Turkish Journal of Agriculture - Food Science and Technology, 12(10): 1826-1834, 2024
DOI: https://doi.org/10.24925/turjaf.v12i10.1826-1834.6869

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Property-Wound Healing Relationship of Manuka-, Anzer- and Chestnut-Honey: Characterization, Antibacterial Properties and Cell Culture Applications

Ayşe Ezgi Ünlü[1,a], Fatma Altunkılıç[2,b], Yelda Asgari[2,c], Sena Demirtaş[2,d], Şeyda Gökyer[2,e], Fadime Kıran[3,f], Pinar Yilgor Huri[2,g,*], Serpil Takaç[1,h,*]

[1]*Department of Chemical Engineering, Faculty of Engineering, Ankara University, Ankara, Türkiye*
[2]*Department of Biomedical Engineering, Faculty of Engineering, Ankara University, Ankara, Türkiye*
[3]*Pharmabiotic Technologies Research Laboratory, Department of Biology, Faculty of Science, Ankara University, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 16.05.2024
Accepted : 25.08.2024

Keywords:
Anzer honey
Chestnut honey
Manuka honey
Wound healing
Antioxidant activity

ABSTRACT

Honey is known to be a natural food product with antibacterial and wound-healing properties. Among its different types, some attributed more with medical applications including Manuka honey. In this study, some wound healing-related parameters were systematically investigated on two different types of Turkish honey (Chestnut and Anzer), in comparison with Manuka honey. Chestnut honey had considerable amount of hydrogen peroxide (5 µg/mL) whereas Manuka and Anzer honey did not contain any. Additionally, Chestnut honey had the highest acidity among all tested samples (24.50 meq/kg), as well as higher antioxidant activity (IC50 ; 2.45 mg/ml) than those of Anzer (13.02 mg/ml) and Manuka honey (6.27 mg/ml). It was also demonstrated that Chestnut honey displayed a considerable antimicrobial activity against selected pathogens. On the other hand, in the scratch assay performed as a model for the wound healing process, Anzer honey induced the highest cell proliferation corresponding to almost total wound closure for all concentrations tested. Overall, the results showed that the Turkish Chestnut and Anzer honey samples had distinguished characteristics for wound healing that is comparable to Manuka honey, and therefore are both promising candidates for use in clinical applications.

[a] aeunlu@eng.ankara.edu.tr https://orcid.org/0000-0001-6942-5777
[b] fbaltunkilic@gmail.com https://orcid.org/0009-0008-8975-0778
[c] yeldasgari@gmail.com https://orcid.org/0009-0006-6492-3770
[d] senabal98@gmail.com https://orcid.org/0000-0001-7844-3731
[e] seydagokyer@gmail.com https://orcid.org/0000-0003-1177-4565
[f] fkiran@science.ankara.edu.tr https://orcid.org/0000-0002-4536-2959
[g] phuri@ankara.edu.tr https://orcid.org/0000-0002-4912-0447
[h] takac@eng.ankara.edu.tr https://orcid.org/0000-0003-3722-9151

Introduction

The introduction of antibiotics to clinical use was a great advancement for medical therapies, especially in the cure of bacterial infection-related diseases. However, their misuse and overuse lead to the formation of antibiotic resistance, which reduces the effectiveness of the therapy (Komolafe, 2004; Shallcross and Davies, 2014; Denny et al., 2020). In fact, antimicrobial resistance has become a global healthcare issue and is suspected to cause a death rate comparable to cancer worldwide. This issue is among the reasons making wound management difficult, and fuels the need for new treatment options (Nolan et al., 2020; Puca et al., 2021). Especially, chronic wounds are still challenging to treat due to the polymicrobial features as well as drug-resistant organisms that are present in the wound site (Mihai et al., 2019; Abd El-Aziz et al., 2022; Foschi et al., 2022).

