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Comparison of the bioactive potential of floral honey from the Western Ghats of India

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Abstract The bioactive compounds and antioxidant activities of three floral honey samples produced by honey bees *Apis dorsata*, *Apis indica* and *Trigona iridipennis* in two locations in southwest India (the west coast and the Western Ghats) were investigated. Bioactive compounds are assessed, including total phenolics, tannins, flavonoids and vitamin C. The antioxidant potential evaluated included, total antioxidant activity, reducing power, ferrous ion-chelating capacity and free radical-scavenging activity. The current study showed a higher content of total phenolics and flavonoids in *A. indica* and *T. iridipennis* than *Apis dorsata*. Total phenolics, flavonoids and vitamin C in Western Ghats honey samples are higher than in many monofloral and multifloral honey samples evaluated in different parts of the world. In the present study, honey samples of *A. dorsata* and *T. iridipennis* possessed substantial antioxidant activity, proving their ethnic or traditional uses for therapeutic purposes. Further investigation on the beneficial properties of the honey samples for medicinal, bioactive and nutritional purposes from the Western Ghats and its vicinity is warranted.

Keywords: Antioxidant activity, Bioactive compounds, Floral honey, Honey bees, Nutraceutical potential

Introduction

Floral honey has an extended history of applications and serves as an essential ingredient in various recipes as a sweetener, flavour enhancer and for its medicinal significance. Honey is produced by the insects belonging to the order Hymenoptera (bees, wasps and bumblebees) and is highly valued in rural communities as a rich source of energy, medicinal potential and diverse secondary products (Zamudio *et al.*, 2010; Khan *et al.*, 2014; Puścion-Jakubik *et al.*, 2020). *Apis indica* produces blossom honey or floral honey using the nectars of flowers, while honeydew honey is another type prepared from the secretion of honeydew by plant-sucking insects e.g., aphids (Havsteen, 2002;

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Adebiyi *et al*., 2004). Bees convert the flower nectar into honey through regurgitation, followed by evaporation and storage in wax combs as a primary nutritional source in an organized manner within the beehive. The flavour, nutritional valve and medicinal properties of honey differs depending on the kinds of flowers from which the nectar was drawn by the honey bees (Arawwawala and Hewageegana, 2017; Viteri *et al*., 2021; Amor *et al*., 2022; Pham *et al*., 2022; Salomon *et al*., 2022).

As a natural product, honey has wide applications in medicine as well as in nutraceutical sources. During the past decade, there has been a tremendous upsurge in the application of honey in traditional systems of medicine (Thomas *et al.,* 2001; Wilkinson and Simpson, 2001; Alia ño-Gonz âlez *et al*., 2019). The therapeutic uses of honey as a folk remedy as well as its recent applications have been studied by several researchers (e.g., Aljadi and Kamaruddin, 2004; Wang *et al*., 2015; Chen *et al.*, 2019). Apitherapy, or the medicinal use of honey bee products, has recently focused attention on preventive medicine in treating specific disease conditions to promote overall human health and wellbeing (Gheldof *et al*., 2002; Brighenti *et al*., 2019; Salomon *et al*., 2022).

Floral honey is one of the important natural products used to treat or cure a variety of diseases like arthritis, cancers (breast, cervical and colon), cough, diabetes, hepatic steatosis, influenza, mucositis, osteoporosis and ulcers (see Puścion-Jakubik *et al*., 2020). Investigations have shown that honey stimulates immunity within a wound, which leads to anti-inflammation activity (Tonks *et al*., 2003; Medhi *et al*., 2008; Islam *et al.*, 2017; Salomon *et al*., 2022). Al-Waili (2004) demonstrated that ingested honey has anti-inflammatory properties. They have also demonstrated that honey prevents the reactive oxygen species (ROS)-induced oxidation of low-density lipoprotein (LDL) *in vitro*, resulting in cardiovascular protection. Honey is well known for its antineoplastic function against bladder cancer (Moundo *et al*., 2001; Sewllam *et al*., 2003; Hegazi and El-Hady, 2009; Ahmed *et al.*, 2018). Owing to the higher quantities of flavonoids and quercetin in the honey samples, it has a promising effect in treating cardiovascular diseases (Khalil and Sulaiman, 2010).

