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Full length article

Total Phenolic, Flavonoid Content and Antioxidant Activity of Selected Yemeni Honeys Compared with Manuka Honey

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ABSTRACT

This study evaluated the total phenolic content (TPC), total flavonoid content (TFC), vitamin C concentration, and antioxidant activity (DPPH IC_{50}) in several Yemeni honey varieties, with New Zealand Manuka honey serving as a reference. Five Yemeni honeys were analyzed: Sorab and Acacia from Dhamar governorate, Jaden from Raymah governorate, Sidr from both Dhamar and Hadramout governorates (monofloral), and Maraei from Hadramout governorate (multifloral). Standard analytical methods were employed to determine TPC, TFC, vitamin C, and DPPH IC_{50} values. Results revealed that Yemeni honeys contained TPC ranging from 33.40 to 163.66 mg GAE/100 g, TFC between 1.27 and 4.07 mg QE/100 g, vitamin C levels from 3.29 to 16.32 mg/100 g, and DPPH IC_{50} values between 30.84 and 42.13 mg/ml. In comparison, Manuka honey showed TPC of 108.83 mg GAE/100 g, TFC of 6.07 mg QE/100 g, vitamin C of 10.83 mg/100 g, and the strongest antioxidant activity with the lowest IC_{50} value (11.07 mg/ml). Among the Yemeni samples, Sidr honey exhibited the highest phenolic content (163.66 ± 7.26 mg/100 g) and vitamin C concentration (16.32 ± 0.73 mg/100 g). In conclusion, Yemeni honeys are rich in antioxidant bioactive compounds, underscoring their potential therapeutic value. Future research should expand to other Yemeni honey types and utilize advanced techniques such as HPLC-based profiling to identify individual phenolic constituents.

Keywords: Antioxidant activity, Yemeni honey, DPPH IC_{50}, Vitamin C, Total phenol, Flavonoid, Manuka honey.

INTRODCTION

Honey is a complex natural product containing more than 180 biochemical compounds from diverse molecular families. This mixture is formed after bees process floral nectar within their abdomen (Feknous & Boumendjel, 2022). The physicochemical properties of Yemeni honey are influenced by multiple factors. Dowman et al. (2023) reported that the concentration of bioactive compounds in Yemeni honey depends largely on honey type, floral source, environmental conditions, and processing methods, all of which shape its biological activities.

Several studies have examined Yemeni honeys. For instance, Wabaidur et al. (2020) reported total phenolic content (TPC) values ranging from 32.28 to 49.07 mg GAE/100 g in Jujube (monofloral), Cactus, and multifloral honeys, while Alzahrani et al. (2012) documented a TPC of 62.76 mg GAE/100 g in Acacia honey. The levels of phenolics, flavonoids, antioxidant activity (DPPH), and pollen-reducing power were found to be strongly influenced by botanical origin. Antioxidant activity measured by DPPH ranged between 9.16 and 66.11 mg EAQ/100 g of honey (Silva et al. 2020). Similarly, Zivkovic et al. (2019) observed radical scavenging activity in linden and forest honeys at 0.45 and 2.75 mmol Trolox equivalents/kg, respectively, concluding that darker honeys generally exhibit higher phenolic content and stronger antioxidant activity.

The antioxidant properties of honey are attributed to a diverse set of constituents, including ascorbic acid, catalase, glucose oxidase, proteins, amino acids, organic acids, flavonoids, phenolic acids, carotenoid derivatives, and melanoidins formed during the Maillard reaction. Flavonoids, particularly flavones present in honey, are known for therapeutic benefits such as anticancer and

immunosuppressive effects (Wilczynska & Zak, 2024). Bereksi-Reguig et al. (2024) further demonstrated that bioactive compound content plays a key role in antibacterial properties, with certain honeys showing promise as therapeutic alternatives.

Phenolic compounds are especially critical, as highlighted by Becerril-Sánchez et al. (2021), who emphasized their importance in biological and functional activities, linking phenolic profiles to floral source, geography, and sensory characteristics. Rehman & Majid (2020) also noted that flavonoids and polyphenols are central to honey's therapeutic value in various diseases. Moreover, phenolic acids and flavonoids serve not only as major contributors to antioxidant activity but also as natural markers of botanical origin (Sergiel et al. 2014).

