Antioxidant activity of Natural Comb and Local Market Honey of Khyber Pakhtunkhwa Pakistan

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Abstract

Free radicals scavenging activity of Natural comb and local market honey of methanolic extract were assess against DPPH free radicals by using honey samples of various concentration 100-600µg/ml. Spectrophotometeric method was used for the determination of antioxidant activity. In Natural comb honey, at the concentration of 600µg/ml (NCH1) Sample show high scavenging activity (84.77±1.12), while at 100µg/ml concentration sample (NCH4) show lowest activity (10.35±1.34).In Local Market honey, at the concentration of 600µg/ml (LMH4) Sample show high scavenging activity (82.23±1.33), while at 100µg/ml concentration sample (LMH4) show lowest activity (10.35±1.34). The percent scavenging activity increases due to increases in compound concentration. Antioxidant activity was observed at all evaluated honey samples. However the natural comb honey has better activity as compare to local market honey. Thus purposely honey might be used as substitute natural antioxidant in various formulations for pharmaceutical industries and food.

Key Words: Antioxidant activity, Spectrophotometer, Honey.

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1. Introductions

Honey is natural, sweet viscous nectar of the flower produce by *Apismellifera*. It has a complex mixture mostly composed on carbohydrates, fructose, maltose, sucrose and glucose [1-2]. Honey also contains thiamin, vitamins, riboflavin, ascorbic acid and pyridoxine [3]. Honey used as effective medicine from prehistoric time at different ancient civilization [4].

The physiological character of honey has been accredited to formation of hydrogen peroxide produced by the variety of enzymes, low pH value, glucose oxidase, osmotic action and antioxidant contents [5]. Antimicrobial and anti-inflammatory property is the intrinsic feature of honey due to which it is used for a long time without becoming spoilage [6, 7]. The reduction of wounds inflammation of honey is due to the presence of reactive oxygen species [8, 9]. In numeral studies it is firmly mentioned that honey is effective medicine for wounds and burns [10].

Among other sweetener, consumption of honey controlled blood sugar level and insulin sensitivity; provide proper fueling to liver for glucose metabolism during sleep. It's considered cough suppressant for children more effective then dextromethorphan. Honey improve immunity, although artificial honey and sugar have very small or negative beneficial effects, natural honey are effective in reduction of homocysteine 6%, triglycerides 2%, blood sugar 6% and total cholesterol l7% [11-12]. In various molecules the oxidative damage caused through free radicals such as protein, lipids and nucleic acids. Antioxidants have free radical chain reaction infringement property. Which considerably prevent oxidation of oxidizible substrate even present in small quantity together with each type of molecule originate in vivo [13]. Honey also contains a number of pollen, and wax which contain high amount of anti-oxidant [14]. It mainly consist of polyphenoles and phenolic acids i.e. caffeic acids, ellagic acid, vanillic acids, benzoic acid , protocateqic acid, chlorogenic acid, cinnimic acid and hydrobenzoic acid [15], though in honey the major flavonoids contents are chrysin, lutelolin, kaempferol, galangin, pinocembrin and naringenin [16]. The flavonoids provide color, flavor to honey and have stronger anti-fungal and antibacterial activity [17]. During high intellectual, physical and emotional stress, honey act is antidepressant due to antioxidants. Various antioxidant (polyphenols) are reported in honey have develop as capable pharmacological agents in cancer curing [18]. Keeping in observation the consequence of honey the current study was designed to evaluate natural comb and local market honey samples for its antioxidant activity collected from Dir lower KPK Pakistan.

2. Materials and methods

2.1. Collection of samples

Eight types of honey samples were chosen from Dir Lower region Khyber Pakhtunkhwa Pakistan, four were purchased from local market and four types were collect directly from honey comb. The collected samples were brought to (PCSIR Labs complex Peshawar) keep in refrigerator in sealed container till analysis.

2.2. Chemical and reagents

1, 1- diphenyl-2-picryl hydroxyl (DPPH) (Sigma Aldrich Germany), Methanol (Scharlau Spain) analytical grade chemicals and reagents were used.

2.3. Antioxidant activity

The free radical scavenging activity of methanolic extract was determined against DPPH on reported method [19-20]. Each extract of 0.01g from stock solution were dissolved in 1mL ethanol and further make five different dilutions (100-600µg/mL). Same dilution was making for ascorbic acid as standard. Mixed meticulously 1mL of each dilution with freshly prepared solution of DPPH, and then keep it for 10 minutes in dark at room temperature. For the determination of antioxidant activity by scavenging of free radical of DPPH of each dilution, determined UV absorbance at 517nm wavelength. Then compared the scavenging competence of sample with control (2ml DPPH+1ml methanol)

The percent inhibition expression of free radical scavenging activity for each sample, using the particular equation

DPPH activity percent inhibition= {(AB-AS)/AB)} ×100%

The representation of blank sample absorbance is; AB and absorbance of test sample is; AS. For the calculation of EC50 value, curve was plotted among percent inhibition against sample concentration where the scavenging reaches to 50% [21-22].