Honey has been used for wound healing since ancient times, as the oldest use dates back to ca. 5500 BC, by the evidence found in the archaeological sites on papyruses in Egypt (Kuropatnicki et al., 2018; Minden-Birkenmaier and Bowlin, 2018; Maruhashi, 2020). These ancient cultures includes Roman, Ancient Chinese, Ancient Arab and Ancient Indians Evidence showed that honey was used to treat conditions such as aches of ears, smallpox lesions, fever, eye disease, respiratory infections, etc. (Blair and Carter, 2005).

To date, honey has been shown to be highly effective in such cases and for heavily infected wounds, reducing the risk that the antibiotics possess (Napavichayanun and Aramwit, 2017; Eroglu et al., 2018; Yilmaz and Aygin, 2020; Scepankova et al., 2021). Moreover, honey has been shown to exhibit inhibitory behavior against some specific pathogens (Oryan et al., 2016; Brudzynski, 2021) and even

antibiotic-resistant bacteria (Gobin et al., 2018; Minden-Birkenmaier and Bowlin, 2018; Combarros-Fuertes et al., 2020; Bazaid et al., 2022). The great potential of honey for wound treatment stems from its antimicrobial, antioxidant, anti-inflammatory and anti-fungal properties.

The high sugar content of the undiluted honey creates an osmotic effect on the wound site and causes osmotic pressure on bacteria. Because of this pressure, bacteria begin to dehydrate and shrink and, therefore, cannot survive in the hypertonic environment (Almasaudi, 2021). Meanwhile, this high sugar content serves as a rich glucose source for the proliferating fibroblasts and endothelial cells effective in wound repair (Krishnakumar et al., 2020; Scepankova et al., 2021; Tashkandi, 2021). It has been also shown that diluted honey inhibits bacterial growth (Saikaly and Khachemoune, 2017; Albaridi, 2019a; Hossain et al., 2022). Its high viscosity also provides a moist environment, which accelerates the wound healing process and behaves like a barrier that prevents the entry of the pathogens to the site. The typically low pH value of honey (in the range of 3.0-5.4) also creates an environment that prevents the growth of most microorganisms (Albaridi, 2019b). In addition, the acidic wound site accelerates the healing by increasing the release of oxygen species (Maruhashi, 2020; Yupanqui Mieles et al., 2022; Chijioke et al., 2023).

On the other hand, diluted honey contains hydrogen peroxide (H_2O_2) that provides its antibacterial efficacy (Oryan et al., 2016; Almasaudi, 2021). It is formed by glucose oxidase (GO) in honey that is added to the nectar by bees, in inactivated form, that is substantially activated by the presence of oxygen in diluted form to catalyze H_2O_2 formation from glucose. There are some honey that show antibacterial activity even with low levels of H_2O_2. This is suggested to be due to its non-peroxide activity.

Another advantage of honey is reported to be the lack of any bacteria that could improve resistance against honey. This property of honey makes it an important topical antimicrobial agent (Bernstein, 2013; Combarros-Fuertes et al., 2020).

Natural compounds of honey, which are transferred from plants, contain some polyphenols that are comprised of a broad range of molecules from simple to complex structure. Flavonoids, phenolic acids, lignans and stilbenes are subgroups of polyphenols. A considerable amount of polyphenols is comprised of flavonoids that have different phenolic structures, having benzo-γ-pirone derivatives with various substituents (Di Carlo et al., 1999). They are basically classified according to their difference in the chemical structures such as flavones, flavan-3-ols, flavonols, flavonones, etc. Their most significant property is the scavenging of the free radicals. Therefore, flavonoids are significant groups of molecules for the treatment of wounds (Menezes et al., 2017; Subramanian et al., 2023). The hydroxyl groups in their structure is the key to lead to the hydroxylation process resulting in their antioxidant, anti-inflammatory, anti-bacterial properties (Pushpavalli et al., 2010; Feng et al., 2016; Carvalho et al., 2021). Polyphenols have a major role in wound healing (Güneş et al., 2017) through their antioxidant activities (Özkök et al., 2010; Pasupuleti et al., 2020; Mssillou et al., 2022). Therefore, there is a significant correlation between the antioxidant activity of honey and the content of total phenolics of honey (Oryan et al., 2016; Pasupuleti et al., 2020; Tashkandi, 2021). Phenolic acids contribute to the antioxidant activity significantly and flavonoids contribute to both anti-inflammatory and antioxidant activity (Aker and Nisbet, 2020). When the amount of antioxidants is low in the wound site, it causes free radicals to increase and delays wound healing (Al-Waili et al., 2011).

