Pharmacological And Antioxidant Evaluation of Natural Honey Samples

Shivani[#1], Ruchika[2], Alka Patel[3], Vinod Kumar Gupta[4]

[#1]*Project Trainee at Rapture Biotech International Pvt. Ltd., Noida (UP), India.*
[2]*Project Trainee at Rapture Biotech International Pvt. Ltd., Noida (UP), India.*
[3]*Project Trainee at Rapture Biotech International Pvt. Ltd., Noida (UP), India.*
[4]*Technical Head (life science division) at Rapture Biotech International Pvt. Ltd., Noida (UP), India*

Abstract - *This study was undertaken to evaluate the phytochemicals and antioxidant activity of natural honey samples collected from Uttarakhand district [H1] and Roorkee [H2]. The existence of phytochemicals such as saponins, tannins, flavonoids, alkaloid, reducing sugar, carbohydrates, monosaccharides, phytosterols, terpenes, anthocynins, quinines and cardiac-glycosides were analyzed by qualitative test. The result of phtyochemical identification illustrated that both honey samples have postiviely presented as containing saponins, tannins, flavonoids, protein and carbohydrates as compared to other components. The total phenolic content, flavonoids content, protein content, carbohydrates content and tannin content of honey were analyzed by using quantitative method. It was observed the protein content in sample H1 and H2 were detected at higher level which ranged 53.2mg/ml and 19.8mg/ml as compared to other phytochemicals respectively. It is also showed that honey samples have antioxidant properties were estimated by determining 1, 1-diphenyl-2-picryl-hydrazyl [DPPH] quantity where sample H1 and H2 average percentage are 41.38% and 40.69% respectively. The current study indicates that honey is an effective source of nutrition which is helpful to cure untreated aliments.*

Keywords- *Phytochemicals, honey, antioxidant activity, DPPH*

I. INTRODUCTION

Honey is a natural sweet collected from the nectar of flowers through the honeybees [1]. Honey has content of 80-85% carbohydrates, 15-17% water, 0.1%-0.4% protein and minor quantities of amino acids, proteins and vitamins are also an integrant of honey [2,3,4]. The composition of honey dependent on its geological, floral and zoological source, some external factors such as environmental constituent and seasonal plays an important role [5]. Honey is a dense complex contains phytochemical and antioxidant property affected by the presence of organic acids, vitamins, flavonoids, phenolic acid, protein and carotenoids [5,6]. Fructose and glucose are main source of sugars which is present in honey. Depending on its physical, chemical and microbiological property ensure the quality of honey [5]. The identification of honey quality has been defined by the EC Directive 2001/110 [7]. Antimicrobial and antibacterial activity also present in honey. The difference in honey colour is based on distinct plant sources and presence of pale yellow by amber to a darkish brown to black. The darkness of honey may be depending on the changes of temperature [8].

India is proliferates for pollen resources and nectar [9]. Above 100 kinds of bee vegetation was identified and also considered to be 65,000 metric tones of total honey production [10]. The vital bee species in India are Indian bee [*Apis cerana indica F.*], little bee [*Apis florae F.*], rock bee [*Apis dorsata F.*], stingless bee [*Trigona iridipennis smith*] and European bee [*Apis mellifera L.]* [9]. It also has a secondary products and nutritional values used to cure medicinal effects includes health, food and face. Honey can be used to treat ailment, such as respiratory infections and gastrointestinal which stated from Huda [11]. It has been used for its therapeutic effects and also contains 200 substances. The therapeutic qualities of honey are changing depends on the variety of honey [12]. In the current study, natural honey samples collected from Uttarakhand and Roorkee. The area is surrounded by the green cultivated land and herbal trees. This study aim was to evaluate the secondary metabolites and biological activity in natural honey samples through qualitative and quantitative phytochemical analysis.

II. MATERIAL AND METHODS

A. Collection of sample

In this study, two types of natural honey Samples were collected from Uttarakhand District and Roorkee. They were marked as natural honey samples [H1] and [H2] respectively. The samples were stored in room temperature.

B. Qualitative methods for phytochemical analysis

The phytochemical screening such as saponins, tannins, flavonoids, reducing sugar, protein, alkaloids, carbohydrates, monosaccharides, phlobatanins, phytosterols, terpenes, anthocynins,

quinones, cardiac-glycosides, hydroanthraquinones and phenolics for qualitative test carried out by standard laboratory procedures [13,14,15].

1) Analysis of Saponins

To 2 ml of natural honey samples was added with 2 ml of distilled water and shaken by vortex for 30 seconds. Presence of foam for 1 minute illustrates the presence of saponins.

2) Analysis of Tannin

2 ml of natural honey samples was placed into a test tube and titrated with 2-3 drops of 1% ferric chloride. The dark blue and greenish black color indicates the presence of tannin compounds [11].

3) Analysis of Flavonoids

2 ml of natural honey samples, 2 ml of sodium hydroxide was added. Then few drops of concentrated HCl were added. The positive discolourised indicates the presence of flavonoids.

