2321-8045 Science Park Research Journal Vol-2, Issue-8, 4th Sept 2014
Impact Factor : 1.6200[UIF-2013]

Original Article

Comparative Study On The Phytochemical Constituents And Invitro Antioxidant Activity Of Four Selected Honey From Different Origin

Aruna.G [1], Anjum.A[1] and C.M. Noorjahan [2]

ABSTRACT

Honey is well known as a natural dietary antioxidant. The components responsible for the redox properties of honey are likely to be phenolic acids, flavonoids, vitamins, and enzymes, as well as a small amount of mineral/other organic content. The redox properties might also be attributed to the combined activity of these minor components through synergistic effects. Numerous studies have reported that most chronic diseases such as diabetes, cancer, coronary, and neurological degeneration are a consequence of oxidative damage. It is also proven that the therapeutic potential of honey is always associated with antioxidant capacity against reactive oxygen species. Therefore, in recent years, studies have been focused on the composition of honeys and their biological properties. The present study was carried out to assess the phytochemical constituents and invitro antioxidant activity of four selected honey using a combination of test including total phenolic content, flavonoids, total antioxidants, free radical scavenging activity and anti lipid peroxidation. Correlation between all the analysed parameters were evaluated. The highest antioxidant contents and the lowest IC50 value were recorded in Manuka followed by Indian honey. A linear positive relationship existed between antioxidant activity and total phenolics/flavonoids. The results showed that all four honeys possess antioxidant activity in appreciable amount with Manuka honey showing the highest followed by Indian honey.

Keywords:
Honey, Total antioxidants, DPPH assay, Antioxidant activity, Lipid peroxidation

Aruna.G [1], Anjum.A[1] and C.M. Noorjahan [2]

From
[1]Asst. Professor, Dept of Biochemistry, J.B.A.S College for Women (Autonomous), Teynampet, Chennai
[2]Asst. Professor, PG and Research Department of Zoology, J.B.A.S College for Women (Autonomous), Teynampet, Chennai.

The Article Is Published On September 2014 Issue & Available At
www.scienceparks.in

DOI:10.9780/23218045/1202013/49

Introduction

Honey a natural sweet product produced by Apis mellifera from nector of plants is claimed to cure a multitude of ailments due to its different medicinal properties like anti-microbial, anti-inflammatory, antioxidant, anti-mutagenic, anti-tumor and anti-diabetic effects(1,2). Since the underlying disturbance in cellular function in the above mentioned are due to the oxidative stress and that the beneficial role of honey is attributed to the presence of antioxidants, it is claimed that honey is effective in preventing the detoriative oxidation reactions. Honey is reported to contain about 200 substances both enzymatic such as catalase, glucose oxidase, peroxidase and non-enzymatic substances such as ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, organic acids, Maillard reaction products and more than 150 polyphenolic compounds containing flavonoids, phenolic acids, catechins, cinnamic acid derivatives etc. (3,4).

Several studies have been carried out to prove the speculation that these substantial antioxidant compounds present in honey may strengthen the organ's defences and consequently prevent oxidative stress (5). In recent years there has been an increasing interest in determination of antioxidant activity of honey. Many studies have indicated that antioxidant activity of honey varies widely depending on the floral source. The botanical origin of

honey has the greatest influence on its antioxidant activity whole processing, handling and storage affect honey antioxidant activity only to a minor degree (6,7). The present study was carried out to assess the in-vitro antioxidant activity of 4 selected honey from different botanical and geographical origin. The selected samples are Manuka honey from New Zealand, Eucalyptus honey from Australia, Indian honey (multifloral) from Kolli Malai hills and commercial honey. The antioxidant effects were assessed by combination of tests and the correlation between the analysed parameters are evaluated.

Materials And Methods

Honey samples: Four selected honey samples were procured from different regions for the present investigation. and transferred to the laboratory. Manuka honey was used as a standard for comparison as it is extensively studied. All the samples were stored in airtight plastic containers at 4°C until further analysis.

