed using Mean±SD and GraphPad prism software.

Results and Discussion

Presence of HMF in honey. Hydroxy methyl furfuryl (HMF) test for the eleven samples of honey were performed. Intensity of the colour obtained showed the adulterations and freshness level of honey. Sherry red colour is the indication of more adulterants in honey samples as compared to other colour presence. For all sample the HMF level was found lower than 60 mg/Kg. Lokat, Baker and Shaftal showed low level of HMF, while Anar, Palosa, Robinia and Marosa had the same colour changes which showed low freshness as compared to Lokat, Baker and Shaftal. The most dark sherry red colour occurred in Beera, Spinazghaki, Malta, showed more freshness and adulteration as mentioned in (Table 2 and Fig. 1).

Moisture content in honey. Moisture in honey samples was determined which illustrated the result that maximum moisture was present in sample S4 (3.73%) and low moisture was present in S5 honey sample 1.89%. All other honey samples had the moisture round

about 2%. The difference in moisture between different honeys was not greater and ranged from 1.89 to 3.73% (Fig. 2).

pH analysis of honey. pH values ranged from 3.43 to 6.00 showing acidic nature of all samples. S8 sample had 3.43 lowest value of pH and S6 sample showed the highest value of pH 6.00. S4 and S9 had pH in the range 5.95 and 5.50, while S1, S2, S3, S5, S7, S10 and S11 had pH values 3.40, 4.32, 4.48, 4.71, 4.23, 4.30 and 4.36 respectively (Fig. 3).

Acidity of honey. Eleven samples of honey were tested for acidity and the results indicated a range of 2.415 meq/Kg to 0.345 meq/Kg. The average of all samples was 1.11 meq/Kg, while the honey sample had a standard deviation of 60 meq/Kg. The result showed that maximum acidity was found in S4 i.e. 2.415 meq/Kg and minimum value in S6 i.e. 0.345 meq/Kg shown in (Fig. 4).

Pollen analysis. The pollen types observed under microscopic of 11 selected honey samples are given in (Table 3 and 4). Total seventeen (17) pollen in eleven samples of honey were observed. One type of pollen was present in S1, S2, S4, S5, S6, S7 honey samples. The analysed pollen showed that these honey types are

Table 2. Colour detection tests for honey obtained from various sources

Sample code	Botanical names	Common name	Colour
S1	*Robinia Pseudoacacia*	Robinia	Light sherry red
S2	*Eriyobotrya japonica*	Lokat	Faint pink
S3	*Justacia adhatoda*	Baker	Faint pink
S4	*Zizyphus jujuba*	Beera	Dark sherry red
S5	*Calathea allouia*	Marosa	Light sherry red
S6	*Punica Granatum*	Anar	Light sherry red
S7	*Otostegia limbata*	Spin azghkay	Dark Sherry red
S8	*Accasia modesta*	Palosa	Light sherry red
S9	*Thymus linearis*	Spirky	Dark sherry red
S10	*Citrus sinensis*	Malta	Dark sherry red
S11	*Trifolium resupinatum*	Shaftal	Faint pink