4) Analysis of Reducing Sugar

Benedict's test: Mixed 2 ml of natural honey samples and 2 ml of benedict's reagent in test tube, heat in the water bath for 5 minutes. Reducing sugar is present in the appearance of red, green and yellow color [1].

5) Analysis of Protein

Xanthoproteic Test: To 2 ml of natural honey samples were treated with a few drops of concentrated nitric acid. The yellow color indicates the presence of protein.

6) Analysis of Alkaloids

Mayer's Test: 2 ml of natural honey samples was added with 2 ml of concentrated hydrochloric acid. Few drops of Mayer's reagent was added in the test tube. Formation of green color or white precipitate indicates the presence of alkaloids.

Wagner's test: The natural honey samples were acidify with hydrochloric acid [1.5% v/v] and added a few drops of wagner's reagent in the test tube. Reddish brown precipitation indicates the presence of alkaloids.

7) Analysis of carbohydrates

Fehling's test: 1 ml of natural honey samples were piped out in the test tube. Then add 1 ml each of fehling solution A [copper sulphate] and B [sodium potassium tartrate] and boiled on water bath for few seconds. Formation of red precipitates indicated the presence of carbohydrates.

Molisch's test: 1ml of natural honey samples were dissolved with 0.5 ml of 10%, α-naphthol in ethanol. Then 200 ml of sulphuric acid was added. Presence of violet ring at the junction of two liquid indicates the presence of carbohydrates [1].

8) Analysis of monosaccharide

Barfoed's test: 1ml of natural honey samples were piped out in the test tube and dissolved in 200 ml of distilled water. Then mix 13.3g of copper acetate and 1.8 ml of glacial acetic acid solution. Heat for 1-2 minutes in boiling water bath and cool it. Formation of red precipitate indicates the presence of monosaccharide [1].

9) Analysis of Phlobatannins

3 ml of distilled water was piped out in a test tube. Then 2 drops of natural honey samples were added with 1.5 ml of 70%, hydrochloric acid. Presence of red precipitation indicates the presence of phlobatannins.

10)Analysis of Phytosterols

3-9 drops of natural honey samples were added with 1ml of distill water then 1 ml of chloroform and 0.5ml of concentrated sulphuric acid was piped out drop wise in the test tube. Appearance of red layer is chloroform and the concentrated sulphuric acid layer appears greenish yellow fluorescence which indicates the presence of phytosterols.

11)Analysis of Terpenes

0.5ml of natural honey samples, 0.5ml of chloroform and 0.5ml of concentrated sulphuric acid was added in the test tubes. The result indicates the upper layer shows yellow color which is the presence of terpenes.

12)Analysis of Anthocynins

0.5ml of natural honey samples were added with a few drops [0.2ml] of 10%, NaOH in the test tube. When the pH is more than 8 it shows the yellow color formation which is illustrates the presence of anthocynins.

13)Analysis of Quinones

1 ml of natural honey samples and 10%, sulphuric acid were added in the test tube. Presence of yellow precipitation indicates the presence of quinones.

14)Analysis of Cardiac-Glycosides

5 ml of natural honey samples, 2 ml of glacial acetic acid and 0.05ml ferric chloride were added in the test tube. Then add 1ml of concentrated sulphuric acid dropwise in the mixture of sample. Formation of reddish brown ring at the upper surface indicates the positive result for cardiac-glycosides.

15)Analysis of Hydroanthraquinones

1ml of natural honey samples mixed with few drops 0.2ml of 10% KOH. Formation of red color indicates the presence of hydroanthraquinones.

16)Analysis of Phenolics

0.5ml of natural honey samples were piped out in the test tube and add 2 ml of ferric chloride. Formation of

blue and green color shows the positive result for phenolics.

C. Phytochemical analysis from quantitative assay in natural honey samples

Quantitative analysis is used to the determination for quality of component which is present in sample. It may be referred through the concentration and mass. In this process, chemical and physical methods involved through density, adsorption of light, oxidation and precipitation. It also identified the presence of radioactive compounds.

1) Estimation of total Phenolic content through Folin-Ciocalteu's assay:

Phenolic refers biological activities including anti-inflammatory, bacteriostatic, anticarcinogenic and analgesic effect in plant product [16]. It is a subgroup of phytochemicals and has an antioxidant capacity to use for human health. Gallic acid and catechin are the standard solution of FC reagent formed from a mixture of phosphotungstic acid or phosphomolybdic acid determined by colorimetric method. The total phenolic content was measured by using the method of Folin Ciocalteu's assay. Gallic acid was used as a standard [1 mg/ml] and total phenolic compound was determined as mg/ml Gallic acid equivalent. Initially, prepare the standards of gallic acid solutions[0.01, 0.02, 0.025, 0.05, 0.1ml] and H1,H2 samples [0.05ml] was mixed with distilled water up to volume 0.5ml followed by adding 2ml of 0.2N, Folin-Ciocalteu reagent. After incubation for 5 min at room temperature, added 2ml of 7.5%, sodium carbonate solution [Na_2CO_3] in each test tubes. The mixture was further incubated at room temperature for 1 hour and the absorbance of the solution was measured at 765 nm with UV-Visible spectrophotometer against a blank with distilled water and reagents [17,18].