Preliminary Phytochemical Screening

All the four samples were screened for presence or absence of various phytochemicals like tannins, saponins, flavonoids, alkaloids, proteins, steroids, quinones, terpenoids, and cardioglycosides using standard methods of Harbone (1973) (8).

Total phenolic content

The total phenolic acid content was determined by modified Singleton and Rossi (1965) method (9). Briefly 5.0g of honey was treated with 50ml of distilled water, mixed and filtered through a qualitative filter paper. 500µl of this solution was mixed with 2.5ml Folin-Ciocalteau reagent (0.2N) for 5 mins and then 2ml of Na2co3 solution (75g/l) was added. The samples were incubated at room temperature in the dark for 2 hours and the absorbance of the samples measured at 760nm. For blank solution, methanol was used in place of honey and for the calibration curve, a stock solution of Gallic acid (1mg/ml) was prepared for further dilution. The linearity obtained was $Y=0.004X +0.0461$, $R^2=0.9985$. Results were expressed as mg Gallic acid equivalent (GAE) per 100g of sample.

Flavonoid determination

The amount of flavonoids present was determined using modified Meda et al.,2005 (10). 1ml of honey solution (1mg/ml) was mixed with 0.5ml of Aluminium chloride (1.2%) and 0.5ml of Potassium acetate (120mM). The samples were mixed well and incubated for 30 minutes. The absorbance was read at 510nm. Quercetin was used to calculate the standard curve. A linearity of $Y=0.0031X +0.037$, $R^2 = 0.999$ was obtained and results were expressed as mg Quercetin equivalent (QE) per 100g of sample.

Total antioxidant assay

The total antioxidants were determined by Prieto et al., 1999; method (11) (phosphomolybdenum method). To 100µl of honey sample (1mg/ml), 1ml of reagent solution (0.6m H2SO4; 4mM ammonium molybdate, 28mM sodium phosphate) was added and incubated at 95°C for 90 minutes. Absorbance of the green phosphomolybdate complex was measured at 695nm. Ascorbic acid was used to calculate the standard curve. The linearity obtained was $Y=0.005X - 0.028$, $R^2 = 0.988$ and results were expressed as mg ascorbic acid equivalent (AAE) per gram of sample.

Estimation of antioxidant activity

DPPH assay

The free radical scavenging activity is measured according to the method of Blois., 1958 (12). 1ml of varying concentration (1-5mg) of honey solutions was mixed with 1ml of 0.004% methanol solution of DPPH. The mixture was vigorously and allowed to stand for 30 minutes at room temperature in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 517nm. The procedure was repeated for the blank and control. The radical scavenging activity was calculated using the equation:

DPPH scavenging effect (%)=(A blank - A sample)/Ablank X 100

Extract concentration providing IC_{50} was calculated from the graph plotting inhibition percentage against extract concentration. Ascorbic acid was used as positive control.

Lipid peroxidation assay

Lipid peroxidation inhibition assay was performed by the method of Okhawa et al., 1979 (13). The quantification of the end products of lipid peroxidation, specifically malonaldehyde (MDA), is the most commonly used test to evaluate lipid peroxidation. Lipids were preincubated with honey samples, lipid peroxidation was induced with FeSo4 and Potassium dihydrogen phosphate. The volume was made upto 3ml then, tricholoroacetic acid and 2-thiobarbituric acid was added. The reaction mixture was boiled for 30 mins and centrifuged at 3500rpm for 10 mins. The absorbance was measured at 534 nm using UV Spectrophotometer. MDA was used as standard. The extent of lipid peroxidation was expressed as nm MDA/gm.

Statistical Analysis of Data

The experimental data were reported as mean ± S.D of three parallel measurements. Linear regression analysis was performed quoting the correlation coefficient. The measure of strength and direction of the linear relationship between variables (Total phenolic content, flavonoids, total antioxidants and antioxidant activity) was calculated and interpreted using Pearson's correlation coefficient (r).

