ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

ANTIOXIDANT POTENTIAL OF *APIS FLOREA* HONEY FROM DRYLAND ECOSYSTEM IN WESTERN INDIA.

Jigisha Vaghela and A. Sankar Reddy.

B R Doshi School of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India.

Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 26 January 2016
Published Online: February 2016

Key words:
Antioxidant activity, *Apis florea*, *Prosopis juliflora*, Phenolic content, Kachchh desert.

Corresponding Author

A. Sankar Reddy.

Abstract

Apis florea bees are found in abundance in the dryland ecosystem of Kachchh; producing highest quantity of honey in Gujarat chiefly contributed by Prosopis juliflora nectar. Aim of the present study was to evaluate the antioxidant potential of this honey. Raw and processed honey samples were assessed for their in vitro antioxidant potential and correlated with proline content, total proteins, total phenolics, total flavonoids and color intensity. Analysis revealed Kachchh honey to be a good source of antioxidants. Reduced amount of proline, total proteins and total flavonoids were found in processed honey whereas total phenolics and color intensity were found least affected. Correlation values indicated high influence of total phenolic content on ferric reducing /antioxidant power (FRAP) (r = 0.85), radical scavenging activity (RSA) (r = 0.81) and ascorbic acid equivalent antioxidant content (AEAC) (r = 0.80). Also, significant correlations between antioxidant properties and total flavonoids, total proteins and proline content were observed. Pigment content (color intensity) correlated well with phenol (r = 0.77) and flavonoid (r = 0.77) contents. Present study indicated major influence of total phenolic content on the antioxidant power of the Kachchh honey.

1 Introduction:-

Kachchh, the largest district of Gujarat occupying the area of 45,612 km², is situated at western part of Indian subcontinent between 23.13° to 24.68° N latitude and 68.10° to 71.80° E longitude (GSI, n. d.). This extremely arid district has long costal area, large grassland 'Banni' and more than half region occupied by desert predominated by xerophytic vegetation making it a unique biodiversity. This ecosystem is invaded by invasive *Prosopis juliflora* (Sw.) DC. over the period of time, due to which much of the indigenous vegetation has been replaced (Saxena, 1998; Shah and Somusundaram, 2010; Patel et al., 2012). It has become a threat for the indigenous plant species found in that unique ecosystem. Quite the opposite, the spread of *P. juliflora* has become advantageous to the honey bees found in this region. According to Varshney (1996), the abundance of *Prosopis* is proven to be beneficial to the dwarf bees *Apis florea*, which could establish and survive in the peculiar climate of Kachchh. Our observations of pollens found in Kachchh honey also revealed *P. juliflora* as the chief source of nectar for honey production in the district (data unpublished). According to Gujarat State Forest Development Corporation Ltd (GSFDC) records, this production contributes to around 90% of the total honey production of the Gujarat state, generating around one lakh man-days of labour. Kachchh honey is in a great demand round the year. As a food and as a therapeutic medicine, it is largely used in local folklore practices.

Honey was the first bee product used by prehistoric man. It is the prime product of beekeeping and naturally concentrated form of sugar; not only used as a sweetener or food but this complex mixture of carbohydrates and various minor substances is also used as medicine due to its therapeutic and curative properties. Honey contains fructose and glucose as two major constituents (60-85%) besides a variety of minor components such as phenolic

compounds, minerals, proteins, free amino acids, enzymes, vitamins etc. (Ortiz, 1996; Perez et al., 2007). Many researchers have 'rediscovered' the applications of honey in the treatment of burns, gastrointestinal disorders, asthma, chronic wounds, skin disorders, eye ailments etc. (Molan, 1992; Marcucci, 1995; Castaldo and Capasso, 2002; Orhan et al., 2003; Ferreira et al., 2009). According to Ferreira et al. (2009), some of these diseases are the consequence of oxidative damage; therefore the therapeutic properties of honey seem to be partly due to its antioxidant capacity.

The studies of recent past (Gheldof and Engeseth, 2002; Berreta et al., 2005) proved that the honey is not only a health-promoting dietary supplement, but also a promising antioxidant. Antioxidants play an important role in food preservation and human health by combating damage caused by oxidizing agents (Meda et al., 2005). Both enzymatic and non-enzymatic substances present in the honey are responsible for its antioxidant activity. There is a synergistic influence of pigments, polyphenols, Maillard reaction products, amino acids and peptides, tocopherols, ascorbic acid, reduced glutathione and enzymes such as catalase, superoxide dismutase and glucose oxidase on antioxidant activity of honey (Al-Mamary et al., 2002; Gheldof and Engeseth, 2002; Vallianou et al., 2014). In several studies antioxidant activity of honey has shown strong correlation with its phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids) and flavonoid components (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin) and carotenoids (Al-Mamary et al., 2002; Beretta et al., 2005; Bertoncelji et al., 2007; Sangsrichan and Wanson, 2008; Ferreira et al., 2009; Krpan et al., 2009; Alvarez-Suarez et al., 2010; Vallianou et al., 2014). Kesic et al. (2009) explained the linear correlation between L-ascorbic acid content and total antioxidant activity of bee-honey. Radical scavenging activity of honey also correlates with some free amino acids (Wu et al., 2003; Perez et al., 2007). Some authors have demonstrated the antioxidant activity of honey mainly due to its proline content (Meda et al., 2005; Saxena et al., 2010).