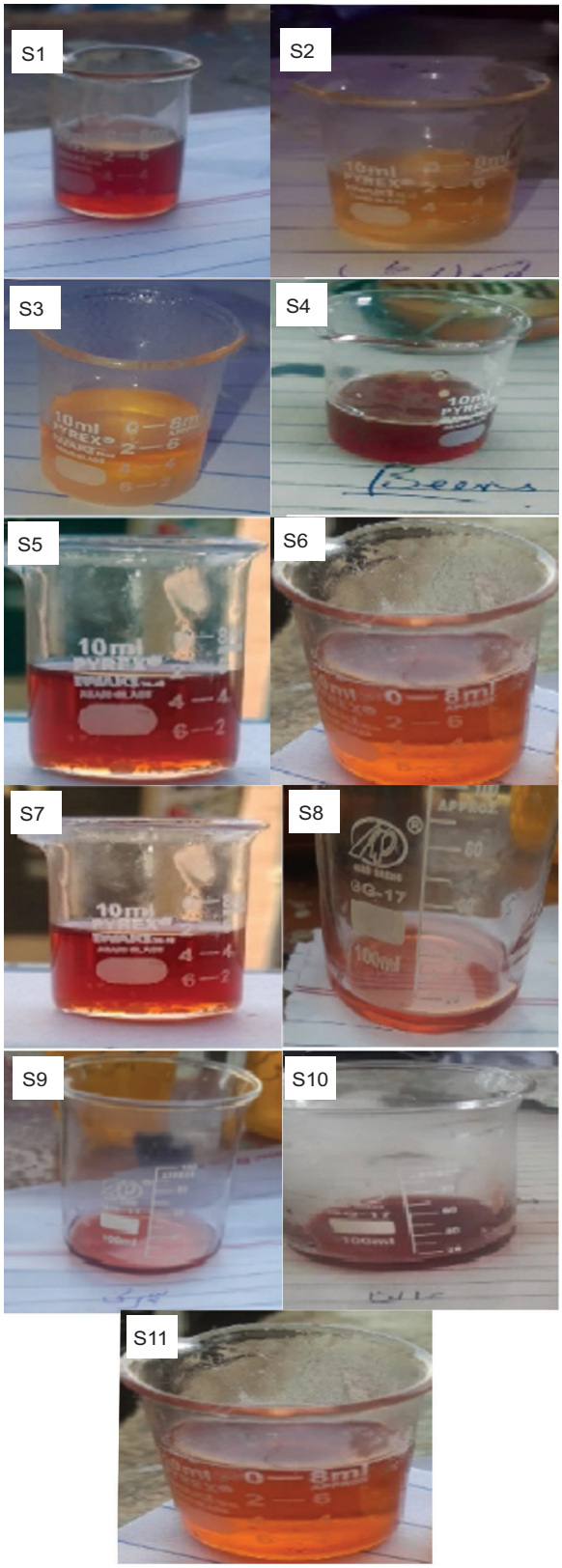

Fig. 1. HMF tests for honey obtained from various sources.

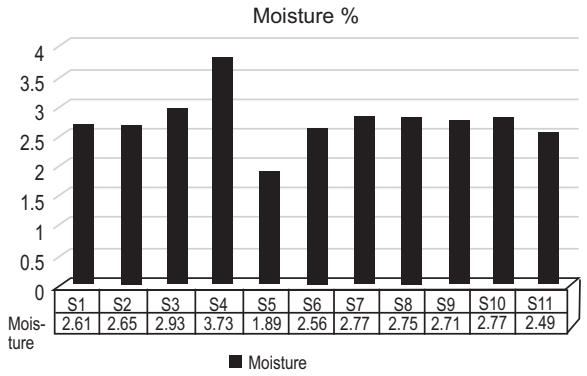

Fig. 2. Moisture content analysis in honey obtained from different sources.

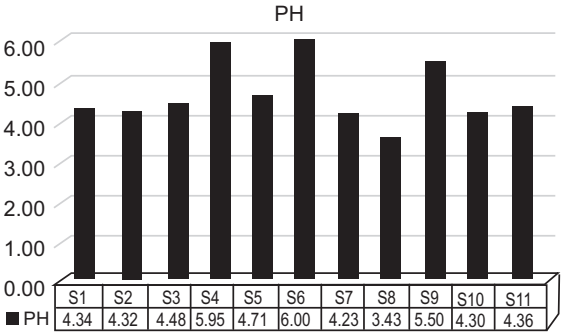

Fig. 3. pH analysis of honey obtained from different sources.

Table 3. Unifloral honey pollen analysis under microscopic review

	Pollen type	Family
Robinia S1 *(Robinia pseudoacacia)*		
Pollen observed	*Robinia pseudoacacia*	Fabaceae
Lokat S2 *(Eriyobotrya japonica)*		
Pollen observed	*Eriyobotrya japonica*	Rosaceae
Beera S4 *(Zizyphus jujuba)*		
Pollen observed	*Zizipus jujube*	Rhamaceae
Marosa S5 *(Calatheae allouia)*		
Pollen observed	*Passiflora edulis*	Passifloraceae
Anar S6 *(Punica granatum)*		
Pollen observed	*Punica granatum*	Puniaceae
Spin azghkay S7 *(Otostegia limbata)*		
Pollen observed	*Passiflora edulis*	Passifloraceae

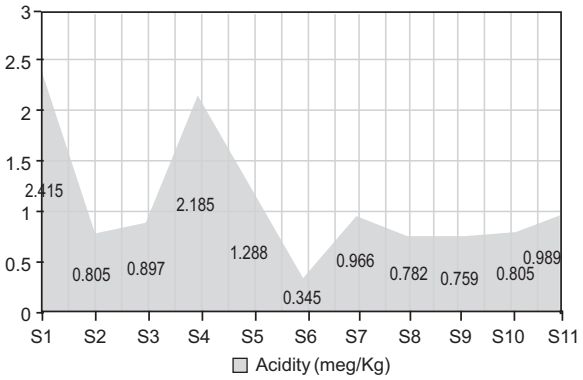

Fig. 4. Analysis of acidity of honey obtained from different sources.

