

Antioxidant, anticholinesterase, and tyrosinase enzyme inhibitory profiles of nine Saudi honeys revealed by multivariate analysis

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Abstract: Honey is well known for its nutritional and therapeutic potential since ancient time. The current study was designed to investigate the chemical composition, antioxidant and enzyme inhibitory activities of nine Saudi honey types. Three extracts, ethanol, methanol and aqueous, were prepared from each type. Results of fructose, glucose, sucrose and 5-hydroxymethylfurfural contents in the tested Saudi honey types indicated their good quality with the honey of Sider Bisha and Sider Albaha occupied the highest position. Polar extracts of honey recorded the highest TPC and TFC values and the best values were recorded from the aqueous extract of Sider Bisha (197.67 mg GAE/100 g) and Talh Hail (67.45 mg RE/100 g) respectively. All honey extracts showed significant antioxidant activity and the MeOH extract of Sider Bisha displayed the highest anti-ABTS (809.42 mg TE/g) and chelating (98.71 mg EDTAE/g) properties and that of Sumra Bisha recorded the best anti-DPPH activity (30.67 mg TE/g). The aqueous extract of the latter and that of Talh Hail revealed the best Fe⁺⁺ (248.41 mg TE/g) and Cu⁺⁺ (256.72 mg TE/g) reducing capacity. All honey types exerted remarkable enzyme inhibitory activity; all extracts of Sumra Bisha, EtOH and aqueous extracts of Sider Bisha and Sider Tehamh and Majra as well as aqueous extract of Talh Tehamh and MeOH extract of Talh Hail exerted remarkable anti-acetylcholinesterase effect (9.64–10.23 mg GALAE/g; $p \geq 0.05$) while aqueous extract of Sumra Bisha and Sider Albaha exhibited the highest anti-butrylcholinesterase effect (16.46 and 15.13 mg GALAE/g; $p \geq 0.05$). MeOH extract of Qatada honey recorded the highest anti-Tyr effect (260.95 mg KAE/g). These findings indicated that the nine Saudi honey types are of good quality, high bioactive molecules and could play a significant role in oxidative stress.

Keywords: Saudi honey, sugar, 5-hydroxymethylfurfural, total phenol, antioxidant, enzyme inhibition

1 Introduction

Honey is one of the first sweeteners used by humans in ancient time and has many nutritional, therapeutic and medicinal benefits (Al-Ghamdi & Ansari, 2021). It is a constituent of a complex mixture of sugars (mainly fructose and glucose 31%) with minor constituent compounds such as various organic acids, proteins, amino acids, vitamins, polyphenols and minerals (Escuredo & Seijo, 2019). Honey is classified according to food source of bees into nectar or blossom honey and honeydew honey. Nectar honey is obtained when bees fed on plant nectar while honeydew

honey is produced when bees depend on the secretions of living parts of the plants or on the excretions of Hemiptera insects (Alarjani & Mohammed, 2024). Honey is used since ancient time in folk medicine to treat many diseases including skin rashes, wounds, asthma, respiratory and vascular diseases, infertility, anemia and even cancer (Yan et al., 2023). In Sudan, it is used to treat the symptoms of COVID-19 (Yagi & Yagi, 2021).

In Saudi Arabia, there are various types of honey produced by beekeepers and South Saudi Arabia regions such as Asir and Al-Baha produce more than 70% of the bee colonies and different types of honey. According to the floral origins, honey can be classified into mono-floral or polyfloral and the most locally produced honey are Sidr honey like Sidr Asir and Sidr Albaha obtained from floral nectar of *Ziziphus Spina-christi* L and Talih honey like Sumra and Talh Medina produced from the floral nectar of many *Acacia* species (Alqarni et al., 2016; Harakeh et al., 2022). Previous studies in selected local Saudi honey revealed that they possess significant antibacterial, antitumor

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and antioxidant activities (Al-Ghamdi & Ansari, 2021; Alarjani & Mohammed, 2024; Nashawi et al., 2017; Ahmed et al., 2018; Halawani, 2021; Alshammari et al., 2022). An *in vivo* study demonstrated that the two Saudi honey; Wadi and Talh reduced gastric ulcer induced by indomethacin in male albino rats (Harakeh et al., 2022). Saudi honey, like other honey types from different countries, is rich in phenolic acids like gallic acid, protocatechuic acid, ellagic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid and caffeic acid and in flavonoids like apigenin, catechin, galangin, genistein, isorhamnetin, kaempferol, luteolin, myricetin, pinobanksin, pinocembrin and quercetin (Al-Ghamdi & Ansari, 2021; Alarjani & Mohammed, 2024).

In the majority of previous studies the antioxidant activity of honey was correlated to its phenolic content, however, a recent study revealed that in addition to phenolics, short peptides which originated from honeybee proteins participated significantly in the antioxidant activity of honey (Alarjani & Mohammed, 2024). Different types of Saudi honey varied in their quality, nutritional and phytochemical composition and hence their biological properties due to many factors like floral nectar origin, honeybees' species and storage condition among others (Alotibi et al., 2018). The current study was designed to evaluate the phenolic, sugar and 5-hydroxymethylfurfural contents in nine Saudi honey types as well as their antioxidant (radical scavenger, reducing power and metal chelation) and enzyme (acetylcholinesterase, butyrylcholinesterase, tyrosinase) inhibition activities.

2 Materials and Methods

2.1 Honey Samples

A total of nine honey samples were collected from the apiaries in Abha, Kingdom of Saudi Arabia (KSA). Names of honey types and botanical origin of floral nectar are depicted in Table 1.