Honey gathered by *A. florea* bees largely from *P. juliflora* flowers in arid land of Kachchh is a valuable natural resource. Similar kind of honey predominantly from *P. juliflora* nectar is reported from Oman (Sajwani et al., 2007), Brazil (de Novais et al., 2014) and Andhra Pradesh in India (Kalpana and Ramanujam, 1990). However, to our knowledge there is no valid information available on its antioxidant potential. The present study was carried out to evaluate *in vitro* antioxidant activity of *A. florea* bee hive honey collected from Kachchh. Further, the color intensity (ABS_{450}), proline, total proteins, total phenolics and total flavonoids were estimated to understand their correlations with the antioxidant potential of this honey. Variation of these properties in the processed honey was analyzed comparing it with raw honey.

2 Materials and methods:-

2.1 Sample collection:-

There were thirty two raw and ten processed honey samples of *Apis florea* honey combs collected from Kachchh were procured during 2009, 2010 and 2011 (Table 1). Raw honey samples were procured as such that it represents the samples from most of the regions of the district (Fig 1). KA samples were procured from sixteen honey collection centers of eight *Talukas*. KB samples were collected from nine bee hives of *A. florea* in the wild habitat. Raw samples were strained by sterile cheese cloth. Processed honey samples collected from Kachchh, were procured from the honey processing unit of GSFDC, Vadodara.

Honey processing includes mainly warming at 40°C, followed by pressure filtration through 150 µm pore size filter unit to remove pollens and other debris; and then heating it at 60°C for a few minutes, in continuous batches in automated processing unit, before bottling. All samples were stored in the sterile bottles in dark at 0°C until analyzed.

2.2 Color intensity (ABS_{450}):-

Color intensity of the honey was determined according to Beretta et al. (2005). 50% (w/v) of honey sample diluted in warm (45-50°C) milli Q water was filtered through a cellulose filter of 0.45 µm pore size (Millipore India Pvt. Ltd, Bangalore) to avoid all coarse particles. The net absorbance (expressed as mAU) was obtained as a difference between the spectrophotometric absorbance at 450 nm and 720 nm.

2.3 Biochemical analyses:-

2.3.1 Proline content:-

Proline present in the honey was estimated by referring the method of Ough (1969) as adapted by Bogdanov (2002). 0.5 ml of (0.05 g/ml) honey solution was mixed with 1 ml of concentrated formic acid and 1 ml of 3% ninhydrin solution (3% in ethylene glycol monomethylether); and shaken vigorously in capped tubes for 15 minutes. The mixture was placed in the boiling water bath for 15 minutes and incubated to the water bath at 70°C for next 10 minutes. 5 ml of 50% 2-propanol solution was added to the mixture and left for cooling for 45 minutes. The absorbance was read at 510 nm. Distilled water was used as a blank. Proline content was determined with the help of the proline standard curve (0-32 µ/ml) and results were expressed as mg/kg of honey.

2.3.2 Total protein content:-

Lowry's method (Thimmaiah, 2006) was used to determine total protein content of honey. 50% w/v dilution of honey in chilled sodium phosphate buffer (pH = 6.8) was centrifuged and 0.1, 0.2, 0.3 aliquots were obtained in three different test tubes. Fourth tube was serving as a blank. Volumes were made up to 1 ml with distilled water in all four tubes. Later 5 ml of alkaline copper solution was added to each tube and kept for incubation at room temperature for 10 minutes. Further 0.5 ml of Folin-Ciocalteu Reagent (FCR) was added and mixed thoroughly in all the tubes, followed by 30 minutes incubation in dark at room temperature. Absorbencies were read against blank at 660 nm. Standard curve of 0-200 µg/ml of Bovine Serum Albumin (BSA) was used to determine the total protein content which was expressed as mg/100g.

2.3.3 Total flavonoid content:-

The Dowd method as adapted by Arvouet-Grand et al. (1994) and Meda et al. (2005) was followed to determine total flavonoid content. 5 ml of 2% of methanolic aluminium trichloride solution was added to 5 ml of 0.4 mg/ml methanolic honey solution and absorbance was read at 415 nm after 10 minutes against the blank; which was prepared by adding 5 ml methanol in 5 ml of 0.4 mg/ml methanolic honey solution. Standard curve was prepared using 0.2-1 mg/20 ml of quercetin (QE), and results were expressed in mg of QE/100 g of honey.

