ORIGINAL RESEARCH PAPER

Biotechnology

INVESTIGATION OF NUTRACEUTICALS OF NATURAL APIS HONEY FROM KOLAR OF KARNATAKA, INDIA.

KEY WORDS: *Apis* honey, honey quality, nutraceuticals, total ash content.

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ABSTRACT

Natural honey is a sweet, flavourful liquid food of high nutritional value (Bogdanov *et al.,* 2008), and immense health benefits (Ajibola *et al.,* 2007). Honey is a remarkable food product of honeybees with high-calorific value. The nutraceuticals of natural honey is highly variable with the species of honeybee, the geographical area and botanical origin. The most medicinal properties of honey along with its flavour depend on its mineral content. In the current study, honey samples of *Apis florea, Apis mellifera, Apis dorsata* and *Apis cerana* were collected from Kolar district of Karnataka. Total ash content was estimated by Ivanov and Chevanakova method (1984). The quantitative mineral analysis of honey samples were performed by Atomic absorption spectrophotometer and flame photometer (A. Mbiri *et al* 2011). The total ash content ranged between 0.11 to 0.26 per cent. The F-test and analysis of variance values of total ash content parameter of honey samples were significant at 5% levels. The mineral nutrients with high frequency were; Ca 3.87± 0.05, Cr 0.09±0.001, Cu 0.02±0.001, Fe 0.04±0.003, Mg 0.18±0.007, Mn 0.13±0.04, P 0.84 ±0.01, K 49.21±0.01, Na 2.94±0.01 and Zn 0.07±0.004 per cent. All the mineral contents were within range and varied significantly at $p < 0.05$ levels. From present study, it is observed that the Indian honey is good in its quality.

INTRODUCTION

Honey is one of the oldest and best loved sweetening agent for foods and over the centuries, it has still retained a "natural" image (Aparna and Rajalakshmi, 1999). The raw material for the production of "Floral" honey is nectar, a dilute solution of sugars found in the nectaries of flowering plants. Honey is an easily digestible food stuff that contains a range of nutritionally important compounds (Celechovoska and Vorlova, 2001). The major components of honey include various saccharides, water, amino acids, minerals, proteins, vitamins and unstable compounds such as enzymes (Qiu *et al.,* 1999). Thus, honey is generally considered as a natural and healthy product (Reybroeck, 2003).

Several different surveys have been compiled on the nutritional and health aspects of honey (Al-Quassemi et al., 2003; Molan P., 2001). However, the nutritional value and medicinal properties of natural honey are too numerous and highly inexhaustible to be comprehensively documented by these manuscripts. The records of honey as functional health food and uses of other honey-bee products are still incipient. Thus, the need to review some relevant materials on natural honey becomes imperative. This present study reveals that the natural honey values as a nutrient food and encourage the economic importance of natural honey production and other apicultural practices.

Karnataka produces 800 to 900 tons of honey annually. The characteristics of honey from different floral sources influence the commercial value and the consumer preferences (Shripad and Rangaswamy, 2001). Honey also contains minerals and heavy metals, which play important role in determining honey qualities. The mineral content varies, ranging from 0.04% in pale honey to 0.20% in darker honey (Bogdanov *et al.,* 2007). Darker honey has comparatively higher mineral content than the lighter honey. The major minerals are mainly derived from the soil and nectar-producing plants, but they may also come from anthropogenic sources, such as environmental pollution. It has been reported that micro- or trace minerals originating from organic or plant sources are important for maintaining human health, while those which originate from inorganic or metallic sources, such as heavy metals, can be toxic (Hernandez *et al.,* 2005; Pohl 2009). Honey has also been used as an indicator for a variety of environmental contaminants, including heavy metals, low-level radioactivity, and pesticides (Nalda *et al.*, 2005).

The aim of present investigation is to determine essential metals and heavy metals like Ca, Cr, Cu, Fe, Mg, Mn, P, K, Na and Zn in *Apis* honey samples collected from various regions of Kolar district of Karnataka, India.

