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STUDIES ON ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF HONEY

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ABSTRACT

In the present study, the analysis of four honey samples viz., one natural honey and three commercial honey were characterised by UV-VIS and FT-IR. From the Spectroscopic results, the natural honey showed good absorbance value. Therefore, this honey were evaluated against pathogenic bacteria (*E.coli and B.subtilis*) and an opportunistic yeast pathogen (*C.albicans*) for the antimicrobial activity by Resazurin microtitre assay. It was then assessed for antioxidant activity by DPPH assay. The natural honey exhibited both antimicrobial and antioxidant activity

Keywords: Honey, UV-VIS, FT-IR, antimicrobial, antioxidant.

I. INRTODUCTION

Honey has been in use for its healing, nutritional and therapeutic properties since ancient times, and currently it has been proved experimentally that honey possesses anti-bacterial, anti-inflammatory and anti-oxidant properties, which may be beneficial in combating multi-drug resistant bacteria as well as in preventing many chronic inflammatory processes[1]. The use of honey in folk medicine is thought to be as old as civilization, but in recent times there has been a renaissance in interest in its use as a medicinal product. The colour of honey can vary from clear to dark amber according to its floral source and mineral content and it has a close relationship with its flavour and quality[2,3]. The bactericidal effect of honey was reported to be dependent on concentration of honey used and the nature of the bacteria [4,5]. The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent[6,7].The objective of the present study was to chose best honey sample from the four different types of honey by using UV-VIS and FT-IR Spectroscopy methods. The chosen honey sample was subjected to antimicrobial and antioxidant activity.

II. MATERIALS AND METHODS

2.1 Materials

One natural honey(H_A) and three commercial honey(H_B, H_C & H_D) has taken for the research work. All the glasswares used for the work were washed well, rinsed with double distilled water and dried in hot air oven.

2.2 Analysis of honey samples

Four honey samples were taken for the work in which H_A was a natural one and the H_B, H_C & H_D were Commercial branded honey. These four samples were characterised under UV-Vis and FT-IR spectroscopy in order to study the nature of the honey.

S.NO	HONEY SAMPLE
1.	H_A
2.	H_B
3.	H_C
4.	H_D

2.3 Antimicrobial activity

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay:

Procedure for antimicrobial activity

Test was carried out in a 96 well Plates(labelled) under aseptic conditions. A volume of 100 µL of sample was pipetted into the first well of the plate. To all other wells 50 µL of nutrient broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of bacterial suspension was added to each well. Similarly for the antifungal activity, 10 µL of fungal suspension was added to each well and each

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plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 24 hrs. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

2.4 Antioxidant activity by dpph free radical scavenging assay

The percentage of Antioxidant Activity (AA %) of each substance was assessed by DPPH free radical scavenging assay. Different concentrations of sample were added to all the tubes except blank. Then 3 mL of ethanol and 0.3 mL of 0.5 mM DPPH radical solution in ethanol was added. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Absorbance was read at 517 nm after 30 min of reaction. The scavenging activity percentage (AA %) was calculated.

III. RESULTS AND DISCUSSION

3.1 UV – visible spectroscopy analysis

The Honey samples were characterized by UV – Visible Spectroscopy. One drop of honey were dissolved in distilled water was analysed in the range between 200 – 800 nm. The Fig 1, 2, 3 and 4 shows the UV – Visible Spectrum of Honey H_A, H_B, H_C and H_D respectively. From the analysis, it is observed that except H_A , all the commercial samples are in similar pattern which shows peak at 285 nm. In H_A , it shows a strong peak at 210 nm. A strong absorption peak at 290.50 nm is attributed to the presence of phenols and flavonoids. The higher absorbance value indicates the rich composition of polyphenols and flavonoids in the sample[8]. Therefore, we have chosen H_A for the antimicrobial activity and antioxidant activity due its potent nature.