Manuka honey is the honey produced in New Zealand from endemic flowers (Kwakman et al., 2011). Due to its non-peroxide activity, anti-bacterial and antioxidant properties, this honey has been used as an agent on the wound dressing material for chronic wounds (Kirkpatrick et al., 2017; Johnston et al., 2018; Girma et al., 2019; El-Senduny et al., 2021). Early studies carried out on some wound infecting bacteria, which are known to have developed resistance to antibiotics, reported that these bacteria could be inhibited by Manuka honey due to its unique factors (Willix et al., 1992). After the efficacy and clinical success of Manuka honey for wound healing was realized, more advanced studies have been performed in recent years (Yang et al., 2017; Frydman et al., 2020; Kapoor and Yadav, 2021). On the other hand, for many years, Anzer and Chestnut honey have been used to treat wounds, respiratory diseases such as asthma, ulcers and viral or bacterial infections by people (Kolaylı et al., 2008; Dağ et al., 2017; Gençay Çelemli et al., 2017; Güneş et al., 2017; Malkoç et al., 2019).

The aim of the present study is to reveal the significant charateristics of Chestnut and Anzer honey from Black Sea Region of Turkey, present their physical-chemical properties, total phenol and total flavonoid contents, antioxidant, antibacterial and wound healing properties and also compare the results with Manuka honey to feature usability of Turkish honey for wound healing in clinical applications. This is the first study to reveal the different types of properties in one article both in terms of physical-chemical and wound healing properties. On the other hand this study is significant in terms of the disclosure of a Turkish honey that has the potential to compete with a worldwide known honey.

Materials and Methods

Materials

Folin-Ciocalteu reagent (9252), sodium hydroxide (06203), methanol (24229), gallic acid (G7384), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (D9132) and fibroblast growth factor (FGF) (F9786) were purchased from Sigma Aldrich (Darmstadt,Germany). Sodium nitrite (237213), brain heart infusion broth (1.10493.0500) and aluminium chloride (237051) were obtained from Merck (Darmstadt, Germany). Sodium carbonate (SO0116) was purchased from Scharlau (Scharlab, S. L., Barcelona, Spain). Gibco Dulbecco's Modified Eagle Medium (DMEM) high glucose (01-052-1A), fetal bovine serum (04-127-1A), penicillin/streptomycin (03-031-1B), phosphate-buffered saline (PBS) (02-020-1A) were purchased from Biological industries (Sartorius, Germany).

All other chemicals and solvents used were of analytical grade and used as received unless otherwise is stated.

Honey Samples

Seven different honey types were tested. Manuka honey (MH) was purchased from Forest Gold (New Zealand). Three

different samples of Chestnut honey from three different beekeepers, named as CH1, CH2, and CH3 were purchased from Zonguldak Beekeepers Association (Turkey). Samples of Anzer honey were purchased from three different local suppliers in the Black Sea Region of Turkey and named as AH1, AH2, AH3. Honey samples were stored in the dark and cool place for a maximum of three months.