2) Estimation of Tannin through Van Burden and Robinson:

This method was measured by Van Burden and Robinson method in 1981. Tannic acid was used as a standard [1mg/ml]. The volume of standard tannic acid [0.05, 0.1, 0.25 and 0.5ml] and H1,H2 samples [0.5ml] was taken up to 5ml distilled water into each test tubes and mixed with 1ml of 0.1M, ferric chloride[$FeCl_3$] in 0.1N, hydrochloric acid [HCl] and 0.008M, potassium ferrocyanide [$K_4Fe(CN)_6$]. A blank with 5ml distilled water and reagents was also prepared. After 10 min incubation at room temperature, the absorbance was measured at 605nm [19].

3) Estimation of Reducing Sugar through DNS method

This method was determined by Dinitrosalicylic acid method. Reducing sugar refers the presence of free carbonyl group [C=O] in chemical reaction. The highly used reagent for detection of reducing sugar is 3, 5-Dinitrosalicylic acid [DNSA]. In this process, DNSA is reduced to 3-amino-5 nitrosalicylic acid in alkaline condition which produced reddish brown colour complex [20]. Dextrose solution was used as a standard and analyzed as 10 mg/ml. An aliquot the dextrose standard solution [0.01, 0.05, 0.1, 0.5, 1ml] and H1,H2 samples [0.1ml] in different test tubes and mixed with up to volume of 3ml distilled water. Then added 2ml of DNS reagent in all test tubes and mix it properly. The mixture was incubating in the water bath for 15min. After that allow cooling at room temperature, the absorbance was measured at 540nm using UV-Visible spectrophotometer, against blank with 3ml distilled water and 2 ml reagent [21].

4) Estimation of Protein concentrations through Folin's Lowry method:

In this method, under alkaline condition divalent copper ions[Cu^{2+}] reacts with peptide bond followed by reduction of cuprous ions [Cu+] and the radical groups of tyrosine, tryptophan and cysteine reacts with Folin Ciocaltaeu's reagent that forms unstable complex which has been reduced to sodium molybdenum/ tungsten blue[22].

The BSA was used as a standard and the concentration of BSA is 1mg/ml. Firstly, prepare different volume of BSA[Bovine serum albumin] [0.03, 0.06, 0.12, 0.24, 0.48ml] and H1,H2 samples[0.025ml] in separate test tubes were mixed up to 1ml distilled water. Then added,4.5ml of reagent consists of 2%, Na_2CO_3 in 0.1M, NaOH, 1%, sodium tartrate and 0.5%, copper sulphate in all test tubes and mix it properly. After incubation at room temperature for 10-15min, add 0.5ml of 2N, Folin-Ciocaltaeu's reagent in it. The mixture was allowed to incubate in the dark for 30min. Then the absorbance was measured at 660nm in UV-Visible spectrophotometer against blank with distilled water and reagents [23].

5) Estimation of Flavonoid through Aluminium Chloride Calorimeter method:

Flavonoids contain C-4 groups and C-3 or C-5 hydroxyl group of flavones and flavonols where $AlCl_3$ appears stable complex. At the A or B ring of flavonoids it also forms acid complex with the orthodihydroxyl group [24]. The quercetin was used as a standard and the concentration of quercetin analyzed as 0.1 mg/ml. the mixture consisting standard solution of quercetin [0.2, 0.4, 0.6, 0.8, 1ml] and H1,H2 samples [0.1ml] up to the volume of 5ml distilled water in a different test tubes followed by adding 0.3ml of 5%, sodium nitrate[$NaNO_2$] solution. After incubation for 5min at room temperature, 0.3ml of 10%, aluminium chloride [$AlCl_3$] solution was added and allowed to incubate for 5min at room temperature. Thereafter, added 2ml of 1M, sodium hydroxide[NaOH] in each test tubes and mix it properly. The absorbance was measured at 510nm against blank with distilled water and reagents[25].

D. Antioxidant activity through dpph radical scavenging activity assay

The DPPH method determined hydrogen atom donating activity and give an estimation of antioxidant activity through free radical scavenging. A stable free radical has purple color which was reduced into the yellow colored diphenylpicryl hydrazine through the spectrophotometer and the color intensity measured at 517 nm.

2 ml of DPPH [24mg in 100 ml of methanol] solution was added with 0.95 concentrations of natural honey samples and incubated at 25°C for 20 minutes. After incubation, the absorbance of mixture was measured at 517 nm. The DPPH radical scavenging activity was illustrated percentage inhibition and calculated through the given equation:

$$\% \text{ of inhibition} = [A_{\varphi} - A_1]/A_{\varphi}\ 100$$

Where A_{φ} was the absorbance of the control (without sample) and A_1 was the absorbance in the presence of the sample [26, 27].