Table 4. Multifolral honey pollen analysis under microscopic review

	Pollen type	Family
Palosa S8 *(Accasia modesta)*		
Pollen observed	*Borassus flabellifer (S8a)*	Borassaceae
	Vitis quadrangularis (S8b)	Vitaceae
Palosa S8 *(Accasia modesta)*		
Pollen observed	*Acacia auriculiformis (S8c)*	Fabaceae
	Fagus sylvatica (S8d)	Fagaceae
	Chamerion angustifolium (S8e)	Conagraceae
Spirky S9 *(Thymus linearis)*		
Pollen observed	*Calycanthus fertilis (S9a)*	Calycantaceae
Spirky S9 *(Thymus linearis)*		
Pollen observed	*Salix issatissensis (S9b)*	Salicaceae
Malta S10 *(Citrus sinensis)*		
Pollen observed	*Mimosa bimucrunta (S10a)*	Fabaceae
	Citrus sinensis (S10b)	Rutaceae
Shaftal S11 *(Trifolium resupinatum)*		
Pollen observed	*Tylophora sylvatica (S11a)*	Apocynaceae
	Trifolium repens (S11b)	Fabaceae

Uni-floral honey as shown in (Fig. 5). S8, S9, S10, S11 had pollen more than one type, which illustrated that these honey samples are poly-floral honey as shown in (Fig. 6). Pollen types belong to various plant families including Fabaceae, Rosaceae, Rhamaceae, Passifloraceae, Fagaceae, Borassaceae, Vitaceae, Apocynaceae, Rosaceae, Puniaceae, Conagraceae and Borassaceae. However, no pollen was observed in S3. The pollen presence showed that honey was originated

from plant source and also illustrated main source of different health benefits.

Electrical conductivity. Results mentioned in (Fig. 7) showed that highest electrical conductivity (EC) was observed in S4, while lowest electrical conductivity (EC) was recorded in S1. However, statistically same values were recorded in S3, S6 and S7. The mean value of all the samples was 0.77 µS/cm, while the SD value was 0.66 µS/cm. The result showed that honey samples electrical conductivity was because of floral origin.

Antibacterial activity. Antimicrobial activities of eleven samples of honey were determined against the four bacterial strains *(Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeroginosa)* at 5% and 10% concentration honey solution. *S. aureus* was more resistant, while *E. coli* was sensitive against honey samples. S6 sample showed maximum inhibition 87.50% (35.0±0.00) against *E. coli* as compared to all other samples with control value 40.00 mm in minimum concentration of 5% honey solution.

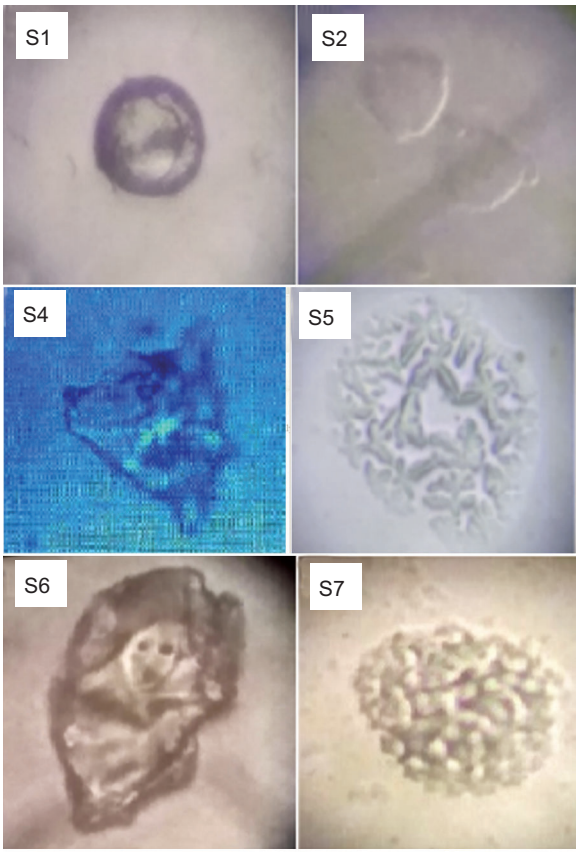

Fig. 5. Pollen analysis of uni-floral honey obtained from different sources.