One of the remarkable properties of honey is its antibacterial activity (Bueno-Costa *et al.,* 2016; Pham *et al*., 2022). Natural honey possesses bactericidal activity against many pathogens, like *Escherichia coli*, *Helicobacter pylori, Pseudomonas aeruginosa*, *Salmonella*, *Staphylococcus sureus* and *Shigella* (Chowdhury, 1999; Alvarez-Suarez *et al*., 2010; Stobener *et al.,* 2019; Pham *et al*., 2022). Six honey samples collected from the Western Ghats of India possess antibacterial activity against Gr+ve and Gr-ve bacteria (Shubharani *et al*., 2013). Honey serves as an excellent prebiotic owing to its

composition (e.g., maltulose, maltotriose, turanose, panose, melezitose, raffinose, inulobiose and ketostose) to stimulate the probiotic microbes in the gastrointestine (Leite *et al*., 2000; Mei *et al*., 2010). Being a nutraceutical source (minerals, vitamins, dietary fibre, carbohydrates and metabolites), honey has several applications in nutrition, functional foods (e.g., prebiotics) and medicine. The objective of research finding was to analyze the bioactive potential of three honey samples collected from the southwest coast as well as the Western Ghats of India.

Materials and methods

Honey samples

Three wild floral honeys formed by *Apis dorsata, A. indica* and *Trigona iridipennis* were sampled in three replicates from the Sirsi region of the Western Ghats of Karnataka (Uttara Kannada District; 14 °28-29'N, 74 °43-45'E; 1700-1744 m asl) during summer (February-April, 2020). During the summer, the same wild honey samples were collected in triplicate from the Bantwala region on Karnataka's west coast (Dakshina Kannada District; 12 °41'N, 75 °3-4'E; 118-121 m asl) during summer (February-April, 2020). They were preserved in glass containers at laboratory temperature (26-28 ℃) and assessment was performed within one month of sampling.

Bioactive components

Total phenolics

The total phenolic content of honey was evaluated using the procedure developed by Rosset *et al*. (1982). The honey sample (1-3 mg) and alcohol (50%, 10 ml) were added by mixing, kept in a hot water bath (95 ℃, 10 min) and after cooling, centrifuged (2000 rpm, 20 min) to get the supernatant. The extraction was repeated, pooled and the volume was made to be 20 ml. The extract (0.5 ml) was diluted with 0.5 ml distilled water before being incubated for 10 minutes at laboratory temperature (26±1 ℃) with sodium carbonate (in 0.1 N NaOH, 5 ml). Added the Folin-Ciocalteau's reagent (diluted 1:2, 0.5 ml) and read the absorbance (765 nm; UV-VIS Spectrophotometer-118, Systronics, Ahmedabad, Gujarat, India). Similarly, the distilled water was added to honey samples and processed as standard. The total phenolics were noted as mg gallic acid (standard) equivalents per gram (mg GAEs/g).

Tannins

The tannin component of honey was evaluated based on Burns (1971). The honey sample was added (1-3 mg) in 50% alcohol (10 ml) for tannin extraction using a rotating shaker (28 ℃, 24 hr), followed by centrifugation (1500 rpm) to get the supernatant. Added the vanillin hydrochloride (5 ml: 4% in methanol + 8% concentrated HCl in methanol at the ratio of 1:1) to 1 ml extract and incubated for 20 min at laboratory temperature (26±1 ℃) to measure the absorbance at 725 nm. Similarly, the distilled water was added to the honey samples. The tannic acid content in methanol as the standard was noted as mg tannic acid (standard) equivalents (mg TAEs/g).

Flavonoids

The flavonoid was assessed using the colorimetric method by Chang *et al*. (2002). Honey sample (1-3 mg) was extracted using alcohol (1.5 ml) and aliquots (0.5 ml) were blended with aluminium chloride (10%, 0.1 ml) as well as potassium acetate (1M, 0.1 ml). The volume was made up to 3 ml using distilled water, followed by incubation (30 min, laboratory temperature, 26±1 ℃). Similarly, the distilled water was added to honey samples and processed. The quercetin dihydrate served as a standard and absorbance was measured (415 nm) to note the flavonoid content in mg (mg QEs/g).

Vitamin C

The vitamin C content of honey was assessed by Roe (1954). The honey sample (1-3 mg) was extracted by trichloroacetic acid (TCA, 5%, 10 ml) and the aliquot of extract (0.2 ml) was made up to 1 ml by TCA (5%) and blending, followed by addition of chromogen (1 ml) (dinitrophenyl hydrazine thiourea copper sulphate solution: five parts of 5% thiourea + five parts of 0.6% copper sulphate + ninety parts of 2% 2,4-dinitrophenylhydrazine in H_2SO_4). After 10 minutes in a boiling water bath, the mixture was cooled, sulphuric acid (65%, 4 ml) was added. It was incubated at laboratory temperature (26±1 ℃) for 10 minutes, and absorbance at 540 nm was measured. Similarly, the distilled water was added to honey samples and processed. Ascorbic acid and vitamin C are measured in mg of ascorbic acid equivalents per gram (mg AAEs/g).

