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First report of some chemical and biological properties of two wild honey types collected from a remote area in Southern Darfur, Sudan

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Abstract

Natural honey's physico-chemical and biological qualities vary by origin, variety and nectar source. Wild honey may provide health benefits. Biomedical research reveals its advantages. Little is known about Sudanese wild honey and Southern Darfur honey. In this investigation, the antioxidant, antibacterial, and physicochemical characteristics of two varieties of Sudanese wild honey were studied. Ziziphus and acacia honey ranged in amber color, density, pH, ash, electrical conductivity and free acidity. Both honey samples included vital nutrients and no heavy metals. Both honey samples exhibit exceptional antioxidant activity, but Acacia honey's antioxidant capacity is greater. Both honey samples demonstrated outstanding antibacterial efficacy against all gram-positive and gram-negative bacteria, except *Pseudomonas aeruginosa.* Ziziphus honey was more antibacterial than acacia honey. The MIC and MBC data exhibited bacteriostatic and bactericidal activity against the investigated microorganisms, indicating a synergistic effect of honey's overall contents against pathogens. These results imply that ziziphus and acacia honey samples from Southern Darfur, Sudan, have antibacterial and antioxidant properties and could be used to improve health and as a food supplement.

1. Introduction

Since ancient times and till nowadays, natural compounds, which are organic substances produced by living organisms, have been an important source of pharmaceuticals, and more than half of all medications in clinical use around the world are derived from natural compounds and their derivatives, including non-synthetic antibiotics and a number of currently used pharmaceutical treatments. According to estimates by the World Health Organization, almost 80% of people in undeveloped countries depend on natural products and plants for basic health care (Abdallah, 2011; Mohapatra *et al.*, 2011; Newman and Cragg, 2016; Seo *et al*., 2020). Research shows that people prefer natural pharmaceuticals over synthetic ones. However, the effects of this preference on the choice of medications in the medical field are not well understood, so scientific verification of natural product bioactivity is needed (Bucar *et al*., 2013; Meier and Lappas, 2016).

Honey has a long history as the earliest functional and nutritional food consumed by humans. Honey was referenced in the texts of Egypt, India, and China as early as 5500 B.C. The use of honey in treatment is recorded in 5,000-year-old Egyptian texts; the Papyrus Ebers is filled with compliments for honey's healing virtues. Ancient physicians, including Aristotle, Aristoxenus, Hippocrates, Cornelius Celsus, Dioscorides, Galen, Porphyry, and Arab physicians El Basry and El Mad Joussy wrote about honey's curative and medical properties (Crane, 1999; Israili, 2014). Until now, honey has maintained its appeal and found a place in contemporary medicine (Eteraf-Oskouei and Najafi, 2013). Honey is a by-product of floral nectar and the bee's upper aerodigestive tract. It has a complex chemical makeup that changes depending on the plant source. It is concentrated by a dehydration process inside the beehive, and most of it is a sugar solution that is very concentrated (Rossi and Marrazzo, 2021). Although

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sugars in natural honey are the major constituents and mostly fructose, glucose, and fructo-oligosaccharides, it also has more than 300 other bioactive molecules (Ajibola, 2015). These bioactive molecules include flavonoids and phenolic acids, ascorbic acid, some enzymes, amino acids, organic acids, proteins, carotenoids, and Maillard reaction products (Gómez-Caravaca *et al*., 2006). Honey's active molecules are thought to have two health-promoting benefits: one is curative, such as antioxidant, antimicrobial, antiparasitic, antidiabetic, anticancer, and wound-healing characteristics (Abdallah and Hamed, 2019; Nabti and Lazhari, 2022); and the other is protective on the gastrointestinal, cardiovascular, respiratory, and nervous systems (Cianciosi *et al*., 2018; Ismail *et al*., 2021). Moreover, some honey sugars are highly beneficial; fructo-oligosaccharides, which are non-digestible substances, may boost digestive function as they're a rich source of prebiotics, which promote probiotics in the human intestines and improve gut-microbial balance (Mohan *et al*., 2017; Ramsay *et al*., 2019).

