IN VITRO ANALYSIS OF PHYSIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF HONEY FROM CORIANDER ECOSYSTEM

USHA RANI, B.[1] – MADHU VANDHI, G.[1] – SURESH, K.[2*]

[1]Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India (e-mails/phones: usharani.b@tnau.ac.in/+91-948-844-8760; madhuvandhigv@gmail.com/+91-962-619-2687)

[2]Indian Council of Agricultural Research – Krishi Vigyan Kendra, TNAU, Madurai, Tamil Nadu, India

**Corresponding author*
e-mail: suresh.k@tnau.ac.in; phone: + 91-99-652-88760; ORCID: 0000-0003-0311-0283

(Received 24th Aug 2024; accepted 18th Dec 2024)

Abstract. Honey is a nutrient-rich, naturally sweet substance with medicinal properties. Its chemical composition depends on several factors such as the source flower, bee species, storage etc. Hence an in vitro analysis was conducted to assess the physiochemical and antioxidant properties of honey collected from the hives placed in coriander ecosystem. The honey collected from little bee, *Apis florea* F. and Stingless bee, *Tetragonula iridipennis* S., colonies placed in coriander ecosystem, was compared with multifloral Indian Bee, *A. cerana indica* honey and *T. iridipennis* honey purchased from bee keeping farms and, *A. florea* honey collected from natural hive. The results revealed that all the physiochemical properties were significantly different from each other and they were in the range of pH- 3.53- 4.98, EC- 1.82- 0.56 mS/cm, ash content- 34.4- 65.6%, moisture content- 18.38- 25.15% proteins- 37.32- 56.00 mg/100 g, free amino acids- 0.21- 0.33 mg/g, phenols (122 mg GAE/100 g) and flavonoids- 2.29- 5.51 mg QE/100 g and all the values were high in the case of stingless bee honey from coriander field except flavonoids. The flavonoid content was maximum in the honey of *A. florea* collected from coriander field (5.51 mg QE/100 g). Antioxidant properties like AEAC, RSA and FRAP of honey samples had a range of 24.54- 43.86 mg/100 g, 28.28- 81.14% and 0.26-0.66 ABS/700 nm, respectively. The antioxidant properties were more in stingless bee honey from coriander field. Specifically, this honey exhibited superior levels of antioxidant activity, as measured by assays such as AEAC (Antioxidant Equivalent Activity), RSA (Radical Scavenging Activity), and FRAP (Ferric Reducing Antioxidant Power). These results suggest that the honey from the coriander field has enhanced potential for neutralizing harmful free radicals and protecting against oxidative stress, which may contribute to its medicinal and health-promoting properties.
Keywords: *antioxidant activity, Apis florea, floral source, Tetragonula iridipennis, physiochemical properties*

Introduction

Honey is sweet stable, high density and high energy food produced by bees from the nectar flowers by converting the sucrose in the nectar to glucose and fructose. Honey is a supersaturated solution of sugars (about 78%), that contains mainly fructose, glucose, sucrose. The sugars make honey hygroscopic nature (moisture absorbing) and viscous, and the sugar concentration plus other factors including minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals makes honey antimicrobial and prevent microbial growth Terrab et al. (2002).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

In Vedas, the honey was considered as one of the nature's most precious gift. This stands a high place in the society because of its great value, as medicinal effect for human beings and for its use in a number of ceremonial occasions. However, the development of scientific bee keeping and the availability of apiary honey in plenty, the usage of honey in the daily routine diet is increased. Honey is a complex, natural product synthesized by honeybees through the transformation of plant nectar into a stable, nutrient rich substance. The color, flavor, and composition of honey are influenced by various factors, including the floral sources visited by the bees, regional climatic and environmental conditions, the specific bee species involved, and storage practices (Guler et al., 2007). This intricate process not only results in a high energy food source for bees but also creates a product valued by humans for its nutritional, medicinal, and culinary applications.

Beekeeping practices, such as colony management, hive placement, and the selection of specific plant ecosystems, can significantly affect honey production. Different bee species, for ex. may exhibit varying foraging behaviors and preferences for particular plants, leading to differences in honey characteristics.