Antioxidant activity

Samples of honey (0.1-0.5 mg) were extracted with methanol (30 ml) using a rotating shaker (150 rpm, 48 hr). After centrifugation, the supernatant was transferred to a pre-weighed Petri dish to evaporate at laboratory temperature (26±1 ℃). The weight of the extract was determined

gravimetrically and dissolved in a known quantity of ethanol (1 mg/ml) and distilled water (1 mg/ml) to evaluate various antioxidant properties.

Total antioxidant activity

The total antioxidant activity (TAA) was evaluated by the procedure developed by Prieto *et al*. (1999). Added a mixture of reagents (28 mM sodium phosphate + 4 mM ammonium molybdate in 0.6 M sulfuric acid) to the alcoholic extract and distilled water extract of honey (1-0.5 mg/ml; 0.1 ml), followed by 90 min incubation in a hot water bath (95 ℃). The absorbance was assessed (695 nm) and TAA was noted in μM equivalents of ascorbic acid (μM AAEs/g).

Reducing power

The reducing power (RP) of the extract was evaluated using the method proposed by Oyaizu (1986) with a minor modification. The alcohol extract and distilled water extract in varied concentrations (0.1-0.5 mg/ml) in phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%, 2.5 ml) were added, followed by incubation in a hot water bath (50 ℃, 20 min). Later, added TCA (10%, 2.5 ml) to the mixture, followed by centrifugation (3000 rpm, 10 min). The supernatant (2.5 ml) was blended with an equal quantity of double distilled water and ferric chloride (0.1%, 0.5 ml). Absorbance was assessed (700 nm).

Ferrous ion-chelating capacity

The ferrous ion-chelating capacity (FCC) was evaluated by the method developed by Hsu *et al*. (2003). Alcohol or distilled water extract (0.5-2.5 mg/ml) was blended with 2 mM ferrous chloride (0.1 ml) as well as 5 mM, ferrozine (0.2 ml). The volume was made up to 5 ml using methanol, incubated (laboratory temperature, 26±1 ℃, 10 min) and the absorbance was measured (562 nm). Ther sample without the addition of the extract served as a control to note the ferrous ion-chelating capacity.

$$\text{Ferrous ion chelating activity (\%)} = \left(1 - \frac{A_{s562}}{A_{c562}}\right) 100$$

where A_s, the absorbance of sample; and A_c, the absorbance of control.

Free radical-scavenging activity

The DPPH radical-scavenging activity (RSA) of samples was assessed on the method by Singh *et al*. (2002). Different concentrations of extracts (0.5-2.5 mg/ml) were made up to 1 ml by alcohol or distilled water and the reagent was added (0.001 M DPPH in methanol, 4 ml). The contents were blended, followed by incubation in the dark (laboratory temperature, 26±1 ℃, 20 min).

Control consisted of reagents without extract and the absorbance was measured (517 nm) to note the DPPH RSA:

$$\text{Free radical-scavenging activity (\%)} = \left(\frac{A_{c517} - A_{s517}}{A_{c517}}\right) 100$$

where, A_c, the absorbance of control; and A_s, the absorbance of the sample.

Data analysis

Using Statistica version # 8.0 (StatSoft, 2008) of a Student's T-test was determined the difference in bioactive compounds and antioxidant activities between alcohol and distilled water extracted samples (StatSoft, 2008).

Results

Bioactive components

Total phenolics

The total phenolics of honey on the west coast and in the Western Ghats were higher in alcohol compared to the aqueous extract, except for *T. iridipennis* of the Western Ghats (Figure 1). At 3 mg/ml, total phenolics were the highest in the alcohol extract of *T. iridipennis* (19.6 mg GAEs/g) of the Western Ghats, followed by *A. indica* (18.5 mg GAEs/g). On the west coast, it was the highest in *A. indica*, and followed by *A. dorsata* (15.8 and 14.8 mg GAEs/g, respectively).

Tannins

The tannin content was higher in alcohol compared to the aqueous extract in all the honey samples except for *T. iridipennis* of the Western Ghats (Figure 2). At 3 mg/ml concentration, it was the highest in the *A. dorsata* of the Western Ghats, and followed by the same species on the west coast of 5.9 and 5.8 mg TAEs/g, respectively.