Due to the large diversity of honey depending on floral origin, it is difficult to come to a consensus on the chemical markers of honey gathered from various sources (Kaškonienė and Venskutonis, 2010). Manuka honey (mono-floral honey) is produced by honeybees in New Zealand and Australia from the nectar of *Leptospermum scoparium*. According to published studies, Manuka honey contains several medicinal advantages, including antibacterial and antioxidant activities (Alvarez-Suarez *et al*., 2014). There have also been reports that honey samples from different countries have different degrees of antibacterial activity. About 24 honey samples, 16 from different parts of Oman and 8 from other countries in Africa were tested to evaluate their antibacterial efficacy. The honey samples had different levels of antibacterial effects (Al-Jabri *et al*., 2003). Variations in honey's antioxidant activity were also noticed, and scientific data indicates that honey, taken alone or in conjunction with conventional medication, could serve as a unique antioxidant in the treatment of chronic ailments often linked with oxidative stress (Erejuwa *et al.*, 2012). Sudan is a massive, agriculture-based country with substantial arable territory, an abundance of water and natural resources, and a number of agro-ecological zones. Numerous plants grow naturally or with traditional techniques and do not need chemical fertilizers (Abdalla and Abdel Nour, 2001). The Darfur area of Sudan is rich in natural resources but has been neglected for decades owing to the continued conflict (Karamalla-Gaiballa and El-Kafafi, 2021). As honey's chemical and medicinal properties vary a lot depending on where beehives are grown because of how the climate, environment, and plant species change and are diverse (Abdallah, 2016), and since little is known about the chemical and biological properties of natural honey grown wild in Darfur (Sudan), this study was carried out to evaluate the physicochemical, antioxidant and antibacterial properties of two famous wild kinds of honey known as Sunt honey (Acacia honey) and Sidir honey (Ziziphus honey) collected from Am-Dafok city, southern Darfur.

2. Materials and methods

2.1 Honey samples

The two honey samples from monofloral botanical origins were manually collected by local honey collectors in Am-Dafok City, southern Darfur, Sudan (Figure 1). Acacia honey, known locally as sunt honey, whose plant origin is predominantly *Acacia nilotica*, and ziziphus honey, known locally as sidir honey, whose plant origin is mainly *Ziziphus spina-christi*. The samples were taken in December 2021 and kept in dark glass vials with tight lids and stored at 4-5°C until they could be investigated.

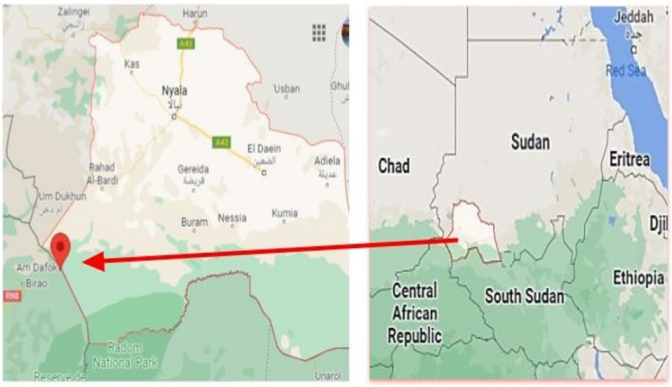

Figure 1. Location of Am-Dafok city, southern Darfur, where honey samples were collected. Source: Google Map data © 2022).

2.2 Microorganisms

A total of five referenced bacterial strains were used in this investigation, representing referenced gram-positives (*Staphylococcus aureus* ATCC BAA-1026 and *Bacillus cereus* ATCC 10876), and gram-negatives (*Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 9637 and *Salmonella enterica* serovar Typhimurium ATCC 14028). All bacterial isolates were obtained from the stock culture of the Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia.

2.3 pH values

The pH of the honey samples was determined using a standardized approach using a pH meter Martini, Mi 180 Bench Meter (Omafuvbe and Akanbi, 2009).

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2.4 Density

The density of the honey samples was determined using the pycnometer technique using the formula below (Aljohar *et al*., 2018).

$$\text{Density} = W_2 - W_1 / V$$

Where W_1 = mass of the pycnometer when empty, W_2 = mass of the pycnometer filled with honey sample and V = volume of the

2.5 Electrical conductivity

Electrical conductivity was measured at 20°C in solutions of honey samples. Approximately 20 g of honey was solved in 100 mL distilled water and measured by a conductometer (Thermo Scientific, Orion Star A212). The method of measuring is prescribed by Lazarus *et al*. (2021).

