Regular Article

Journal of Medicinal Herbs and Ethnomedicine 2015, 1(1): 38-44
http://scienceflora.org/journal/jmhe/
doi: 10.5455/jmhe.2015-07-013

Phytochemical screening and antioxidant activity of different bee honeys

V. Krishnasree*, P. Mary Ukkuru

Department of Home Science, College of Agriculture, Kerala Agricultural University, Vellayani, Trivandrum, Kerala, India

Received: 15.07.2015
Accepted: 27.07.2015
Published: 28.07.2016

***Address for Correspondence:**
V. Krishnasree, Department of Home Science, College of Agriculture, Kerala Agricultural University, Vellayani - 650 522, Trivandrum, Kerala, India.
E-mail: krishnasree.arun@gmail.com

ABSTRACT

The present study aimed at elucidating the protective role of honeys produced by the five bee species that prevail in Kerala namely, *Apis cerana indica* F., *Apis mellifera* L., *Apis dorsata* F., *Apis florea* F. and *Trigona iridipennis* S. in their raw and processed form against oxidative stress and free radicals. The major phytochemicals screened were polyphenols, flavonoids, and flavonols. The antioxidant potency of the honeys was analyzed using *in vitro* antioxidant assays namely, 2, 2 diphenyl-1-picrylhydrazyl assay and nitric oxide scavenging assay. The results indicated that the honeys in pure form were having a higher rate of antioxidant activity compared to processed honeys as processing reduce the thermolabile phytochemicals.

KEY WORDS: Antioxidant activity, honey, phytochemicals, 2, 2 diphenyl-1-picrylhydrazyl assay, nitric oxide scavenging assay

INTRODUCTION

Honey has a valued place in traditional medicine for centuries. However, it has a limited use in modern medicine due to lack of scientific support. For a long time, it has been observed that honey can be used to overcome liver, cardiovascular, and gastrointestinal problems (Lin *et al.*, 2010). Honey is a natural product that has been widely used for its therapeutic effects. It has been reported to contain about 200 substances. The therapeutic properties of honey are variable and depend on the type of honey used (Alvarez-Suarez *et al.*, 2010).

Honey, a natural product formed from nectar by honeybees, has been a subject of renewed research interest in the last few years. Evidence indicates that honey can exert several health-beneficial effects such as gastroprotective (Gharzouli *et al.*, 2002), hepatoprotective (Al-Waili *et al.*, 2006), reproductive (Mohamed *et al.*, 2012), hypoglycemic (Erejuwa, 2012), antioxidant antihypertensive, antibacterial, anti-fungal, and anti-inflammatory effects (Ienco *et al.*, 2011). It consists of primarily sugars such as monosaccharides, disaccharides, oligosaccharides, and polysaccharides. It contains enzymes such as glucose oxidase, diastase, invertase, catalase, and peroxidase. Honey also contains other bioactive constituents such as organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins, and Maillard reaction products (Bogdanov *et al.*, 2008).

Natural antioxidants can be phenolic compounds (tocopherol, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid (Halliwell, 2011). Phenols are very efficient scavengers of peroxyl radicals because of their molecular structures which include an aromatic ring with hydroxyl groups containing mobile hydrogens. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion which catalyze lipid peroxidation (Uttara *et al.*, 2009). Therefore, the present study concentrated on analyzing the health boosting components in honey namely the polyphenols, flavonoids, and flavonols. The efficacy of these phytochemicals in enhancing the therapeutic function of honey was determined using the *in vitro* antioxidant assays.

MATERIALS AND METHODS

Sample Collection

A total of five honey samples (in triplicates each) of, the Indian hive bee, *Apis cerana indica* F. (Apidae) (Ac), the

European or Italian bee, *Apis mellifera* L. (Apidae) (Am), the rock bee, *Apis dorsata* F. (Apidae) (Ad), the little bee, *Apis florea* F. (Apidae) (Af) and *Trigona irridipennis* S. (Stingless bee) (Ti) were collected from the local beekeepers of different areas of southern zone of Kerala in raw as well as processed form. The processed form of Af bee honey was not available due to the paucity of inventory. The samples collected were stored in half liter pet containers duly labeled with name codes and date of collection. All chemicals used in this study were of analytical grade.