III. RESULTS AND DISCUSSION

A. Qualitative analysis of different types of natural honey samples:

To check the quality and freshness of the natural honey samples, some tests were carried out. The outcomes of these experiments confirmed that these samples were pure and fresh. The qualitative phytochemicals screening had been performed on the two culled natural honey samples from a different locality, among which active medicinal component i.e. Carbohydrates, Phenolics, Proteins, Saponins, Tannins ($FeCl_3$), Flavonoids, Terpenes, Anthocyanins, Quinones present in both the natural honey samples. Saponins are present in H2 in higher as compare to H1. Tannins ($K_2Cr_2O_7$), Phlobatannins, Hydro-anthraquinones, alkaloids were absent in H1 and H2 and phytosterols, cardiac glycoside either present or absence in both samples are represented in [table no.1].

TABLE I
QUALITATIVE PHYTOCHEMICAL ANALYSIS OF NATURAL HONEYS

Sr.No.	Phytochemical components	H1	H2
1.	Saponins	+	++
2.	Tannin $FeCl_3$	+	+
3.	Tannin $K_2Cr_2O_7$	-	-
4.	Flavonoids	+	+
5.	Alkaloids	-	+
6.	Terpenes	+	+
7.	Quinines	+	+
8.	Anthocynines	+	+
9.	Phenolics	++	++
10.	Phytosterols	+/-	+/-
11.	Phlobatannins	-	-
12.	Hydroanthraquinones	-	-
13.	Cardiac glycosides	+/-	+/-
14.	Protein	++	++
15.	Benedicts test for reducing sugar	++	++
16.	Fehling test for reducing sugar	+	+
17.	Molisch's test for carbohydrates	+++	+++
18.	Barfoed's test for monosaccharides	+	+

Note: + low concentration, ++ moderate concentration, +++ high concentration, - not detected, +/- may be present or absent.

The presence or absence of these secondary metabolites is totally dependent upon the region, geographical changes and climatic changes [28]. This phytochemical screening shows that both the samples are rich in many bio active compounds. These bioactive compounds have a few therapeutic properties against many microbes [29] and also used for curing many diseases [30] the concentration of saponins, flavonoids, and tannins show the aromatic property of honey [31]. Saponins is a heterogeneous group i.e. triterpenoids and steroids which show those biological and pharmacological activities such as anti-inflammatory, anti-hepatotoxic, wound healing, vein tonic, hypoglycemic, antimicrobial, antiviral and also affect the immune systems against cancer and lower cholesterol levels. High saponin diet inhibits the dental caries platelet aggregation, hypercalciuria in humans and used in the food industry [32]. Terpenes are presented in both the samples show significant pharmacological activities, i.e. antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-tumor activities [33].

B. Quantitative analysis of different types of natural honey samples:

The result of quantitative phytochemical analysis of natural honey samples are described in [table no.2 and 3].

TABLE 2
QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF SAMPLE H1

Sr. No.	Parameters	Natural honey sample H1	
		Amount of Concentration in sample	Concentration (mg/ml)
1.	Flavanoid	0.032mg/0.1ml	0.32mg/ml
2.	Phenolic	0.079mg/0.05ml	1.58mg/ml
3.	Tannin	0.101mg/0.5ml	0.202mg/ml
4.	Carbohydrate	0.224mg/0.1ml	2.24mg/ml
5.	Protein	1.33mg/0.025ml	53.2mg/ml

TABLE 3
QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF SAMPLE H2

Sr. No.	Parameters	Natural honey sample H2	
		Amount of Concentration in sample	Concentration (mg/ml)
1.	Flavonoid	0.051mg/0.1ml	0.51mg/ml
2.	Phenolic	0.107mg/0.05 ml	2.14mg/ml
3.	Tannin	0.107mg/0.5ml	0.214mg/ml
4.	Carbohydrate	0.222mg/0.1ml	2.22mg/ml
5.	Protein	0.495mg/0.025 ml	19.8mg/ml

The result shows that the flavonoid content in H1 and H2 are 0.32mg/ml and 0.51mg/ml respectively. It has been documented that flavonoids are essential dietary nutraceutical elements [34]. High flavonoid content demonstrates antioxidant properties [11] thus, H2 shows more antioxidant properties than H1. Among plants, animals and bacteria, flavonoids play multiple biological activities [35] including anti-cholinesterase, anti- inflammatory [36], anti-fungul [37], anti-alergenic [38], antiviral [39], vasodilating actions [40], anti- mutagenic [41], anti-carcinogenic [42].