2.6 Ash content

The dry ash technique was used to determine the ash content (Braga *et al*., 2019). In the blazing crucibles, ten grams of each sample were inserted. The crucibles were heated for two hrs in an Electric Muffle Furnace (JISICO J-FM38, South Korea). The following formula is used to compute the proportion of ash in g/100 g of honey.

$$\text{Ash content} = A_1 - A_2 / A_0$$

Where A_0 = weight of honey taken, A_1 = weight of crucible + ash and A_2 = weight of the crucible

2.7 Free acidity

The acidity of honey was determined by the volumetric method (Kayode and Oyeyemi, 2014). Ten grams of honey were dissolved in 75 mL of distilled water and the solution was titrated with 0.1 M NaOH to pH 8.30. Acidity is expressed in milliequivalents/kg honey (mEq/kg).

2.8 Element analysis

Elemental analysis was carried out using Thermo Fisher Scientific ARL QUANT'X XRF spectrometer instrument model: AA83811. The plastic cups were fixed at one end with a film, and then, 1 g of two honey samples was placed in special cups. After, the cups were placed inside the spectrometer chamber facing down toward the X-ray tube (Farooq Khan and Maqbool, 2008).

2.9 Preparation of honey extracts

Sample (1 g) and 15 mL 50% aqueous ethanol were stirred for 3 mins in a 25-mL universal bottle at 25,000 rpm using a homogenizer (IKA, Germany). Samples were then centrifuged at 4,750×*g* for 10 mins using a mini centrifuge (Thermo-line, China) and the supernatants were used for further analyses.

2.10 Determination of total phenolic content

The total phenolic content (TPC) was estimated using the Folin-Ciocalteu method. TPC is expressed as µg gallic acid equivalents (GAE) per 100 g samples following the procedure of Slinkard and Singleton (1977).

2.11 Ferric reducing antioxidant power

FRAP determination was carried out according to the method of Benzie and Strain (1999) The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (µmol TE/100 g).

2.12 Cupric reducing antioxidant capacity

Cupric reducing antioxidant capacity (CUPRAC) determination was carried out according to Elshaafi *et al*. (2020). The calculation of results was based on the equation generated by Trolox standard curve. Results were expressed as milligrams of Trolox equivalents per 100 g of dry sample (µmol TE/100 g).

2.13 DPPH radical scavenging activity

Based on the method of Musa *et al*. (2011) the antioxidant activity was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH was freshly prepared by dissolving 40 mg DPPH in 1000 mL methanol to obtain the absorbance of 1.00±0.01 unit at 517 nm wavelength using a spectrophotometer (Epoch, Biotek, USA). Approximately 100 µL sample with mix with 1 mL DPPH solution and kept in the dark for 2 hrs. The DPPH scavenging activity was determined based on the following equation: DPPH scavenging activity (%) = (Absorbance of DPPH without sample –Absorbance of DPPH with sample) / Absorbance of DPPH without sample × 100.

2.14 Well-diffusion assay

The antibacterial activity of the two wild honeys was first tested using the modified Agar-well diffusion assay (Abdellatif *et al*., 2022). As a positive control, a conventional antibiotic (chloramphenicol, 2.5 mg/mL) was utilized. The bacterial strains were sub-cultured and adjusted to 0.5 McFarland turbidity (10^7 CFU/mL) prior to antibacterial screening. A group of 25 mL glass bottles containing autoclaved nutrient agar were placed in pre-sterilized Petri plates and allowed to harden at room temperature. On the surface of the plates, three wells

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were drilled using a sterile 6-mm cork-borer. The agar plates were smeared with 100 µL of the adjusted culture (10^7CFU/mL). About 50 µL of raw honey samples (100%) and the positive control were placed into the wells (2 wells on each plate). The 50 µL of chloramphenicol (2.5 mg/mL) was poured into another well on the same plate. At 35°C, the seeded plates were incubated overnight. The inhibition zone was determined after incubation. The diameter of the inhibitory zone (in mm) was used to indicate antibacterial activity, and the mean of two replicates (in mm ± standard deviation) was calculated. If the zone was less than 10 mm, the antibacterial activity was regarded to be weak, between 10 and 13 mm for moderate, and more than 13 mm for strong antibacterial activity.