Total Polyphenols

Phenols react with the phosphomolybdic acid in Folin–Ciocalteau reagent in alkaline medium and produce molybdenum blue complex. According to this principle, various concentrations of the prepared extracts when react with the Folin–Ciocalteau reagent and 10% Na_2Co_3 solution give shades of blue color which was measured at 725 m. Gallic acid was used as a standard. All the tests were performed in quadruples. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) (Slinkard and Singleton, 1977).

Total Flavonoids

Total flavonoids contents were estimated in the methanolic extract of honey by the method of Zhishen *et al*. (1999). The absorbance of the reaction matrix was measured at 510 nm using ultraviolet (UV) - visible spectrophotometer. The flavonoid content determined from the calibration curve was expressed as mg quercetin (QE)/g.

Flavonols

The concentration of total flavonols was measured using the p-(dimethylamino) cinamaldehyde (p-DMACA) method. DMACA condensates are detected at 640 nm thus offering greater over conventional UV detector setting. The DMACA assay measure flavonols, dihydrochalcones and proanthocyanidens (Di Stefano *et al*., 1989).

2, 2 diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical is long-lived organic nitrogen radical and has a deep purple color. It is commercially available and does not have to be generated before assay. In this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by electron spin resonance or by monitoring the absorbance decrease at 515-528 nm until the absorbance remains stable in organic media. This widely used method was first reported by Chen *et al*. (2000).

Nitric Oxide (NO) Scavenging Activity

NO was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. The absorbance of the chromophore formed was read at 546 nm (Marcocci *et al*., 1994; Sreejayan and Rao, 1997).

RESULTS AND DISCUSSION

Total Polyphenols

The total polyphenols for raw honey analyzed in the present study were in the order of Ad honey (1168 mg) > *Trigona iridipennis* honey (1144.25 mg) > Af honey (1084.5 mg) > Ac honey (1053.75 mg) > Am honey (905.25 mg). On processing a reduction in the phenols content was evident in all the honeys analyzed and it was highest in processed Am honey as the polyphenols had reduced to 384.5 mg from 905.25 mg with a percentage reduction of 57.52%. The phenol content of the processed *Trigona* honey was observed to be least effected as the processed Ti honey had phenolics content of 1016.25 mg which ranked the highest among the processed honeys. Processed Ac honey and Ad honey followed Ti honey with phenolics content of 666.75 mg and 525.75 mg, respectively. All the raw and processed honeys exhibited significant difference with respect to polyphenols at $P < 0.025$ [Table 1].

A comparatively lower level of polyphenols was reported for litchi honey (35.4 mg gallic acid/100 g) by Das *et al*. (2013). Relatively lower total polyphenol content of Thai honeys ranging from 10 and 14.4 mg gallic acid/100 g have

Table 1: Total polyphenols (mg/kg sample) of raw and processed bee honeys

Species	Total polyphenols (mg/kg sample)		
	Raw honey	Processed honey	Change in polyphenols (%)
Ac	1053.75 (32.49)	666.75 (5.07)	−36.72
Am	905.25 (30.03)	384.5 (4.73)	−57.52
Ad	1168 (34.15)	525.75 (5.17)	−54.98
Af	1084.5 (32.89)	-	-
Ti	1144.25 (33.81)	1016.25 (5.52)	−11.18
CD (0.025)	2.741	0.184	

Values in the parenthesis are transpose values, Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*

also been reported by Sangsrichan and Wanson (2008). Manuka honey from New Zealand was reported to have a total phenolic level of 43.4 mg gallic acid/100 g (Khalil *et al*., 2011).