Flavonoids

The flavonoids were higher in alcohol compared to the aqueous extract in all honey samples from the west coast and the Western Ghats, except for *A. dorsata* from the Western Ghats (Figure 3). At 3 mg/ml concentration, it was the highest in *A. indica* of the west coast, and followed by *T. iridipennis* of the west coast of 1.3 and 1.2 mg QEs/g, respectively.

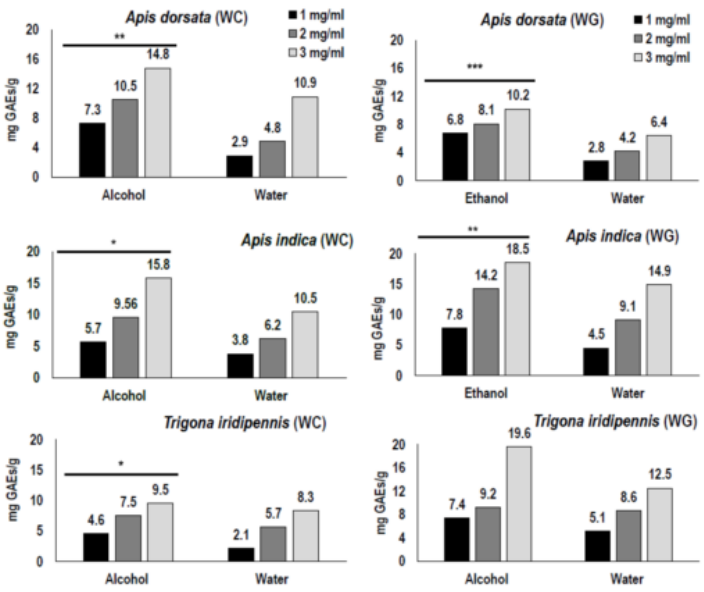

Figure 1. Total phenolics in honey samples at different concentrations extracted in alcohol and distilled water (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; WC, west coast; WG, Western Ghats)

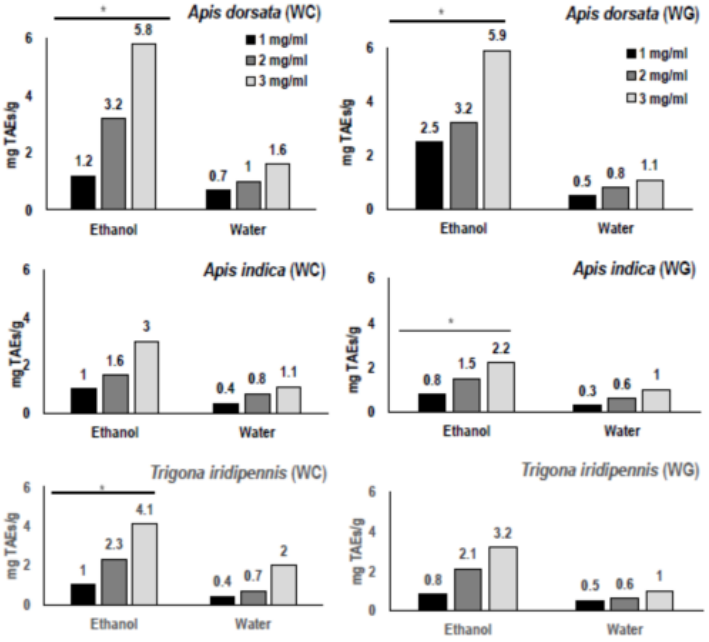

Figure 2. Tannin content in honey samples at different concentrations extracted in alcohol and distilled water (*, $p<0.05$; WC, west coast; WG, Western Ghats)