2.2 Preparation of Extracts

Three solvents including water, methanol and ethanol were used for the extraction of different honey test. Water, ethanol

and methanol extracts were prepared according to the procedure described by Al Qahtani et al. (2022) with few modification. 3 g of honey was dissolved in 20 mL of each solvent and was kept in orbital shaker for 4 h at 120 rpm at room temperature (25–30°C). Then solution was centrifuged at 2000 g for 20 min, the supernatant was recovered, filtered through Whatman No. 4 filter paper. Organic extracts were concentrated using a rotary evaporator under reduced pressure till dryness. Aqueous extracts were freeze-dried and then all extracts were stored at 6°C until analysis. Sugar profile and HMF experiments were performed on entire honey samples as well as their methanol, ethanol and water extracts.

2.3 Sugar Content

Sugar content determined using (HPLC-RID) following International Honey Commission protocols (IHC, 2009). 10 gm of honey sample was weighed through an electric balance (AE Adam-UK) in beaker and then dissolved in 50 mL of deionized water, and transferred to a 100 mL volumetric flask and deionized water up to 75 mL. 25 mL of (HPLC grade methanol) was added, stir the sample and then filter it took 1 mL of the sample and place it in sample vials and then injected into the HPLC-RID. Standard solutions of fructose, glucose and sucrose were prepared in distilled water (10 g pure sucrose), add 5 mL hydrochloric acid (ca. 36.5% w/w pure HCl) and dilute with water to about 100 mL. Store this acidified solution for several days at room temperature (ca.7 days at 12°C to 15°C or 3 days at to 20°C to 25°C) and then dilute to 1000 mL (NB acidified 1% invert sugar remains stable for several months). Neutralize a suitable volume of this solution with 1 M sodium hydroxide solution (40 g/L) immediately before use and dilute to the required concentration (2 g/L) for the standardization.

2.4 Hydroxymethylfurfural (HMF) Content

The HMF measured according to the method of the international honey commission using the HPLC (IHC, 2009; Jeuring & Kupperts, 1980). Precisely 10 gm of honey sample was weighed through an electric balance (AE Adam-UK), and diluted with ultrapure water in a 50 mL volumetric flask. Filtrate collected through a 0.45 µM filter is directly injected into the HPLC-DAD system to find out the content of HMF. Quantification is performed by comparing the peak area with the calibration curve derived from standards of known concentration. standard solution is determined using an UV spectrophotometer at 285 nM in 1 cm quartz cells with water in the blank cell.

2.5 Assay for Total Phenolic and Flavonoid Content

Previous studies have established standardized methodologies for quantifying total phenolic and flavonoid content. In these analytical procedures, gallic acid (GA) and rutin (R) were employed as reference standards. The resultant data were subsequently expressed in terms of gallic acid equivalents (GAE) and rutin equivalents (RE) for comparative analysis (Zengin et al., 2016).

Table 1. Types of the investigated Saudi honey and botanical origin of floral nectar

Sample name	Botanical origin of Floral nectar
Dhuhyana	<i>Acacia asak</i>
Majra	<i>Hypoestes forskoolii</i>
Qatada	<i>Acacia hamulosa</i>
Sider Albaha	<i>Ziziphus spina-christi</i>
Sider Bisha	<i>Ziziphus spina-christi</i>
Sider Tehamh	<i>Ziziphus spina-christi</i>
Sumra Bisha	<i>Acacia tortilis</i>
Talh Hail	<i>Acacia gerrardii</i>
Talh Tehamh	<i>Acacia gerrardii</i>

2.6 Assays for In Vitro Antioxidant Capacity

As documented in Grochowski et al. (2017) antioxidant activity was evaluated using multiple assays, including FRAP, CUPRAC, DPPH, and ABTS. The results were quantified and expressed as trolox equivalents (TE) per gram of sample. Additionally, the phosphomolybdenum (PBD) assay was employed to assess total antioxidant capacity, with values reported in millimoles of TE per gram of extract. For metal chelating activity (MCA), measurements were conducted using EDTA equivalents (EDTAE). All experimental details are given in the supplemental materials.

2.7 Inhibitory Effects against Some Key Enzymes

Enzyme inhibition effects were evaluated in accordance with the spectrophotometric protocol established by Grochowski et al. (2017). Cholinesterase inhibition potentials (AChE and BChE) were standardized against galanthamine hydrobromide and expressed as milligram galanthamine equivalents per gram dry extract (mg GALAE/g). Tyrosinase inhibition capacity was reported as milligram kojic acid equivalents per gram (mg KAE/g). All experimental details are given in the supplemental materials.

2.8 Statistical Analysis

Each experiment was conducted three times, and statistical analysis across the extract groups was performed through a one-way ANOVA, followed by Tukey's post hoc multiple comparison test. The statistical assessments were executed using GraphPad Prism (version 9.2), with $p < 0.05$ deemed statistically significant. The Pearson correlation test was used to examine the relationship between total phenolic/flavonoids in the tested extracts and biological (antioxidant and enzyme inhibitory) activities.

3 Results and Discussion

3.1 Sugar Profiling and 5-Hydroxymethylfurfural (HMF) Contents

The content of fructose, glucose and sucrose as well as the HMF content in the nine Saudi honey types were determined and results are presented in Figure 1. The fructose content ranged between 35.61% and 46.79% and the samples ranked as follows: Dhuhyana > Talh Hail > Sumra Bisha > Majra > Qatada > Sider Bisha > Sider Albaha > Sider Tehamh > Talh Tehamh. The glucose content ranged between 23.73% and 48.16% and they ranked as follows: Majra > Qatada > Dhuhyana > Sumra Bisha > Talh Tehamh > Talh Hail > Sider Albaha > Sider Tehamh > Sider Bisha. Variation in sugar content in different samples was mainly attributed to nectar sources consumed by the bees, regional and climatic conditions and storage circumstances (Ghramh et al., 2020). Furthermore, samples of the same floral origin varied according to geographical origin, seasonal and climatic conditions (Anklam, 1998). Previous studies also demonstrated that fructose and glucose were the major monosaccharide in honey and usually the fructose content exceed the glucose (Ghramh et al., 2020; Mesallam & El-Shaarawy, 1987; Habib et al., 2014; Khan et al., 2016). In the present study it was observed that all samples except the Majra and Qatada had higher fructose content than glucose. Sucrose was not quantifiable in 7 samples and detected only in Talh Tehamh (8.34%) and Sider Tehamh (6.14%). In fact, sucrose is considered as a key quality parameter to identify the adulteration in honey samples and it should not exceed 1% of the dried mass of natural honey (Cotte et al., 2003; Guler et al., 2007; Saxena et al., 2010) A previous study in a number of Saudi honey types found that the sucrose content did not exceed 11.7% (Mesallam & El-Shaarawy, 1987).