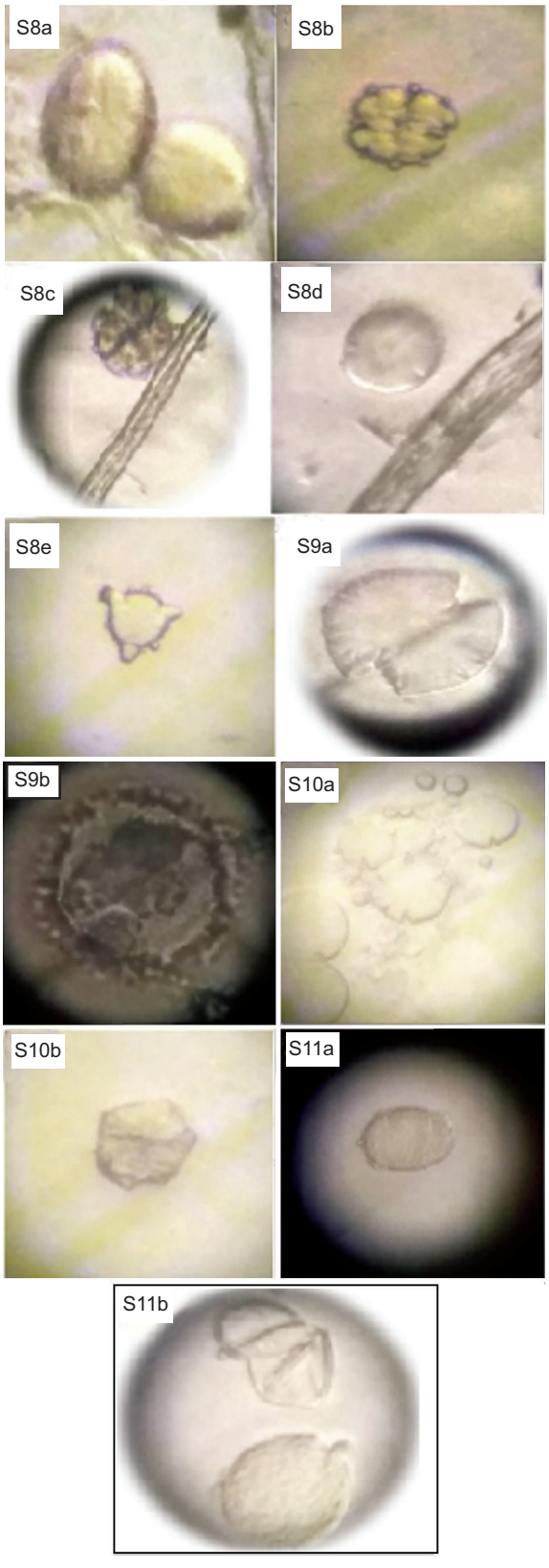

Fig. 6. Pollen analysis of poly-floral honey obtained from different sources.

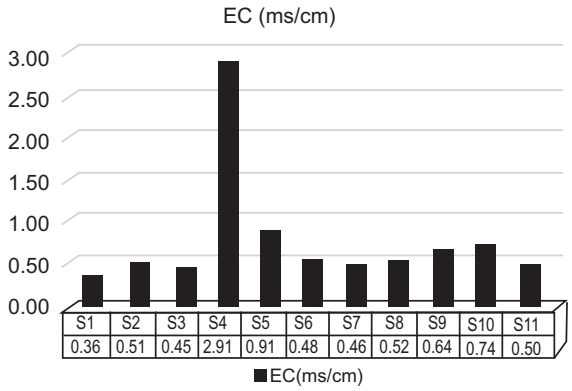

Fig. 7. Analysis of Electrical Conductivity of honey obtained from different sources.

S. typhi was found sensitive against S6 showing significant inhibition of 76.5% (30.33±0.58), 27.7% (11.00±0.00) with control being 39.67 mm. S4 honey sample showed significant result 52.9% (21.33±0.58), 66.9% (27.00±0.00) with control being 40.33 mm in 5% and 10% concentration of solution. Low significant result was showed by honey sample S7 57.5% (23.00±0.00) with control being 40 mm and showed no result in 10% concentration of solution. S5 and S10 honey sample showed result 45.3% (19.33±0.58), 49.2% (21.00±0.00) with control being 40.00 mm and 35.8% (14.33±1.53), 23.3% (09.33±0.33) with control being 40.00 mm in 5% and 10% concentration of solution as shown in (Table 5).

Honey sample S6 showed highest significant result against *S. aureus* 74.38% (30.00±0.00) in 5% and showed no result in 10% concentration of solution with control being 40.33 mm. S7 honey sample showed significant result in 45.93% (20.67±0.58) in 5% and no result in 10% concentration of solution with control value 45.00 mm. S5 honey sample showed low significant result 24.79% (10.00±0.00), 23.14% (09.33±0.58) with control 37.00 mm in 5% and 10% solution of honey. S1, S2, S3, S4, S8, S9, S10 and S11 displayed non-significant result in 5% and 10% concentration solution of honey. Honey sample S6 showed highly significant in minimum concentration of solution as compared to other honey sample against *S. aureus* bacteria as shown in (Table 6).