In this context, the coriander ecosystem is of particular interest. Coriander flowers offer a distinctive nectar the coriander ecosystem is of particular interest source with unique aromatic and medicinal qualities, contributing to honey with a distinctive flavor and composition. The selection of this plant ecosystem is justified by its potential to produce honey with high commercial value, particularly for its flavor profile and potential health benefits

Limited number of studies had been conducted to find out the medicinal properties of honey collected from different cropping system. Hence, the present study was conducted to evaluate the physiochemical and antioxidant properties of honey collected from the hives placed in the coriander ecosystem.

Materials and methods

To study the physiochemical properties in honey, respective bee colonies viz., *Apis florea* and *Tetragonula iridipennis* colonies were placed at five per cent flowering during night time in the coriander ecosystem of 0.20 ha (half acre) field during *Rabi* season 2019-2020 at Lalapuram village of Kallikudi block, Madurai district, Tamil Nadu, India. The design was RBD and treatments were replicated thrice.

Multifloral Indian Bee, *A. cerana indica* honey and Stingless bee, *T. iridipennis* honey were purchased from bee keeping farms and little bee honey, *A. florea* was collected from natural hive. The honey from natural hive was collected in a sterilized polythene bottle and filtered through hygienic fine cloth to remove dirt's, pollen *etc*., and later all honey samples was stored in airtight container at ambient temperature. Coriander honey was collected from installed honey bee hive after completion of coriander season.

The inclusion of a third species, with honey collected from a natural hive, was intended to provide a comparative baseline for evaluating the physiochemical properties of honey produced by *Apis florea* and *Tetragonula iridipennis* colonies. This comparison allows for a better understanding of the variations in honey characteristics that may arise from different bee species and natural foraging conditions

The following physiochemical properties of all honey samples was worked out. Potential of Hydrogen (pH), Electrical conductivity (EC), Moisture content, Ash

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

content, total proteins Lowry et al. (1951) amino acid estimation (Moore and Stein, 1948), total phenol content (Laaroussi et al., 2020; Bakour et al., 2018), total flavonoids Melo et al. (2005) and antioxidant properties like Ascorbic acid equivalent antioxidant contents (AEAC), Radical scavenging activity (RSA) and Ferric reducing antioxidant power assay (FRAP) Saxena et al. (2010). The laboratory study data were compared by Least Significant Difference (LSD) at 5 percent probability with CRD design to assess the physiochemical properties of honey by using Webagri State Package 2.0 software.

Results

Physiochemical properties of honey

The physiochemical property of honey was presented in the *Table 1*. The pH of stingless bee honey from coriander field was recorded maximum with a value of 4.98 in the Indian bee it was least 3.53. The range of pH in the honey samples was 3.53-4.98. The Electrical Conductivity (EC) of various honey was in the range of from 0.56-1.82 mS/cm. EC of stingless bee honey from coriander field was found maximum (1.82 mS/cm) than stingless bee honey purchased collected from multifloral honey sample (1.62 mS/cm). Ash content was maximum in stingless bee honey collected from coriander filed (65.6%) and minimum in Indian bee honey (34.4%). Moisture content was in the range of 18.38-25.15 per cent and found maximum in Indian bee honey (25.15%).

Table 1. *Physiochemical and antioxidant properties of honey samples unifloral (Coriander ecosystem) and multifloral honey*

Sl. No.	Treatments	pH*	EC* (mS/cm)	Ash*** (%)	Moisture** (%)	AEAC*** (mg/100 g)	RSA*** (%)
1.	Unifloral *A. florea* honey from coriander ecosystem	4.63 (0.66)^{c}	1.30 (1.14)^{c}	56.2 (48.54)^{b}	21.76 (27.79)^{b}	36.92 (37.40)^{b}	55.70 (48.25)^{c}
2.	Unifloral *T. iridipennis* honey from coriander ecosystem	4.98 (0.69)^{a}	1.82 (1.34)^{a}	65.6 (54.06)^{a}	18.38 (25.38)^{c}	43.87 (41.46)^{a}	81.14 (64.23)^{a}
3.	Multifloral honey from *A. cerana indica*	3.53 (0.54)^{e}	0.56 (0.74)^{e}	34.4 (35.89)^{c}	25.15 (30.08)^{a}	24.54 (29.68)^{d}	28.28 (32.11)^{e}
4.	Multifloral honey from *A. florea*	4.45 (0.64)^{d}	1.15 (1.07)^{d}	54.2 (47.39)^{b}	21.58 (27.66)^{b}	31.56 (34.17)^{c}	46.96 (43.24)^{d}
5.	Multifloral honey from *T. iridipennis*	4.82 (0.68)^{b}	1.62 (1.27)^{b}	64.8 (53.58)^{a}	17.85 (24.98)^{d}	38.34 (38.24)^{b}	66.94 (54.88)^{b}
	S Ed	0.003	0.007	0.043	0.084	0.55	0.82
	CD (p = 0.05)	**0.007**	**0.016**	**0.091**	**0.176**	**1.16**	**1.71**