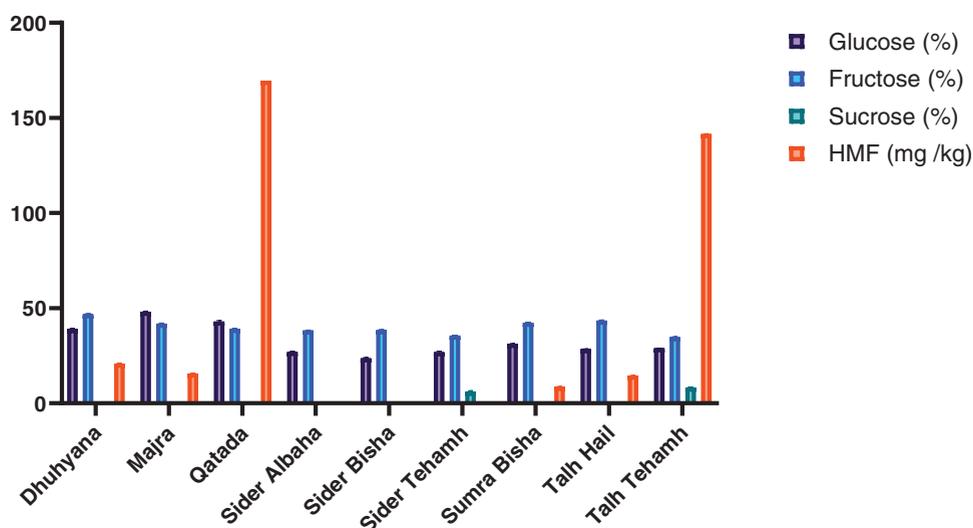


Figure 1. Sugar and 5-hydroxymethylfurfural (HMF) contents in nine Saudi honey types. Results were expressed as the mean of triplicates \pm standard deviation (S.D.)

The profile of HMF in the different samples varied between high content, obtained from Qatada (169.56 mg/kg) and Talh Tehamh (141.56 mg/kg), and low content recorded from Dhuhyana (20.86 mg/kg), Majra (15.63 mg/kg), Talh Hail (14.6 mg/kg) and Sumra Bisha (8.66 mg/kg) while the three Sider honey did not reveal the presence of HMF. However, HMF is considered as an important element to estimate honey freshness and/or overheating. Fresh honey samples are usually devoid of HMF and its presence is correlated to storage time (Lee & Nagy, 1990). Other factors included the temperature, pH, content of simple sugars, acids and minerals as well as nectar source of honey influence also the level of HMF in honey (Zaldivar-Ortega et al., 2024). Arida et al. (2012) found that the Saudi honey samples namely Acacia, Orange, Seder, and Dorm types did not contain quantifiable HMF while values of 41, 140, and 34 mg/kg were recorded in Blossoms, Samra (Elshoka), and multi-floral Saudi honey samples respectively. Overall the results of sugar and HMF indicated the good quality of the studied Saudi honey types and Sider Bisha and Sider Albaha occupied the highest position. However, Qatada and Talh Tehamh

showed high HMF content indicated that storage at high temperature. Generally, sucrose content and HMF are the most important indicators of quality honey. According to Codex Alimentarius (Alimentarius-Codex) HMF should be below 40 mg/kg and sucrose content is less than 5%. Our values (Qatada and Talh Tehamh honey samples) un acceded within the (Alimentarius-Codex), higher HMF concentration is indicative of poor storage conditions (Ghramh et al., 2020). Also, it was observed that sucrose was detected only in Talh Tehamh (8.34%) and Sider Tehamh (6.14%). Addition of sucrose syrups or over-feeding of bees with sucrose or overheated honey samples may occasionally have sucrose more than 5% (Aljohar et al., 2018).

3.2 Total Phenolic (TPC) and Flavonoids (TFC) Contents

The TPC and TFC of the different extracts from the nine Saudi honey types were determined and results are presented in Table 2. The TPC was ranged between 14.27 and 197.67 mg GAE/100 g with aqueous extract of Sider Bisha honey recorded the highest significant ($p < 0.05$) content. This followed in descending order as; aqueous extract

Table 2. Total phenolic and flavonoids contents of different extracts from the nine Saudi honey types