The total phenolic content of gelam and coconut honeys was found to be 21.4 (±1.29) and 15.6 (±1.05) mg/g honey, respectively. Although, different methods for extraction and determination of total phenolic contents in the present study, the results are in agreement with that reported by Ferreres *et al*. (1992) and Martos *et al*. (1997) who found that the total phenolic contents of honeys were between the ranges of 500-2000; 700-2000 and 20-2400 mg/100 g honey, respectively. Since, different plants contain different phenolic compounds and show variation in their total phenolic content (Zheng and Wang, 2001).

Total Flavonoids

The flavonoid content ranged from 185 to 545.75 mg QE for raw honeys and from 108.75 to 143 mg QE for processed honeys under the study. Similar to the polyphenols content of the raw honeys the highest flavonoid content was also observed in raw Ad honey of 545.75 mg QE and this was followed by raw *Trigona* honey with 263.25 mg QE of flavonoids. The flavonoid content of raw Am honey was viewed to be 202 mg of QE, whereas, the raw Ac and Af honeys had almost nearby levels of flavonoid content of 185 mg and 188.25 mg of QE, respectively. Processing had found to have a negative impact on the levels of flavonoids too. The lowest flavonoid content was noticed in processed Ad honey of 108.75 mg QE, which was followed by processed Am honey of 113 mg QE. The highest flavonoid content was observed in *Trigona* honey with 143 mg QE and processed Ac honey followed Ti honey among the processed honeys containing higher amounts of flavonoid content (132.5 mg QE). A gradual reduction in the flavonoids content was noticed in all the honeys after processing with the highest being in Ad honey were the flavonoid content of the raw Ad honey reduced to 108.75 mg QE. The least percentage change of flavonoid content after processing was observed in Ac honey (−28.37%). All the honeys under the study varied significantly in their flavonoid content ($P < 0.025$) [Table 2].

Similar to the flavonoids content of the honeys in the present study, tualang honey contained the highest amount (65.65 mg/kg) of flavonoids while, the flavonoid content in acacia honey (21.95 mg/kg) Croatian acacia honey (43.66 mg/kg) and Burkina Fasan acacia honey (61.4 mg/kg) was lower (Krpan *et al*., 2009). This could be due to the different floral and geographical origins of the honey sources.

Flavonols

The flavonol content of five different raw bee honeys ranged from 4.6 to 17.6 mg of catechin. The highest flavonol content was detected in raw Ti honey of 17.6 mg followed by raw Ad and Ac honey with 12.9 mg and 10.12 mg of catechin. The raw honeys Am, and Af had equivalent levels of flavonols of 4.64 mg and 4.6 mg, respectively. Hence, the raw honeys Am and Af did not exhibit any significant difference at $P < 0.025$ in their flavonol content whereas, all the other raw honeys varied significantly in their flavonol content.

As observed [Table 3] in other phytochemicals analyzed, the flavonol content was also observed to decrease on processing of the raw honeys under the study. The flavonol content of the processed honeys ranged from 12.17 to 2.67 mg of catechin. The lowest flavonol content was viewed in Am honey of 2.67 mg, and the highest flavonol content was observed to be in Ti honey with 12.17 mg of catechin. All the processed honeys differed significantly ($P < 0.025$) in with respect to their flavonol content. Similar to the flavonol profile of raw honeys among the processed honeys also the Ad and Ac honeys acquired similar levels of flavonols of 8.67 mg and 7.27 mg of

Table 2: Flavonoid content (mg QE/kg sample) of raw and processed bee honeys

Species	Flavonoid content (mg QE/kg sample)		
	Raw honey	Processed honey	Change in flavonoid (%)
Ac	185 (13.60)	132.5 (6.16)	−28.37
Am	202 (14.22)	113 (6.11)	−44.05
Ad	545.75 (23.35)	108.75 (5.77)	−80.07
Af	188.25 (13.72)	-	-
Ti	263.25 (16.18)	143 (5.95)	−45.67
CD (0.025)	1.415	0.123	

Values in the parenthesis are transpose values. Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*, QE: Quercitin

Table 3: Flavonol content (mg catechin/kg sample) of raw and processed bee honeys