3. Results and discussion

Methanol extract of local market honey and natural comb honey samples was examine for their DPPH radical scavenging activity of various dilution of honey samples (100, 200, 300, 500 & 600µg/ml). Percent activity of methanolic extract with vitamin C control is represented (Table 1 and 2). These scavenging behavior were comparative to the extract concentration. The percent scavenging activity raise with enhance in concentration of compound and when scavenging attain to 50 percent it's

the EC_{50} value. However the percent scavenging is inversely allied to EC_{50}. The sample having higher antioxidant activity illustrates lower EC50 value [23]. (Table 3) represents the EC_{50} value for evaluated honey samples. It this scrutiny it was observed that free radical scavenging increase with honey concentration.

DPPH produce stable free radical which dissolves in methanol, presently used for the determination of radical scavenging activity of different compounds. It has particular purple color and a characteristic 517nm absorption wavelength. When antioxidants provide protons to this radical, the purple color of DPPH test solution turn into light yellow ensuing in a decline in absorbance. The decline in absorbance is taken as a measure of the amount of radical scavenging [24-26].

Natural comb honey show admirable DPPH radical scavenging activity as weigh against to local market honey samples. In Natural comb honey, at the concentration of 600µg/ml (NCH1) Sample show high scavenging activity (84.77±1.12) among four honey samples, while the lower activity (10.35±1.34) was observed at concentration (100µg/ml) in sample NCH4 (Table 1). In Local market honey, sample LMH4 illustrate high antioxidant activity (82.23±1.33) at concentration (600 µg/ml) along with all honey samples, where the lowly activity (19.23±1.20) was examine at concentration of (100 µg/ml) in sample LMH1.

Table 1: Natural comb honey scavenging activity against DPPH freee radicals

Concentration (µg /ml)	NCH1	NCH2	NCH3	NCH4	Control (Vitamin C)
100	14.11±1.14	17.44±1.13	11.22±1.11	10.35±1.34	40.33±1.14
200	30.26±1.15	34.23±1.13	29.35±1.12	26.14±1.15	64.66±2.00
300	47.22±1.16	44.11±1.13	41.55±1.12	42.13±1.33	67.22±1.77
400	58.33±1.23	61.33±1.26	56.11±1.21	55.16±1.41	80.22±1.30
500	73.12±1.17	75.11±1.22	72.33±1.32	71.42±1.26	84.14±1.55
600	84.77±1.12	83.22±1.25	82.13±1.11	83.13±1.16	86.11±1.63

NCH: Natural comb honey, Mean ± S.D (n=3)

Table 2: Local market honey scavenging activity against DPPH free radicals

Concentration (µg / ml)	LMH1	LMH2	LMH3	LMH4	Control (Vit C)
100	19.23±1.20	24.22±1.23	23.33±1.11	25.18±1.13	40.33±1.14
200	27.22±1.18	37.18±1.17	35.36±1.14	40.44±1.23	64.66±2.00
300	36.22±1.12	50.44±1.33	42.25±1.47	53.34±1.18	67.22±1.77
400	42.56±1.17	63.11±1.19	57.23±1.33	65.45±1.46	80.22±1.30
500	50.37±1.29	74.22±1.36	62.35±1.30	77.24±1.32	84.14±1.55
600	61.11±1.35	79.33±1.55	80.22±1.42	82.23±1.33	86.11±1.63

LMH : Local Market Honey,Mean ± S.D (n=3)

Table 3: DPPH radical scavenging activity (EC_{50}) for Natural Comb Honey

Sample	NCH1	NCH2	NCH3	NCH4	Control (Vit C)
Natural Comb Honey	98	79	99	21	160

Table 4: DPPH radical scavenging activity (EC_{50}) for Local Market Honey

Sample	LMH1	LMH2	LMH3	LMH4	Control (Vit C)
Local Market Honey	120	98	126	144	160

From the above data it is exemplify that increase the concentration of honey samples decrease the DPPH initial absorbance. It was renowned that phenolic contents including isoflavonides, phenolic acids, flavonols, catechins and flavons were also present in honey [13].

In natural comb honey samples the high EC50 values (99) was calculated for NCH3 sample. Moderate EC50 values (79) and (98) were calculated for NCH2 and NCH1 sample correspondingly, whereas NCH4 sample show the lower value (21) represented (Table 3). In Local Market honey; the high EC50 values (144) was calculated for LMH4 sample. Moderate EC50 values (120) and (126) were calculated for LMH1 and LMH3 samples respectively; whilst LMH2 sample show the lower value (98) represented (Table 4). A lower EC50 value gave indication of higher antioxidant activity. Natural comb honey EC50 value was lower than samples of local market honey.

4. Conclusion

All the selected honey samples assess showed antioxidant activity. Natural comb honey presented superior antioxidant activity as compared to local market honey. Therefore expressly honey may compose of appropriate source and could be used as option natural antioxidant in variety of

formulations for the preparation of food and pharmaceutical products, which is specially confirmed by the present work.

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6. References

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