Each value is a mean of five replications
Figures in the parentheses are transformed values (*log transformation, **square root transformation, ***Arcsine transformation)
In a column, means followed by same letter(s) are on par by LSD (p = 0.05)

Protein content of honey was maximum in stingless bee honey from coriander field and ranges from 37.32- 56.00 mg/100 g (*Fig. 1*). Free amino acids also had the same scenario with a range of 0.21- 0.33 mg/g (*Fig. 2*). This was followed by honey of little bee honey collected from multifloral and coriander ecosystem. The total phenol content in honey sample was presented in *Figure 3*. The phenol content was maximum in stingless bee honey from coriander field (122 mg GAE/100 g). This was followed by little bee honey from coriander field (103.8 mg GAE/100 g). But multifloral Indian bee

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

honey had minimum amount of total phenol content (73.8 mg GAE/100 g). The flavonoid content in honey samples varied significantly from each other. The value was maximum in little bee honey from coriander field (5.51 mg QE/100 g). The range was about 2.29- 5.51 (*Fig. 4*).

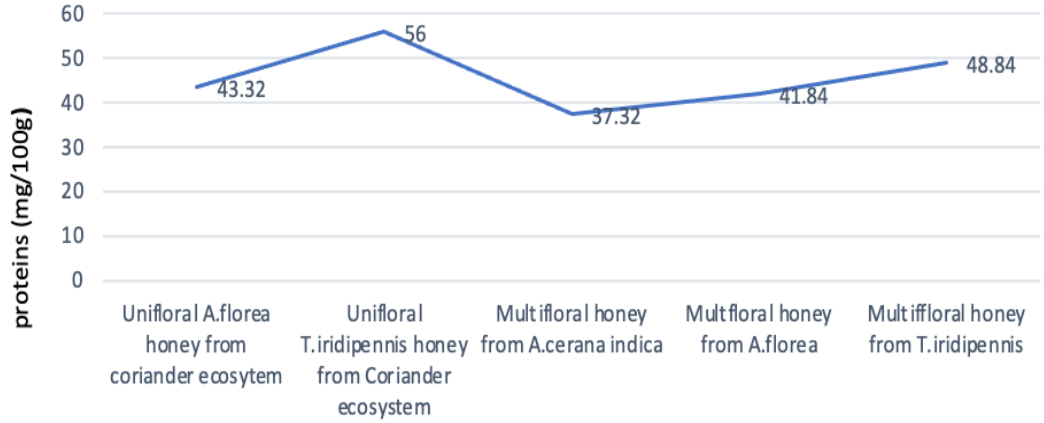

Figure 1. *Protein content of honey from unifloral (coriander) and multifloral ecosystem*

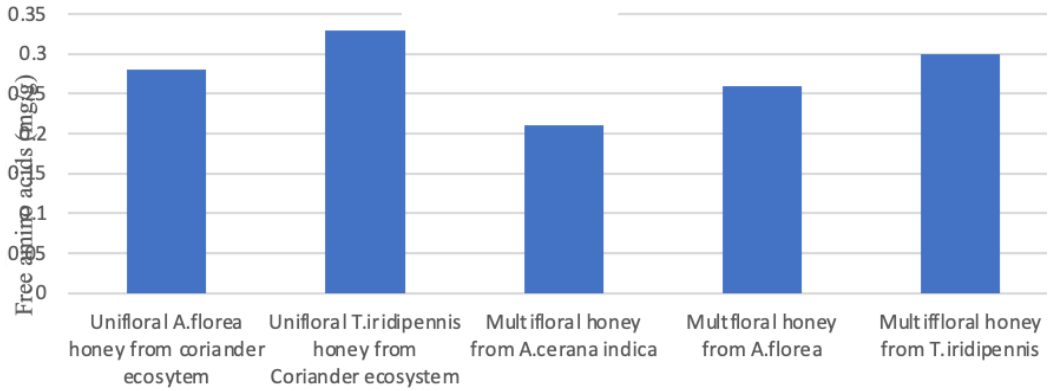

Figure 2. *Total amino acid content in unifloral (coriander) and multifloral honey*