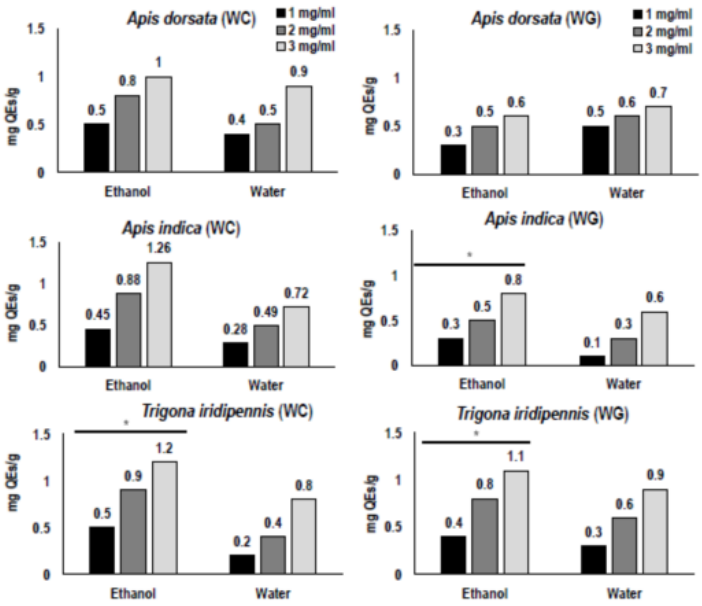

Figure 3. Flavonoids in honey samples at different concentrations extracted in alcohol and distilled water (*, p<0.05; WC, west coast; WG, Western Ghats)

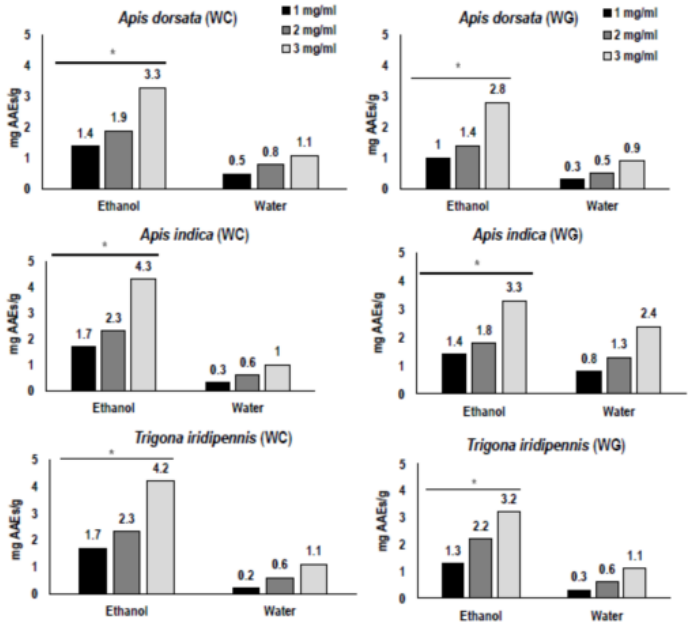

Figure 4. Vitamin C content in honey samples at different concentrations extracted in alcohol and distilled water (*, p<0.05; WC, west coast; WG, Western Ghats)

Vitamin C

The vitamin C content was higher in alcohol compared to the aqueous extract of all the honey samples from the west coast as well as the Western Ghats (Figure 4). At 3 mg/ml concentration, it was the highest in *A. indica*, followed by *T. iridipennis* of 4.3 and 4.2 mg AAEs/g, respectively.

Antioxidant potential

Total antioxidant activity

The total antioxidant activity (TAA) was higher in the aqueous than the alcohol extract in honey (Figure 5). At 0.4 mg/ml concentration, TAA was the highest in *A. dorsata*, and followed by *T. iridipennis* of the west coast of 0.27 and 0.24 mM AAEs/g, respectively.

Reducing power

The reducing power (RP) was higher in the aqueous extract compared to the alcohol extract in all the honey from the west coast and Western Ghats (Figure 6). At 0.5 mg/ml concentration, RP was the highest in *A. dorsata* of the west coast and *T. iridipennis* of the Western Ghats in absorbance of 0.27 and followed by *T. iridipennis* of the west coast in absorbance of 0.24.

Ferrous ion-chelating capacity

The ferrous ion-chelating capacity (FCC) was higher in the alcohol compared to the aqueous extract in all honey except for *T. iridipennis* of the Western Ghats (Figure 7). The FCC was the highest at 2.5 mg/ml concentration in *A. dorsata*, and followed by *A. indica* of the Western Ghats of 80.3 and 471.2%, respectively.

Free radical-scavenging activity

The DPPH radical-scavenging activity (RSA) was higher in the alcohol than the aqueous extract in all honey samples (Figure 8). The DPPH RSA was the highest at 2.5 mg/ml concentration in *A. dorsata*, and followed by *T. iridipennis* of the west coast of 44.1 and 43.2%, respectively.