2.15 Minimum inhibitory concentration assay

The bacteria that exhibited significant sensitivity to the raw honey samples in the agar diffusion assay were submitted to the minimum inhibitory concentration (MIC) assay using a modified version of the microdilution technique (Gulluce *et al*., 2006). In a 96-well plate, 100 µL of raw honey was loaded into the first well, followed by 100 µL of sterile normal saline (0.9%) and mixed with an Eppendorf pipette with a single-use sterile tip. then, 100 µL was transferred to the other well and repeated in a series of twofold dilutions for each of the two honey samples (using sterile normal saline) in descending concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 % v/v, respectively. Another 100 µL from the last well (the final dilution) was removed to equalize the volume of dilutions in each well to 100 µL. Then, 95 µL of sterile Double-strength nutrient broth and 5 µL of the adjusted bacterial suspensions (10^7 CFU/mL) were added to each row of wells. Approximately 200 µL is the final volume in each well. Each MIC test was conducted three times to eliminate the possibility of human error and obtain the precise MIC. The same technique was done with two more sets of negative control (broth + normal saline + bacteria) to compare and adapt the experiment, and positive control (chloramphenicol 2.5 mg/mL), was serially diluted. The plates were then incubated at 37°C overnight. By measuring absorbance at 959 nm with the iMark Absorbance 4 Bioinorganic Chemistry and Applications Microplate Reader, bacterial growth was detected. The inhibitory effect was determined by absorbance at 959 nm using the iMark Absorbance Microplate Reader.

2.16 Minimum bactericidal concentration assay

The minimum bactericide concentration (MBC) was determined by subculturing 10 µL from each microplate well of the MIC test and incubating them separately on a Nutrient Agar (Oxiod, UK) plate and incubated at 37°C for 18 hrs and then inspected for bacterial growth. The MBC value is defined as the lowest concentration in the medium that did not produce any growth (Hamad Al-Mijalli *et al*., 2022). After obtaining MBC values, MBC/ MIC ratios were determined.

2.17 Statistical analysis

The analysis of variance (ANOVA) test was used to analyze the data, followed by Tukey's HSD test. Values with $p < 0.05$ were considered significant. Whenever possible, data was presented as mean ± standard deviation (SD). In this investigation, the SPSS Statistical Analysis System program was used (SPSS software version 21).

3. Results and discussion

3.1 Physico-chemical properties

Table 1 shows the findings of the physicochemical characteristics investigated. The color of honey varies from Light amber for sample 1 to dark amber for sample 2 (Figure 2). The color difference is caused by differences in the proportions of dissolved substances in the plant's origin, such as pigment compounds that are transported through nectar and are high in chlorophyll and carotene, and the proportion of minerals and mineral salts, which varies depending on the type of plant. The hue of honey is also affected by temperature and storage conditions (Machado De-Melo *et al*., 2018). There is a stronger association between the color of honey and its overall flavonoid concentration. The darkest honey has the highest levels of total flavonoid concentration (Becerril-Sánchez *et al*., 2021). The pH values of honey samples were within international specifications, ranging from 4.1 to 4.4. Honey is an acidic medium, and the differences in acidity values between honey samples are due to a variety of factors, including the period of maturity, the type of flowering, as well as the chemical composition of honey, mineral concentrations, phenols in it, and the percentage of acids in addition to mineral concentrations (de Almeida-Muradian *et al*., 2013). The density values obtained for honey samples are in accordance with international standards. The values varied from 1.1 to 1.12, with the lowest value for honey

Table 1. Physio-chemical properties of the five honey samples.

Honey Samples	Color	Density (g/cm^3)	pH	Ash content (%)	Electrical conductivity (mS/cm)	Free Acidity (mEq/kg)
Ziziphus honey	Light Amber	1.12	4.4	1.01	0. 5	31
Acacia honey	Dark amber	1.1	4.1	0.96	0. 4	30.8

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sample 2 and the highest value for sample 2, and the variance is attributable to storage or failure to shut storage containers affecting the density. The ash content of honey samples was within the acceptable range (0.96 - 1.1%) mentioned in previous research (Svečnjak *et al*., 2019). According to the data in Table 1, the conductivity of the samples varied from 0.4 to 0.5 mS/cm, with sample 1 having the greatest conductivity because bees feed on its blooms, which are located in saline locations. Furthermore, as compared to the places where sample 2 is found, sample 1's mineral content is substantially higher than sample 2, which has a direct impact on its conductivity (Ruoff *et al*., 2006). In two of the samples evaluated, free acidity was less than 50 mEq/kg. These findings came from the examination of two types of honey. The acidity levels in the samples studied ranged from 1.5 to 30 mEq/kg. When compared to other honey varieties, the results showed that acacia honey has a low acidity while forest honey has a high acidity (Boussaid *et al*., 2018). Similar previous study on Ethiopian honey (Harenna forest honey) showed that reducing sugar, sucrose, ash, pH, hydroxymethylfurfural (HMF) contents and electrical conductivity and specific rotation were found to be 69.48±1.72 g/100 g, 2.43±1.02 g/100 g, 0.19±0.09 g/100 g, 3.87±0.16, 0.84±0.46 mg/kg and 0.70±0.04 mS/cm (Belay *et al*., 2013).