Result against *P. aeroginosa* of honey sample showed that highest significant result was present in honey sample S6 76% (36.33±0.47), 27.50% (11.00±0.00) in 5% and 10% solution. S4 sample of honey showed highly significant result 72.32% (27.00±0.00) in 5% and also showed result in 10% solution 57% (21.33±0.47) with control value 37.33 mm. S7 showed low significant result 58% (23.00±0.00) in 5% and showed no result in 10% concentration pf solution. Other significant result showed by honey sample S5 and S10 36% (14.33±1.24), 23.14% (9.33±0.58), 52% (19.33±0.47), 56.25% (21.00±0.00) with control value 40.33 mm in 55 and 10% concentration of solution. Non-significant result was found in S1, S2, S3, S8, S9 and S11 honey sample in both % concentration of solution the overall result showed that honey S6 showed effective result in minimum concentration of solution against *P. aeroginosa* as shown in (Table 7).

Result of various honey samples showed that *E. coli* was sensitive to S6 honey sample showed highest significant result 87.50% (35.00±0.00), 38% (15±00.00) withy control being 40.00 mm in 5% and 10% doses. Significant result of honey sample S7 77.65% (22±3.50) with control being 28.33 mm in 5% dose and showed no result in 10% dose. S5 showed low significant result 30.005 (12±2.60), 19% (7.67±0.58) with control being 40.00 mm in 5% and 10% doses. *E. coli* showed resistant to S8 honey sample also displayed significant result 24.32% (9±0.00) with control being 37.00 mm and showed no result in 10% concentration of solution. Honey samples S1, S2, S3, S4.S9 and S10 showed no significant result in 5% and 10% concentration of solution against *E. coli.* Honey sample S6 showed highest inhibition even in low percent concentration of solution as shown in (Table 8).

Antifungal activity. Antifungal activity of eleven honey sample against four fungal strains *(Aspergillus niger, Aspergillus terreus, Fusarium oxysporum* and *Alternaria alternate)* showed that *A. niger* was resistant and *F. oxysporum* was found sensitive to honey samples as compared to other fungal strain. The result were the following;

Fungus *A. niger* was sensitive to honey sample S9 in 10% concentration of solution showed significant zone of inhibition 86.11% (10.33±1.03) with control being 12.00 mm, while showed no result in 5% concentration of solution. Significant result was followed by S10 showed same result in 5% and 10% concentration of solution 82.00% (06.83±0.62) with control value 08.00 mm. Significant result was also showed by S5 honey sample which was same in both 5% and 10% concentration of solution 81.25% (06.50±0.47) with control 08.50 mm.

Table 5. Antibacterial activity of honey samples against *Salmonella typhi*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	40.33	-	-	-	-
S2	40.00	-	-	-	-
S3	40.33	-	-	-	-
S4	40.33	21.33±0.58**	52.9%	27.00±0.00**	66.9%
S5	40.00	14.33±1.53	35.8%	09.33±0.33	23.3%
S6	39.67	30.33±0.58***	76.5%	11.00±0.00	27.7%
S7	40.00	23.00±0.00**	57.5%	-	-
S8	40.00	-	-	-	-
S9	37.00	-	-	-	-
S10	42.67	19.33±0.58*	45.3%	21.00±0.00*	49.2%
S11	43.67	-	-	-	-

Table 6. Antibacterial activity of honey samples against *Staphylococcus aureus*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	37.00	-	-	-	-
S2	43.67	-	-	-	-
S3	43.00	-	-	-	-
S4	41.33	-	-	-	-
S5	40.33	09.33±0.58	23.14%	10.00±00	24.79%
S6	40.33	30.00±0.00***	4.38%	-	-
S7	45.00	20.67±0.58*	45.93%	-	-
S8	41.33	-	-	-	-
S9	36.00	-	-	-	-
S10	35.00	-	-	-	-
S11	19.67	-	-	-	-

Table 7. Antibacterial activity of honey samples against *Pseudomonas aeroginosa*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	41.33	-	-	-	-
S2	37.67	-	-	-	-
S3	47.00	-	-	-	-
S4	37.33	21.33±0.47*	57%	27.00±0.00**	72.32%
S5	40.33	14.33±1.24	36%	09.33±0.58	23.14%
S6	40.00	30.33±0.47***	76%	11.00±0.00	27.50%
S7	40.00	23.00±0.00*	58%	-	-
S8	39.33	-	-	-	-
S9	40.00	-	-	-	-
S10	37.33	19.33±0.47	52%	21.00±0.00*	56.25%
S11	40.67	-	-	-	-

Low significant result as compared to other honey sample was showed by S6 honey sample 60.00% (07.00±0.00) with control 11.67 mm in 5% and 10% concentration of solution. Least significant result was showed by S3 honey sample 58.82% (06.67±0.47), 52.94% (06.00±1.29) control value 11.33 mm honey samples S1, S2, S4, S7, S8, S11 showed no result in 5% and 10% concentration of solution as shown in (Table 9).