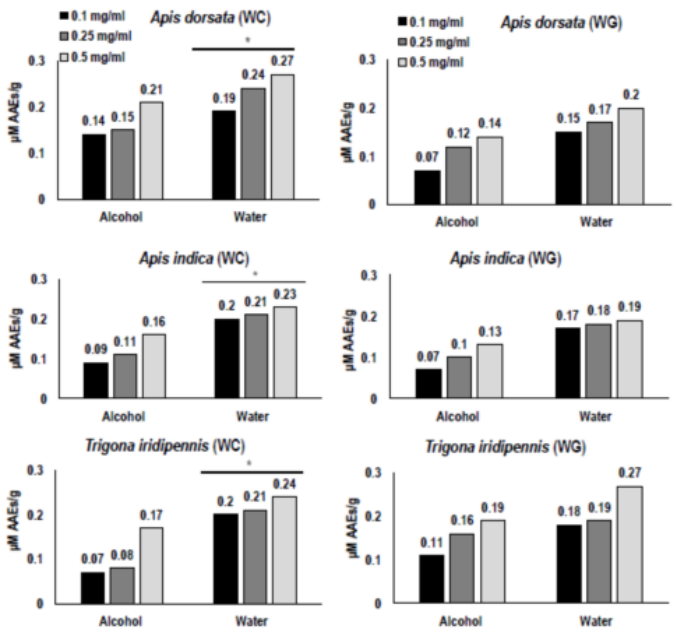

Figure 5. Total antioxidant activity (TAA) of honey samples at different concentrations extracted in alcohol and distilled water (*, p<0.05; WC, west coast; WG, Western Ghats)

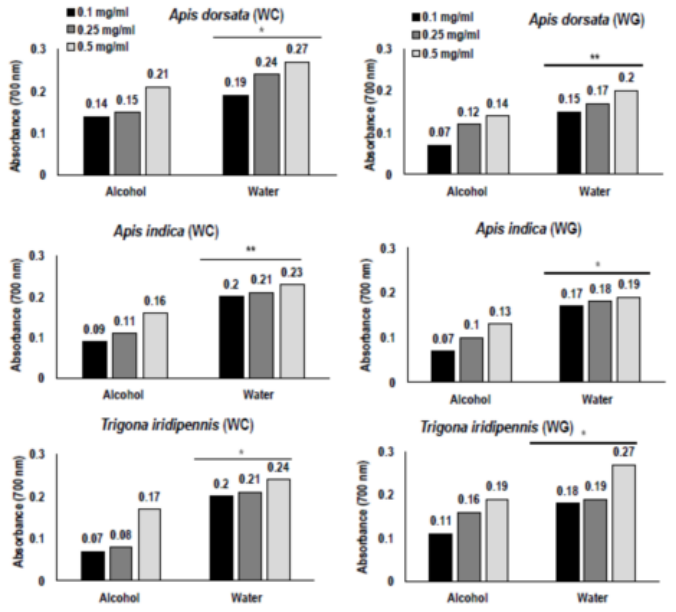

Figure 6. Reducing power (RP) of honey samples at different concentrations extracted in alcohol and distilled water (*, p<0.05; **, p<0.01; WC, west coast; WG, Western Ghats)

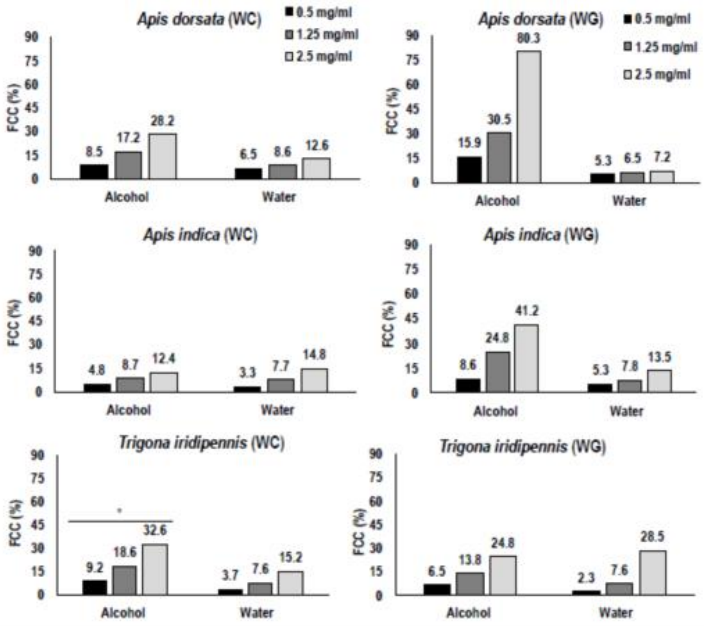

Figure 7. Ferrous ion-chelating capacity (FCC) of honey samples at different concentrations extracted in alcohol and distilled water (*, $p<0.05$; WC, west coast; WG, Western Ghats)