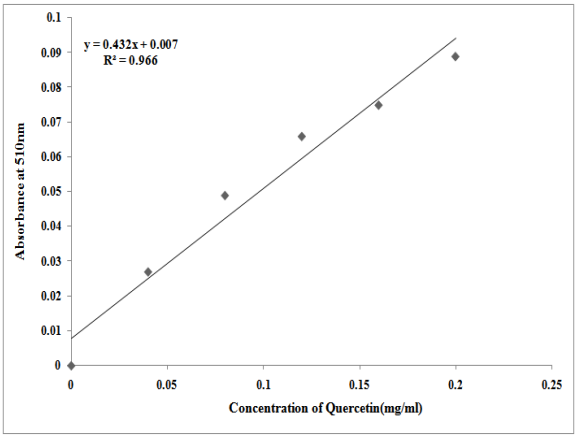

Fig. 1 Concentration of flavonoid in different natural honey samples

Phenolic compounds are among the plant metabolites's largest and most ubiquitous group [43]. The total phenolics content in H1 and H2 are 1.58mg/ml and 2.14mg/ml respectively. H2 shows higher phenolics content as compare to H1. Phenolic groups posses many biological activities such as antioxidant, antiageing, anticarcinogen [44], antiapoptosis [45], anti- inflammation, anti-atherosclerasis, inhibition of angiogenesis and cell proliferation activities, osteoporosis and neurodegenerative diseases [46,47], antimicrobial,anti-thrombotic [48], cardiovascular protection [49].

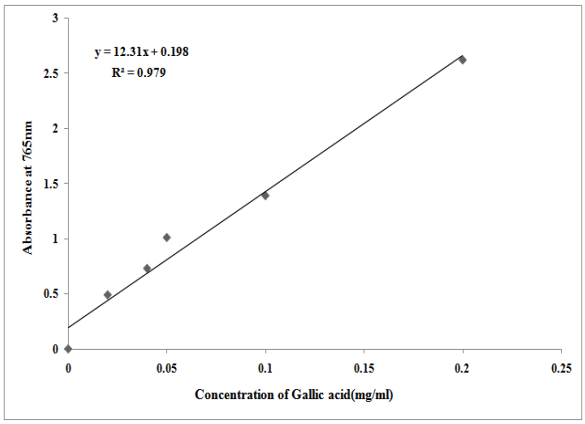

Fig. 2 Concentration of phenolics in different natural honey sample

The Tannin content was found high amount in H2 (0.214mg/ml) than H1 (0.202mg/ml). Tannin is responsible for decreasing the feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in animals, Hence, tannin-rich foods are known to have poor nutritional benefits [50] and also recorded in anti-oxidant [51], anti-microbial [52], anti-obesity and anti-diabetes, anti-inflammatory and inhibition of itching in atopic dermatitis activities [53].

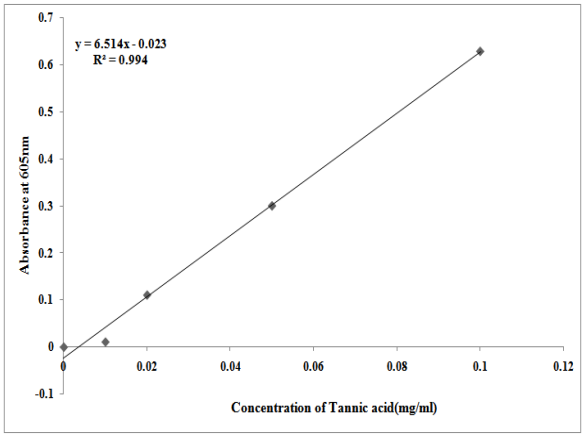

Fig. 3 Concentration of tannin in different natural honey samples

The carbohydrate content in H1 and H2 are 2.24mg/ml and 2.22mg/ml respectively. Carbohydrate is very important in innate immune system [54]. The carbohydrate present in honey is fructose and glucose and glucose is ideal energy source for human beings and fructose is more sweater than sucrose [55].

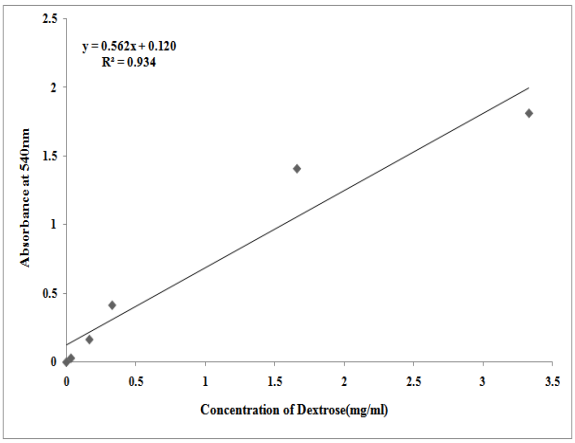

Fig. 4 Concentration of carbohydrate in different natural honey samples

The protein content in H1 and H2 are 53.2mg/ml and 19.8mg/ml respectively. Denaturation of proteins is lost quaternary, tertiary and secondary structure by applying external stress or through chemical compounds, though when most of the denatured proteins probably lose their biological function. Due to denaturation of protein caused artherities disease [56].