2.3.4 Total phenolic content:-

The Folin-Ciocalteu method described by Singleton et al. (1999) and followed by Meda et al. (2005) was used to determine total phenolic content. Honey sample of 0.1 g/ml concentration was prepared. 30 µl of this solution was dissolved in 2.37 ml of milli Q water to which 150 µl of FCR was added. After thorough mixing, it was incubated at an ambient temperature for two minutes. 450 µl of 0.2 g/ml sodium carbonate solution was added to this mixture and further incubated for 2 hours at ambient temperature. Absorbance was measured at 765 nm. Standard curve of 0-200 mg/l gallic acid was used to determine the total phenolic content and results were expressed in mg of gallic acid equivalents (GAE) /100 g of honey.

2.4 Antioxidant properties:-

Adapting the following three commonly applied *in vitro* antioxidant assays, Kachchh honey samples were analyzed for determining antioxidant properties.

2.4.1 Ascorbic acid equivalent antioxidant content (AEAC):-

It was determined as per the method followed by Saxena et al. (2010) by referring Meda et al. (2005). Honey sample was dissolved in methanol to get 0.03 g/ml methanolic honey solution. 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) solution was prepared by dissolving 0.02 mg/ml DPPH in methanol. 0.75 ml of methanolic honey solution was mixed with 1.5 ml of DPPH solution. Blank was prepared by mixing 0.75 ml of methanolic honey solution with 1.5 ml of methanol. The mixtures were incubated at room temperature for 15 minutes. Absorbance was measured with the use of spectrometer at 517 nm against the blank. Standard curve of ascorbic acid plotted with 0-10 µg/ml concentrations was used to determine AEAC which was expressed as mg AEAC/100 g of honey.

2.4.2 Radical scavenging activity (RSA):-

DPPH radical scavenging activity (RSA) was measured according to Chen et al. (2007) and Saxena et al. (2010). 100 µl aliquot of honey solution (0.1 g/ml) was diluted to 500µl with 70% ethanol. 400 µl of 0.2 mM DPPH solution prepared in absolute ethanol was mixed vigorously with this solution. After 15 minutes incubation at room temperature, the absorbance of the solution (T1) was measured at 517 nm. Sample blank (B1) was prepared by adding 400 µl of DPPH in 600 µl of 70% ethanol. DPPH blank (B2) was prepared by 100 µl of honey sample, 500 µl of 70% ethanol and 400 µl of absolute ethanol. RSA (%) was calculated by the formula: [1-{(T1-B2)/B1}] x 100.

2.4.3 Ferric reducing /antioxidant power (FRAP) assay:-
FRAP assay was carried out referring Saxena et al. (2010) following Oyaizu (1986). 1 ml aliquot of 10% (v/v) honey extracted in ethanol was mixed with 0.2 M phosphate buffer of pH 6.6 and 2.5 ml of 1% potassium ferri-cyanide. After incubating it at 50°C for 20 min, 2.5 ml of 10% trichloroacetic acid was vigorously mixed with the solution. The mixture was centrifuged for 10 minutes at 3000 rpm to obtain the supernatant of which 2.5 ml was mixed with equal amount of milli Q water and 0.5 ml of 0.1% of ferric chloride. The absorbance of the mixture was measured at 700 nm. Ascorbic acid (0.1 mg/ml) was used as a reference standard.

All analyses were performed in triplicates. Results are expressed as the mean values with standard deviation (SD). Data were processed using SPSS 12.0 software for Windows. Pearson Correlation coefficients (r) with two tailed test of significance between two variables were calculated for antioxidant activity and influencing factors. For all analyses $p < 0.01$ was considered significant.

3 Results and discussion:-
The results of color intensity (ABS_{450}) and biochemical analyses have been summarized in Table 2; those of antioxidant properties are précised in Table 3. Differences (%) in various parameters of processed honey in comparison with raw honey are exhibited in Fig 2.

3.1 Color intensity (ABS_{450}) :-
The color of honey partly reflects the content of (carotenoids, flavonoids, etc.) pigments with antioxidant properties (Beretta et al., 2005). Net absorbencies in raw samples were ranging from 23.00 to 85.67 mAU, showed similarity to those of *Acacia* honey of Slovenia ranging from 44 to 95 mAU (Bertoncelj et al., 2007). ABS_{450} found in processed samples were between 36.00 to 58.33 mAU. There was no major difference (16.67%) found between the ABS_{450} of processed and raw samples (Fig 2).

3.2 Biochemical analyses:-
3.2.1 Proline content:-
This predominant free amino acid present in honey was determined with the help of a standard curve of proline (R^2 = 0.998). Its content (249.69 mg/kg ± 27.04**)** in the raw honey was comparable to that (254.9 mg/kg ± 68.82) of *Robinia pseudoacacia* honey from Croatia (Saric et al., 2008). However, proline content ranges from 437.82 to 2169.37 mg/kg in Burkina Faso honey (Meda et al., 2005) and 465.3 to 827.6 mg/kg in honey from Southwest of Kef, Tunisia (Jilani et al. 2008) which is higher than that of the Kachchh honey (204.62-310.75 mg/kg). Considerable difference (48.24%) in the proline content of processed honey was seen as compared to that of raw honey (Fig 2).