Honey types	Extract	Total phenolic (mg GAE/100 g)	Total flavonoid (mg RE/100 g)
Dhuhyana	EtOH	22.32 ± 0.16 ⁿ	8.06 ± 0.08 st
	MeOH	84.79 ± 1.75 ^e	10.92 ± 0.14 ^{op}
	H ₂ O	67.57 ± 0.43 ^f	16.29 ± 0.47 ^k
Majra	EtOH	16.69 ± 1.67 ⁿ	7.33 ± 0.06 ^{tu}
	MeOH	39.99 ± 0.37 ^{hij}	9.04 ± 0.02 ^{fs}
	H ₂ O	41.22 ± 0.94 ^{hi}	13.14 ± 0.24 ^m
Qatada	EtOH	18.28 ± 0.06 ⁿ	9.49 ± 0.08 ^{gr}
	MeOH	31.93 ± 0.70 ^{kl}	14.58 ± 0.14 ^l
	H ₂ O	34.22 ± 0.61 ^{ijkl}	21.46 ± 0.44 ⁱ
Sider Albaha	EtOH	31.14 ± 0.99 ^{lm}	8.36 ± 0.07 st
	MeOH	102.95 ± 1.58 ^d	11.73 ± 0.07 ^{no}
	H ₂ O	170.56 ± 3.43 ^b	31.03 ± 0.10 ^f
Sider Bisha	EtOH	33.20 ± 0.15 ^{ijkl}	9.96 ± 0.07 ^{pqr}
	MeOH	34.83 ± 0.61 ^{hijkl}	30.61 ± 0.01 ^f
	H ₂ O	197.67 ± 0.74 ^a	33.55 ± 0.19 ^e
Sider Tehamh	EtOH	14.27 ± 2.91 ⁿ	7.20 ± 0.03 ^u
	MeOH	32.78 ± 0.84 ^{ijkl}	12.14 ± 0.02 ^{mn}
	H ₂ O	64.88 ± 0.54 ^{fg}	27.07 ± 0.22 ^g
Sumra Bisha	EtOH	21.14 ± 0.48 ⁿ	10.23 ± 0.04 ^{pq}
	MeOH	33.83 ± 0.42 ^{ijkl}	40.08 ± 0.13 ^d
	H ₂ O	42.28 ± 0.37 ^h	50.48 ± 0.11 ^b
Talh Hail	EtOH	23.79 ± 0.05 ^{mn}	12.10 ± 0.07 ^{mn}
	MeOH	117.94 ± 0.31 ^c	41.46 ± 0.10 ^c
	H ₂ O	103.90 ± 0.85 ^d	67.45 ± 0.10 ^a
Talh Tehamh	EtOH	35.38 ± 2.35 ^{hijkl}	8.87 ± 0.02 ^{fs}
	MeOH	38.87 ± 0.75 ^{hijk}	17.85 ± 0.04 ^j
	H ₂ O	59.40 ± 1.90 ^g	24.78 ± 0.15 ^h

Note: Results were expressed as the mean of triplicates ± standard deviation (S.D.), GAE: Gallic acid equivalent, RE: Rutin equivalent. Different letters indicate significant differences in the tested samples (ANOVA, Tukey's test, $p < 0.05$).

Table 3. Antioxidant activity of different extracts from nine Saudi honey types

Honet types	Extracts	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	MCA (mg EDTAE/g)
Dhuhyana	EtOH	8.12 ± 0.28 ^{efgh}	28.19 ± 2.40 ⁿ	79.24 ± 1.27 ^{ijk}	84.73 ± 1.54 ^{hjk}	22.44 ± 2.75 ^{mno}
	MeOH	11.54 ± 0.04 ^{de}	174.53 ± 0.69 ^h	87.60 ± 0.93 ^{hij}	99.8 ± 0.89 ^g	63.01 ± 0.35 ^d
	H ₂ O	6.46 ± 0.07 ^{gh}	117.48 ± 0.87 ⁱ	93.16 ± 1.56 ^h	123.05 ± 1.18 ^{ef}	22.96 ± 0.18 ^{lmno}
Majra	EtOH	6.38 ± 0.07 ^h	11.21 ± 1.28 ^{no}	63.66 ± 3.77 ^{mno}	68.61 ± 0.22 ^{no}	34.05 ± 1.41 ^{ghi}
	MeOH	9.64 ± 0.07 ^{efgh}	101.55 ± 0.97 ^{ij}	69.37 ± 0.35 ^{klm}	80.49 ± 1.46 ^{jkl}	49.23 ± 0.34 ^e
	H ₂ O	5.87 ± 0.09 ^h	82.58 ± 3.08 ^{jk}	62.15 ± 0.38 ^{mno}	75.27 ± 0.80 ^{klmno}	20.40 ± 0.36 ^o
Qatada	EtOH	5.98 ± 0.23 ^h	21.59 ± 1.01 ^{no}	67.16 ± 3.33 ^{lmn}	79.88 ± 0.48 ^{ijklm}	27.47 ± 1.06 ^{klmn}
	MeOH	6.09 ± 0.19 ^h	78.41 ± 0.33 ^{kl}	70.03 ± 0.23 ^{klm}	79.48 ± 0.56 ^{ijklmn}	30.26 ± 0.02 ^{hijk}
	H ₂ O	8.99 ± 0.07 ^{efgh}	194.49 ± 2.71 ^h	85.56 ± 0.32 ^{hij}	94.32 ± 0.57 ^{gh}	21.56 ± 0.67 ^{no}
Sider Albaha	EtOH	10.53 ± 0.09 ^{defg}	13.43 ± 1.30 ^{no}	58.02 ± 0.42 ^{no}	69.25 ± 0.66 ^{mno}	22.50 ± 0.17 ^{mno}
	MeOH	7.26 ± 0.11 ^{efgh}	227.62 ± 1.69 ^g	76.69 ± 0.45 ^{ijkl}	95.70 ± 0.74 ^{gh}	65.53 ± 0.35 ^{cd}
	H ₂ O	8.16 ± 0.07 ^{efgh}	313.83 ± 3.72 ^f	107.77 ± 0.65 ^g	172.15 ± 0.62 ^{8c}	28.04 ± 0.14 ^{ijklm}
Sider Bisha	EtOH	9.61 ± 0.06 ^{efgh}	57.58 ± 1.13 ^{lm}	83.53 ± 3.34 ^{hij}	92.03 ± 1.68 ^{ghi}	29.00 ± 0.22 ^{ijkl}
	MeOH	23.80 ± 1.31 ^b	809.42 ± 7.50 ^a	155.58 ± 1.31 ^e	174.33 ± 2.01 ^c	98.71 ± 0.21 ^a
	H ₂ O	8.45 ± 0.99 ^{efgh}	381.16 ± 4.78 ^e	137.87 ± 0.16 ^f	151.70 ± 2.84 ^d	38.64 ± 0.26 ^{fg}
Sider Tehamh	EtOH	8.76 ± 0.04 ^{efgh}	na	55.47 ± 0.43 ^o	65.72 ± 1.13 ^o	20.07 ± 2.07 ^o
	MeOH	9.56 ± 1.26 ^{efgh}	375.68 ± 5.73 ^c	86.62 ± 0.63 ^{hij}	112.90 ± 1.14 ^f	71.60 ± 2.00 ^c
	H ₂ O	7.14 ± 0.44 ^{gh}	223.95 ± 1.01 ^g	89.68 ± 1.29 ^{hi}	127.74 ± 0.66 ^e	27.53 ± 0.23 ^{ijklmn}
Sumra Bisha	EtOH	9.5 ± 0.095 ^{efgh}	57.97 ± 2.67 ^{lm}	78.55 ± 0.81 ^{ijk}	93.13 ± 1.24 ^{ghi}	33.08 ± 0.07 ^{ghij}
	MeOH	30.67 ± 1.34 ^a	757.15 ± 4.63 ^b	218.86 ± 0.59 ^b	200.72 ± 2.11 ^b	81.78 ± 0.28 ^b
	H ₂ O	17.22 ± 1.25 ^c	567.73 ± 6.56 ^d	204.52 ± 1.58 ^c	248.41 ± 1.06 ^a	35.22 ± 0.43 ^{efgh}
Talh Hail	EtOH	11.32 ± 0.04 ^{def}	54.39 ± 1.12 ^m	81.87 ± 2.47 ^{hij}	93.02 ± 2.81 ^{ghi}	33.41 ± 0.21 ^{ghij}
	MeOH	24.43 ± 1.17 ^b	622.06 ± 3.43 ^c	186.08 ± 1.36 ^d	176.76 ± 2.51 ^c	78.33 ± 0.60 ^b
	H ₂ O	22.36 ± 0.69 ^b	751.38 ± 3.54 ^b	256.72 ± 3.54 ^a	196.17 ± 4.76 ^b	41.08 ± 0.17 ^f
Talh Tehamh	EtOH	11.57 ± 0.05 ^{de}	12.37 ± 0.09 ^{no}	55.17 ± 0.74 ^o	69.52 ± 0.28 ^{lmno}	12.72 ± 0.95 ^p
	MeOH	13.77 ± 0.19 ^{cd}	87.33 ± 4.27 ^{jk}	85.01 ± 2.19 ^{hij}	90.43 ± 1.63 ^{ghij}	28.71 ± 0.12 ^{ijkl}
	H ₂ O	5.76 ± 0.01 ^h	113.3 ± 1.96 ⁱ	77.11 ± 0.04 ^{ijkl}	82.19 ± 0.24 ^{ijk}	24.96 ± 0.125 ^{klmo}