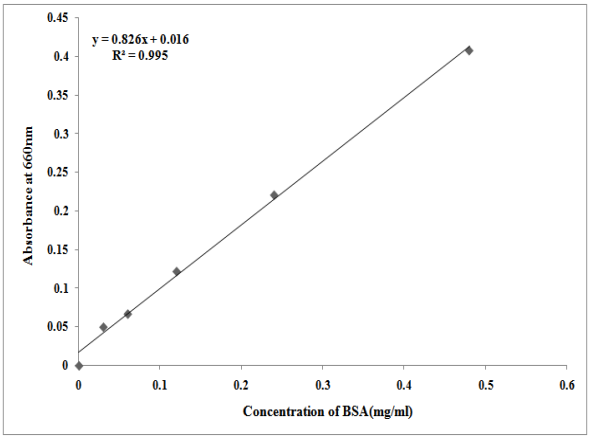

Fig. 5 Concentration of protein in different natural honey samples

C. Antioxidant activity in natural honey

TABLE 4
ANTIOXIDANT ACTIVITY IN NATURAL HONEYS

Sr.no.	Sample	Absorbance (517nm)	DPPH activity (%)
1.	B	1.010	-
2.	H1	0.599	40.69%
3.	H2	0.592	41.38%

The scavenging activity of natural honey samples are measured by DPPH assay with stable organic radical 1,1- diphenyl-2-picrylhydrazyl,measured as IC50: antioxidant amount required to decrease the initial DPPH concentration 50%. DPPH's unpaired electron produces a pair of hydrogen donated via honey through free radical scavenging antioxidant and hence turns the purple colour odd electron DPPH into yellow in its reduced form. The lower the IC50 value the higher the neutralizing capacity of honey, because it required less amount of free radical from the honey to reduce DPPH [57,58]. The antioxidant percentage of H_1 and H_2 are 40.69% and 41.38% respectively [table 4]. This study showed that, the H_1 have higher scavenging activity i.e, because it requires less amount of radical scavenger to reduce DPPH from honey however, the H_2 have lower potential for antioxidant activity.

IV. CONCLUSION

This studies showed that the qualitative and quantitative phytochemical analysis in different varieties of honey samples recorded the mixture of secondary metabolites such as protein, flavonoid, tannin, saponin, carbohydrate, etc. having many therapeutic properties, biological activities and also helpful in drug development. Our study considered, the DPPH assay express that the H2 has dominant antioxidant activity. The study also concluded that the honey sample can be considered as significant natural source of nutritional values.

REFERENCE

[1] K. Urmila, A. Sanjay, "Comparative pharmacognostical evaluation of honey of marketed products", International journal of pharmacy & life sciences, vol.1, iss.2, pp.109-112, 2010.

[2] National Honey Board, "Honey: Health and Therapeutic Qualities", Longman 28, 2003.

[3] M. Blasa, M. Candiracci, A. Accorsi, M. P. Piacentini, M. C. Albertini, E. Piatt, "Raw Millefiori honey is packed full of antioxidants", Food Chem, vol.97, pp.217-222, 2006.

[4] M. I. Khalil, M. Moniruzzaman, L. Boukraa, M. Benhanifia, et. al., "Physicochemical and Antioxidant properties of Algerian Honey, Molecules", vol.17, iss.9, pp.11199-11215, 2012.

[5] M. Moniruzzaman, M. I. Khalil, S. A. Sulaiman, S. H. Gan, "Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera", BMC Complementary and Alternative Medicine, vol.13, iss.43, pp.2-12,2013.

[6] N. Gheldof, N. J. Engeseth, "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", Journal of Agriculture and Food Chemistry, vol.50, iss.10, pp.3050-3055,2002.

[7] Council Directive of the European Union, "Council Directive 2001/110/EC of 20 December 2001 relating to honey", Official Journal of the European Communities, L10, pp.47-52, 2002.

[8] O. M. Atrouse, S. A. Oran, S. Y. Al- Abbadi, "Chemical analysis and identification of pollen grains from different Jordanian honey samples", International journal of food science and amp;technology, vol.39, iss.4, pp.413-417, 2004.

[9] V. Krishnasree, P. M. Ukkuru, "Nutrient and antioxidant profile of bee honey from Kerala", Indian Journal of Entomology, vol.80, iss.3, 2018.

[10] NHM (National Horticulture Mission), http://nhm.gov.in/area-pro/database-2011, pdf, 2011.

[11] A. Yelin, Kuntadi, "Phytochemical identification of honey from several regions in Java and Sumbawa", International

conference on biology and applied science, vol.2120, iss.1, pp.080024-1-080024-5, 2019.

[12] J. M. A. Suarez, S. Tulipani, S. Romandini, E. Bertoli, M. Battio, "Contribution of honey in nutrition and human health: A review, Mediterranean", Journal of nutrition and metabolism, vol.3, iss.1, pp.15-23, 2010.

[13] S. Asokan, Jayanthi, "Phytochemical analysis of various honey samples obtained from Theni district, South India", International journal of current research, vol.9, iss.1, pp. 45387-45390, 2017.