3.2.2 Total protein content:-
With the help of the standard curve (R^2 = 0.999), the total protein content obtained in the raw honey samples was 322.84 mg/100g ± 55.21, whereas it was found considerably less (36.51%) in processed samples (Fig 2). Total protein content of both raw and processed honey justified the interpretation of Kaakeh and Gadelhak (2005) which indicates that the total protein content in honey averages about 0.17% of its weight, but can vary widely (between 0.02% and 1.0%). The total protein content of raw Kachchh honey was comparable to that of some monofloral honey samples of *Eucalyptus* (370 mg/100g ± 0.2) and *Capparis* sp. (380 mg/100g ± 0.6) from Algeria (Ouchemoukh et al., 2007) but higher than Brazilian honey of *Borreria verticillata*, which is 223.6 mg/100g (Azeredo et al., 2003). Even the total protein content range of processed honey (175.78-267.45 mg/100g) was better compared to that of some reputed commercial Indian honeys; ranging from 48 to 229.3 mg/100g (Saxena et al., 2010).

3.2.3 Total flavonoid content:-
The standard curve of quercetin (R^2 = 0.946) was used to measure the flavonoid content of the honey samples. Flavonoid content of raw honey was 6.27 mg of QE/100g ± 2.34 which was more than that of processed honey (4.15 mg of QE/100g ± 0.57) with a substantial difference of 33.81% (Fig 2). Kachchh honey showed higher flavonoid content as compared to that of Burkina Faso honey, which is estimated to be 2.57 ± 2.09 mg of QE/100 g of honey (Meda et al., 2005).

3.2.4 Total phenolic content:-

Total phenolic content was determined by using standard curve of gallic acid (R^2 = 0.994). It was 42.57 mg GAE/100g ± 7.50 in raw honey and 37.03 mg GAE/100g ± 3.95 processed honey indicating no significant difference (13.01%) between them (Fig 2). Phenolic content of Kachchh honey was found coincident with commercial Buckwheat honey (48.22 mg GAE/100g ± 0.24) and pure tropical Burkina Faso honey (28.74 - 59.52 mg GAE/100g) (Beretta et al., 2005), amber honey of *Echium vulgare* from Northeast Portugal (40.62 mg GAE/100g ± 1.72) (Ferreira et al., 2009) and *Trigona carbonaria* honey from Australia (48.53-63.43 mg GAE/100g) (Oddo et al., 2008).

3.3 Antioxidant properties:-

3.3.1 Ascorbic acid equivalent antioxidant content (AEAC):-

AEAC of the honey samples was estimated with the help of a standard curve of ascorbic acid (R^2 = 0.991). There was no much difference (16.37%) seen (Fig 2) in the AEAC of raw (16.62 mg AEAC/100g ± 3.75) and processed (13.90 mg AEAC/100g ± 3.52) honey. These results were similar to those of commercial Indian honeys (Saxena et al., 2010), that are ranging from 15 to 30 mg AEAC/100g, and were also comparable to the results of Burkina Fasan honey (Meda et al., 2005), ranging from 10.20 to 37.87 mg AEAC/100g.

3.3.2 Radical scavenging activity (RSA):-

DPPH radical scavenging activity of the raw honey was found to be ranging from 39.95 to 84.35%. Processed honey showed RSA ranging from 35.07 to 49.47%. Thus, RSA was 27.1% less in processed samples as compared to that of raw samples (Fig 2). These results were comparable to the DPPH radical scavenging activity (44 to 71%) of some commercial Indian honeys analyzed by Saxena et al. (2010). Out of sixteen KA honey samples studied, nine showed RSA greater than 50% whereas in KB samples, out of nine only three samples showed RSA greater than 50%. In case of processed (KC) samples, RSA (%) was low, and thus none of the samples showed RSA more than 50%.

3.3.3 Ferric reducing /antioxidant power (FRAP) assay:-

The reference standard (0.1 mg/ml ascorbic acid) showed an average absorbance value of 0.64 for FRAP assay at 700nm. Absorbance value of raw honey varied from 0.13 to 0.57. In processed honey, it was ranging from 0.27 to 0.39. Here, there was no significant difference (3.03%) of FRAP value seen in processed honey as compared to raw honey (Fig 2). The higher absorbance value indicates higher reducing power (Saxena et al., 2010) as ferric ions are converted to ferrous ions by the antioxidants present in the honey that increase the absorbance. Saxena et al. (2010) evaluated absorbance values of FRAP assays from 0.38 to 0.59 in some commercial Indian honeys.

It becomes evident from the outputs of the discussed analyses that the processed honey contained lesser amount of all the studied components as compared to raw honey. Higher difference of values was found in proline, total protein and total flavonoid contents. However, in case of total phenolic content and pigments (color intensity), such difference was not much significant. From this comparison, it can be derived that the processing of honey may lead to decreased amount of proline, total protein and total flavonoid contents. Total phenolic content and pigments are the least affected factors due to processing. In case of antioxidant properties, higher difference was seen in RSA (%) followed by AEAC and least was noted in FRAP of processed samples compared to raw samples.