MATERIALS AND METHODS

Study area

The present study area of Karnataka was Kolar district.

Procurement of *Apis* honey samples

The honey samples (n=130) of *Apis florea, Apis mellifera, Apis cerana Apis dorsata,* were harvested from various geographical areas of Kolar, Karnataka during 2020. Each honey sample was first filtered with a sterile mesh to remove debris. All the samples were collected and transported in sterile sealed bottles or screwed cups with authentic labels. Four replications of bottles for each sample were kept under storage at 2-8 ^{0}C until tested as per the method proposed by Nzeako and Hamdi (2000).

Determination of total ash content in honey

5-10g of honey was accurately weighed in a silica or platinum dish whose weight was pre-determined. Few drops of pure olive oil were added to prevent the spattering of honey. Then, it was heated carefully over a low flame until swelling ceases. It was then kept in the muffle furnace at 600 ± 20 ^{0}C till white ashes obtained. The dish was cooled in a desiccator and weighed (Ivanov and Chevanakova, 1984).

The ash content in honey sample was calculated by using the following formula:

$$\text{Ash (\% by mass)} = \frac{100 \times (m_2 - m)}{(m1 - m)}$$

m= mass in g. of empty dish
m1= mass in g. of dish with 5-10ml of honey
m2=mass in g. of dish with ash

The results were expressed as mg/Kg.

Analysis of minerals or nutraceuticals in Honey Samples

5 g of each sample was weighed using an analytical balance, transferred into a beaker, digested using nitric/perchloric acid and filtered into a 50 ml volumetric flask. Distilled water was used to make the solution to the mark. For the determination of calcium, strontium was added to reduce interferences from aluminum and phosphorous (A. Mbiri *et al*

2011). Potassium and Sodium was determined using flame photometer. Calcium, Iron, Zinc, Copper, Phosphorus, Manganese and Chromium were determined using Atomic absorption spectrometer (Mudasar Manzoor *et al.,* 2013 and Rodriguez-otero *et al., 1*994). Data of all mineral contents of honey samples were analyzed by Analysis of Variance (ANOVA) along with F test, highly significant values were determined by using F table (p≤.005).

RESULTS

The nutraceutical composition of *Apis* honey samples of Kolar of Karnataka showed significant variations. The total ash content of *Apis* honey samples of Kolar district ranged from 0.11 to 0. 26 per cent (Fig.1). The honey samples of *Apis florea* of Kolar district ranged from 0.11 to 0.26 per cent, *Apis cerana* between 0.11 and 0.26 per cent, *Apis mellifera* from 0.12 to 0.26 per cent and *Apis dorsata* from 0.13 to 0.26 per cent.

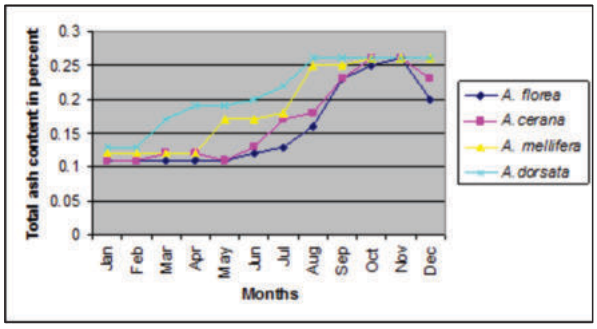

Fig.1. Showing the total ash content of Apis honey samples from Kolar during 2020.

The F-test and analysis of variance values of total ash content parameter of honey samples from Kolar were significant at 5% levels.

Table. 1 – Nutraceutical Constituents of Apis honey samples from Kolar, Karnataka during 2020.