Species	Flavonol content (mg catechin/kg sample)		
	Raw honey	Processed honey	Change in flavonol (%)
Ac	10.12 (3.25)	7.27 (1.43)	−28.19
Am	4.64 (2.26)	2.67 (1.48)	−42.38
Ad	12.90 (3.65)	8.67 (1.33)	−32.76
Af	4.6 (2.25)	-	-
Ti	17.6 (4.25)	12.17 (1.28)	−30.87
CD (0.025)	0.180	0.003	

Values in the parenthesis are transpose values. Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*

catechin, respectively. Similar phenolic contents were reported for Croatian (126-905.7) (Piljac-Zegarac *et al.*, 2009), Burkina Fasan (325.9-1147.5) (Meda *et al.*, 2005), and Portuguese honeys (132.1-727.7) (Bertoncelj *et al.*, 2007).

All the honeys within the raw and processed categories showed significant differences in terms of phytochemicals analyzed *viz.*, total polyphenols, flavonoids, and flavonols content. The experiment confirmed huge differences in all the analyzed phytochemicals when the honeys between the raw and processed categories were compared.

DPPH Assay

On elucidating the protective role of raw and processed bee honeys in the present study it was noticed that both raw, as well as processed honeys, had a particular amount of scavenging capacity which was exhibited over a range of concentrations in which those honeys were taken for the analysis.

From Table 4 it was clear that in the raw honeys analyzed (at 100 µg/ml) the Ac and Am honeys obtained equivalent levels of percentage scavenging of 78.65% and 78.55%, respectively. Hence, raw Ac and Am honeys did not show any significance difference ($P < 0.025$) in their antioxidant activity against DPPH radical. On the other hand, the processed Ac and Am honeys had different levels of scavenging activity with 61.95% and 73.37%, respectively. Among the raw honeys analyzed the least radical scavenging activity was observed in Ad honey with 25.9% and the Af honey had percentage scavenging of 31.98% against DPPH radical. The processed Ad honey had 21.28% of radical scavenging activity. On processing the radical scavenging activity of raw *Trigona* honey (83.76%) had reduced to 79.67% even then the processed Ti honey continued to have higher levels of scavenging activity among the processed honeys. All the processed honeys varied significantly at $P < 0.025$ level in their scavenging activity against DPPH radical [Table 5].

The DPPH radical scavenging activity of raw and processed honeys under the present investigation was compared with a standard using a synthetic antioxidant QE. Although the honey samples under the study showed a decreased antioxidant capacity, it could be noted that certain honeys specifically Ti, Ac, and Am had relative activity with the moderate difference from the standard.

The highest inhibitory concentration 50 (IC_{50}) value was observed for raw and processed Ad and Af honeys. The IC_{50} value of raw and processed Ad honeys was viewed to be 432.2 µg/ml and 524.4 µg/ml respectively. This was followed by raw Af honey with an IC_{50} value of 434.9 µg/ml. The least IC_{50} value was noticed in raw Ti honey of 59.73 µg/ml. On processing Ti honey had exhibited a slight increase in the IC_{50} value and was noted to be 62.19 µg/ml. Relatively lesser amounts of IC_{50} values were observed among the raw and processed Ac (63.15, 79.73 µg/ml), and Am (63.58, 67.93 µg/ml) honeys [Figure 1].

Table 4: DPPH scavenging activity of raw honeys

Species	Concentration (µg/ml)				
	Percentage of DPPH scavenging activity of raw honeys				
	100	200	300	400	500
Ac honey	78.69	82.43	85.76	90.82	95.55
Am honey	78.55	80.71	83.19	88.98	90.33
Ad honey	25.9	39.30	42.26	48.47	53.07
Af honey	31.98	36.21	43.81	48.57	53.77
Ti honey	83.76	88.09	90.68	94.39	97.21
CD (0.025)	0.903	1.17	0.843	0.662	0.692
QE	87.81	90.19	93.51	95.59	97.48

Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*, DPPH: 2, 2 diphenyl-1-picryl hydrazyl, QE: Quercitin