Manuka honey, derived from the manuka tree (*Leptospermum scoparium*) native to New Zealand and Australia, has received considerable attention for its antioxidant properties (Alvarez-Suarez et al. 2014). Despite these findings, there remains a notable gap in the literature regarding comprehensive profiling of bioactive compounds and antioxidant activity across the wide diversity of Yemeni honey types. In particular, comparative studies between premium Yemeni honeys such as Sidr and internationally recognized antioxidant-rich honeys like Manuka are scarce. Such comparisons are essential to establish the functional and commercial potential of Yemeni honeys in both local and global markets.

Accordingly, the present study was designed with two main objectives: (1) to determine the total phenolic content (TPC), total flavonoid content (TFC), vitamin C concentration, and DPPH radical scavenging activity (IC_{50}) of five Yemeni honey types—Sorab, Acacia, Jaden, Sidr, and Maraei; and (2) to compare these parameters with those of Manuka honey.

MATERIALS AND METHODS

Study sitting

This study was conducted in Department of Biotechnology and Food Technology, Faculty of Agriculture, Thamar University, Yemen**.**

Samples collection

Yemeni honey samples were collected from different governorates as described in Table 1. and Manuka honey was purchased from Jordan. All samples were stored in dark glass containers at room temperature until they were analyzed.

Table (1) Samples of honey investigated in this study

No	Honey name	Floral origin	Region	Year	Country
1	**Jaden**	*Kleinia odora*	Raymah-Jadajid	2021	**Yemen**
2	**Sidr**	*Ziziphus spina-Christi*	Dhamar- AL-Manar Doa´ani - Hadramout	2021 2024	
3	**Acacia**	*Acacia gerardi*	Dhamar – Dawran	2021	
4	**Sorab**	*Hypoestes forskaolii*	Dhamar – Dawran	2021	
5	**Maraei** (Pasture)	Multi-flora and sugar-fed honey.	Hadramout	2021	
6	**Manuka** 263NGO (10UMF)	*Leptospermum scoparium*		2021	**New Zealand**

Manuka honey sample

Sorab plant (*Hypoestes forskaolii*).

Jaden plant (*Kleinia odora*).

Bioactive compounds and antioxidants activity determination

Total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu reagent as described by Singleton et al. (1999) with minor modification. Briefly, A 0.5 mL of honey solution (0.1 g/mL) was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent and incubated for 5 min. Then, 2 mL of sodium carbonate (75 g/L) was added and incubated for 2 hrs. After incubation, the absorbance was measured against methanol as blank at 760 nm using a UV-Vis spectrophotometer (Varian Cary 50, Australia). The calibration curve was constructed using gallic acid (0 - 150 µg/ml). The measurement was performed in triplicate and the total phenolic content was expressed as mg gallic acid equivalent (GAE)/100g of honey.

Total flavonoid content (TFC)

The flavonoid contents were determined using a colorimetric method adapted from Sant'ana et al. (2012). Aluminum chloride ($AlCl_3$), 2 ml were mixed with 2 ml of honey solution (500 mg/ml) and 2 ml H_2O: MeOH (1:1). The mixture was then incubated for 30 min. at 25 ˚C. The absorbance was measured at 415 nm. A calibration curve was prepared using a quercetin standard (0 – 50 µg/ml). The results were expressed as mg quercetin/100g of honey.

Vitamin C

Vitamin C (ascorbic acid) content was determined by iodine titration method with starch as indicator according to technique described by Al-Mosa et al. (2019) and Satpathy et al. (2021). In brief, 20 ml of honey solution (5 g in 100 ml distilled water) was transferred into 250 ml beaker and 1 ml of cooled, filtered starch solution (0.5%) was added. Slow titration with iodine (0.005 M) with stirring was carried out to reach the end point and dark blue color appeared. The volume of iodine used in titration was recorded and the vitamin C content was calculated as follow:

$$\text{Vitamin C (mol/l)} = \frac{\text{ml of iodine} \times \text{molarity of iodine}}{\text{Sample volume (ml)}}$$

The amount of vitamin C in (mg/100g) was calculated using the following equation: Vitamin C (mg/100g) = Vitamin C (mole/l) × M.W. of Ascorbic acid × 100.