Results

Preliminary phytochemical screening revealed the presence of various components. Flavonoids, Alkaloids, Proteins and Terpenoids were the most prominent ones present in all the samples. Saponins, Quinones, Steroids and Cardio glycosides were reported in some of the samples. (Table1)

Table 1. Phytochemical screening

S.No	Contents	Name of the test	Manuka honey	Eucalyptus honey	Indian honey	Commercial honey
1.	Tannins	Ferric Chloride test	-	-	-	-
2.	Saponin	Foam test	+	-	-	-
3.	Flavonoids	Shinoda test	++	+	+	-
4.	Alkaloids	Wagners test	+++	+	++	+
5.	Proteins	Biuret test	+	+	++	+
6.	Steroid	Libermann Burchard test	+	-	-	-
7.	Quinones	Borutrager's test	+	-	-	++
8.	Terpenoid	Salkowitch test	++	+	+	+
9.	Cardio glycosides	3,5 Dinitro benzoic acid test	-	+	-	-

The total phenol content of the different honeys was investigated using modified Folin Ciocalteau method. The results of the polyphenolic content for the four types of honey are expressed as GAE/100g of sample. This reveals that the polyphenolic content was higher in Manuka (86.47±3.25), closely followed by the Indian honey (73.03±1.19) and then Commercial honey (56.7±1.50). The Eucalyptus honey was found to have the least content of polyphenols (41.6±2.67).

The Flavonoid contents are expressed as Quercitin equivalents per 100g of the sample. Table 2 showed that the flavonoid content ranged from 22.65±1.46 to 51.70±1.37 with

Manuka honey having highest and Commercial honey having the least.

The total antioxidant capacity of the samples was tested and expressed as mg/g of Ascorbic acid Equivalent. Among the the four samples the maximum antioxidant capacity was shown by Manuka honey which was equivalent to 148.7 ± 1.92 followed by Indian honey with the mean value of 133.2±2.13 and Eucalyptus honey with a mean value of 125.5±1.32. The commercial honey was found to have least amount of total antioxidants with a mean value of 92.6±1.67. (Table 2)

Table 2. Levels of Phenolic content, Flavonoids and Total antioxidants in selected honey

Samples	Total phenolic content mgs GAE/100g	Flavonoids mgs QE/100g	Total antioxidants mg/g AAE
Manuka honey	86.47±3.25	51.70±1.37	148.7±1.92
Eucalyptus honey	41.6±2.67	26.04±1.04	125.5±1.32
Indian honey	73.03±1.19	48.5±1.35	133.2±2.13
Commercial honey	56.7±1.50	22.65±1.46	92.6±1.67

The values are expressed as Mean of triplicate measurements ± Standard deviation

Table 3. Percentage inhibition of DPPH radical by selected honey samples

Samples	1mg	2mg	3mg	4mg	5mg
Ascorbic acid	84.36±2.51	91.8±1.9	94.1±1.9	96.3±1.6	99.6±0.57
Manuka honey	49.6±0.57	62.2±1.9	78.2±1.1	84.8±1.2	91.5±2.2
Eucalyptus honey	41.9±2.7	56.2±1.1	70.7±2.1	71.6±1.5	74.9±2.5
Indian honey	45.4±1.5	57.7±2.5	64.9±2.9	77.3±2.1	83.2±2.2
Commercial honey	41.6±3.1	53.5±2.8	62.4±2.6	69.9±2.0	72.2±2.1

The values are expressed as Mean of triplicate measurements ± Standard deviation and Ascorbic acid is used as the positive control.

The free radical scavenging assay is based on the reduction of 1,1 diphenyl-2-picrylhydrazyl (DPPH). The ability of the samples and the standard ascorbic acid to scavenge the free radical at different concentrations (1mg -5mg) and pair off the odd electron was shown in this assay. It was observed that all the samples showed increase in percentage of inhibition as the concentration increases. However, the highest percentage of inhibition was recorded with Manuka honey (91.5%), followed by Indian honey (83.2%), Eucalyptus (74.9%), and Commercial honey (72.2%), when compared with ascorbic acid with 99.6 % at a concentration of 5mg/ml. The IC_{50} values were 1mg/ml for Manuka honey and 2mg/ml for Eucalyptus, Indian & Commercial honey. (Table 3)