A. terreus was found sensitive against S10 honey sample 81.48% (07.33±0.58), 85.19% (07±0.58) with control value 9.00 mm. S9 and S8 honey samples also showed highly significant result 76.67% (07.67±0.58), 83.33% (08±0.58) with control value 10.00 mm, 81.48% (07.33±0.58), 77.78% (07.00±1.00) control value 9.00 mm in 5% and 10% concentration of honey solution. 80.65% (08.33±0.58) in 10% solution of honey was also highly significant result in S2 honey sample. This was followed by significant result of honey sample S11 79.41% (09.00±0.00) control being 11.33 mm. Significant result was also showed by S7 honey sample 76.92% (06.67±0.58) with control value 8.67 mm in 5% solution and showed no result in 10% concentration of solution. S5 honey sample showed significant result in 5% and 10% concentration of solution as like S7 honey sample 73.08% (06.33±0.58), 76.92% (06.67±0.58) control being 8.67 mm. Honey sample S3 displayed result 75.86% (07.33±0.58), 68.97% (06.67±0.58) control being 9.67 mm in 5% and 10% solution of honey. S4 honey sample showed result 72.41% (07.00±1.00), 65.52% (06.33±0.58) control value 9.67 mm low significant result as compared to other honey sample showed by S6 honey sample 67.65% (07.67±0.58), 70.59% (08.00±0.00) in 5% and 10% concentration of honey solution. The result showed that effective honey sample S10 showed highest significant result against *A. terreus*. It was also showed the result of honey samples that significant was increased in 10% concentration. *A. terreus* was found resistant to honey sample S6 as compared to other honey samples as shown in (Table 10).

F. oxysporum was found most sensitive to S8 honey sample 88.57% (10.33±0.58), 74.29% (08.67±0.58) with control being 11.67 mm similar result was also showed by S10 honey sample 87.10% (09.00±0.00), 83.87% (08.67±0.58) with control value 10.33 mm, S1

Table 8. Antibacterial activity of honey samples against *Escherichia coli*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	40.333	7.67±1.20	19.01%	-	-
S2	35.333	-	-	-	-
S3	40.000	-	-	-	-
S4	43.667	-	-	-	-
S5	40.000	12±2.60	30.00%	07.67±0.58	19%
S6	40.000	35±0.00***	87.50%	15±00.00	38%
S7	28.333	22±3.50**	77.65%	-	-
S8	37.000	09±0.00	24.32%	-	-
S9	43.000	-	-	-	-
S10	42.000	-	-	-	-
S11	38.667	-	-	-	-

Table 9. Antifungal activity of honey samples against *Aspergillus niger*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	7.33	-	-	-	-
S2	8.33	-	-	-	-
S3	11.33	6.67±0.47*	58.82%	06.00±1.29	52.94%
S4	6.00	-	-	-	-
S5	8.50	6.50±0.47**	81.25%	06.50±0.41**	81.25%
S6	11.67	7.00±0.00**	60.00%	07.00±0.00*	60.00%
S7	10.67	-	-	-	-
S8	9.67	-	-	-	-
S9	12.00	-	-	10.33±1.03***	86.11%
S10	8.00	6.83±0.62**	82.00%	06.67±0.47**	80.00%
S11	11.00	-	-	-	-

and S7 showed same result in 5% solution 86.67% (08.67±0.58) with same control 10.00 mm, S1 honey sample showed 60% (06.00±0.00) in 10% solution of honey. Highly significant result showed by S4 and S9 honey samples 83.33% (10.00±0.00), 75.00% (09.00±0.00) with control being 12.00 mm and 83.78% (10.33±0.58), 70.27% (08.67±0.58) with control being 12.33 mm, S2 honey sample showed significant result 82.76% (08.00±0.00) with control value 09.67 mm in 10% concentration of solution and showed no result in 5% solution of honey. Significant result was followed by S6 honey sample 79.41% (09.00±0.00), 76.47% (08.67±0.58) with control 11.33 mm, low significant result showed by S5 honey sample 63.33% (06.33±0.58), 66.67% (06.67±0.58) with control being 10.00 mm. Non-significant result was observed in honey sample S3 which showed no result in both concentration of solution. So, *F. oxysporum* was found resistant against S3 honey sample as shown in (Table 11).