Table 5: DPPH scavenging activity of processed honeys

Species	Concentration (µg/ml)				
	Percentage of DPPH scavenging activity of processed honey				
	100	200	300	400	500
Ac honey	61.95	65.23	70.86	75.19	82
Am honey	73.37	76.67	80.24	84.42	88.18
Ad honey	21.28	27.10	36.05	41.31	48.17
Ti honey	79.67	84.78	88.94	91.27	92.56
CD (0.025)	1.510	1.695	0.986	0.986	1.202
QE	87.81	90.19	93.51	95.59	97.48

Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*, DPPH: 2, 2 diphenyl-1-picryl hydrazyl, QE: Quercitin

Nitric Oxide Scavenging Activity

Percentage nitric oxide radical scavenging activity exhibited by methanolic extracts of raw and processed honeys were concentration dependent manner. As the concentration of honey in the assay medium increases the percentage of $NO^{\bullet}$ scavenging increases. The overall $NO^{\bullet}$ scavenging activity varied from 38.01% to 77.19% among the raw honeys analyzed in the present investigation, whereas the scavenging activity of the processed honeys were in range of 15.27-53.92% at concentrations 100-500 µg/ml. On increasing the concentration of raw honeys to 500 µg/ml the increase in the activity of Af was only up to 65.35% whereas a larger increase in the activity was observed in the $NO^{\bullet}$ scavenging activity of Am honey with 77.19% which was followed by Ad with 68.22% of

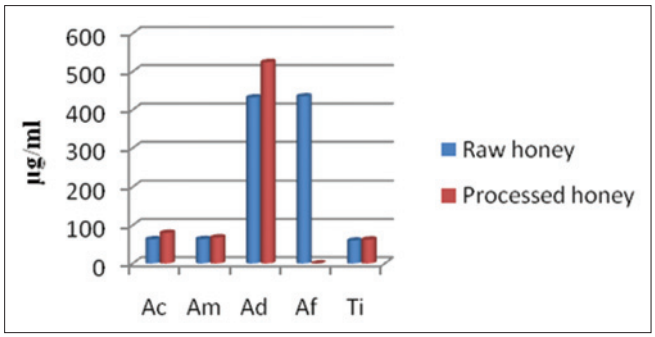

Figure 1: Inhibitory concentration 50 values for 2, 2 diphenyl-1-picryl hydrazyl scavenging activity of raw and processed honeys

scavenging activity. The raw *Trigona* honey ranked fourth in the NO• scavenging activity at 500 µg/ml with 64.59% of scavenging this was followed by Ac honey with 54.56%. No significant difference was observed between the raw Af (65.35%) and Ti (64.59%) honeys [Table 6].

Processed honeys exhibited significant differences at all concentrations from 100 to 500 µg/ml. Whereas among the raw honeys in spite of the presence of significant differences *Trigona* honey exhibited non-significance with Af and Am honeys. The efficacy of the NO• radical scavenging activity of the honeys under the study was compared with a standard antioxidant butylated hydroxyl toluene (BHT). The radical scavenging activity of BHT over the concentrations 100-500 µg/ml were observed to be from 49.38% to 83.32% and it could be noted that the raw Af, Am, and *Trigona* honeys had comparatively equivalent antioxidant capacity in relation to the synthetic antioxidant BHT [Table 7].

The antiradical activity of the honeys analyzed in the present study were compared and found to higher. Turkish red pine honey produced by *Marchalina hellenica* reported to scavenge DPPH effectively, suggestive of its antiradical activities from 31.1% to 86.09% (Akbulut *et al.*, 2009). Some Saudi Arabian honey samples were demonstrated to exhibit antioxidant activities from 50.4% to 96.8% (Al-Hindi *et al.*, 2011). Similar antioxidant properties were also reported for Peruvian honey (80.44%) (Rodríguez-Malaver *et al.*, 2009). Australian honey produced by the stingless bees *Trigona carbonaria* was reported to have high antioxidant properties (89.5%) (Oddo *et al.*, 2008). Malaysian Tualang honey produced by the giant Asian bees Ad has been shown to exhibit good antioxidant (28.48-36.94%) and antiradical (273.46 and 292.34 µM Fe(II)/kg) activities (Kishore *et al.*, 2011). Antioxidant activities have also been documented for American buckwheat honey (4.0 µg/ml) (Van den Berg *et al.*, 2008), Croatian oak honeydew (IC_{50} at 420.3 µg/ml)