Mineral Type	Honey Samples			
	Apis florea **(ppm±S.E)**	***Apis cerana*** **(ppm±S.E)**	***Apis mellifera*** **(ppm±S.E)**	***Apis dorsata*** **(ppm±S.E)**
Calcium	1.1±0.01	1.9±0.06	2.43±0.03	3.87±0.05
Chromium	0.003±0.001	0.005±0.001	0.008±0.002	0.09±0.001
Copper	0.01±0.001	0.01±0.001	0.02±0.001	0.02±0.001
Iron	0.04±0.04	0.02±0.01	0.03±0.01	0.04±0.03
Magnesium	1.09±0.03	1.06±0.01	1.01±0.01	1.01±0.002
Manganese	0.03±0.001	0.09±0.001	0.10±0.005	0.13±0.004
Phosphorus	0.48±0.03	0.66±0.04	0.84±0.01	0.69±0.03
Potassium	30.05±0.01	34.22±0.02	38.62±0.02	49.21±0.01
Sodium	2.49±0.05	2.33±0.06	2.79±0.01	2.94±0.01
Zinc	0.02±0.001	0.04±0.001	0.03±0.001	0.07±0.004

Significant at p< 0.05 levels

DISCUSSIONS

In the present analysis of total ash content in *Apis* honey ranged from 0.11 and 0.26 percent. Similar findings were reported by Bonvehi and Coll (1993) reported 0.06 to 0.39 percent of average ash content in French lavender honey of Spain. Anass *et al* (2003) analysed average ash content with 0.16 to 0.44 percent in Eucalyptus honey.0.64 ±0.11 to 1.67±0.25 per cent was reported by Kambai *et al.,* 2015 in the Honey of Nigeria.

The major minerals of honey recorded were potassium, iron, manganese, magnesium, calcium, sodium, phosphorus, zinc and chromium. The highest content being potassium with 49.21±0.01 ppm in *Apis dorsata* honey and least with 30.05±0.01 ppm in *Apis florea* honey. The values of K in this study were less than the values recorded in earlier studies where mean values in honey were found to be 2310, 1774 and 3166 ppm from Kicevo, Zulia and Tenerife respectively (Frias *et al.*, 2008; Betzabé Sulbarán de Ferrer *et al.,* 2004). The mean K contents of 965 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia. The lower values of K content in present samples may be influenced by climatic factors, storage conditions or lowest values of NPK in that particular area (Fakhri *et al.,* 2019).

The mineral calcium content is highest in *Apis dorsata* honey with 3.87±0.05ppm and 1.1±0.01ppm was recorded least in *Apis florea* honey samples. 3.67 ±0.70 to 7.20±0.70 per cent was reported by Kambai *et al.,* 2015 in the Honey of Nigeria. The highest Ca value of present honey samples may be influenced by the time of extraction from the comb in relation to ripening process by the bees, temperature conditions, seasons or geographical zone. The mean Ca contents of 85.2 mg/kg were reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral chromium content is also highest in *Apis dorsata* honey with 0.09±0.001 ppm and 0.003±0.001ppm was recorded least in *Apis florea* honey samples. Similar correlated results were reported by Mossel (1998), Karbournioti and Drimjias (1997) in Australia, Greek and grass land honey respectively. The mean Cr contents of 0.0077 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral copper content is highest in *Apis dorsata* and *Apis mellifera* honey with 0.02±0.001ppm and 0.01± 0.001ppm was recorded least in *Apis florea and Apis cerana* honey samples. The highest concentration of Cu was recorded in honey sample *Apis dorsata* from Western Ghats of Tamil Nadu with a value of 0.624 ppm while the lowest concentration of Cu was recorded from honey sample *Apis mellifera* from Jammu and Kashmir with value of 0.275 ppm (Mudasar Manzoor *et al.,* 2013). The concentration of Cu in present samples were lower, compared to the values recorded in Swiss and Tenerife honey which were 0.88 and 1.28 ppm (Stefan *et al.,* 2007) and has a closer values reported by A. Mbiri,*et al (*2011) ranging between 0.02 and 0.03. The mean Cu contents of 0.2 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral iron content is highest in *Apis dorsata* honey with 0.04±0.003 ppm and 0.02±0.001ppm was recorded least in *Apis cerana* honey samples. The Fe content was recorded highest in the honey sample *Apis mellifera* with a value of 2.800 ppm from hills of Jammu and Kashmir and the lowest level was recorded in the honey sample *Apis cerana* from hills of Tamil Nadu with a value of 0.69 ppm (Mudasar Manzoor *et al.,* 2013). In our study the values of Fe recorded were lower than those values of 13.5 and 3.37 ppm reported earlier in Zulia and Tenerife (Frias *et al.,* 2008), Mudasar Manzoor *et al.,* 2013 and closer to values reported earlier by A. Mbiri,*et al* 2011, ranging between 0.08 and 0.59 ppm. The mean Fe contents of 3.1 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral magnesium content is highest in *Apis florea* honey with 1.09±0.03 ppm and 1.01±0.002 ppm was recorded least in *Apis dorsata* honey samples. The highest concentration of Mg was found in *Apis mellifera* from Australia with a value of 28.7mg/kg reported by Natasha *et al.,* 2020.