Honey sample S2 showed highest antifungal activity against *A. alternate* which 88% (12.00±1.00) was with control being 13.67 mm in 10% solution of honey and showed non-significant result in 5% concentration of solution. Honey sample S8 also showed highest significant result 85% (12.00±0.00) with control value 13.33 mm in 5% concentration of solution and showed no result in 10% solution of honey. Highly significant result displayed by honey sample S11 that was 81% (10.67±0.00), 75% (09.00±0.00) control value 12.00 mm in 5% and 10% concentration of solution. Honey sample S5 had significant result 80% (10.67±0.57), 77% (10.33±1.53) control value 13.33 mm in both concentration of solution. The significant result was also followed by honey sample S7 79% (10.33±0.00) 64% (08.33±0.0) with control being 13 mm. S6 honey sample showed 71% (09.67±7.00) with control 13.67 mm in 5% concentration of solution. Low significant result as compared to other honey sample showed by

Table 10. Antifungal activity of honey samples against *Aspergillus terreus*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	12.67	09.33±0.58	73.68%	08.67±0.58	68.42%
S2	10.33	-	-	08.33±0.58**	80.65%
S3	9.67	07.33±0.58*`	75.86%	06.67±0.58	68.97%
S4	9.67	07.00±1.00*	72.41%	06.33±1.15	65.52%
S5	8.67	06.33±0.58*	73.08%	06.67±0.58*	76.92%
S6	11.33	07.67±0.58	67.65%	08.00±0.00	70.59%
S7	8.67	06.67±0.58*	76.92%	-	-
S8	9.00	07.33±0.58**	81.48%	07.00±1.00*	77.78%
S9	10.00	07.67±0.58*	76.67%	08.33±0.58***	83.33%
S10	9.00	07.33±0.58**	81.48%	07.67±0.58***	85.19%
S11	11.33	09.00±0.00**	79.41%	07.00±0.00	61.76%

Table 11. Antifungal activity of honey samples against *Fusarium oxysporum*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	10.00	06.00±0.00	60.00%	08.67±.058***	86.67%
S2	9.67	-	-	08.00±0.00 **	82.76%
S3	10.33	-	-	-	-
S4	12.00	10.00±0.00**	83.33%	09.00±0.00*	75.00%
S5	10.00	06.33±0.58	63.33%	06.67±0.58	66.67%
S6	11.33	09.00±0.00*	79.41%	08.67±0.58*	76.47%
S7	10.00	-	0.00%	08.67±0.58***	86.67%
S8	11.67	10.33±0.58***	88.57%	08.67±0.58	74.29%
S9	12.33	10.33±0.58**	83.78%	08.67±0.58	70.27%
S10	10.33	09.00±0.00***	87.10%	08.67±0.58***	83.87%
S11	12.67	-	-	-	-

S2 honey sample 53% (07.00±0.00) with control being 13.33 mm in 10% concentration of solution and showed no result in 5% solution of honey. Honey sample S1, S4, S9 and S10 showed non-significant result. The result of honey sample S2 showed significant result in 10% and honey sample S8 showed significant result in 5% concentration of solution. The fungus *A. alternate* was very resistant to sample S1, S4, S9 and S1 as shown in (Table 12).

Pharmacognosy being a pharmacological branch refers to a drug study in crude form or natural form. Although therapeutic agents are derived from many other sources, yet generally when pharmacognosy is described, disease control agents of plant origination are considered mostly. The pharmacognostic study is medicinally important they involve phytochemical screening antimicrobial characters, physic-chemical parameters and pharmacological evaluation and so on. The present study of comparative pharmacognostic profiling of honey from different plant sources were showed results, which will be a significant contribution to the pharmacognosy.

HMF is an indicative test through which freshness and adulterants in honey samples are evaluated. Our results indicated that three samples i.e., Lokat, baker and Shaftal appeared light in colour that indicated its freshness. Similar results were reported by (Erbakan *et al*., 2021) who observed that new form honey is poor in colour range due to small value of HMF. On the other hand some samples had dark colour showed less freshness nature of honey samples i.e., Anar, Palosa and Robinia had the same colour changed showed low freshness. HMF level rises due to time duration of storage and handling. The darkest colour occur in Beera, Spinazghaki, Malta and Marosa showed maximum adulterants and low freshness. Various features effect HMF level are hot and cold time, packing situation, pH, type of pollen presence. So, HMF intensity shows high temperature and low level packing environment (Kurtagic *et al*., 2021).

Moisture content is important characteristic of honey. The result different from each other of honey samples show that the honey samples absorb moisture content from air. The current results of moisture contents showed that the result were same as that of Codex standards. Low moistures show mining duration of honey as well as their production time, packing condition and geographical conditions. Minimum value keep safe honey from germs and fermentation (Maric *et al*., 2021).

pH value of honey show the acidity and mineral presence in decrease or increase quantity. pH values in presence study were similar to the result of Nigerian honeys as reported by Ndife *et al*. (2014). The pH values of honeys which was same as that of U.S. standards (3.4-6.1). *Acacia* honey has pH 3.43 value, other higher values was showed by Anar, *Zizipus* and Spirky. The pH values of honey is related to its packing and micro-organisms mounting. The pH value represent the excellence and permanency of honey samples. Change values of pH show fermentation or contamination (Nowak *et al*., 2021).