Physical-Chemical Properties of Honey Samples

The pH values of honey samples (1 g) diluted in 7.5 mL of deionized water were measured at room temperature using a pH-meter (Sartorius PP25, Sartorius Lab Instruments GmbH & Co. KG, Germany) (Kara, 2020). For the total acidity assay, the samples were titrated with 0.05 N NaOH until the pH of 8.3 (AOAC, 1990; Derebaşı et al., 2014). The electrical conductivity of 20% (w/v) honey samples were measured by conductivity meter (Radiometer IonCheck 65, Villeurbanne, Cedex, France). Moisture content was measured using the standard of DIN 10752 (Martin, 1979) and °Brix was measured according to the national standard of TS3036 (Ball, 2006). Protein analysis was performed by Kjeldahl method (Helrich, 1990).

Hydrogen peroxide content was determined using MQuantTM peroxide test strips (Merck, Germany). Briefly, diluted honey samples were kept in 37 °C water bath and immediately transferred to ice-water bath to stop the reaction. The strips were placed into the honey solution for 1 s and evaluated in terms of the degree of the color developed after 15 s. The content of hydrogen peroxide was determined by comparing the color developed with the reference of the manufacturer, in 'mg H_2O_2/L' and presented in the units of µmol/L h (Strelec et al., 2018).

Total Phenolic Content

Honey samples were diluted in metanol at 10% (w/v) and incubated at room temperature (Edmund Bühler TH 30) for 24 h prior to use. The samples were filtered and stored at +4°C for the analyses of total phenolic content, total flavonoid content and antioxidant activity.

Total phenolic content was determined by Folin-Ciocalteu method (Samatha et al., 2012). Briefly, Folin-Ciocalteu reagent (10% -diluted with distilled water) of 1.5 mL were added to 0.5 mL of honey sample and incubated at room temperature for 5 min. After the addition of 2 mL of sodium carbonate (7.5%), the sample was incubated in the dark for a period of 90 min. The absorbance value of the colored sample was determined using UV-VIS spectrophotometer (Shimadzu, UV–1601, Europa, Gmbh) at 725 nm. Total phenolic content of the samples was calculated by the calibration curve prepared using gallic acid as standard. The results were given as mg gallic acid equivalent (GAE) per 100 g honey.

Total Flavonoid Content

Total flavonoid content was determined using aluminum chloride assay (Samatha et al., 2012). Honey sample (0.5 mL) was incubated for 6 min after addition of 2 mL of distilled water and 150 µl of $NaNO_2$ (5%). After 6 min, 150 µL of 10% $AlCl_3$ was added and incubated for an additional 6 min. 2 mL of 4% (w/v) NaOH was added into the mixture and final volume was completed to 5 mL and incubated for 15 min. The absorbance of the samples was determined at 510 nm. The total flavonoid yield of the samples was expressed in mg quercetin equivalents (mg QE) per 100 g honey using the calibration curved prepared using the standard.

Antioxidant Activity

In determination the antioxidant activity, DPPH solution in methanol (100 µM) was used as the radical source. Equal amounts of honey solution and DPPH solution was mixed and incubated in the dark for 50 min. After incubation, the absorbance values were monitored at 517 nm. IC_{50} values of samples, which is defined as milligrams per liter required to inhibit DPPH radical formation by 50%, was calculated from the log dose inhibition curve (Kolaylı et al., 2008).

Antibacterial Activity

Methicillin-resistant *Staphylococcus aureus,* MRSA ATCC 43300, *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus epidermidis* (ATCC 35984) were used in this study as the most important skin pathogens, and were grown in Brain Heart Infusion (BHI, Merck) medium for 24 h at 37°C. The antibacterial activity was assessed by agar well diffusion assay (Onbas et al., 2018). Following the adjustment of each bacterial culture suspension to McFarland 0.5, they were inoculated in a soft medium containing 0.75% agar, and then pour plated on BHI agar (1,5%) surfaces, separately. Honey samples were transferred as 100 µL into the 6-mm-diameter wells opened on agar surfaces. Following the incubation for 24 h at 37°C, the diameters of the inhibition zones around the wells were measured as millimeters.