Note: - na, not active. -Values are reported as mean ± SD. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC: cupric ion reducing antioxidant capacity, FRAP: ferric reducing antioxidant power, MCA: metal chelating activity, TEs, trolox equivalents, EDTAEs, disodium edetate equivalents. Different letters indicate significant differences in the tested samples (ANOVA, Tukey's test, $p < 0.05$).

of Sidr Albaha (170.56 mg GAE/100 g) > MeOH extract of Talh Hail (117.94 mg GAE/100 g) > its aqueous extract (103.90 mg GAE/100 g) = MeOH extract of Sidr Albaha (102.95 mg GAE/100 g) > MeOH extract of Dhuhyana (84.79 mg GAE/100 g) > its aqueous extract (67.57 mg GAE/100 g) > aqueous extract of Sider Tehamh (64.88 mg GAE/100 g) > other extracts (≤ 59.40 mg GAE/100 g). The TFC ranged between 7.20 and 67.45 mg RE/g with the aqueous extract of Talh Hail recorded significantly ($p < 0.05$) the highest content. This followed in the descending order as; aqueous extract of Sumra Bisha (50.48 mg RE/100 g) > MeOH extract of Talh Hail (41.46 mg RE/100 g) > MeOH extract of Sumra Bisha (40.08 mg RE/100 g) > aqueous extract of Sider Bisha (33.55 mg RE/100 g) > its MeOH extract (30.61 mg RE/100 g) > other extracts (≤ 27.07 mg RE/100 g). It was noted that the EtOH recovered the least TPC and TFC in all honey

samples and Qatada honey had relatively low TPC while Majra accumulated low TFC.

Generally, the values of TPC and TFC obtained in the present study were superior to those previously obtained from Saudi honey samples or those collected from other countries (Al Qahtani et al., 2022; Olas, 2020; Kavanagh et al., 2019). It was found that the variation of TPC and TFC in honey samples dose not solely depends on the botanical origin of nectar but also on the country or the region where the honey produced (Becerril-Sánchez et al., 2021). Also, the aqueous and MeOH extracts recorded the highest TPC and TFC values contrary to the results of Al Qahtani et al. (2022). Who demonstrated that the highest TPC and TFC in two Saudi honey types were recovered from the EtOAc extract and both the EtOH and aqueous extracts had the lowest content. However, in the current study it was noted

Table 4. Enzyme inhibitory activity of different extracts from nine Saudi honey types