[14] J. B. Harbone, "Phytochemical methods: A guide to modern techniques of plant analysis, Chapman and hall ltd., New York, 3rd edition", pp.49-188, 1973.

[15] G. Jayapriya, F. G. Shoba, "Screening for phytochemical activity of Urechites lutea plant", Asian Journal of Plant Science and Research, vol.4, iss.6, pp.20-24, 2014.

[16] D. J. Bhuyan, A. Basu, "Phenolic compounds: Potential Health Benefits and toxicity", pp.27-59, 2017.

[17] B. John, V. R. K. Reddy, C. T. Sulaiman, "Total phenolics and Flavonoids in selected Justicia Species", Journal of Pharmacognosy and Phytochemistry, vol.2, iss.4, pp.51-52, 2013.

[18] J. Oliveira, A. Sousa, I. C. F. R. Ferreira, A. Bento, L. Estevinho, J. A. Pereira, "Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green Husks", Food and Chemical Toxicology, vol.46, iss.7, pp.2326-2331, 2008.

[19] O. R. Akinseye, A. E. Morayo, A. S. Olawumi, "Qualitative and quantitative evaluation of the phytochemicals in dry, wet and oil extracts of the leaf of Morinda lucida", Journal of biology, agriculture and healthcare, vol.7, iss.7, pp.22-25, 2017.

[20] M. Garriga , M. Almaraz , A. Marchiaro, "Determination of reducing sugars in extracts of Undaria pinnatifida (harvey) algae by UV-Visible spectrophotometry (DNS method)", Actas de ingenieria, vol. 3, pp.172-179, 2017.

[21] C. Thetsrimuang, S. Khammuang, K. Chiablaem, C. Srisomsap, R. Sarnthima, "Antioxidant properties and cytotoxicity of crude polysaccharides from Lentinus polychrous Lev.", Food chemistry, vol.128, iss.3, pp.634-639, 2011.

[22] https://study.com. Lowery protein Assay: Principle, Protocol and Mechanism. Copyright 2003-2020 study.com.

[23] O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, "Protein measurement with the folin phenol reagent", Journal of biological chemistry, vol.193, iss.1, pp.265-275, 1951.

[24] G. C. Bag, P. Grihanjali Devi, T. Bhaigyabati, "Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three hedychium species of Manipur Valley", International Journal of Pharmaceutical Science Review and Research, vol.30, iss.1, pp.154-159, 2015.

[25] R. Dong, Y. Zheng, B. Xu, "Phenolic profiles and antioxidant capacities of Chinese unifloral honeys from different botanical and geographical sources", Food Bioprocess Technology, vol.6, iss.3, pp.762-770, 2013.

[26] A. H. M. Solaiman, T. Nishizawa, N. Sultana, B. Sarker, et. al., "Antimicrobial and antioxidant activity analysis of some medicinal plants of Bangladesh", Advances in plants and agriculture research, vol 2, iss.4, pp.174-181, 2015.

[27] A. Rebiai, T. Lanez, A. Chouikh, "Physicochemical and biochemical properties of honey bee products in South Algeria", Chemistry and chemical engineering, biotechnology, food industry, vol.16, iss.2, pp.133-142, 2015.

[28] K. Turhan, "Chemical contents and some trace metals of honeys produced in the middle Anatolia region of Turkey", Fresenius environmental bulletin, vol.16, iss.5, pp.459-464, 2007.

[29] Y. Ewnetu, W. Lemma, N. Birhane, "Antibacterial effects of Apis mellifera and stingless bees honeys on susceptible and resistant strains of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae in Gondar, Northwes Ethiopia", BMC Complementary and Alternative Medicine, vol.13, pp.269, 2013.

[30] P. G. Pietta, J. N. Prod, "Flaonoids as antioxidants", Journal of natural products, vol.63, iss.7, pp.1035-1042, 2000.

[31] K. Rahman, A. Hussain, S. Ullah, I. U. M. Zai, "Phytochemical analysis and chemical composition of different branded an unbranded honey samples", International journal of microbiological research, vol.4, iss.2, pp.132-137, 2013.

[32] J. Shi, K. Arunasalam, D. Yeung, Y. Kakwda, G. Mittal, Y. Jiang, "Saponins from edible legume: chemistry, processing and health benefits", Journal of medicinal food, vol.7, iss.1, pp.67-78, 2004.

[33] S. B. Mahato, S. Sen, "Advances in triterpenoid research,1990-1994", Phytochemistry, vol.44, pp.1185-1236, 1997.

[34] L. P. Taylor, E. Grotewolds, "Flavonoids as developmental regulators", Current opinion in plant biology, vol.8, pp.317-323, 2005.

[35] A. Samanta, G. Das, S. K. Das, "Roles of flavonoids in plants", International journal of pharmaceutical science and technology, vol.6, pp.12-35, 2011.

[36] A. G. Lafuente, E. Guillamon, et. al., "Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease", Inflammation research, vol.58, iss.9, pp.537-552, 2009.