3.4 Correlation amongst biochemical parameters and antioxidant properties:-

The correlation matrix (Table 4) reveals highest correlation between total phenolic content and FRAP (r = 0.85), RSA (r = 0.81) and AEAC (r = 0.80) indicated significant contribution of phenolic content to the antioxidant activity of honey. Total flavoniods showed noteworthy correlation (r = 0.85) with RSA (%), whereas good correlation with AEAC (r = 0.62) and FRAP (r = 0.60). Color intensity (pigments), showed good correlation with AEAC (r = 0.58), FRAP (r = 0.57) and RSA (r = 0.70) indicating considerable influence of pigments of the honey on its radical scavenging activity. Color intensity also correlated well with phenolic (r = 0.77) as well as flavonoid contents (r = 0.77). Similar observations were also made by Saxena et al. (2010) for some commercial Indian honeys, where color intensity (ABS_{450}) showed good correlation with phenolic content as well as antioxidant activity of honey. Total protein content correlated well with RSA (r = 0.76) and AEAC (r = 0.73) but less with the FRAP (r = 0.54). Free amino acid proline showed good correlation with RSA (r = 0.72) but the values were comparatively less with AEAC (r = 0.55) and FRAP (r = 38). Though the correlations of color intensity, total flavoniods and total protein were significant to the antioxidant potential of Kachchh honey, total phenolic content was a major influencing factor amongst them.

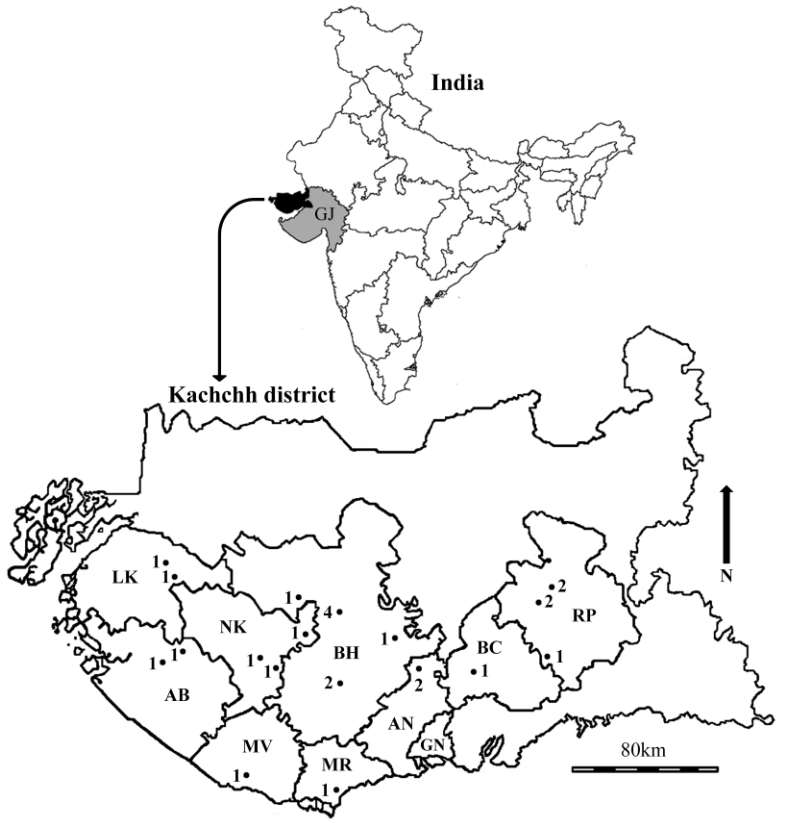

Fig 1 Map shows the location of honey sample collection sites (with dots) in Kachchh district of Gujarat (GJ) in India. Digits indicate the number of the honey samples collected from that place. Names of taluka of district are shown as, AB-Abdasa, AN-Anjar, BC-Bhachau, GN-Gandhidham, LK-Lakhpat, MR-Mundra, MV-Mandavi, NK-Nakhatrana and RP-Rapar.