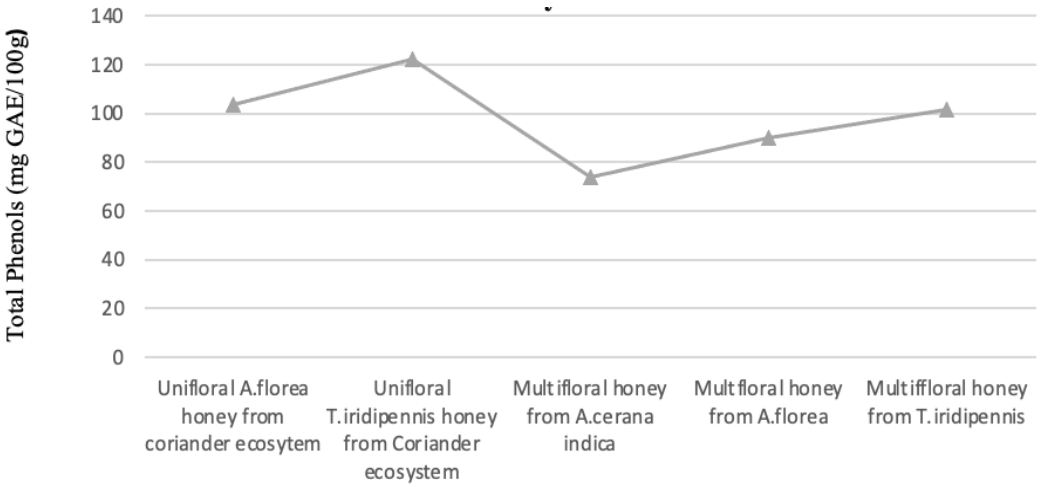

Figure 3. *Total phenol content in unifloral (coriander) and multifloral honey*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

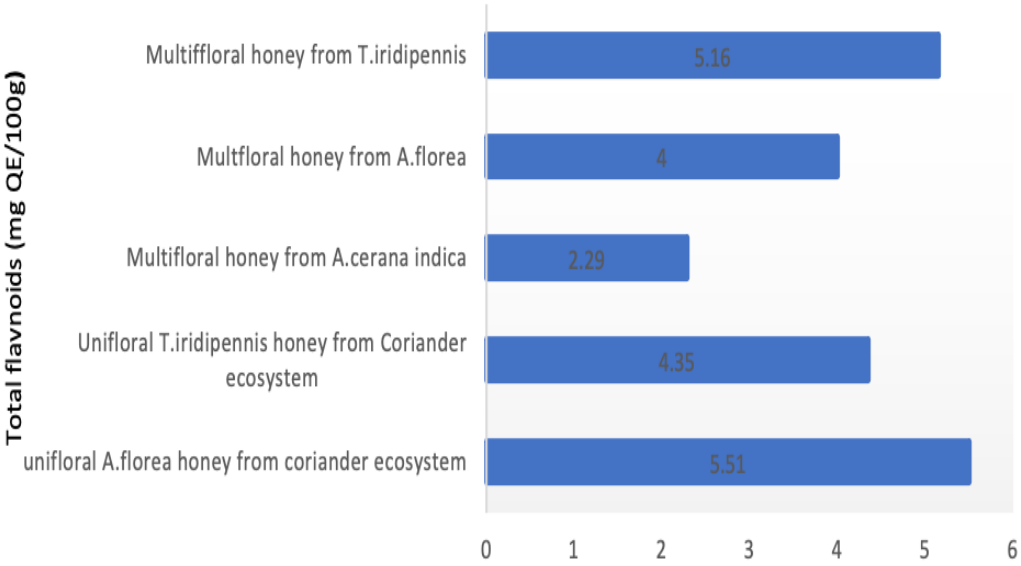

Figure 4. *Total flavonoid contents in unifloral (coriander) and multifloral honey*