Table 6: NO scavenging activity of raw honeys

Species	Concentration (µg/ml)				
	Percentage of NO• scavenging activity of raw honeys				
	100	200	300	400	500
Ac honey	38.01	46.33	48.23	53.02	54.56
Am honey	45.33	50.84	64.81	70.13	77.19
Ad honey	43.53	44.36	47.09	52.12	68.22
Af honey	48.6	54.05	55.93	58.57	65.35
Ti honey	47.42	50.45	53.30	54.24	64.59
CD	1.174	1.325	0.783	0.813	1.054
QE	49.38	54.62	67.08	74.91	83.32

NO: Nitric oxide, Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennisi*, QE: Quercitin

Table 7: NO scavenging activity of processed honeys

Species	Concentration (µg/ml)				
	Percentage of NO• scavenging activity of processed honey				
	100	200	300	400	500
Ac honey	15.27	20.70	24.48	34.99	42.72
Am honey	17.6	24.81	35.03	43.58	50.74
Ad honey	33.9	38.96	42.93	49.37	53.92
Ti honey	28.83	32.25	36.07	41.92	48.10
CD	0.832	0.739	0.493	1.017	1.233
QE	49.38	54.62	67.08	74.91	83.32

NO: Nitric oxide, Ac: *Apis cerana*, Am: *Apis mellifera*, Ad: *Apis dorsata*, Af: *Apis florea*, Ti: *Trigona iridipennis*, QE: Quercitin

honey (Jerkovic *et al.*, 2010), Spanish honey (IC_{50} at 150.6 µg/ml) (Pérez *et al.*, 2007), Portugal honey (IC_{50} at 174.5 µg/ml) (Estevinho *et al.*, 2008), Cuban honey (Alvarez-Suarez *et al.*, 2010), Venezuelan honey (IC_{50} at 249.4 µg/ml) (Vit *et al.*, 2009) and Ecuadorian honey (IC_{50} at 162.8 µg/ml) (Guerrinia *et al.*, 2009).

By comparing these findings with that reported in Gelam and Coconut honeys were shown to contain 24 and 21% water, respectively (Aljadi and Kamaruddin, 2004) it was noted that the free radical scavenging activities of honeys in the present study were higher than the values for gelam and coconut honeys.

As depicted in Figure 2 the IC_{50} value of the raw and processed honeys ranged from 126 to 625.79 µg/ml. Among the raw honeys the 50% inhibition was acquired by Am honey of 126 µg/ml followed by Af (132.7 µg/ml) and Ti (195.78 µg/ml). The Ac and Ad honeys had moderate IC_{50} values of 356.6 µg/ml and 283.15 µg/ml respectively. The IC_{50} value of the standard BHT was observed to be 121.25 µg/ml. Hence, raw Ac honey had almost equivalent IC_{50} value with regards to the IC_{50} value of the standard antioxidant.

On concluding the phytochemical profile of raw and processed honeys was adequate enough to impart

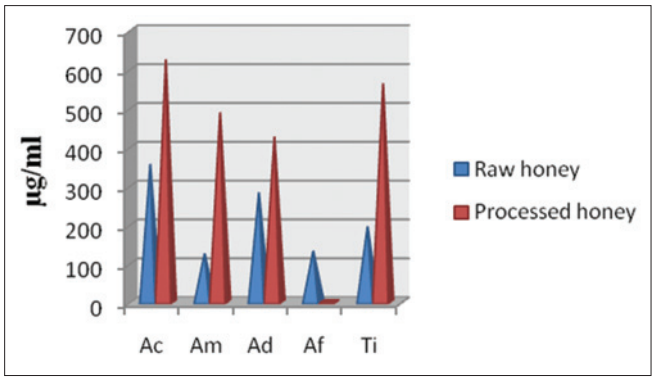

Figure 2: Inhibitory concentration 50 values for NO• scavenging activity of raw and processed honeys