Figure 2. The colors of the honey samples, sample 1: Ziziphus honey, and sample 2: Acacia honey.

Table 2 shows the findings of the element analysis of honey samples using XRF equipment. Potassium, aluminum, calcium, iron, bromide, and chloride were the most prevalent components detected in honey samples evaluated in this study. In general, the mean concentration of these components in sample 1 was greater than the concentration in sample 2 (Lovaković *et al*., 2018). Fe, Mn, and Zn are major and trace elements that are required for a variety of physiological activities and provide nutritional advantages. However, these elements are only safe and sufficient for the body within a certain range of consumption, and excessive exposure can cause acute and chronic toxicity (El-Haskoury *et al*., 2018). Trace elements such as As, Cd and Pb did not appear in the analysis because their presence endangers humanity. Due to its tendency to accumulate in the body and disrupt normal biological activities even at low doses, it can be harmful (Aghamirlou *et al*., 2015).

3.2 Antioxidant properties

The total phenolic compounds of Acacia honey were higher significantly than Ziziphus honey (Table 3). Total phenolic content of Ziziphus honey is higher than the phenolic content of honey reported by other researchers from different countries (Ferreira *et al*., 2009; Khalil *et al*., 2012; Pontis *et al*., 2014) and lower than the results of Al-Farsi *et al*. (2018) who reported the total phenolic of Ziziphus honey collected in Oman to be in a range of 972–1520 mg/kg. However, Acacia honey showed higher total phenolics in comparison to other honey reported. The reducing capacity based on CUPRAC and FRAP was high in Acacia honey in comparison to Ziziphus honey (Table 3). However, Acacia honey was higher than the value of CUPRAC and FRAP reported by de Almeida *et al*. (2016) that the FRAP values were varying from 99.4 to 720.4 µmol TE/100 g honey and reported the CUPRAC values to be between 338.7 to 960.0 µmol TE/100 g honey.

Table 3. Total phenolics and antioxidant activity using different assays.

Test	Ziziphus honey	Acacia honey
TPC (µg GAE/100 g)	1057±25.37^{b}	1665.82±36.18^{a}
CUPRAC (µmol TE/100 g)	775.67±3.93^{b}	1614.28±7.46^{a}
FRAP (µmol TE/ 100 g)	249.31±17.68^{b}	716.23±17.13^{a}
DPPH (%)	50±4^{b}	85±3^{a}

Values are presented as mean±SD. Values with different superscripts within the same rows are statistically significantly different ($p<0.05$). TPC: total phenolic content, CUPRAC: cupric reducing antioxidant capacity, FRAP: ferric reducing/ antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

The antioxidant activity of honey was also tested by

Table 2. Results of element analysis of the two honey samples.

Honey Samples	The percentage of elements analysis (m/m) %									
	K	Al	Ca	Cl	Br	Fe	Zn	Ni	Mn	Rb
Ziziphus honey	43.78	16.69	8.7	2.72	0.097	0.189	0.026	0.031	0.096	0.241
Acacia honey	36.11	24.65	4.73	2.28	0.098	0.213	0.042	Nil	0.148	0.343

Nil: No result

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the DPPH stable free radical assay. The average antioxidant activity of tested honey samples expressed as % of inhibition was 50% Ziziphus honey and 85% for acacia honey. Dzugan *et al*. (2018) reported the DPPH value for 90 samples of Polish honey and the results were lower than the DPPH value of acacia honey in this report. The Ziziphus honey showed only 50% scavenging of DPPH radicals, and it may be considered a medium scavenging activity. The results from this study are higher than those reported by Moniruzzaman *et al*. (2013) for Malaysian honey. Perna *et al*. (2013) reported the radical scavenging activity of Italian honey from 55.06% to 75.37%. Acacia samples showed higher total phenolic content as well as antioxidant activities. Several studies have revealed a stronger significant correlation between honey's total phenol content and its biological activity (Dzugan *et al*., 2018; Becerril-Sánchez *et al*., 2021).