Sample name	Honey extract	AChE inhibition (mg GALAE/g)	BchE inhibition (mg GALAE/g)	Tyrosinase inhibition (mg KAE/g)
Dhuhyana	EtOH	9.10 ± 0.06 ^{cde}	10.02 ± 0.75 ^{def}	231.36 ± 2.21 ^{bcd}
	MeOH	7.25 ± 0.07 ^g	4.73 ± 0.30 ^{ij}	241.61 ± 2.37 ^{ab}
	H ₂ O	na	5.17 ± 0.10 ⁱ	185.28 ± 0.56 ^h
Majra	EtOH	9.62 ± 0.08 ^{abc}	11.03 ± 0.32 ^{cde}	206.8 ± 2.50 ^{fgh}
	MeOH	6.67 ± 0.13 ^{gh}	6.16 ± 0.32 ^{ghi}	226.01 ± 0.36 ^{bcdef}
	H ₂ O	9.99 ± 0.16 ^{ab}	4.43 ± 0.16 ^{ij}	226.65 ± 0.74 ^{bcdef}
Qatada	EtOH	8.58 ± 0.15 ^{ef}	8.35 ± 0.40 ^{fgh}	206.8 ± 4.02 ^{fgh}
	MeOH	6.71 ± 0.16 ^{gh}	4.87 ± 0.23 ⁱ	260.95 ± 4.78 ^a
	H ₂ O	8.88 ± 0.10 ^{de}	2.23 ± 0.18 ^j	234.22 ± 3.63 ^{bc}
Sider Albaha	EtOH	8.96 ± 0.04 ^{de}	8.51 ± 0.15 ^{fg}	214.01 ± 3.13 ^{cdefg}
	MeOH	6.59 ± 0.16 ^h	4.67 ± 0.90 ^{ij}	225.56 ± 6.71 ^{bcdef}
	H ₂ O	na	15.13 ± 0.18 ^{ab}	231.75 ± 1.02 ^{bcd}
Sider Bisha	EtOH	9.64 ± 0.16 ^{abc}	12.13 ± 0.22 ^{cd}	229.00 ± 1.09 ^{bcd}
	MeOH	6.75 ± 0.15 ^{gh}	3.65 ± 0.18 ^{ij}	232.83 ± 0.61 ^{bcd}
	H ₂ O	10.22 ± 0.00 ^a	9.53 ± 0.19 ^{ef}	199.28 ± 2.24 ^{gh}
Sider Tehamh	EtOH	9.82 ± 0.03 ^{ab}	11.34 ± 0.20 ^{cde}	206.46 ± 0.50 ^{fgh}
	MeOH	5.31 ± 0.04 ^l	6.12 ± 0.72 ^{ghi}	230.54 ± 1.77 ^{bcd}
	H ₂ O	10.23 ± 0.00 ^a	13.11 ± 0.10 ^{bc}	220.19 ± 1.38 ^{bcd}
Sumra Bisha	EtOH	10.03 ± 0.05 ^a	7.89 ± 0.34 ^{fgh}	221.16 ± 1.46 ^{bcd}
	MeOH	10.19 ± 0.01 ^a	8.39 ± 0.42 ^{fgh}	232.71 ± 2.51 ^{bcd}
	H ₂ O	10.12 ± 0.00 ^a	16.46 ± 0.04 ^a	213.07 ± 0.83 ^{cdefg}
Talh Hail	EtOH	9.40 ± 0.10 ^{bcd}	9.30 ± 0.19 ^{ef}	208.12 ± 1.68 ^{fg}
	MeOH	9.97 ± 0.03 ^{ab}	5.98 ± 0.25 ^{hi}	233.07 ± 3.20 ^{bcd}
	H ₂ O	na	15.54 ± 0.09 ^{ab}	226.92 ± 1.64 ^{bcd}
Talh Tehamh	EtOH	9.17 ± 0.09 ^{cde}	8.44 ± 0.26 ^{fgh}	217.39 ± 9.12 ^{cdefg}
	MeOH	8.17 ± 0.06 ^f	4.83 ± 0.69 ⁱ	211.26 ± 6.74 ^{defg}
	H ₂ O	10.21 ± 0.01 ^a	5.36 ± 0.05 ⁱ	234.88 ± 0.52 ^{bc}

Note: na, not active. Values are reported as mean ± SD., GALAEs, galanthamine equivalents. KAEs, kojic acid equivalents. Different letters indicate significant differences in the tested samples (ANOVA, Tukey's test, $p < 0.05$).

that there was a significant ($p < 0.05$) difference in the TPC and TFC between the MeOH and EtOH extracts. Indeed, the extraction yield and phenolic content depend on the raw materials and nature of compounds which in turn reflect in the efficiency of extraction solvent (Osorio-Tobón, 2020).

3.3 Antioxidant Activity

The antioxidant activity of the nine Saudi honey types was evaluated using free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP) and metal chelating assays and results are presented in Table 3. All honey extracts showed potent antioxidant activity especially Sider Bisha, Sumra Bisha and Talh Hail which exerted remarkable activity. The anti-DPPH activity of extracts was in the range 5.76–30.67 mg TE/g with MeOH extract of Sumra Bisha recorded the highest effect followed by MeOH extract of Sider Bisha and Talh Hail as well as the aqueous extract of the latter ($p \geq 0.05$). On the other hand the anti-ABTS activity was in the range of not active to 809.42 mg TE/g. It was noted

that at least one extract from the investigated honey samples displayed potent ABTS scavenging activity (≥ 101.55 mg TE/g) and they are ranked as follows; MeOH extract of Sider Bisha > MeOH extract Sumra Bisha = aqueous extract of Talh Hail > MeOH extract of Talh Hail > aqueous extract of Sumra Bisha > aqueous extract of Sider Bisha > aqueous extract of Sider Albaha > MeOH extract of Sider Albaha > aqueous extract of Qatada > MeOH extract of Dhuhyana > aqueous extract of Dhuhyana > aqueous extract of Talh Tehamh > MeOH extract of Majra.