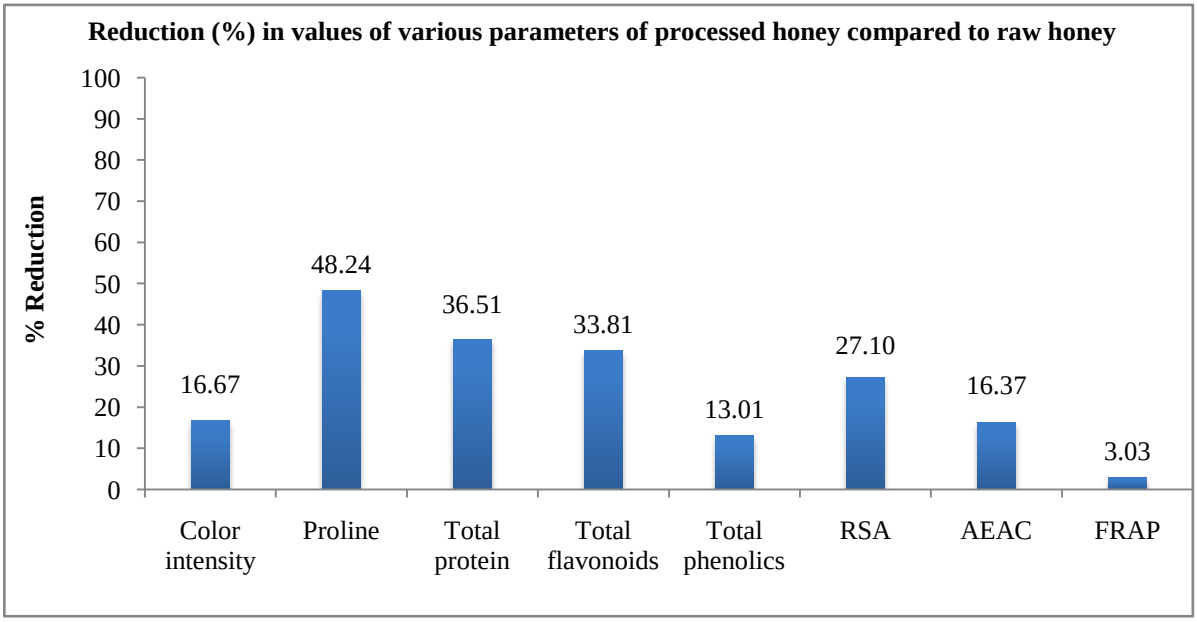

Fig 2 Reduction (%) in color intensity, proline, total proteins, total flavonoids, total phenolics and antioxidant properties (AEAC, RSA and FRAP) of processed honey considering the average values of raw honey as a standard.

Table 1 Brief account of the honey collection.

Honey samples	Collection	Collection period	Denoted by
Raw honey samples	Honey collection centers (n=16)	March 2011	KA1 to KA16
	Bee hives (n=9)	March 2011	KB1 to KB9
Processed honey samples	Honey processing unit (n=10)	August 2009 to July 2010	KC1 to KC10

Table 2 Color intensity (ABS_{450}), proline, total proteins, total flavonoids and total phenolics of tested raw (KA &KB) and processed (KC) honey samples.

Honey Samples		ABS_{450} (mAU)	Proline (mg/kg)	Total protein (mg/100g)	Total flavonoids (mg QE/100g)	Total phenolics (mg GAE /100g)
Raw samples KA (n=16)	Mean ± SD	56.75 ± 15.43	245.42 ± 29.89	309.94 ± 62.19	6.63 ± 2.68	42.29 ± 7.22
	Range	30.00-85.67	204.62-310.75	243.77-437.68	2.62-13.18	30.51-58.82
Raw samples KB (n=9)	Mean ± SD	44.89 ± 17.80	257.28 ± 20.43	345.78 ± 31.13	5.62 ± 1.46	43.06 ± 8.41
	Range	23.00-72.67	230.60-284.60	292.94-394.23	3.23-7.49	28.51-56.19
Average (KA & KB)		**52.48 ± 16.98**	**249.69 ± 27.04**	**322.84 ± 55.21**	**6.27 ± 2.34**	**42.57 ± 7.50**
Processed samples KC (n=10)	Mean ± SD	**43.73 ± 6.21**	**129.24 ± 9.83**	**204.97 ± 31.62**	**4.15 ± 0.57**	**37.03 ± 3.95**
	Range	36.00-58.33	114.62-147.05	175.78-267.45	3.29-5.35	30.09-42.72

Table 3 Antioxidant properties (AEAC, RSA and FRAP) of analyzed raw (KA &KB) and processed (KC) honey samples.

Honey Samples		AEAC (mg/100g)	RSA	FRAP (ABS_{700nm})
Raw samples KA (n=16)	Mean ± SD	15.90 ± 4.05	54.90 ± 12.71	0.30 ± 0.10
	Range	10.26-24.92	39.95-84.35	0.13-0.51
Raw samples KB (n=9)	Mean ± SD	17.89 ± 2.93	49.41 ± 6.23	0.39 ± 0.14
	Range	14.56-21.88	41.50-60.29	0.13-0.57
Average (KA & KB)		**16.62 ± 3.75**	**52.92 ± 11.01**	**0.33 ± 0.13**
Processed samples KC (n=10)	Mean ± SD	**13.90 ± 3.52**	**38.58 ± 4.47**	**0.32 ± 0.04**
	Range	9.50-19.68	35.07-49.47	0.27-0.39

Table 4 Correlation matrix displaying the interrelation amongst color intensity (ABS_{450}), proline, total proteins, total flavonoids, total phenolics, AEAC, RSA and FRAP.