Antioxidant properties in honey

AEAC was maximum in stingless bee honey collected from coriander field (43.87 mg/100 g) followed by stingless bee honey (38.34 mg/100 g) and it was on par with little bee honey (36.92 mg/100 g). The value was minimum in Indian bee honey (24.54 mg/100 g). RSA per cent was maximum in the case of stingless bee honey from coriander field (81.14%). The value ranged from 28.28- 81.14 per cent. FRAP value (*Fig. 5*) was in the range of 0.26- 0.66 (ABS/700 nm) and it was significantly different from all the honey samples.

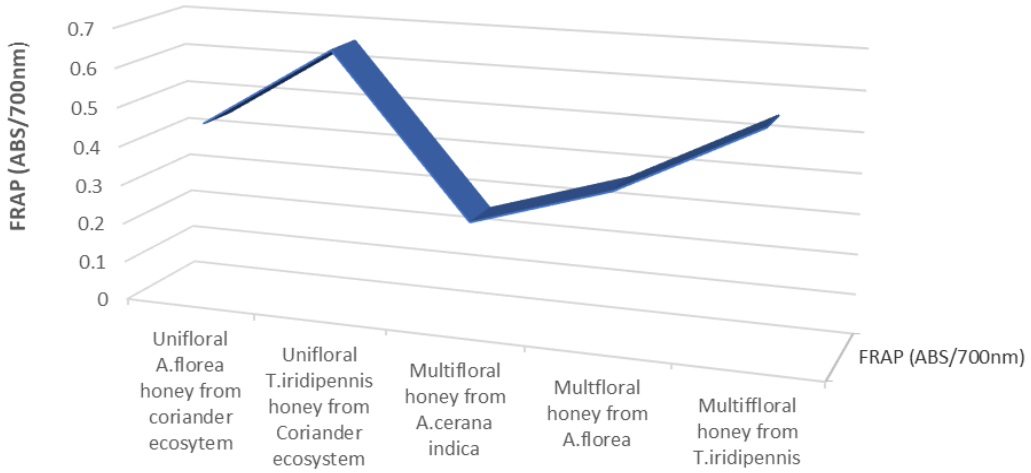

Figure 5. *Estimating the antioxidant properties of unifloral (coriander) and multifloral honey through FRAP assay*

Discussion

In the present study, the pH range observed in the honey samples was 3.53 to 4.98, aligning closely with the findings of previous research conducted by Bezabeh et al.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

(2004) who reported that honey is characteristically acidic in nature, it consists of organic acids, particularly gluconic, pyruvic, malic acid and citric acids, lactones, esters and some inorganic icons such as phosphate and chloride. All honeys are acidic with a pH-value generally lying between 3.5 and 5.5, due to the presence of organic acids that contribute to honey flavor and stability against microbial spoilage (Bogdanov et al., 2004).

The ashes of honey give an indication of geographical origin. The Electrical conductivity (EC) of the honey is related to the concentration of mineral salts, organic acid and proteins and proved to be useful for discriminating honey of different floral origins (Acquarone et al., 2007). Other factors, such as floral source, number of organic acids and proteins, and storage time can also influence the electric conductivity of honey. The results of the present study revealed that EC of stingless bee honey from coriander field was found maximum (1.82 mS/cm). Hence stingless bee honey from coriander field had good medicinal value.

Protein content of honey was maximum in stingless bee honey from coriander field. Previous work conducted by Won et al. (2009), suggested that proteins from honey originate from plant on which the bees forage or honeybees themselves and the amount depend on the species of honeybees as well as environmental factors. The results support the findings of Szeles et al. (2008) who reported that coriander honey had a high protein content of 1084 mg/kg. The findings are in accordance with the reports of Boussaid et al. (2018) where unifloral honey from mint had high protein content.

In the present study, stingless bee honey from coriander flowers exhibited higher phenol content compared to other honey varieties. This observation may be attributed to the darker coloration of the stingless bee honey. Comparable findings were also noted in previous research by Amiot et al. (1989), who stated that dark-colored honeys are reported to contain more phenolic acid derivatives but less flavonoids than light-colored ones.