In vitro Scratch Assay for Wound Repair Model

In vitro scratch assay is a simple technique to model wound repair (Rodriguez et al., 2005). Basically, a scratch or a "wound" is created on a cell monolayer and the extent of cell migration based on chemotaxis is monitored within this scratch area. In this study, the effect of different concentrations of various honey samples were studied on the migration of two different cell types. L929 mouse fibroblasts (ATCC #CCL-1) and primary adipose-derived stem/stromal cells isolated from human subcutaneous fat tissue were used to study the effect of cell type on the honey-induced cell migration capacity.

L929 cells were cultured at T175 flasks within growth medium (DMEM high glucose (BI) supplemented with 10% fetal bovine serum (BI) and 1% penicillin/streptomycin) in a humidified incubator with 5% CO_2 at 37°C. Cell growth was monitored periodically by an inverted light microscope (Zeiss Primovert Inverted, Germany). Detached cells were seeded into 24 well culture plates at a density of 1×10^5 cells/well and allowed to grow up to 70-80% confluence as a monolayer. The monolayer was then gently scratched across the center of the well with sterile 10 µm pipette tip. After scratching, the medium was removed, and the wells were rinsed twice with sterile PBS. The scratched surfaces were treated with growth media in the presence of different concentrations of honey samples at 0.1, 1, 10, and 100 µg/mL for 24 h. The width of scratched surfaces was measured at t=0 and at t=24 h with an inverted light microscope. The size of the scratch zones was assessed using the ImageJ software (NIH). The percentage of scratch closure was calculated according to the following equation:

Scratch closure (%) = ((So ─ St)/So) × 100

where S_O and S_t are the sizes of the scratch zones before and after treatment, respectively.

Tests were also conducted by the use of primary adipose-derived stem/stromal cells (ASCs) isolated from human subcutaneous adipose lipoaspirates according to (Ergene et al., 2020). ASCs were cultured at T175 flasks with growth medium (DMEM high glucose (BI) supplemented with 10% fetal bovine serum (BI), 1% penicillin/streptomycin and FGF) under standard conditions as described above. Cells were then seeded into 24 well cell culture plates at a density of 1×10^5 cells/well and allowed to grow to 70-80% confluence as a monolayer. The scratch assay was conducted as described above.

Statistical Analysis

All analyses were carried out at least in triplicate, and the data were expressed as mean ± standard deviation (SD). The coefficient of variations were 0.022, 0.040 and 0.014 for total phenol content, total flavonoid content and antioxidant activities, respectively (Microsoft Excel TM).

Results

Moisture, Protein, °Brix, pH, Total Acidity and Electrical Conductivity

Physical-chemical properties of the honey samples were found to change between the following values: pH (3.99–5.12); total acidity (21.5-40.0 meq/kg); moisture (17.8-21.4%), protein (0.41-0.98%), Brix (77-82%), electrical conductivity (0.279-1.283 mS/cm**)** (Table 1). Moisture contents of the Anzer honey were found to be lower (17.8% and 18.6%) than those of Chestnut honey (21.0, 21.4 and 21.4%) and Manuka honey (21.4%). AH1 and AH2 had high acidity (low pH) which were close to that of MH. The characteristics of Anzer honey were comparable with Manuka honey with respect to the moisture and total acidity values; however; Chestnut honey samples had more protein than AH samples. Additionally, CH1 and CH3 were found to have higher protein than MH.

Hydrogen Peroxide Content

Hydrogen peroxide contents of the honey samples were found to change between 0-5 µg /mL (Table 2)**.** Chestnut honey samples had higher hydrogen peroxide content than the other honey samples. On the other hand, Manuka and Anzer honey (AH2 and AH3) did not contain any hydrogen peroxide.

Total Phenolic Content

The total phenolic content of honey samples is compared in Figure 1. One of Chestnut honey samples had the highest total phenolic content (CH1; 95.86 ± 4.0 mg GAE/100 g) followed by Manuka honey (MH; 93.23 ± 3.7 mg GAE/100 g) and other samples of Chestnut honey (CH3, CH1).