antioxidant property for each honey under the study. Processing was noticed to reduce the phytochemical content in the honey. Among the raw honeys, highest polyphenols were observed in Ad, Ti and Af honeys. In the same way, the flavonoid content was found to be higher in Ad, Ti and Am honeys. Similar results were obtained on analyzing the flavonol content of honeys. The raw honeys were superior in the antioxidant potency, as the processing causes the denaturation of the bioactive components that impart the antioxidant property. All the raw honeys under the study had antioxidant activity with highest being in *Trigona* honey (97.21% at 500 µg/ml) with IC_{50} value of 59.73 µg/ml in scavenging the DPPH radicals. In the same way, the NO• scavenging activity of the honeys revealed similar results which suggest the higher effectiveness of the raw honeys over the processed honeys in the antioxidant capacity. Among the honeys analyzed Ti honey observed to be rich in phytochemicals with high antioxidant capacity. This might be due to the peculiarity of the bees, collecting nectar from the small wild growing plants, whereas it is difficult for bigger bees to get inside the smaller flowers. The major foraging plant for *Trigona* bees is *Leucas aspera* which is highly medicinal in nature.

CONCLUSION

The health boosting components of honey depends mainly on the geographical area and the climatic conditions form which they were collected. This correlates with the results obtained from the present study as the honeys for the present study were procured from Kerala known for the abundance of medicinal herbs. The phytochemical composition and antioxidant activity of each honey under the study had a significant association. However, each honey had a different amount of phytochemicals and rate of radical scavenging activity even though all the honeys were procured from the same geographical area and climatic conditions. Hence, the present study strongly suggests the influence of certain substances that are added by the bees in the process of conversion of the nectar in to the honey which could be further explored under control conditions.

ACKNOWLEDGMENT

We are grateful to the authority of Kerala Agricultural University, for providing the facilities for conducting this research.

REFERENCES

Akbulut M, Ozcan MM, Coklar H. Evaluation of antioxidant activity, phenolic, mineral contents and some physicochemical properties of several pine honeys collected from Western Anatolia. Int J Food Sci Nutr 2009;60:577-89.

Al-Hindi RR, Bin-Masalam MS, El-Shahawi MS. Antioxidant and antibacterial characteristics of phenolic extracts of locally produced honey in Saudi Arabia. Int J Food Sci Nutr 2011;62:513-7.

Aljadi AM, Kamaruddin MY. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 2004;85:513-8.

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. Contribution of honey in nutrition and human health: A review. Med J Nutrition Metab 2010;3:15-23.

Al-Waili NS, Saloom KY, Al-Waili TN, Al-Waili AN, Akmal M, Al-Waili FS, *et al.* Influence of various diet regimens on deterioration of hepatic function and hematological parameters following carbon tetrachloride: A potential protective role of natural honey. Nat Prod Res 2006;20:1258-64.

Bertoncelj J, Dobersek U, Jamnik M, Golob T. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem 2007;105:822-8.

Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A review. J Am Coll Nutr 2008;27:677-89.

Chen L, Mehta A, Berenbaum M, Zangerl AR, Engeseth NJ. Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem 2000;48:4997-5000.

Das A, Mukherjee A, Dhar P. Characterization of antioxidants and antioxidative properties of various unifloral honeys procured from West Bengal, India. IOSR J Environ Sci Toxicol Food Technol 2013;7:56-63.

Di Stefano R, Cravero MC, Gentilini N. Methods for the study of wine polyphenols, L'Enotecnico 1989;25:83–89.