Radical scavenging activity by DPPH assay

DPPH radical scavenging activity was measured according to Mokaya et al. (2020) with modification. Different concentrations of honey samples were prepared (0 – 25 mg/ml). One milliliter of each concentration was mixed with 4 ml of 0.1 mM methanolic DPPH solution. The tubes were shaken vigorously and incubated at room temperature for 30 min. A control was prepared using 1 ml of methanol instead of the sample and 4 ml of 0.1 mM methanolic DPPH solution. The blank consisted of 1 mL of honey solution with 4 mL of methanol. The absorbance was measured at 517 nm. The percentage of radical scavenging activity was calculated as follows: % Radical scavenging activity $= \frac{A\ control - A\ sample}{A\ control} \times 100$

Where:

A control: Absorbance of control at 517 nm,

A sample: absorbance of the sample at 517 nm.

The IC_{50} (concentration required to inhibit 50% of DPPH radicals) value for each sample was derived from the plot of % radical scavenging activity vs concentration using the linear regression equation: $\boldsymbol{y = ax + b}$, and the IC_{50} was calculated using the equation:

$$IC_{50}\ (mg/ml) = \frac{50 - b}{a}$$

Statistical analysis

All measurements were carried out in triplicate. Data analysis was performed using IBM SPSS Statistics software, version 21.0 (IBM Corp.). A one-way analysis of variance (ANOVA) was applied, followed by Duncan's HSD post-hoc test to assess multiple comparisons. Results are presented as mean values with standard deviation (SD). Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic contents (TPC)

The total phenolic content is widely acknowledged as a key marker of honey's antioxidant capacity, which varies considerably among different honey types due to their floral origin (Zabaiou et al. 2017; Singleton et al. 1999). Phenolic compounds are regarded as the primary contributors to antioxidant activity, and the significant variation in TPC values underscores the influence of botanical source on phenolic composition (Khalil et al. 2012). TPC content of honey samples were determinate as gallic acid equivalent (GAE) using gallic acid standard curve (Fig. 1).

As shown in Table 2, mean TPC values differed significantly ($p < 0.05$) between Yemeni and Manuka honey samples. Sidr honey exhibited the highest TPC (163.66 mg GAE/100 g), while Maraei honey recorded the lowest (33.40 mg GAE/100 g). The overall range observed in Yemeni honey (29.90–163.66 mg GAE/100g) is consistent with earlier studies. For example, Wabaidur et al. (2020) reported TPC values of 49.07, 37.46, and 32.28 mg GAE/100 g for Jujube (monofloral), Cactus, and multifloral Yemeni honeys, respectively. Likewise, Bereksi-Reguig et al. (2024) documented wide variability in TPC (24.17–122.15 mg GAE/100 g) across honey types, attributing this to differences in floral origin. Zivkovic et al. (2019) found forest honey to have the highest phenolic content (138.97 mg GAE/100 g), noting that darker honeys generally contain greater phenolic levels and stronger antioxidant activity.

The relatively low TPC in Maraei honey (33.40 mg GAE/100 g) may be linked to its production method, as this honey is often produced from bees fed sugar solutions rather than natural nectar, resulting in reduced phytochemical content. Comparable findings were reported by Silva et al. (2020), who observed phenolic contents ranging from 27.65 to 97.01 mg GAE/g, with an average of 62.66 ± 20.46 mg GAE/g across honey samples.