	Color intensity	Proline	Total protein	Total flavonoids	Total phenolics	AEAC	RSA	FRAP
Color intensity	1	0.49 (**)	0.54 (**)	0.77 (**)	0.77 (**)	0.58 (**)	0.70 (**)	0.57 (**)
Proline		1	0.89 (**)	0.65 (**)	0.62 (**)	0.55 (**)	0.72 (**)	0.38 (*)
Total protein			1	0.68 (**)	0.71 (**)	0.73 (**)	0.76 (**)	0.54 (**)
Total flavonoids				1	0.81 (**)	0.62 (**)	0.85 (**)	0.60 (**)
Total phenolics					1	0.80 (**)	0.81 (**)	0.85 (**)
AEAC						1	0.67 (**)	0.67 (**)
RSA							1	0.55 (**)
FRAP								1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

4 conclusions:-

Honey of *Apis florea* collected from a wide range of arid zone of Kachchh region in Gujarat, by and large, showed significant *in vitro* antioxidant/radical scavenging activities. Honey contained good amount of total protein, phenols and flavonoids and fair amount of proline content. Thus, our study indicates the good quality of Kachchh honey for consumption as a source of antioxidants. Total phenolic content of honey was found least affected due to processing and also showed highest correlation with antioxidant activity. Pigment content (color intensity) correlated well with phenol and flavonoid contents. Total proteins and flavonoids also showed significant correlation with antioxidant content; whereas with proline content, the correlation of antioxidant properties was fairly significant. Therefore, the total phenolics were chiefly found to be influencing the antioxidant power of the Kachchh honey.

Acknowledgement:-

Authors are thankful to GSFDC, Gujarat for providing assistance in collection of honey samples. They are thankful to Sardar Patel University authorities for providing laboratory facilities and providing financial assistance to the senior author (JV) through DST-PURSE program.

References:-

1. 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.
2. Alvarez-Suarez, J. M., Tulipani, S., Diaz, D., Estevez, Y., Romandini, S., Giampieri, F., Damiani, E., Astolfi, P., Bompadre, S. and Battino, M. (2010): Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem. Toxicol., 48: 2490-2499.
3. Arvouet-Grand, A., Vennat, B., Pourrat, A. and Legret, P. (1994): Standardisation d'un extrait de propolis et identification des principaux constituants. J. Pharm. Belg., 49: 462-468.
4. Azeredo, L. D. C., Azeredo, M. A. A., De Souza, S. R. and Dutra, V. M. L. (2003): Protein content and physicochemical properties in honey samples of Apis Mellifera of different floral origins. Food Chem., 80: 249–254.
5. Beretta, G., Granata, P., Ferrero, M., Orioli, M. and Facino, R. M. (2005): Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal. Chim. Acta., 533: 185-191.
6. Bertoncelj, J., Doberšek, U., Jamnik, M. and Golob, T. (2007): Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem., 105 (2): 822-828.
7. Bogdanov, S. (2002): Harmonized method of the international honey commission, Swiss Bee Research Center, FAM, Liebefeld, CH-3003 Bern, Switzerland.
8. Castaldo, S. and Capasso, F. (2002): Propolis, an old remedy used in modern medicine. Fitoterapia, 73: S1–S6.
9. Chen, H. Y., Lin, Y. C. and Hsieh, C. L. (2007): Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem., 104 (4): 1418-1424.
10. de Novais, J. S., Absy, M. L. and Dos Santos, F. A. R. (2014): Pollen types collected by Tetragonisca angustula (Hymenoptera: Apidae) in dry vegetation in Northeastern Brazil. Eur. J. Entomol., 111(1): 25-34.
11. Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. and Estevinho, L. M. (2009): Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food chem., 114:1438-1443.
12. Gheldof, N. and Engeseth, N. J. (2002): 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. J. Agric. Food Chem., 50 (10): 3050-3055.
13. Jilani, I. B. H., Schweizer, P., Khouja, M. L., Zouaghi, M. and Ghrabi, Z. (2008): Physicochemical properties and pollen spectra of honeys produced in Tunisia (Southwest of KEF). Apiacta, 43: 38-48.
14. Kaakeh, W. and Gadelhak, G. G. (2005): Sensory evaluation and chemical analysis of Apis mellifera honey from the Arab Gulf region. J. food drug anal., 13 (4): 331-337.
15. Kalpana, T. P. and Ramanujam, C .G. K. (1990): Melitopalynology of Prosopis juliflora honeys from Ranga Reddy district (A.P) and its significance. Indian Bee J., 52 (1-4): 44-46.