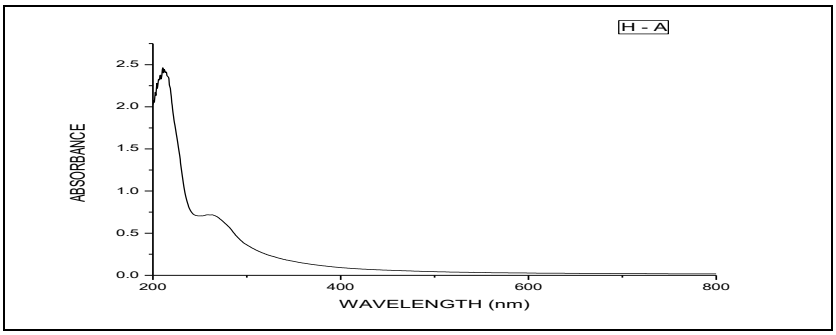

Fig 1: UV – VISIBLE SPECTRUM OF H_A

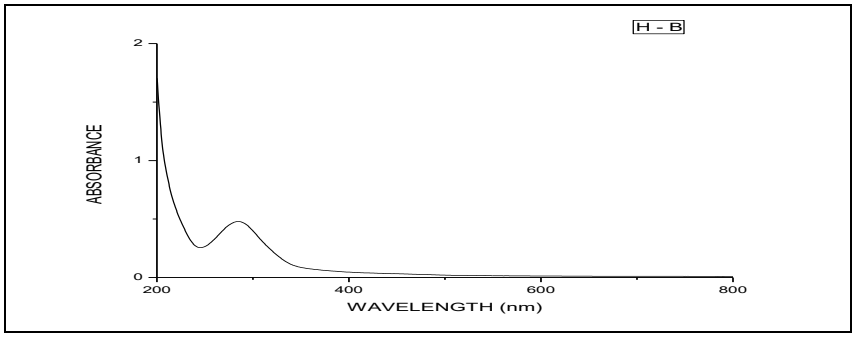

Fig 2: UV – VISIBLE SPECTRUM OF H_B

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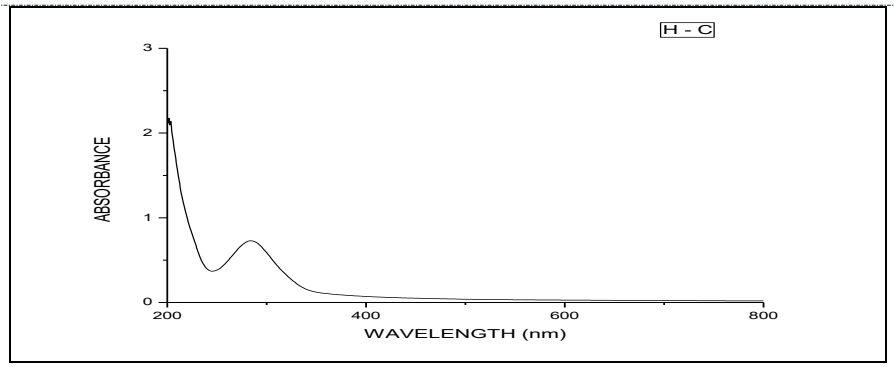