Table (2) Total phenolic compounds content of Yemeni and Manuka honey (mg GAE/100g)

Origin	Honey type	Mean ± SD	Min.	Max.
Yemen	Jaden	98.46 ± 4.11 ^{c}	95.00	103.00
	Sidr	163.66 ±7.26 ^{e}	155.70	169.90
	Acacia	114.66 ±7.24 ^{d}	109.90	123.00
	Sorab	67.36 ± 4.16 ^{b}	62.70	70.70
	Maraei	33.40 ± 3.36 ^{a}	29.90	36.60
New Zealand	Manuka	108.83 ± 2.51 ^{d}	106.50	111.50

Different superscript letters in column indicate statistically significant differences at ($p < 0.05$).

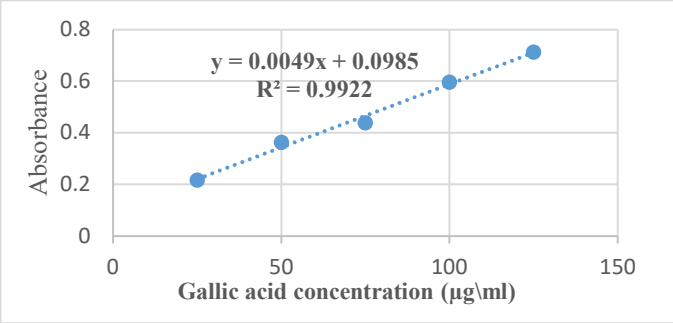

Fig. 1. Standard curve of gallic acid for total phenolic content

Manuka honey exhibited a mean TPC of 108.83, with a range (106.50 to 111.50 mg GAE/100 g), which is higher than previously reported values of 65.79 mg GAE/100g (Bundit et al. 2016) and 85.61 mg GAE/100g (Grabek-Lejko et al. 2024). Previous studies have reported the TPC of Manuka honey to be 10.399 mg GAE/100g (Joshua Boateng and Diunase, 2015), 53.5 mg GAE/100g (Kazmierczak-Baranska et al. 2024), and 89.909 mg GAE/100g (Alzahrani et al. 2012).

Notably, Yemeni Sidr and Acacia honeys exhibited higher TPC than Manuka honey, suggesting that these local honeys possess comparable or superior antioxidant potential.

Total flavonoid content (TFC)

Flavonoids are well known for their strong free radical scavenging activity and play an important role in the functional properties of honey (Beretta et al. 2005; Yao et al. 2003). TFC content of honey samples were determinate as quercetin equivalent (QE) using quercetin standard curve (Fig. 2).

Table 3 summarizes the TFC values of the analyzed honey samples. For Yemeni honeys, TFC ranged between 1.27 and 4.07 mg QE/100 g, which falls within the range (0.07–33.49 mg QE/100 g) reported by Bereksi-Reguig et al. (2024), but is markedly lower than the values (543–1302 mg QE/100 g) documented by Silva et al. (2020). Wabaidur et al. (2020) also reported relatively low TFC values for Yemeni honeys, ranging from 5.29 to 11.61 mg QE/100 g. In contrast, Manuka honey exhibited a higher TFC (6.07 mg QE/100 g) compared with Yemeni samples, consistent with its reputation for strong bioactivity.

Table (3) Total flavonoid content of Yemeni and Manuka honey (mg QE/100 g)

Origin	Honey type	Mean ± SD	Min.	Max.
Yemen	Jaden	4.07 ±0.21 ^{d}	3.90	4.30
	Sidr	2.57 ±0.21 ^{b}	2.40	2.80
	Acacia	1.27 ±0.06 ^{a}	1.20	1.30
	Sorab	3.50 ±0.10 ^{c}	3.40	3.60
	Maraei	1.63 ±0.49 ^{a}	1.30	2.20
New Zealand	Manuka	6.07 ±0.46 ^{e}	5.80	6.60

Different superscript letters in column indicate statistically significant differences at (p < 0.05).