Low value of acidity demonstrate the freshness and high value demonstrate the fermentation of sugar into organic acid. Present acidity study of honey samples showed that the value was not greater than the mean values of 0.01 meq/Kg to 1.2 meq/Kg as reported by the Ethopian Quality and Standard authority (De Beer *et al*., 2021). The difference in the value of honey

Table 12. Antifungal activity of honey samples against *Alternaria alternate*

Sample code	Control(mm)	5% (V/V)		10% (V/V)	
	Mean	Mean±SD	% Inhibition	Mean±SD	% Inhibition
S1	12.67	-	-	-	-
S2	13.33	-	-	07.00±0.00	53%
S3	13.67	-	-	12.00±1.00***	88%
S4	13.33				
S5	13.33	10.67±0.57**	80%	10.33±1.53*	77%
S6	13.67	09.67±0.00	71%	-	-
S7	13	10.33±0.00	79%	08.33±0.00	64%
S8	13.33	12.00±0.00***	85%	-	-
S9	13.67	-	-	-	-
S10	12.33	08.00±0.00	65%	09.67±0.58*	78%
S11	12	10.67±0.00**	81%	09.00±0.00*	75%

samples showed the change in their production time (Wei *et al*., 2021).

Electrical conductivity of honey samples show that honey consist of chemicals and acids which ionize in its moisture condition. The conductivity of honey show floral origination, so value of electrical conductivity depends upon the floral origin. All samples have electrical conductivity property of nectar honey (≤ 0.8 mS/cm). The electrical conductivity of tested honey illustrate that the result is similar to that of at Malaysia honey determined by (Moniruzzaman *et al*., 2013). The electrical conductivity is important for separating honey due to bloomy origination (Guerzou *et al*., 2021).

The present study of eleven samples of honey showed that pollen are present in samples of honey. The honey bees collect pollen from different plant source to nourish (Oyeyemi and Kayode, 2012). The bees collect pollen from insect pollinated and wind pollinated plants (Vossler, 2021). The pollen are of different plants origin but bees focuses on some family pollen more like in the present study most of the Fabaceae pollen are found in honey samples. Baker S3 has no pollen found same to the study of (Elliott *et al*., 2021). In present study 17 pollen types were found in honey sample which is the quality of honey bees and show that honey are originated from different plants sources.

This maximum zone of inhibition and sensitivity of *E.coli* was similar to the result of (Gobin *et al*., 2018), who showed maximum zone of inhibition at concentration of 4%, 5% and 6% honey solution. *S. aureus* was found resistant strain as also reported by (Almasaudi *et al*., 2017).

Antifungal activity for 5% and 10% concentration of solution showed more result as compared to antibacterial zone of inhibition. The most maximum zone of inhibition was present against *F. oxysporum* fungus. The *A. terreus* fungus also show less resistant to honey as more samples of honey has antifungal effect against the *A. terreus* but the zone inhibition was greater in *F. oxysporum* fungus. Similarly other polyfolral honey also showed more zone of inhibition as compared to the unifloral honey because of pollen presence of plant like the study of (Anyanwu, 2012). Antifungal activities from different floral sources of honey were different and can be compared to the study of (Zhang *et al*., 2021). Diverse pollens were found in the present data along with other honey sample including S9, S10, S11 which are also polyfolral honey showed high zone of inhibition in *F. oxysporum* as well as other fungus. The present study also show that honey samples with more pollen will showed more antifungal activeness. Fungus *A. niger* was sensitive to honey sample S9 in 10% concentration of solution showed significant zone of inhibition, similar with the studies of (Feas and Estevinho, 2011). But the overall result showed that as compared to other fungus, *A. niger* was resistant to honey sample, as same observation was found by (Boukraa and Bouchegrane, 2007).

Conclusion

Various honey features led to the conclusion that each sample of honey has its own physical and chemical parameter quantity. The standard values of pH, acidity, moisture content and electrical conductivity showed that, honey samples are according to the international standard. Pollen observation in various honey samples illustrated the surety of plant source. Pollen presence in honey from different plant flowers showed that honey bee collect nectar due to which honey quality has also been changed. Antimicrobial characteristic of honey sample showed that, various honey samples may be used as antibacterial and antifungal agents. The resistant bacteria was *Staphylococcus aureus* to various samples however, *E. coli* was found more sensitive against various honey samples. *Fusarium oxysporum* was sensitive and *Aspergillus niger* was resistant to both uni and multi-floral honey samples. The aforementioned characteristics of various honey samples help in authentication of the honey and prevent these samples form adulteration.

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