Different honey samples exhibited also remarkable ions reducing capacity. The Cu^{++} reducing capacity of honey extracts was in the range of 55.17–256.72 mg TE/g and those recorded values ≥ 107.77 mg TE/g were ranked as follows; aqueous extract of Talh Hail > MeOH extract Sumra Bisha > aqueous extract Sumra Bisha > MeOH extract of Talh Hail > MeOH extract of Sider Bisha > aqueous extract of Sider Bisha > aqueous extract of Sider Albaha. The Fe^{+++} reducing capacity of honey extracts was in the range of

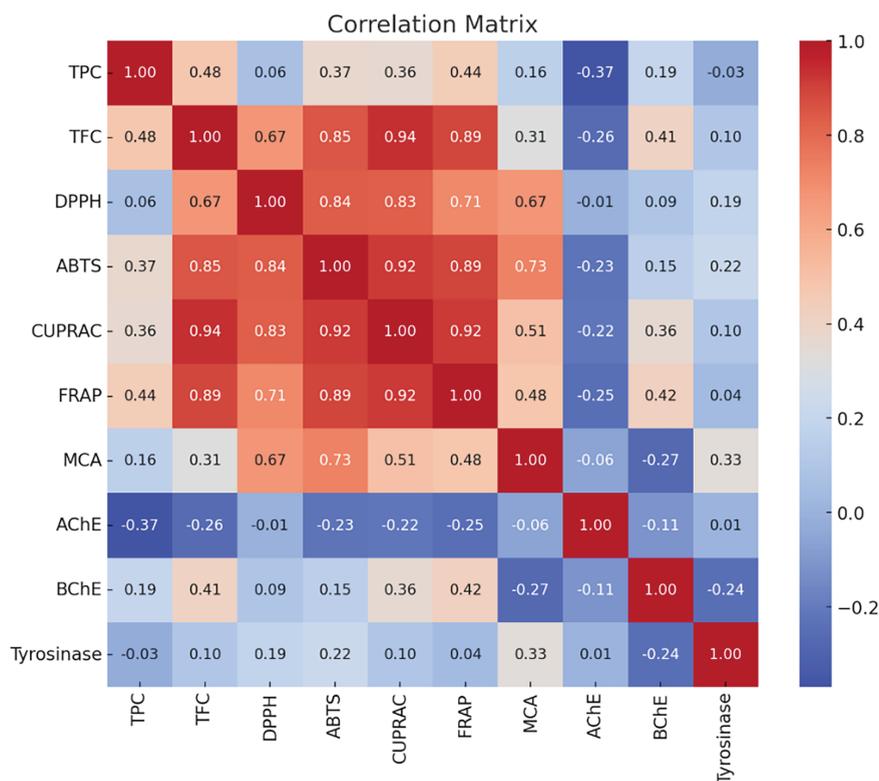


Figure 2. Correlation matrix between the tested biological properties (Pearson's correlation, $p < 0.05$)

65.72–248.41 mg TE/g and those recorded values ≥ 112.90 mg TE/g were ranked as follows; aqueous extract of Sumra Bisha > MeOH extract Sumra Bisha = aqueous extract of Talh Hail > MeOH extract of Talh Hail > MeOH extract of Sider Bisha = aqueous extract of Sider Albaha > aqueous extract of Sider Bisha > aqueous extract of Sider Tehamh > aqueous extract of Dhuhyana > MeOH extract of Sider Tehamh.

All honey samples extracts exerted significant chelating ability in the range 12.72–98.71 mg EDTAE/g with the MeOH extract of Sider Bisha recorded the highest significant ($p < 0.05$) value followed by MeOH extract Sumra Bisha and Talh Hail ($p \geq 0.05$), Sider Tehamh, Dhuhyana and Sider Albaha. Other extracts revealed values ≥ 49.23 mg EDTAE/g.

Overall, it was clear that the different samples of Saudi honey possess remarkable antiradical, ions reducing and chelating properties and it was also observed that the MeOH and aqueous extracts displayed higher antioxidant activity than the EtOH extract. This observation aligned with the TPC which was mainly accumulated in the two former extracts (Džugan et al., 2018). There are many previous studies demonstrating the significant antioxidant properties of honey and it was noted that many factors including the botanical origin and its related environmental conditions, honeybee species and other factors like honey harvest, transport and storage have great influence on the chemical composition of honey and consequently on its antioxidant activity (Al Qahtani et al., 2022; Zaldivar-Ortega et al., 2024; Vujčić Bok et al., 2024; Maringgal et al., 2019; Ya'akob et al., 2019).

3.4 Enzyme Inhibitory Activity

The enzyme inhibitory property of the nine Saudi honey types was evaluated against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase (Tyr) enzymes and results are presented in Table 4. All samples possessed remarkable inhibitory activity towards the three tested enzymes. The anti-AChE was in the range between not active and 10.22 mg GALAE/g with all extracts of Sumra Bisha, EtOH and aqueous extracts of Sider Bisha and Sider Tehamh and Majra as well as aqueous extract of Talh Tehamh and MeOH extract of Talh Hail exerted the highest effect ($p \geq 0.05$). The anti-BChE activity was in the range 2.23–16.46 mg GALAE/g with aqueous extract of Sumra Bisha, Sider Albaha and Talh Hail exhibited the highest inhibitory effect. For the other extracts, except Sider Tehamh, the best anti-BChE was exerted by their EtOH extract. It was also noted that, although the aqueous extract of Dhuhyana, Sider Albaha and Talh Hail was inactive, the aqueous extract of all other honey samples exerted potent anti-AChE activity. Interestingly, the two latter exerted the best anti-BChE activity. Many previous studies on honey samples from different countries demonstrated their significant acetylcholinesterase inhibition (Baranowska-Wójcik et al., 2020; Şahin, 2021; Muhammad et al., 2015; Philip & Fadzelly, 2015; Sz wajgier et al., 2022; Zaidi et al., 2019). They correlated this activity to their high antioxidant activity and TPC. Concerning Saudi honey, only one study reported the anticholinesterase activity of two Saudi honey (Sidr honey and Talih honey) and it was found that both types had anti-BChE