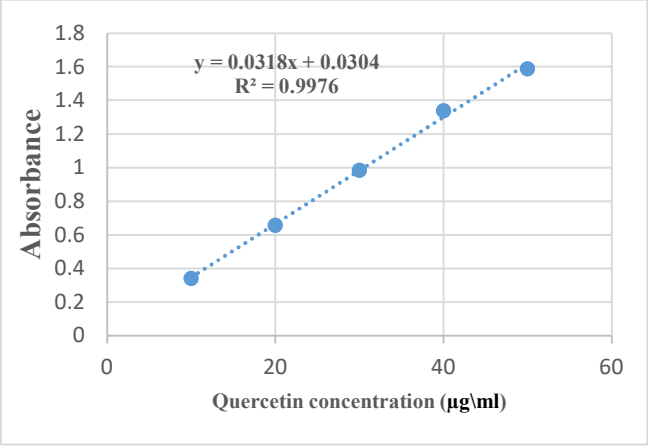

Fig. 2. Standard Curve of Quercetin (µg/ml)

TFC/TPC ratio

Flavonoid and phenolic acids are natural indicators of the botanical origin of some honeys (Sergiel et al. 2014). Fig. (3) shows the ratio of total flavonoid and total phenol content (TFC-TPC) for all honey samples tested. The values were 0.0413, 0.0153, 0.0113, 0.0520, and 0.0483 for Jaden, Sidr, Acacia, Sorab, and Maraei honey samples respectively, while it was 0.0557 for Manuka honey samples.

These results are fall in the range of (Can et al. 2015) who reported that the flavonoid expressed as quercetin equivalents represented 2-10% of the TPC of Turkish monofloral honeys, while, these results are lower than those of Zivkovic et al. (2019) who found that the lowest TFC- TPC ratio of 0.08 was in acacia honey, whereas the highest was recorded in meadow and bee pollen-enriched honey (0.13). Bueno-Costa et al. (2016) reported that honeys from several regions in Brazil possessed TFC of approximately 10% of the average total phenolic content.

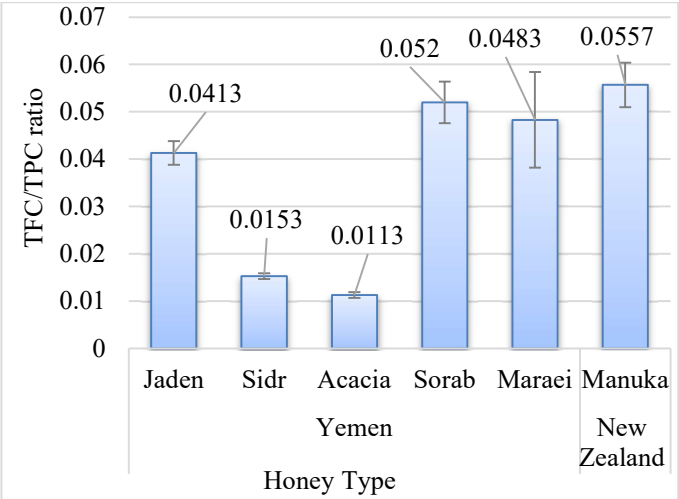

Fig. (3) TFC/TPC ratio in Yemeni and Mauka honey

Vitamin C Content

Although present in smaller quantities than phenolic compounds, vitamin C contributes significantly to the antioxidant potential of honey. Table 4 shows that vitamin C levels varied notably ($p < 0.05$) among the Yemeni and Manuka honey samples analyzed. Sidr honey contained the highest concentration (16.32 mg/100 g), while Maraei honey had the lowest (3.29 mg/100 g). Acacia and Jaden honeys also exhibited relatively high levels, at 11.42 and 9.80 mg/100 g, respectively. The reduced vitamin C content in Maraei honey may be linked to sugar-feeding practices during production rather than natural nectar collection, which limits its phytochemical richness.

Table (4) Vitamin C content of Yemeni and Manuka honey (mg/100 g)

Origin	Honey Type	Mean ±SD	Min.	Max.
Yemen	Jaden	9.8 ±0.41 ^{c}	9.45	10.25
	Sidr	16.32 ±0.73 ^{e}	15.52	16.94
	Acacia	11.42 ±0.72 ^{d}	10.94	12.25
	Sorab	6.69 ±0.42 ^{b}	6.22	7.02
	Maraei	3.29 ±0.34 ^{a}	2.94	3.61
New Zealand	Manuka	10.83 ±0.25 ^{d}	10.60	11.10

Different superscript letters in column indicate statistically significant differences at (p < 0.05).