Fig 3: UV – VISIBLE SPECTRUM OF H_C

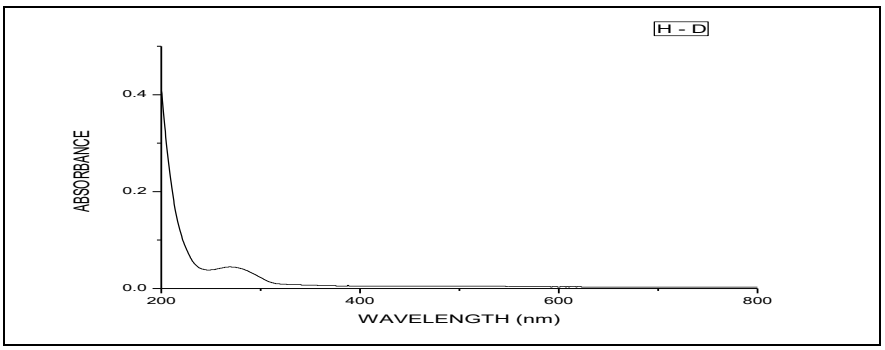

Fig 4: UV – VISIBLE SPECTRUM OF H_D

3.2 FT – IR analysis

FTIR measurement was carried out to identify the possible biomolecules responsible for the pharmacological activities of honey. Peaks at 3471 cm-1, 3431 cm^{-1}, 3371 cm^{-1} corresponds to hydrogen bonded O-H, N-H, C-H stretching vibrations of alcohols, phenols, amides and alkanes. A Peak at 2924 cm^{-1} and 2918 cm^{-1} indicates the presence of very strong C-H stretching vibrations of aromatic rings, methylene, methyl and O-H group of acids. The Peak at 1435cm^{-1} corresponds to asymmetric C=O, C-N of amines. The Peak at1357 cm^{-1} indicates the presence of symmetric C=O, Symmetric C-H, N=O bonds. Peak at 1066 cm^{-1} and 1049cm^{-1} corresponds to fundamental C-C bond peaks. Therefore, FT-IR Spectrum of the Honey sample A, B, C and D shows the presence of fundamental vibrations such as aromatic C-C, C-H, C-O, N-H and O-H bonds which correspond to the presence of alcohols, acids, esters and aliphatic amines[9].

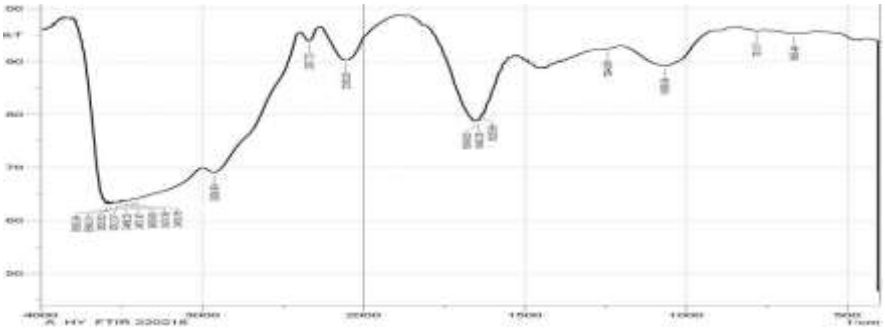

Fig 5: FT-IR SPECTRUM OF H_A

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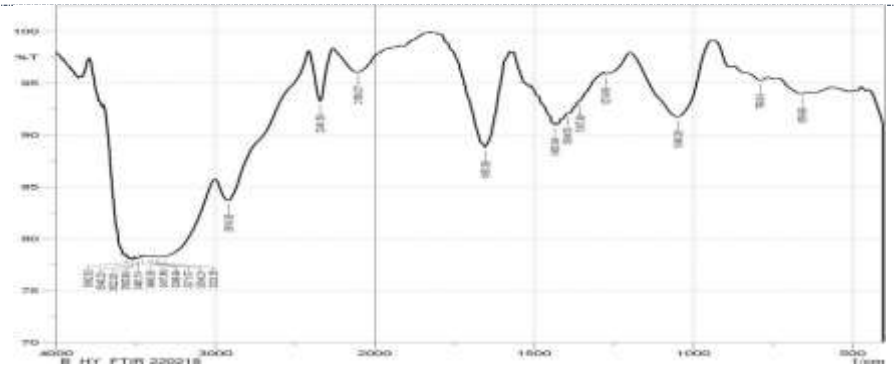