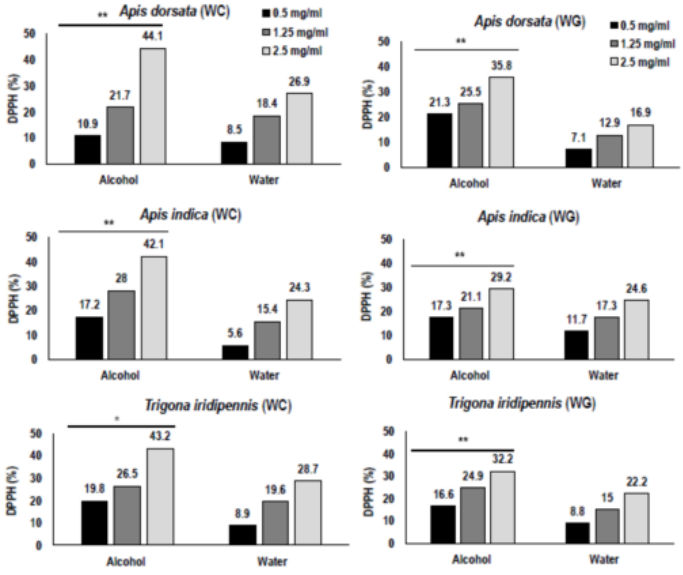

Figure 8. The DPPH radical-scavenging activity (RSA) of honey samples at different concentrations extracted in alcohol and distilled water (*, $p<0.05$; **, $p<0.01$; WC, west coast; WG, Western Ghats)

Discussion

Bioactive components

Several studies have been performed on the nutritional, bioactive components, antimicrobial activities and medicinal values of honey samples obtained from the market, monofloral honey and multifloral honey from different parts of the world (Pham *et al*., 2022; Júnior *et al*., 2022; Nedić *et al*., 2022). Phenolic acids in honey consist of benzoic, chlorogenic, Gallic, caffeic cinnamic acids and other compounds known for their antioxidant function (Moniruzzaman *et al.,* 2014; Nedić *et al*., 2022). The honeys obtained from the *Apis* and *Trigona* are endowed with rich nutrients as well as therapeutic potential (Amin *et al*., 2018). *Trigona* honey is rich in antioxidants and possesses the ability to reduce inflammation as well as microbial infection (Kwapong *et al.,* 2013; Haron *et al*., 2022; Salomon *et al*., 2022).

Total phenolics

The total phenolics from the six honey samples from the Western Ghats were studied by Shubharani *et al*. (2013). The present study showed a higher content of total phenolics than the report by Shubharani *et al*. (2013). Similarly, the total phenolic contents of three types of honey samples in our study were higher compared to the honey of *Apis mellifera* collected worldwide (Durović *et al*., 2022; Viteri *et al*., 2021; Amor *et al*., 2022), eleven floral honey samples from Vietnam (Pham *et al*., 2022), six honey samples from Tunisia (Boussaid *et al*., 2018) and many monofloral as well as polyfloral honey samples from Serbia and Brazil (Júnior *et al*., 2022; Nedić *et al*., 2022). However, our honey samples showed a lesser quantity of total phenolics compared to the honey samples from Turkey (Kaya and Yildirim (2021). The total phenolic content of honey by *T. iridipennis* in our study was higher when compared to the *T. fiebrigi of* Argentina (Salomon *et al*., 2022) as well as *Trigona* sp. from Malaysia (Haron *et al*., 2022).

Tannins

The tannin content was higher in the alcohol extract than the aqueous extract in all the honey samples studied except for *T. iridipennis* in our study. At this concentration, 3 mg/ml, it was the highest in the *A. dorsata* of the Western Ghats compared with the same species on the west coast. Tannin content of honey in our study is significantly lower than the honey samples reported by (Oriolowo *et al.,* 2019), but higher than the honey samples of Ethiopia studied by Amabye and Mekonen (2016) and tannins in ethanolic

extracts are comparable to the eight honey samples from São Paulo (Catelani *et al*., 2016). However, there were no tannins in the 10 monofloral honey samples from Jawa and Sumbawa, as reported by Yelin and Kuntadi (2019). According to Sagona *et al*. (2021), high levels of tannins will have a negative impact on honey bees' immunity as well as metabolism, which leads to a reduction in their lifespan. According to our findings, aqueous extract has lower tannin levels than ethanol extract, which may have a minor impact on honey bees.