Total Flavonoid Content

Total flavonoid content of honey samples is given in Figure 2. The highest flavonoid containing honey was AH1 with 6.30 ± 0.25 mg QE/100 g followed by one of the Chestnut honey samples (CH1; 6.12 ± 0.18 mg QE/100 g) and the other Anzer honey (AH3;5.94 ± 0.12 mg QE/100 g).

Antioxidant Activity

Figure 3 shows the IC_{50} values of the honey samples towards DPPH radical. According to the results, CH1 showed the highest antioxidant activity as IC_{50}= 2. 45 mg/m followed by other Chestnut Honey samples (CH2 and CH3 as IC_{50}= 2.84 and 2.93 mg/mL, respectively). Anzer honey (AH1 and AH2) had also high antioxidant activity except for AH3. However, Manuka honey did not show any considerable antioxidant activity when compared with the others.

Antibacterial activity

Screening for the potential antagonistic activity of honey samples against important skin pathogens was performed using the agar well diffusion assay. According to our results, 50% concentration of honey samples was found effective against the skin pathogens tested (Table 3). It was also demonstrated that CH1 (50%) displayed a powerful antimicrobial activity against all selected pathogens. The most effective inhibition was observed against *S. aureus* with the honey samples of AH3 (50%) and CH1(50%). In addition to CH1 (50%), MH (50%) also exhibited antibacterial activity against MRSA. However, AH1 and AH2 did not show any inhibition zone against selected skin pathogens.

Table 1. Moisture, protein, °Brix, pH, total acidity and electrical conductivity of honey samples

Honey sample	Moisture, %	Protein, %	°Brix	pH	Total acidity, meq/kg	Electrical conductivity, mS/cm
AH1	18.6	0.46	79.95	4.09	31.50	0.303
AH2	17.8	0.63	82.0	3.99	31.00	0.279
AH3	18.6	0.41	80.75	4.34	21.50	0.363
CH1	20.2	0.95	78.25	5.04	24.50	1.258
CH2	21.0	0.76	77.50	5.12	22.00	1.255
CH3	21.4	0.98	77.0	4.86	40.00	1.283
MH	21.4	0.85	77.0	4.25	27.00	0.438

Table 2. Hydrogen peroxide content of the honey samples

Honey sample	H_2O_2 content (µg /mL)
AH1	0.5
AH2	0
AH3	0
CH1	5
CH2	2
CH3	2 - 5
MH	0

Table 3. Inhibition zones diameters (mm) by different honey samples on different skin pathogens.

Honey Type /Inhibition zones (mm)	P. aeruginosa	S. epidermidis	S. aureus	MRSA
AH1(%50)	-	-	-	-
AH2(%50)	-	-	-	-
AH3(%50)	-	-	28 ±0.5	-
CH1(%50)	10±0.1	10 ±0.2	25 ±0.5	18±0.4
CH2(%50)	-	10 ±0.2	-	-
CH3(%50)	-	10 ±0.1	-	-
MH(%50)	-	12±0.4	14 ±0.2	10 ±0.2