16. Kesić, A., Mazalović, M., Crnkić, A., Ćatović, B., Hadžidedic, S. and Dragošević, G. (2009): The influence of L-Ascorbic Acid content on total antioxidant activity of bee-honey. Eur. J. Sci. Res., 32 (1): 95-101.
17. Krpan, M., Markovic, K., Saric, G., Skoko, B., Hruskar, M. and Vahcic N. (2009): Antioxidant activities and total phenolics of Acacia honey. Czech J. Food Sci., 27: S245-S247.
18. Marcucci, M. C. (1995): Propolis: Chemical composition, biological properties and therapeutic activity. Apidologie, 26: 83-99.
19. Meda, A., Lamien, C. E., Romito, M., Millogo, J. and Nacoulma, O. G. (2005): Determination of total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571-577.
20. Molan, P. C. (1992): The antibacterial activity of honey. 1: The nature of antibacterial activity. Bee World, 73 (2): 5-28.
21. Oddo, L. P., Heard, T. M., Malaver, A. R., Perez, R. A., Muino, M. F., Sancho, M. T., Sesta, G., Lusco, L. and Vit, P. (2008): Composition and antioxidant activity of Trigona carbonaria honey from Australia. J. Med. food, 11(4): 789-794.
22. Orhan, F., Sekerel, B. E., Kocabas, C. N., Sackesen, C., Adalioglu, G. and Tuncer, A. (2003): Complementary and alternative medicine in children with asthma. Ann. Allergy Asthma Immunol., 90: 611-615.
23. Ortiz Valbuena, A., Fernandez Maeso, M. C. and Subra Munoz de la Torre E. (1996): Principales caracteristicas de la miel de La Alcarria. Consejeria de Agricultura y Medio Ambiente de Castilla la Mancha, Castilla la Mancha, Spain.
24. Ouchemoukh, S., Louaileche, H. and Schweizer, P. (2007): Physicochemical characteristics and pollen spectrum of some Algerian honey. Food Control., 18: 52-58.
25. Ough, C. S. (1969) : Rapid determination of proline in grapes and wine. J. Food Sci., 34 : 228-230.
26. Oyaizu, M. (1986): Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi, 35:771-775.
27. Patel, R. M., Vijay Kumar, V. and Ashish Kumar (2012): Structure and composition of woody vegetation in western Kachchh. J. biosci. res., 3(3): 163-174.
28. Perez, R., A., Iglesias, M., T., Pueyo, E., Gonzalez, M. and Lorenzo, C. (2007): Amino acid composition and antioxidant capacity of Spanish honeys. J. Agric. Food Chem., 55: 360-365.
29. Sajwani, A., Farooq, S. A., Patzelt, A., Eltayeb, E. A. and Bryant, V. M. (2007): Melissopalynological Studies from Oman. Palynology, 31: 63-79.
30. Sangsrichan, S. and Wanson, W. (2008): The antioxidant capacity of honey samples collected in the north part of Thailand in relationship with its total polyphenol. KMITL Sci. J., 8 (2): 68-73.
31. Saric, G., Matkovic, D., Hruskar, M. and Vahcic, N. (2008): Characterisation and classification of Croatian honey by physicochemical parameters. Food Technol. Biotechnol., 46 (4): 355-367.
32. Saxena, S. K. (1998): Ecology of Prosopis juliflora in the arid regions of India. In: Prosopis Species in the Arid and Semi-Arid Zones of India on November 21-23, 1993 (Chapter II): Proceedings of a conference held at Central Arid Zone Research Institute: Jodhpur, Rajasthan, India.
33. Saxena, S., Gautam, S. and Sharma, A. (2010): Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem., 118 (2): 391-397.
34. Shah, J. and Somusundaram, S. (2010): Preliminary GIS and remote sensing analysis on Banni grasslands, Kachchh. J. Pure Appl. Sci., 18: 15-17.
35. Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods enzymol., 299: 265-275.
36. The Geological Survey of India (GSI) (n. d.): Kachchh Peninsula and the Great Rann. Ministry of Mines, Government of India. [Internet] http://www.portal.gsi.gov.in/portal/page?_pageid=127,693641&_dad=portal&_schema=PORTAL (Accessed May 11, 2015).
37. Thimmaiah, S. K. (2006): Standard methods of biochemical analysis. New Delhi: Kalyani Publishers, pp. 94-96.

38. Tiwari, J. K. and Rahmani, A. R. (1999): An army of mad trees. Down to earth. [Internet] http://www.downtoearth.org.in/node/19669 (Accessed August 2, 2013).
39. Vallianou, N.G., Gounari, P., Skourtis, A., Panagos, J. and Kazazis, C. (2014): Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. Gen. Med., 2: 132.
40. Varshney, A. (1996): Overview of Use of Prosopis juliflora for Livestock Feed, Gum, Honey, and Charcoal as Well as Its Role in Combating Drought and Desertification: Regional Case Studies from Gujarat, India. In: Prosopis: Semiarid fuel wood and forage tree: Proceedings of the workshop on Building consensus for the disenfranchised held at Washington, D.C., U.S.: National Academy of Sciences, pp. 6-40.
41. Wu, H. C., Shiau, C. Y., Chen, H. M. and Chiou, T. K. (2003): Antioxidant activities of carnosine, anserine, some free amino acids and their combination. J. Food Drug Anal., 11: 148-153.