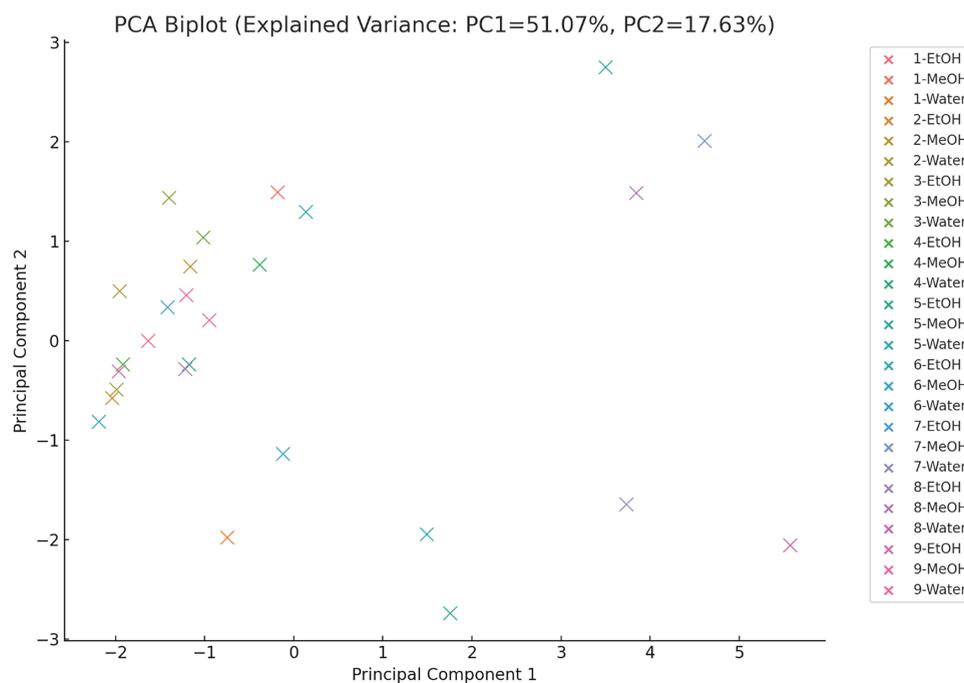


Figure 3. Principal component analysis between the tested honey samples based on the biological properties. 1-Dhuhyana, 2-Majra, 3-Qatada, 4-Sider Albaha, 5-Sider Bisha, 6-Sider Tehamh, 7-Sumra Bisha, 8-Talh Hail, 9-Talh Tehamh



Figure 4. The biological parameters in PCA loadings

activity but sidr honey did not exert anti-AChE activity (Al Qahtani et al., 2022). Contrary to their findings the present study showed that all honey samples (whether sidr or talih type) exerted high anti-AChE and anti-BChE activity.

The anti-Tyr activity was in the range 185.28–260.95 mg KAE/g and hence it was clear that all extracts from the nine Saudi honey samples were potent Tyr inhibitors. Although extracts from Qatada showed the least inhibitory effect towards the two cholinesterase enzymes its MeOH extract recorded the highest anti-Tyr property. Enzyme inhibition has long been approached in pharmaceutical drug development. These inhibitors are synthesized as part of pharmacology and biochemistry. In contrast, previous study on Saudi honey showed inhibition activity against ACh, BuCh, Tyr, α -amylase and α -glucosidase enzymes (Al Qahtani et al., 2022). These findings aligns with previous studies

that reported the anti-Tyr activity of honey obtained from different types of pollen (Al Qahtani et al., 2022; Boutoub et al., 2021; Di Petrillo et al., 2018; Fratianni et al., 2024; Habib et al., 2022; Jantakee & Tragoolpua, 2015; Nguyen et al., 2024).

3.5 Multivariate Analysis

A detailed multivariate statistical examination was conducted on various antioxidant and enzyme inhibitory parameters of honey extracts. The correlation matrix unveiled robust connections among antioxidant analyses, displaying significant positive correlations between TFC, and antioxidant measures like ABTS, CUPRAC and FRAP. This suggests that phenolic and flavonoid compounds considerably enhance the antioxidant capacity of the samples (Figure 2). However, enzyme inhibitory activities (AChE, BChE, Tyrosinase) displayed no correlations with total phenolic/flavonoid contents. Principal Component Analysis (PCA) reduced the complex dataset into three principal components, collectively accounting for the majority of the variance. The PCA biplot displayed distinct sample separation, with extracts such as 1-MeOH and 1-Water differentiated from 1-EtOH and 2-EtOH, underscoring the impact of extraction solvents and sample origin (Figure 3). The loadings plot indicated that parameters like CUPRAC, FRAP, and ABTS contributed significantly to PC1, while enzyme inhibitory activities made notable contributions to PC2 and PC3 (Figure 4).

4 Conclusion

The current study examined the quality of nine Saudi honey types by determining their sugar and HMF contents. Also

their therapeutic potential was evaluated. Results showed that all honey types were of good quality, rich in phenolics and possess remarkable antiradical, chelating and ions reducing properties with Sider Bisha, Sumra Bisha and Talh Hail recorded remarkable effect. Also, the majority of extracts from all the tested honey types displayed potent enzyme inhibitory effect against the acetylcholinesterase, butyrylcholinesterase and tyrosinase enzymes. Thus, these Saudi honey types could play a significant role oxidative stress. Determination of their phenolic composition and isolation of active ingredients are recommended.

Author Contributions

Conceptualization: AA, HE, AAl, SY; methodology: AA, HE, AAl, ME, GZ; software: AA, SY, EM, GZ; validation: SY, GZ; formal analysis: AA, HAE, SY; investigation: AA, HE, AAl, ME; resources: AA, SY, HE, ME, GZ; writing—original draft preparation: AA, SY, ME; writing—review and editing: HE, AAl, GZ; supervision: SY, GZ.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/Records-of-Agricultural-and-Food-Chemistry>.

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