Flavonoids

Kaya and Yildirim (2021) found a wide range of flavonoids in honey samples using a qualitative assay. Our study showed a higher content of flavonoids in honey samples when compared to the total flavonoids of honey samples from the Western Ghats studied by Shubharani *et al*. (2013). The flavonoid contents of honey samples in our study are also higher than several monofloral honey samples from Brazil (Júnior *et al*., 2022), Tunisia (Boussaid *et al*., 2018) and Algeria (Amor *et al*., 2022). Compared to a study of some of the Turkish honey samples by Kaya and Yildirim (2021), the honey of *A. indica* and *T. iridipennis* in our study possess higher quantities of flavonoids. The flavonoid contents of *T. iridipennis* in our study were also higher than *T. fiebrigi* of Argentina which reported by Salomon *et al*. (2022) and *Trigona* sp. in Malaysia (Haron *et al*., 2022). Flavonoids in honey consist of catechin, kaempferol, myricetin and naringenin and those constituents are known for their antioxidant activity (Moniruzzaman *et al*., 2014). The flavonoid content of honey samples from Malaysia was strongly correlated with ferric-reducing activity as well as DPPH radical-scavenging activity (RSA) (Chua *et al*., 2013).

Vitamin C

Water-soluble vitamins in the honey samples e.g., vitamin C was correlated with free radical-scavenging activity (RSA), as reported by Chua *et al*. (2013). Honey samples containing vitamin C are known to prevent the metabolic disorder dyslipidaemia in a rat model (Adeyomoye *et al.,* 2021). Vitamin C content in honey samples is known to be antibacterial owing to triggering the intracellular production of reactive oxygen species (ROS) in bacterial cells (Majtan *et al.,* 2020). The vitamin C content of our honey samples was shown to be higher when compared to the honey samples from Poland studied by Sawicki *et al*. (2020). A substantial quantity of vitamin C in the honey samples in our study indicated its health benefits, such as antioxidant potential as well as antimicrobial activities.

Antioxidant potential

Honey serves as an important functional food due to its antioxidant, anti-inflammatory, antimicrobial, antitumor and anti-mutagenic properties (Otero and Bernolo, 2020). Antioxidant properties of honey such as total antioxidant activity, ferric-reducing capacity, radical-scavenging activity were correlated to the contents of various compounds like flavonoids, ascorbic acid, carotenoids, b-carotene, lycopene and reducing sugars as compared to Aljadi and Kamaruddin (2004), Ferreira *et al*. (2009), and Islam *et al*. 2017). These properties are critically important for the prophylactic capacities of honey and for the treatment of several diseases (Chua *et al*., 2013; Bueno-Costa *et al*., 2016; Ahmed *et al*., 2018; Amin *et al*., 2018).

Free radical-scavenging activity

Kaya and Yildirim (2021) studied the honey samples from Turkey for their antioxidant potential of total antioxidant and DPPH radical-scavenging activities, and RSA. Six honey types collected from the Western Ghats were studied for their antioxidant activities for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging and ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (Shubharani *et al*., 2013). The flavonoid content of honey samples from Malaysia was strongly correlated with ferric ion-reducing capacity (FCC) as well as DPPH RSA (Chua *et al*., 2013).

The DPPH RSA of the honey samples in our study was higher than that of *Apis mellifera* honey which reported by Durović *et al.* (2022), and other honey samples of *A. mellifera* worldwide (Viteri *et al*., 2021). The DPPH RSA of the honey samples were compared with the Western Ghats by Shubharani *et al*. (2013), except for one honey sample, our study showed less in DPPH RSA. Similarly, the DPPH RSA was found to be lower in our honey samples as compared to the honey samples from Turkey which studied by Kaya and Yildirim (2021). Similarly, the reducing power (RP) of all the honey samples at 0.5 mg/ml of aqueous extract was higher than the RP of five honey samples from Turkey (Kaya and Yildirim, 2021).

In summary, the current study was found to be higher levels of total phenolics, flavonoids and vitamin C in Western Ghats honey samples than in many monofloral and multifloral honey samples. The tannin content was relatively low which may not influence the lifespan of the honey bees. The total antioxidant activity and reducing power were shown to be higher in the aqueous extract than the ethanol extract, while it was opposite for ferric ion-chelating and DPPH radical-scavenging activities. The honey samples of *A. dorsata* and *T. iridipennis* from the Western Ghats possess substantial antioxidant activities,

and proved their ethnic uses for therapeutic purposes. Further investigations on the nutritional, bioactive and therapeutic properties of honey samples from the Western Ghats are needed and highly rewarding to understand the nutraceutical and health significance.

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