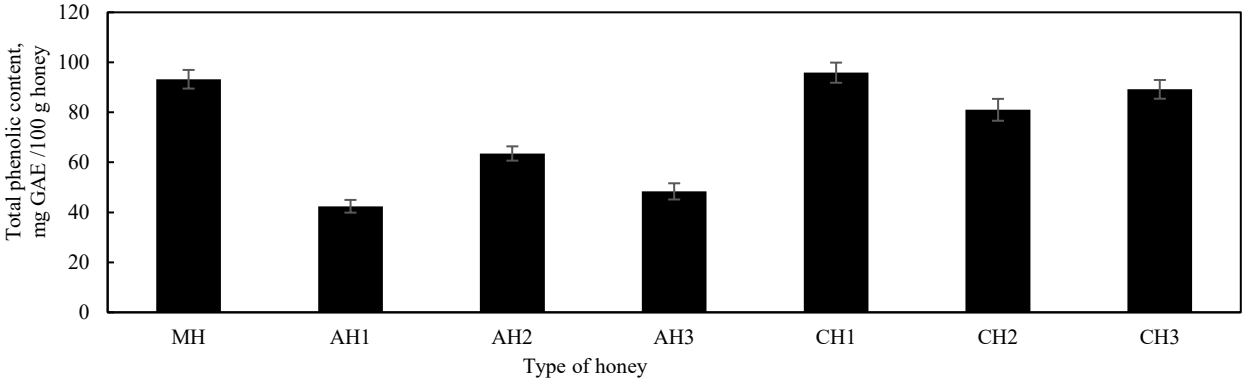
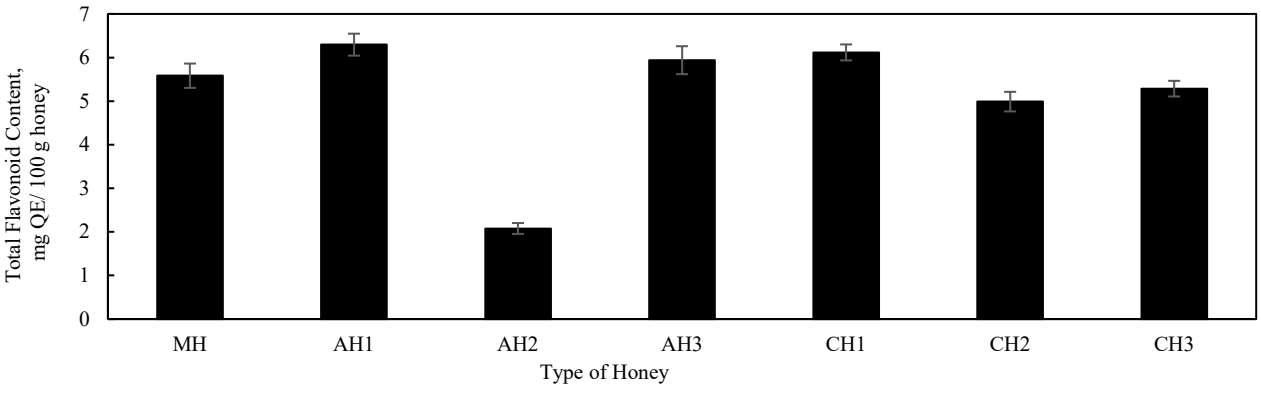


Figure 1. Total phenolic content of the honey samples



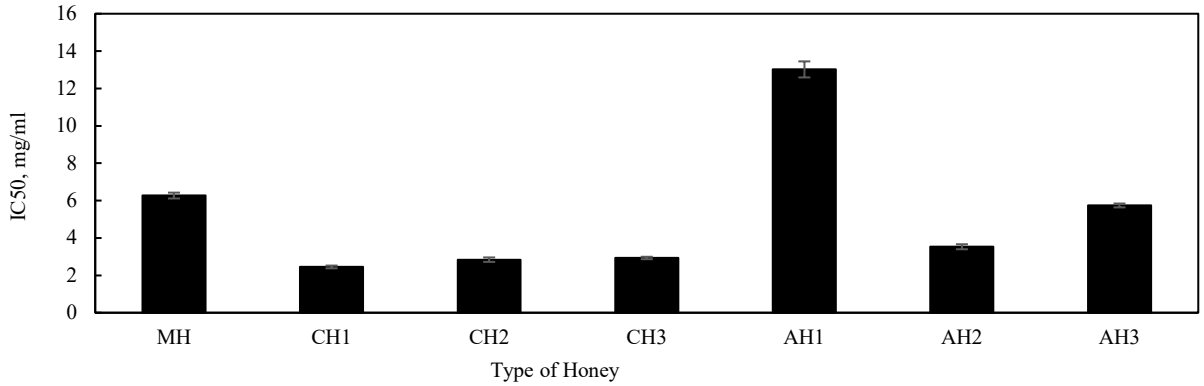


Figure 3. Antioxidant activity (IC_{50}) of the honey samples (radical-scavenging effects on DPPH radicals)