Fig 6: FT-IR SPECTRUM OF H_B

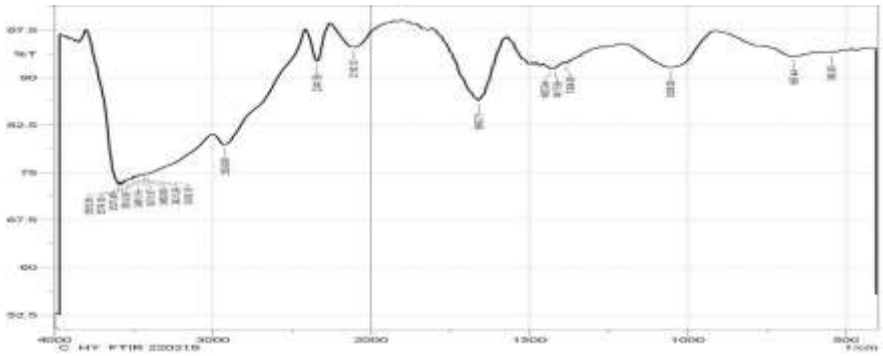

Fig 7: FT-IR SPECTRUM OF H_C

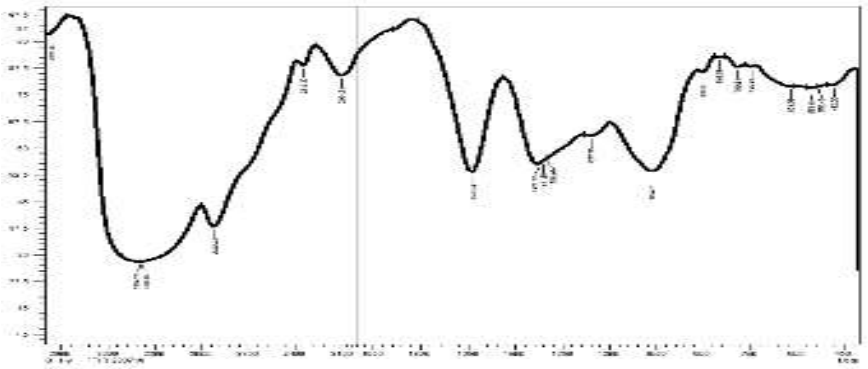

Fig 8: FT-IR SPECTRUM OF H_D

The area of bands with the intense maximum at approximately 1055 cm^{-1} - 1049 cm^{-1} coming from stretching vibrations of both C-O in C-OH and C-C group in the carbohydrate structure also seems to be very interesting. It clearly indicates varied sugar composition of the honey samples. The band from approximately 1500–750 cm^{-1}corresponds to the most sensitive absorption region of the major components of honey, particularly the most suitable region to quantify honey sugar (about 60–75 %) and organic acids. The contribution of sucrose, glucose, fructose, show characteristic bands in the region between 1500 and 900 cm^{-1}. Another important spectral region located at 900–750 cm^{-1} is characteristic for the saccharide configuration. The last region which is very distinctive in the evaluation and description of honey is the band from 890 to 810 cm^{-1}characteristic for the vibration anomeric region of carbohydrates or C–H deformation[10].

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3.3 Antimicrobial activity

Table 1: antimicrobial activity of honey h_a

S.No	Microorganisms/sample	Growth of inhibition										
	H_A Sample volume	50 µl	25 µl	12.5 µl	6.25 µl	3.12 µl	1.56 µl	0.78 µl	0.39 µl	STD Steptomycin 10µg	DMSO Negative control	Culture
1	*Escherichia coli*	-	-	-	-	+	+	+	+	-	+	+
2	*Bacillus subtilis*	-	-	-	-	-	-	-	+	-	+	+
										Amphotericin B 10µg	Negative control	Culture
3	*Candida albicans*	-	-	-	-	-	-	-	+	-	+	+

The sample H_A shows good antibacterial activity against *E.coli* and *B. subtilis* whose MIC values are 6.25 µl and 0.78 µl respectively. It also shows good antifungal activity against *C. albicans* with MIC value 0.78 µl.