Erejuwa OO. Management of diabetes mellitus: Could simultaneous targeting of hyperglycemia and oxidative

stress be a better panacea? Int J Mol Sci 2012;13:2965-72.
Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 2008;46:3774-9.
Ferreres F, Ortiz A, Silva C, Garcı´a-Viguera C, Toma´s-Barberan FA, Toma´s-Lorente F. Flavonoids of "La Alcarria" honey – A study of their botanical origin. Z Lebensm Unters Forschg A 1992;194:139-43.
Gharzouli K, Amira S, Gharzouli A, Khennouf S. Gastroprotective effects of honey and glucose-fructose-sucrose-maltose mixture against ethanol-, indomethacin- and acidified aspirin-induced lesions in the rat. Exp Toxicol Pathol 2002;54:217-21.
Guerrinia A, Brunib R, Maiettia S, Polic F, Rossia D, Paganettoa G, *et al*. Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product. Food Chem 2009;114:1413-20.
Halliwell B. Free radicals and antioxidants - Quo vadis? Trends Pharmacol Sci 2011;32:125-30.
Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford, UK: Calendon Press; 1989. p. 51-7.
Ienco EC, LoGerfo A, Carlesi C, Orsucci D, Ricci G, Mancuso M, *et al.* Oxidative stress treatment for clinical trials in neurodegenerative diseases. J Alzheimers Dis 2011;24:111-26.
Jerkovic I, Marijanovic Z. Oak (*Quercus frainetto* Ten.) honeydew honey – Approach to screening of volatile organic composition and antioxidant capacity (DPPH and FRAP assay). Molecules 2010;15:3744-56.
Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci 2011;76:C921-8.
Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 2011;31:322-5.
Krpan M, Markovi K, Šarić G, Skoko B, Hruškar M, Vahčić N. Antioxidant activities and total phenolics Af acacia honey. Czech J Food Sci 2009;27:246-7.
Lin SM, Molan PC, Cursons RT. The post-antibiotic effect of manuka honey on gastrointestinal pathogens. Int J Antimicrob Agents 2010;36:467-8.
Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun 1994;201:748-55.
Martos I, Cossentini M, Ferreres F, Toma´s-Barberan FA. Flavonoid composition of Tunisian honeys and propolis. J Agric Food Chem 1997;45:2824-9.
Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005;91:571-7.
Mohamed M, Sulaiman SA, Jaafar H, Sirajudeen KN. Effect of different doses of Malaysian honey on reproductive parameters in adult male rats. Andrologia 2012;44:182-6.
Oddo LP, Heard TA, Rodríguez-Malaver A, Pérez RA, Fernández-Muiño M, Sancho MT, *et al.* Composition and antioxidant activity of *Trigona carbonaria* honey from Australia. J Med Food 2008;11:789-94.
Pérez RA, Iglesias MT, Pueyo E, Gonzalez M, de Lorenzo C. Amino acid composition and antioxidant capacity of Spanish honeys. J Agric Food Chem 2007;55:360-5.
Piljac-Zegarac L, Stipcevic T, Belscak A. Antioxidant properties and phenolic content of different floral origin honeys. J ApiProd ApiMed Sci 2009;1:43-50.
Rodríguez-Malaver AJ, Rasmussen C, Gutiérrez MG, Gil F, Nieves B, Vit P. Properties of honey from ten species of Peruvian stingless bees. Nat Prod Commun 2009;4:1221-6.
Sangsrichan S, Wanson W. The antioxidant capacity of honey samples collected in the north part of Thailand in relationship with its total polyphenol. KMITL Sci J 2008;8:1-6.
Slinkard K, Singleton VL. Total phenol analyses: Automation and comparison with manual methods. Am J Enol Vitic 1977;28:9.
Sreejayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997;49:105-7.
Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7:65-74.
van den Berg AJ, van den Worm E, van Ufford HC, Halkes SB, Hoekstra MJ, Beukelman CJ. An *in vitro* examination of the antioxidant and anti-inflammatory properties of buckwheat honey. J Wound Care 2008;17:172-4, 176-8.
Vit P, Rodriguez-Malaver A, Roubik WD, Moreno E, Souza BM, Sancho MT, *et al*. Expanded parameters to assess the quality of honey from Venezuelan bees (*Apis mellifera*). J ApiProd ApiMed Sci 2009;1:72-81.
Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 2001;49:5165-70.
Zhishen J, Mengeheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555.