The study conducted by Meziti et al. (2012) reported that black cumin (*Nigella sativa* L.) is a good source of phenolic compounds and exhibits high antioxidant capacity. They also reported that the high phenolic content in the plant parts of the black cumin (*Nigella sativa, Family: Ranunculaceae*) tree is transferred into the nectar collected by the bees and introduced to the honey, contributing to the high phenolic contents observed in this type of honey. As flavonoids and other phenolic compounds come exclusively from plants, it is clear that their content will be defined by botanical origins of honey (Šedík et al., 2019).

Antioxidant properties like AEAC, RSA and FRAP of honey samples had a range of 24.54- 43.86 mg/100 g, 28.28- 81.14% and 0.26-0.66 ABS/700 nm, respectively. The antioxidant properties were more in stingless bee honey from coriander field. Hence in the present study coriander floral aroma may influenced the flavonoid content of little bee honey. It is inferred that phenolics and flavonoids are responsible for its marked antioxidant activity. This is consistent with several reports that have shown close relationship between total phenolic contents and antioxidative activity (Cheung et al., 2019). The results were also supported by Boussaid et al. (2018) that a positive correlation was observed between the DPPH and Radical scavenging activity and the total polyphenol ($r = 0.945$, $p < 0.01$) antioxidant activities and total flavonoids ($r = -0.866$, $p < 0.01$) as well as total flavonoids and total polyphenols ($r = 0.957$, $p < 0.01$).

Antioxidant activity of aqueous extract of coriander on carotene and linoleic acid oxidation has been studied (Melo et al., 2005). Wong and Kitts (2006) have reported

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

free radical scavenging and antibacterial activity in the extracts of coriander leaves and stem. Free radical scavenging and lipid per oxidation inhibition activity in the dichloromethane and aqueous extracts of coriander leaves and seeds has also been reported by Wangensteen et al. (2004).

Coriander seeds was rich in polyphenolic compounds gallic acid, caffeic acid, ellagic acid, quercetin and kaempferol are the principle component responsible for high antioxidant activity of methanolic extract of coriander seed (Dua et al., 2014). Hence it is concluded that, antioxidant properties of coriander may be imbibed in the honey samples collected from coriander ecosystem.

Conclusion

From the above study, it is concluded that physiochemical properties viz., Ash content (65.6%), proteins (56 mg/100 g), free amino acids (0.33 mg/g) and total phenol were found maximum in stingless bee, *T. iridipennis* honey collected from coriander field but flavonoid content was maximum (5.51 mgQE/100 g) in honey collected from little bee, *A. florea* F. 1787 hives kept in coriander field. Antioxidant properties like AEAC, RSA, FRAP of stingless bee honey samples were found maximum. Hence the honey collected from coriander field had rich medicinal and antioxidant properties. High phenolic content and antioxidant properties in the coriander may transferred into the nectar collected by the bees and introduced to the honey, contributing to the high phenolic contents and antioxidant observed in this type of honey.

Author contributions. Usharani Balakrishnan, Madhuvandhi Gnanavel, Suresh Krishnasamy planned the research work and designed the methodology including treatments. Conceptualization and formulation of project, field design, measurements, curation of data. All authors have written various chapters of the research paper, reviewed and approved.

Acknowledgements. All authors are thankful to Agriculture College and Research Institute, Madurai. Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for the facilities provided to carry out the research. The authors are thankful to Tamil Nadu State Council for Science and Technology, Student Project Scheme (SPS) for funding for thesis.

Conflict of interests. The authors declare no competing interests.

REFERENCES

[1] Acquarone, C., Buera, P., Elizalde, B. (2007): Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. – Food Chemistry 101(2): 695-703. https://doi.org/10.1016/j.

[2] Amiot, M. J., Aubert, S., Gonnet, M., Tacchini, M. (1989): Phenolic composition of honeys: preliminary study on identification and group quantification. – Apidologie 20: 115-125. DOI: 10.1051/apido:2004047.

[3] Bakour, M., Najoua, S., Nawal, H., Hinde, E. F., Abderrazak, A., Amal, T., Abdellaoui, A., Al-Waili, N., Lyoussi, B. (2018): The antioxidant content and protective effect of argan oil and Syzygium aromaticum essential oil in hydrogen peroxide-induced biochemical and histological changes. – International Journal of Molecular Sciences 19(2): 610. DOI: 10.3390/ijms19020610.