Manuka honey contained 10.83 mg/100 g of vitamin C, which is lower than the values reported by Bundit et al. (2016), who found 106.737 mg/100 g in Manuka honey. Similarly, Al-Mosa et al. (2019) reported much higher vitamin C levels in Ziziphus and Acacia honeys (239.2 and 260.4 mg/100 g, respectively). In contrast, the Yemeni honeys analyzed in this study showed higher vitamin C content than that reported by Alwaseai et al. (2022), who found only 0.65 mg/100 g in Yemeni honey. Overall, these variations in vitamin C content highlight the strong influence of plant

source, processing conditions, and geographical region on the nutritional composition of honey (Silva et al. 2009; Alwaseai et al. 2022).

DPPH IC_{50} Scavenging activity

DPPH IC_{50} Scavenging activity of each honey sample was derived from the plot of % radical scavenging activity vs sample concentration (mg/ml) using the linear regression equation: $y = ax + b$ (Fig. 4). The antioxidant activity assessed using the DPPH assay showed no significant variation ($P<0.05$) among the honey samples analyzed (Table 5). As is well established, a lower IC_{50} value reflects stronger antioxidant capacity. Manuka honey demonstrated the highest radical scavenging activity, with the lowest IC_{50} value (11.07 mg/ml). Among the Yemeni honeys, Sidr honey exhibited the strongest activity (IC_{50} = 30.84 mg/ml), while Jaden honey showed the weakest (IC_{50} = 42.13 mg/ml).

These results are in line with previous reports. Alzahrani et al. (2012) documented IC_{50} values of 13.46 mg/ml for Manuka honey and 13.62 mg/ml for Acacia honey. Similarly, Bereksi-Reguig et al. (2024) reported a broad range of IC_{50} values (22.91–98.58 mg/ml) across different honey types. The comparatively lower antioxidant activity of Yemeni honeys relative to Manuka may be attributed to differences in floral sources and phenolic composition.

According to Sakika et al. (2022), honey samples with IC_{50} values between 10 and 50 mg/ml are classified as having strong antioxidant activity, those between 50 and 100 mg/ml as intermediate, and values above 100 mg/ml as weak. Based on this classification, all Yemeni honey samples in the present study fall within the strong antioxidant activity category.

Table (5) DPPH IC_{50} scavenging activity of Yemeni and Manuka honey (mg/ml)

Origin	Honey type	Mean ± SD	Min.	Max.
Yemen	Jaden	42.13 ±6.55 [b]	37.61	49.64
	Sidr	30.84 ±5.90 [ab]	25.88	37.37
	Acacia	32.68 ±6.03 [ab]	25.88	37.38
	Sorab	37.89 ±28.02 [ab]	21.71	70.24
	Maraei	36.34 ±16.0 [ab]	21.38	53.22
New Zealand	Manuka	11.07 ±0.53 [a]	10.52	11.57

Different superscript letters in column indicate statistically significant differences at ($p < 0.05$).

Importantly, the DPPH IC_{50} of Sidr honey (30.84 mg/ml) was within the range of values reported for many commercial honeys with acknowledged bioactivity. This finding supports the traditional use of Yemeni Sidr honey as a health-promoting product.

The results demonstrated variations in the antioxidant activity among the honey samples. These variations reflect differences in botanical origin and highlight the diverse quality attributes of the honey samples.