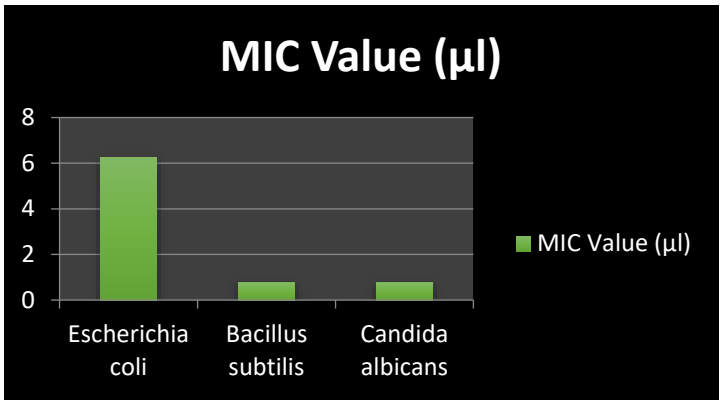

Fig 9: Antimicrobial activity of Honey H_A

3.4 Antioxidant activity of H_A

The anti-oxidant capacity of the synthesized silver nano particles was assessed by its DPPH (1, 1–diphenyl-2-picryl hydrazyl) radical scavenging ability[11]. Deep violet coloured DPPH showed an absorbance maximum at 517nm and its absorbance decreases due to pairing up of odd electron present in DPPH. The extent of discoloration and decrease in absorbance was stoichiometric with the number of electrons captured[8]. It was compared with the anti-oxidant ability of the standard Butyl Hydroxy Toluene. % antioxidant potential is calculated using the formula,

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Blank}}} \times 100$$

The honey H_A showed 77.1% of the antioxidant activity at the concentration level of 500 µg.

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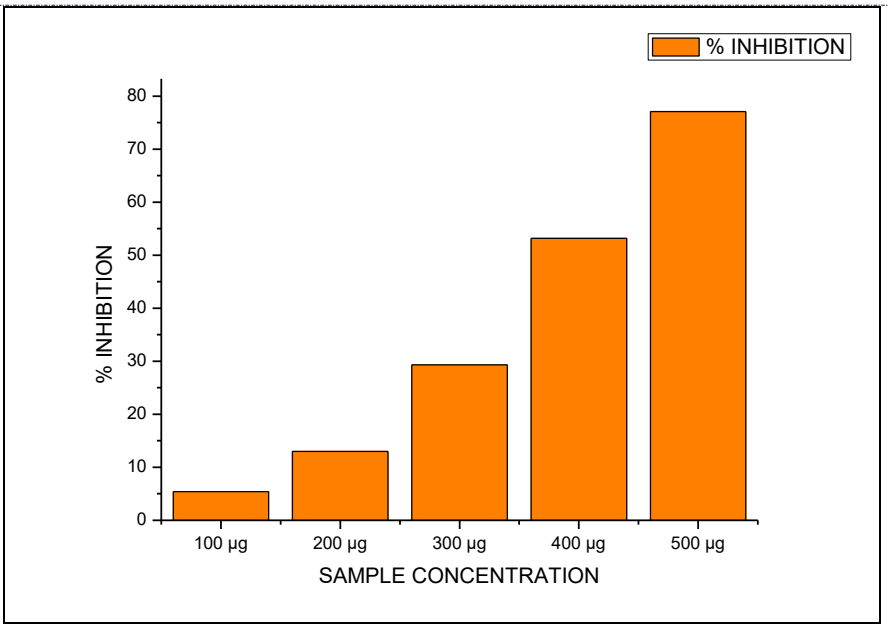

Fig 10: Antioxidant activity by DPPH radical scavenging method

IV. CONCLUSION

From the present study, the four honey samples were characterised by UV-VIS and FT-IR spectroscopic analysis. The UV-VIS spectrum shows good absorption peak for H_A. From the FT-IR analysis, it clearly indicates that the characteristic bands for the major components of Honey. Hence, the chosen natural honey H_A shows good antimicrobial activity against *E.coli, B.subtilis* and *C.albicans.* It also act as a good source of antioxidant due its 77% inhibition nature. Due to the cost-effective, easily available nature of honey, it can be effectively used for the biomedical applications in future. New studies based on the pharmacological and clinical approach must be conducted.

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