The mineral manganese content is highest in *Apis dorsata* honey with 0.13±0.004 ppm and 0.02±0.01 ppm was recorded least in *Apis cerana* honey samples. The highest concentration of Mn was found in *Apis dorsata* from Western Ghats of Tamil Nadu with a value of 1.126 ppm reported by Mudasar Manzoor *et al.,* 2013. Mahmood Ahmed *et al.,* (2016) reported 0.73 to 0.97 ppm Mn in the Pakistan honey samples. The mean Mn contents of 3.8 mg/kg was reported by Natasha *et al.,* 2020 in

honey samples of *Apis mellifera* of Australia.

The mineral phosphorus content is highest in *Apis mellifera* honey with 0.84±0.01 ppm and 0.48±0.03 ppm was recorded least in *Apis florea* honey samples. The range is in accordance with the results reported by Bhushanam and Madhusudan (2017). The mean P contents of 51.5 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral sodium content is highest in *Apis florea* honey with 2.94±0.01 ppm and 2.33±0.06 ppm was recorded least in *Apis cerana* honey samples. 122.8 to 181.7 ppm was reported by Mahmood Ahmed *et al.,* (2016) in the Pakistan honey samples. Mudasar Manzoor *et al.,* (2013) reported 25.17 ppm of Na in *Apis cerana* honey samples from Tamilnadu and Kashmir. The mean Na contents of 99.7mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

The mineral zinc content is highest in *Apis dorsata* honey with 0.07±0.004 ppm and 0.02±0.001 ppm was recorded least in *Apis florea* honey samples. In *Apis* honey samples, Ciobanu Radukscu (2016) reported 0.987 mg/kg of Zn, Berinde and Michnea (2013) reported 1.09 to 1.39 range of Zn in *Apis* honey. The mean Zn contents of 6.0±16.7 mg/kg was reported by Natasha *et al.,* 2020 in honey samples of *Apis mellifera* of Australia.

Mahajan (1984) analyzed honey from Shimla and reported that dark colored honey have more minerals than light colored honey of *Apis cerana*. Though, the quantity of minerals was less, they play a vital role in determining the color, medicinal and nutritional value of honey. From present study, it is observed that the Indian honey is good in its quality.

CONCLUSIONS

The present study reported that the nutrient analysis of honey is essential for nutritional quality and safety of honey with regards to the contents of major, minor and trace elements. The study has provided comprehensive data on elemental levels of Kolar *Apis* honey, which would be useful for assessing the quality of honey for its nutritional importance. Because of the health benefits, there has been an increased interest in the consumption of honey products. Of all the minerals tested in the honey samples, *Apis dorsata* honey from Kolar have high contents of K, Fe, Mn, Na, Ca, Cr, Cu and Zn as compared to the rest of the honey samples. While the P content is high in *Apis mellifera honey*. The results of the present study reveal that Indian honey quality with respect to the concentration of these mineral elements in safety baseline levels for human consumption of products. The study also ensure confidence in the usage of honey for medicinal applications.

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