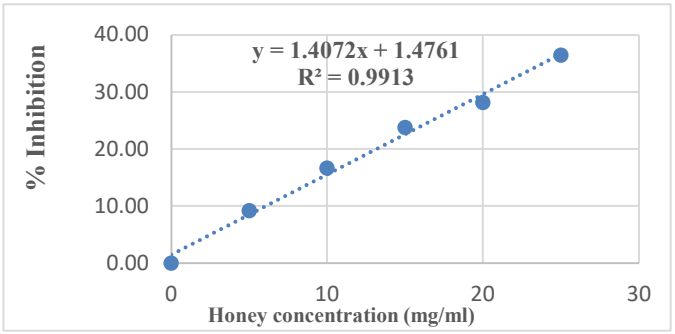

Fig. (4) DPPH scavenging activity curve of honey sample (mg/ml)

CONCLUSIONS

This study demonstrates that Yemeni honeys, particularly Sidr and Acacia honeys, contain substantial amounts of TPC and vitamin C, which contribute to their antioxidant activity. Yemeni Sidr and Acacia honey contains TPC and vitamin C amount higher than Manuka honey, supporting its value as a bioactive natural product. Significant variations in bioactive compound content among Yemeni honey samples highlight the influence of geographical origins and botanical source. Future studies should include more Yemeni honey types using modern techniques such as HPLC-based profiling of individual phenolic compounds.

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AUTHOR CONTRIBUTIONS

HHA Al-Khawlani, AM Alwaseai, MM Alsharhi authors equally contributed on protocol proposal, collection, processing, analyzed, interpretation of data and wrote first & final version of Manuscript. All Authors have approved this version of the manuscript.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ETHICAL STANDARDS

The study received approval from the Faculty of Agriculture, Thamar University.

DATA AVAILABILITY:

All data generated and analysed during this study are included in this published article.

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الفينولات الكلية والفلافونيدات والفعالية المضادة للأكسدة في أنواع من العسل اليمني مقارنةً بعسل المانوكا

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الملخص

هدفت الدراسة الى تقدير الفينولات الكلية والفلافونيدات وفيتامين C والفعالية المضادة للأكسدة مقدرة في صورة DPPH IC_{50} في أنواع من العسل اليمني ومقارنتها بعسل المانوكا. شملت الدراسة خمسة أنواع من العسل اليمني: (عسل احادي الزهرة) صورب واكاسيا من محافظة ذمار وجعدن من محافظة ريمة وسدر من محافظتي ذمار وحضرموت) و(عسل متعدد الزهرة) مراعي من محافظة حضرموت ونوع واحد من عسل (المانوكا من نيوزيلاندا). تم تقدير كلاً من الفينولات الكلية والفلافونيدات وفيتامين C والفعالية المضادة للأكسدة DPPH IC_{50} في عينات العسل. أظهرت النتائج ان الفينولات الكلية كانت تتراوح ما بين 33.40 الى 163.66 (مليجرام 100/GAE جرام)، والفلافونيدات الكلية كانت ما بين (1.27 الى 4.07 مليجرام 100/QE جرام) وفيتامين C ما بين (3.29 الى 16.32 مليجرام/100 جرام)، والفعالية المضادة للأكسدة DPPH IC_{50} (30.84 الى 42.13 مليجرام/مل) في العسل اليمني، في حين كانت الفينولات الكلية (108.83 مليجرام 100/GAE جرام) والفلافونيدات الكلية (6.07 مليجرام 100/QE جرام)، وفيتامين C (10.83 مليجرام/100 جرام)، والفعالية المضادة للأكسدة DPPH IC_{50} (11.07 مليجرام/مل) في عسل المانوكا. كما أظهرت النتائج ان عسل السدر اليمني كان يحتوي على اعلى نسبة من الفينولات الكلية وفيتامين C حيث بلغت (163.66 ± 7.26 مليجرام 100/GAE جرام) و (16.32 ± 0.73 مليجرام/100 جرام) على التوالي مقارنةً بكافة عينات العسل التي شملتها الدراسة، في حين حقق عسل المانوكا اعلى فعالية مضادة للأكسدة (أقل قيمة لـ IC_{50} = 11.07 مليجرام/مل)، كذلك كان لعسل السدر اليمني فعالية مضادة للأكسدة مقاربة للفعالية لعسل المانوكا مما يؤكد قيمته العالية كمنتج ذات فعالية حيوية عالية.

الكلمات المفتاحية: مضادات الاكسدة، العسل اليمني، DPPH IC_{50}، فيتامين C، الفينولات الكلية، الفلافونيدات، عسل المانوكا.

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