Based on the results of this study, honey may serve as a source of antioxidants. Dzugan *et al*. (2018) suggested that antioxidant activity be used as a biomarker of different varieties of honey. The high phenolic content and antioxidant activities in the Acacia honey and Ziziphus honey from Darfur, Sudan confirmed their good quality. Different values of phenolic content and antioxidant activities may be due to the different geographical and botanical sources of honey.

3.3 Antibacterial properties

The well-diffusion test has been used to qualitatively evaluate the honey samples' potential growth inhibition capacity against selected bacterial strains. However, this simple and easy-to-use technique is only appropriate for diffusive test materials (Dwivedi *et al*., 2017). Figure 3 shows the results of the agar-well diffusion method used to evaluate the antibacterial activity of the two Sudanese honey samples. The findings indicated that both samples had varying degrees of antibacterial activity. However, as compared to chloramphenicol, Ziziphus honey demonstrated significantly more substantial antibacterial activity (Figure 3 and Figure 4).

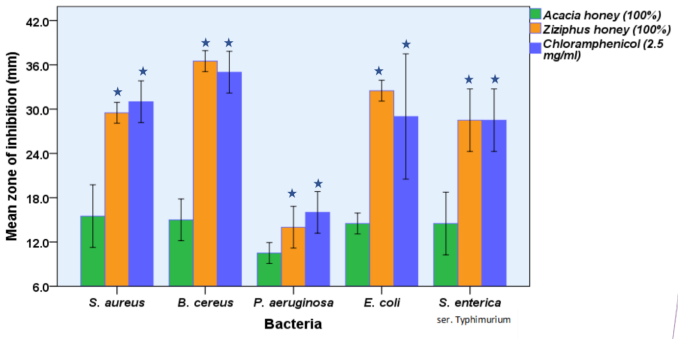

Figure 3. The mean zone of inhibition of Acacia honey and Ziziphus honey (from southern Darfur) compared with the chloramphenicol. Bars with notations above are statistically significantly different $p \leq 0.05$.

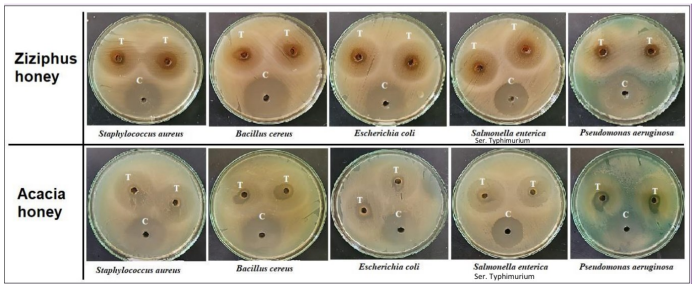

Figure 4. The zone of bacterial growth inhibition on the nutrient agar medium. T: the tested honey 100%, C: the positive control, chloramphenicol 2.5 mg/mL.

Regarding the Acacia honey, the inhibition zones of the susceptible bacteria were ranging between 15.5±2.1 to 10.5±0.7 mm. Whereas, for the Ziziphus honey, which showed significant antibacterial activity, the most susceptible bacterium was *Bacillus cereus* (36.5±0.7 mm), followed by *E. coli* (32.5±0.7 mm), *S. aureus* (29.5±0.7 mm), *S. enterica* ser. Typhimurium (28.5±2.1 mm) and *P. aeruginosa* (14.0±1.4 mm), respectively. The higher activity of the Ziziphus honey is attributed to the high level of the total phenolic content compared to the Acacia honey. The accumulation of phenolic compounds at the surface of bacteria is the most widely observed mechanism of action of phenolics against bacteria. The interaction can be described as a destructive effect of the phenolic compounds on the bacterial cell wall (Negi, 2012; Bouarab-Chibane *et al*., 2019).