[4] Bezabeh, A., Adgaba, N., Radloff, S., Hepburn, R. (2004): Multivariate morphometric analysis of honeybees (Apis mellifera) in the Ethiopian region. – Apidologie 35(Suppl. 1): S4-S17. DOI:10.1051/apido:2003066.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964

[5] Bogdanov, S., Ruoff, K., Persano Oddo, L. (2004): Physico-chemical methods for the characterisation of unifloral honeys: a review. – Apidologie 35(2004) S4–S17. DOI: 10.1051/apido:2004047.
[6] Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsì, F., Ferrari, G., Hamdi, S. (2018): Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. – Arabian Journal of Chemistry 11(2): 265-274. https://doi.org/10.1016/j.arabjc.2014.08.011.
[7] Cheung, Y., Meenu, M., Yu, X., Xu, B. (2019): Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources. – International Journal of Food Properties 22(1): 290-308. https://doi.org/10.1080/10942912.2019.1579835.
[8] Dua, A., Agrawal, S., Kaur, A., Mahajan, R. (2014): Antioxidant profile of Coriandrum sativum methanolic extract. – International Research Journal of Pharmacy 5(3): 220-224. DOI: 10.7897/2230-8407.050347.
[9] Guler, A., Bakan, A., Nisbet, C., Yavuz. (2007): Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (Saccharum officinarum L.) syrup. – Food Chemistry 105(3): 1119-1125. doi.org/10.1016/j.Food Chemistry 2007.02.024.
[10] Laaroussi, H., Toufik, B., Meryem, B., Driss, O., Badiaa, L. (2020): Physicochemical properties, mineral content, antioxidant activities, and microbiological quality of Bupleurum spinosum Gouan honey from the middle atlas in Morocco. – Journal of Food Quality 1-12. https://doi.org/10.1155/2020/7609454.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. – Journal of Biological Chemistry 193(1): 265-275.
[12] Melo, E. A., Filho, J. M., Guerra, N. B. (2005): Characterization of antioxidant compounds in aqueous coriander extract (Coriandrum sativum L.). – Lebensmittel Wissenschaft & Technologie 38(1): 15-19.
[13] Meziti, A., Meziti, H., Boudiaf, K., Mustapha, B., Bouriche, H. (2012): Polyphenolic profile and antioxidant activities of Nigella sativa seed extracts in vitro and in vivo. – International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnologial Engineering 6(4): 109-117.
[14] Moore, S., Stein, W. H. (1948): Analysis of Amino Acids. – In: Colowick, S. P., Kaplan, N. O. (eds.) Methods in Enzymology. Academic Press, New York, pp. 468-471.
[15] Saxena, S., Gautam, S., Sharma, A. (2010): Physical, biochemical and antioxidant properties of some Indian honeys. – Food Chemistry 118(2): 391-397.
[16] Šedík, P., Pocol, C. B., Horská, E., Fiore, M. (2019): Honey: food or medicine? A comparative study between Slovakia and Romania. – British Food Journal 121(6): 1281-1297. DOI: 10.1108/BFJ-12-2018-0813.
[17] Szeles, E., Luca, M., Czipa, N., Prokisch, J. (2008): Determination of free amino acids in honey by gas chromatography. – Cereal Research Communications 36: 1903-1906.
[18] Terrab, A., Díez, M. J., Heredia, F. J. (2002): Characterisation of Moroccan unifloral honey by their physicochemical characteristics. – Food Chemistry 79(3): 373379.https://doi.org/10.1016/S0308-8146(02)00189-9.
[19] Wangensteen, H., Samuelsen, B. A., Malterude, E. K. 2004: Antioxidant activity in extracts from coriander. – Food Chemistry 88(2): 293-297. DOI: 10.1016/j.foodchem.2004.01.047.
[20] Won, S. R., Li, C. Y., Kim, J. W., Rhee, H. I. (2009): Immunological characterization of honey major protein and its application. – Food Chemistry 11(4): 1334-1338. DOI: 10.1016/j.foodchem.2008.08.082.
[21] Wong, P. Y. Y., Kitts, D. D. (2006): Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. – Food Chemistry 97(3): 505-515. DOI: 10.1016/j.foodchem.2005.05.031.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29572964