Table 4. Lipid peroxidation assay

Samples	nm MDA/gm
Manuka honey	171 ± 1.25
Eucalyptus honey	353 ± 1.12
Indian honey	353 ± 0.98
Commercial honey	716 ± 1.8

The values are expressed as Mean of triplicate measurements ± Standard deviation

Lipid peroxidation assay is used for putative antioxidants which diffuse freely in lipids and all honey samples had strong antioxidant efficiency to inhibit lipid peroxidation. The reduction in peroxidation is represented by the amount of MDA. The amount of MDA formed in Indian and Eucalyptus honey were high when compared to Manuka and Commercial honey was observed to possess lowest activity. (Table 4)

Discussion

Phytochemical analysis is of paramount importance in identifying the source of therapeutically and industrially valuable compounds. Out of the four honey samples Manuka and Indian honey showed maximum number & amount of plant constituents such as saponins, flavonoids, alkaloids, proteins, steroids, quinones and terpenoids. These phytochemicals has been found to possess a wide range of medicinal properties, example alkaloids protect against chronic diseases, saponins protect against hypercholesterolemia, steroids show analgesic properties etc. (14). The findings are in agreement with that of Erejuwa et al (15).

Polyphenols are the major compounds commonly found in natural sources and they have been reported to have multiple biological effects, including antioxidant activity. Their antioxidant activity is mainly due to their redox properties, ability to act as hydrogen donors and serve as singlet oxygen quenchers. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are predominant compound found in honey that are claimed to have antioxidant activity. Several studies have shown that it can protect lipids from oxidation (16). Apart from these compounds honey is also said to possess other antioxidants like ascorbic acid, carotenoids, tocopherol, organic acids and their derivatives. Along with phenolic compounds these nonphenolic reducing compounds can also be assessed by making them react with the mixture of phosphor tungstic acid and phosphor molybdic acid (17). From the results obtained in the present study it has been observed that a significant amount of antioxidants are found in Indian honey than the other varieties, when compared with that of the standard manuka honey. The present findings were almost agreeable with Singh and Bath, 1997 (18).

In living organisms free radicals are constantly generated from numerous physiological and biochemical processes (19). These radicals are responsible for damage to cellular biomolecules leading to many disease conditions (20). Though all living organisms possess protective antioxidant systems against the free radicals causing oxidative damage, sometimes it is not adequate to prevent all the possible damage. Thus, the use of antioxidants to provide enhanced and greater protection against oxidative damage is increasingly practised. The results demonstrated that all four varieties of honey exhibited considerable amount of free radical inhibition effect with the IC 50 values of 1mg for manuka and 2mg for other honey samples. A strong positive correlation was observed between the TPC, Flavanoid (r = 0.923), Total antioxidants (r = 0.83) and their free radical scavenging ability. Though the IC 50 values were same for Indian, Eucalyptus and Commercial honey the percentage of inhibition was significantly higher for Indian honey than the other two varieties and the percentage of inhibition was also close to that of manuka honey. The results obtained are agreeable with Saxena et al 2010 (21).

Lipid peroxidation is a natural & common mechanistic pathway which if uncontrolled may lead to precipitation of chronic diseases (22). In the present study lipid peroxidation was measured as nmol/g of MDA which is an important secondary metabolite formed due to oxidation of lipids. Decrease in the MDA value indicates lower lipid peroxidation and higher membrane stability. The results obtained in the present study was in close agreement with Manuela Blasa et al 2007 (23).

Conclusion

On the basis of the results obtained in the present study, Indian honey has been shown to possess the antioxidant markers and antioxidant test values closest to that of Manuka honey when compared to other honey varieties. This indicates that the Indian (natural mountain) honey has significant antioxidant activity. Therefore their supplementation in diet might be helpful in combating the progression of various diseases with oxidative stress.

Recommendations

Further research to establish the molecular events underlying to combat oxidative stress is recommended.

Acknowledgement:

We acknowledge the help of Ms. Summera Rafiq, Head of the Department of Microbiology JBAS College for Women for helping us get Manuka honey and eucalyptus honey from Australia and SK. Jasmine Shahina, Assistant Professor for her help in completing the paper.