Interestingly, Acacia honey showed antibacterial activity against both the gram-positive and the gram-negative bacteria. However, *P. aeruginosa* (gram-negative) showed weak activity compared to the other bacteria tested. *Pseudomonas aeruginosa* is a highly resistant bacterial pathogen that can prevent antibacterial agents through a variety of mechanisms, including mutational changes or transfer of resistance genes, generation of antibiotic-inactivating enzymes, low outer membrane penetrability, and expression of efflux pumps that discharge antibacterial agents from the cell (Pang *et al*., 2019). The remarkable resistance of *P. aeruginosa* to the phenolics in Ziziphus honey is thought to be caused by the latter mechanism. Previous studies on Ziziphus honey from different localities support our findings, a study from Pakistan cited that all gram-positive and gram -negative bacteria exhibited significant inhibitory zones in response to samples of Ziziphus honey (Fahim *et al*., 2014), Another study from Saudi Arabia indicated that Ziziphus honey was more antibacterial than Manuka honey (Halawani, 2021). Likewise, acacia honey also exhibited a substantial antibacterial effect against several gram-positive and gram-negative bacteria, equivalent to

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manuka honey (Luka, 2021). Finally, in order to develop new natural alternatives to conventional antibacterial drugs that are losing their effectiveness, there is an intrinsic need to boost global interest and support research efforts in natural product investigations (Abdallah, 2017). Therefore, antibacterial ingredients in honey could be used as a food supplement to improve the health of patients.

Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of acacia and Ziziphus honey. With low MIC and MBC values, the results matched with the findings of the agar well-diffusion test meaning that both honey samples have antibacterial activity. However, values were similar with some bacterial strains with MIC and MBC tests. As a consequence, we firmly believe that acacia honey was more viscous than Ziziphus honey, which influenced several well-diffusion test findings. This notion has been mentioned in the literature before (Dwivedi *et al*., 2017).

For acacia honey, *P. aeruginosa* was the most susceptible bacteria, with MIC 12.5%, MBC 50% and MBC/MIC ratio was 8.0. Other microorganisms examined recorded similar MIC, MBC and MBC/MIC that were 6.25%, 50% and 8.0, respectively. The antibacterial efficiency of Ziziphus honey was greater than that of acacia honey with certain bacteria, such as *S. aureus* and *E. coli. Bacillus cereus* and *P. aeruginosa* showed bactericidal activity with Ziziphus honey.

In fact, the MBC/MIC ratios that are listed in Table 4 were computed to determine the nature of the antibacterial compounds. If the compound tested was less than 4, indicating that it is bactericidal against the microorganism. When the MBC/MIC ratio is 4, the compound examined is considered bactericidal. However, when the MBC/MIC ratio is > 4, the compound tested is considered bacteriostatic. Accordingly, Ziziphus honey is more bactericidal and acacia honey is more bacteriostatic. Suggested that the phenolic compounds which were higher in the acacia honey might have a bacteriostatic effect against bacteria, and the bactericidal activity with the Ziziphus honey could be attributed to the phenolic compounds in parallel with other mechanisms, such as high osmolarity (low pH level and high sugar content), compositional differences in the phytochemical compounds of honey samples, acidity and hydrogen peroxide content (Mandal and Mandal, 2011; Combarros-Fuertes *et al*., 2020; Almasaudi, 2021).

4. Conclusion

Since ancient times, honey has been utilized and prescribed in traditional medicine. Various industrialized locations on Earth have a significant impact on agriculture and, therefore, natural products such as honey in the current era. Therefore, pollutant-free rural areas might be a significant source of highly effective and therapeutic products. The current study found that Ziziphus and acacia honey collected from a remote area in Southern Darfur, Sudan, had high levels of phenolics, minerals, antioxidants, and antibacterial potential. Interestingly, our results showed that the phenolic content of Ziziphus and Acacia honey is higher than the phenolic content of similar honey types reported by many other researchers from different countries. This is the first report about Ziziphus honey and acacia honey from this remote area. Moreover, their physico-chemical values satisfied all national and international guidelines and were free of harmful heavy metals. Therefore, the studied honey samples from this remote area are strongly recommended for use as a dietary supplement or in other pharmaceutical applications.

Conflict of interest

The authors declare no conflict of interest.

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Table 4. MIC, MBC and MBC/MIC values of the acacia and ziziphus honeys.

Microorganism	Acacia honey			Ziziphus honey		
	MIC %	MBC %	MBC/MIC	MIC %	MBC %	MBC/MIC
Staphylococcus aureus	6.25	50	8	6.25	50	8
Bacillus cereus	6.25	50	8	12.5	25	2
Pseudomonas aeruginosa	12.5	50	4	25	25	1
Escherichia coli	6.25	50	8	6.25	50	8
Salmonella enterica ser. Typhimurium	6.25	50	8	6.25	25	4

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