[37] M. Candiracci, B. Citterio, G. Diamantini, et. al., "Honey flavonoids, natural antifungul agents against Candida albicans", International Journal of Food Properties, vol.14, pp.799-808, 2011.

[38] M. Kawai, T. Hirano, S. Higa, et. al., "Flavonoids and related compounds as anti-allergic substances", Allergology international, vol.56, iss.2, pp. 113-123, 2007.

[39] K. Zandi, B. T. Teoh, et. al., "Novel antiviral activity of baicalein against dengue virus", BMC complementry and alternative medicine, vol.12, pp.214, 2012.

[40] A. N. Panche, A. D. Diwan, S. R. Chandra, "Flavonoids: an overview", Journal of nutritional science, vol.5, pp.1-15, 2016.

[41] P. W. Snijman, S. Swanevelder, E. Joubert, et. al., "The antimutagenic activity of the major flavonoids of rooibos (Aspalathus line-aris): some dose–response effects on mutagen activation–flavonoid interactions", Mutation research, vol.631, iss.2, pp.111–123, 2007.

[42] T. M. LeJeune, H. Y. Tsui, L. B. Parsons, et. al., "Mechanism of action of two flavone isomers targeting cancer cells with varying cell differentiation status", Public library of science ONE, vol.10, iss.11, e0142928, 2015.

[43] J. Dai, R. J. Mumper, "Plant phenolics: extraction, analysis and their antioxidant and anticancer properties", Multidisciplinary Digital Publishing Institute, vol.15, iss.10, pp.7313-7352, 2010.

[44] M. I. Khalil, S. A. Sulaiman, L. Boukraa, "Antioxidant properties of honey and its role in preventing health disorder", The open nutraceuticals journal, vol.3, pp.6-16, 2010.

[45] N. Orsolic, A. Knezevic, L. Sver, et. al., "Influence of honeybee products on transplantable murine tumors", Veterinary and comparative oncology, vol.1, iss.4, pp.216-226, 2003.

[46] X. Han, T. Shen, H. Lou, "Dietary polyphenols and their biological significance", International journal of molecular sciences, vol.8, iss.9, pp.950-988, 2007.

[47] S. Sreeja, R. Raphael K. , "Qualitative and quantitative phytochemical analysis and DPPH radical scavenging activity of different extracts of flower of Bauhinia acuminata linn", International journal of current pharmaceutical research, vol.9, iss.6, pp.23-26, 2017.

[48] S. Anand, M. Deighton, G. Livanos, et. al., "Antimicrobial activity of Agastache honey and characterization of its bioactive compounds in comparision with important commercial honeys", Frontiers in microbiology, vol.10, art.263, 2019.

[49] M. I. Khalil, S. A. Sulaiman, "The potential role of honey and its polyphenols in preventing heart disease:a review, African journal of traditional", complementary and alternative medicines, vol.7, iss.4, pp.315-321, 2010.

[50] K. T. Chung, T. Y. Wong, C. I. Wei, W. Y. Huang, Y. Lin, "Tannins and human health:a review", Critical reviews in food science and nutrition, vol.38, iss.6, pp.421-464, 1998.

[51] R. Padma, N. G. Parvathy, V. Renjith, K. P. Rahate, "Quantitative estimation of tannins, phenols and antioxidant activity of methanolic extract of Imperta cylindrical", International journal of research in pharmaceutical sciences, vol.4, iss.1, pp.73-77, 2013.

[52] H. G. Shete, M. P. Chitanand, "Bioconversion of naturally occurring tannins into a value added pharmaceutical intermediate gallic acid a new approach", International journal of current microbiology and applied sciences, vol.4, iss.11, pp.597-604, 2015.

[53] S. Ogawa, Y. Yazaki, "Tannins from Acacia mearnsii de wild bark: tannin determination and biological activities", Molecules, vol.23, pp.837, 2018.

[54] T. Wang, X. C. Hu, Z. P. Cai, J. Voglmeir, L. Liu, "Qualitative and quantitative analysis of carbohydrate modification on glycoproteins from seeds of Ginkgo biloba", Journal of agricultural and food chemistry, vol.65, iss.35, pp.7669-7679, 2017.

[55] M. A. Kamal, P. Klein, "Determination of sugars in honey by liquid chromatography, Saudi", Journal of biological sciences, vol.18, pp.17-21, 2010.

[56] G. R. Angel, B. Vimala, B. Nambisa, "Antioxidant and anti-inflammatory activities of protein isolated from eight Curcuma species", Phytopharmacology, vol.4, iss.1, pp.9-105, 2013.

[57] J. A. Pontis, L. A. M. A. D. Costa, S. J. R. D. Silva, A. Flach, "Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil", Food Science and Technology, vol.34, iss.1, pp.69-73, 2014.

[58] G. Beretta, P. Granata, M. Ferrero, M. Orioli, R. M. Facino, "Standardization of antioxidant properties of honey by a combination of spectrophotometric/ fluorimetric assays and chemometrics", Analytica Chimica Acta, vol.533, pp.185–191, 2005.