References

1.Allsop, K.F. and Miller J.B. 1996. Honey revisited: a reappraisal of honey in pre–industrial diets. Br J Nutr., 75(4): 513–520.
2.Othman, N.H. 2012. Honey and Cancer: Sustainable Inverse Relationship Particularly for Developing Nations: A Review. Evid Based Complement Alternat Med., 12(2): 10.
3.Erejuwa, O.O., Sulaiman, S.A. and Wahab, M.S. 2012. Honey: A Novel Antioxidant. Molecules., 17(4): 4400–4423.
4.Al-Mamary, M., Al-Meeri, A. and Al-Habori, M. 2002. Antioxidant activities and total phenolics of different types of honey. Nutr Res., 22(9): 1041–1047.
5.Gheldof, N. and Engeseth, N.J., 2002. Antioxidant activities of honey from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of invitro lipoprotein oxidation in human serum samples. J. Agric. Food Chem., 50: 3050-3055.
6.Aljadi, A.M. and Kamaruddin, M.Y., 2004. Evaluation of the phenolic contents and the antioxidant capacities of two Malaysian floral honeys. Food Chem., 85: 513-518.
7.Allsop, K.F. and Miller J.B. 1996. Honey revisited: a reappraisal of honey in pre–industrial diets. Br J Nutr., 75(4): 513–520.
8.Harborne, J.B. 1973. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd.London. Pp.49 - 188.
9.Singleton, V.L and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. Am J Enol. Viti Cult, 16: 144-158.
10.Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571–577.
11.Prieto, P., Pined, M and Aguilav, M. 1999. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the Vitamin E. Anal. Biochem. 269: 337-341.
12. Blios, M.S. 1958. Antioxidant determinations by use of stable free radical. Nature. 181,1199-1200.
13.Okhawa, H., Hoyishi, N and Yagi, K. 1979. Assay of lipid peroxides in animal tissues by Thiobarbituric acid reaction. Anal Biochemistry. 95 (2) : 351 – 358.
14.Kumar, A., Illavarasan, P., Jayachandran, T., Decaraman, M., Aravindan, P., Padmanapan, N and Krishnan, M.R.V. 2009. Phytochemical investigation on tropical plant Syzygium cumini from Kottapalayam. Pak J Nutr. 8: 83 – 85.
15.Erejuwa, O.O., Gurtu, S., Sulaiman, S.A. Ab Wahab, M.S, Sirajudeen, K.N, Salleha, M.S. 2010. Hypoglycemic and antioxidant effect of honey in STZ induced diabetic rats. Int. J. VitamNutr. Res. 80: 74 – 82.
16.Kücük, M., Kolayh, S. and Karaoglu, S. 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem., 100: 526–534.
17.George, S., Brat, P., Alter, P and Amiot, M. 2005. Rapid determination of plant phenols and Vitamin C in plant derived products. Journal of Agricultural and Food Chemistry. 53: 1370-1373.
18. Singh, N and Bath, P.K. 1997. Quality evaluation of different types of Indian honey. Food Chem.58: 129-133.
19. Jain, P.K and Agarwal, R.K. 2008. Antioxidant and free radical scavenging properties of developed mono and polyherbal formulations. Asian Journal of Experimental Science., 22(3): 213-220.
20. Valko, M., Rhodes C.J., Monocol J., Izakovic M and Mazur M. 2006. Free radicals, metals

and antioxidants in oxidative stress induced cancer. Chemico Biological interactions. 160: 1-40.
21. Saxena, S., Gautam, S and Sharma, A. 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118: 391-397.
22.Takeshi Nagaia , Mizuho Sakaia, Reiji Inouec, Hachiro Inouec and Nobutaka Suzukia. 2001. Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chemistry 75(2) : 237–240
23.Manuela Blasa, Manila Candiracci, Augusto Accorsi, Maria Piera Piacentini and Elena Piatti. 2007. Honey flavonoids as protection agents against oxidative damage to human red blood cells. Food Chemistry. 104 (4) : 1635–1640.

Aruna.G
Asst. Professor, Dept of Biochemistry, J.B.A.S College for Women (Autonomous), Teynampet, Chennai