Scratch Assay

In the L929 cell line, Anzer and Chestnut Honey groups without CH3 were observed to induce higher cell proliferation that corresponds to more scratch closure than the control group and MH1. The difference in honey concentrations did not significantly affect the extent of scratch closure.

In the observation with primary ASC cells, the difference in honey concentrations did not cause a significant change in scratch closure for AB1 and AB2 groups. However, it was observed that scratch closure decreased as the concentration increased in AB3 groups.

In vitro scratch analysis of L929 cell line is illustrated in Figures 4 and 5. According to the results, AH3, CH1 and CH2 promoted the closure of the wound while CH3 and MH inhibited the migration of the cells for all concentrations tested (0.1-100 µg/mL).

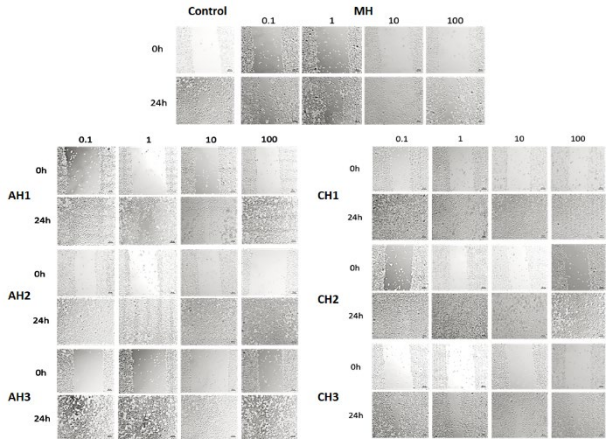


Figure 4. Analysis of L929 cells migration by in vitro scratch assay

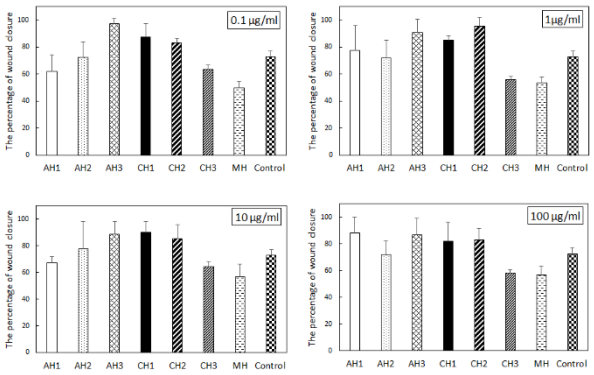


Figure 5. Wound healing activity of different honey samples on L929 cells at different concentrations

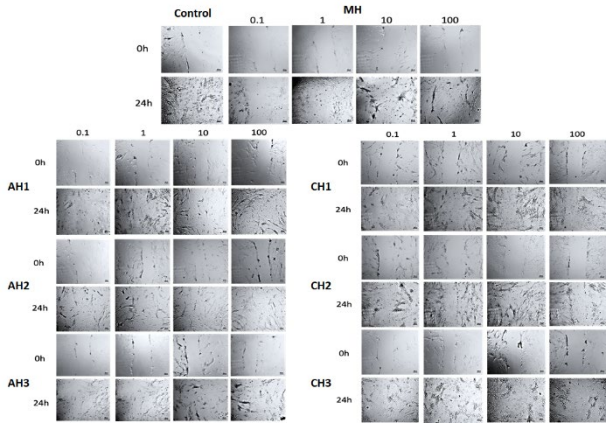


Figure 6. Analysis of ASC cells migration by in vitro scratch assay

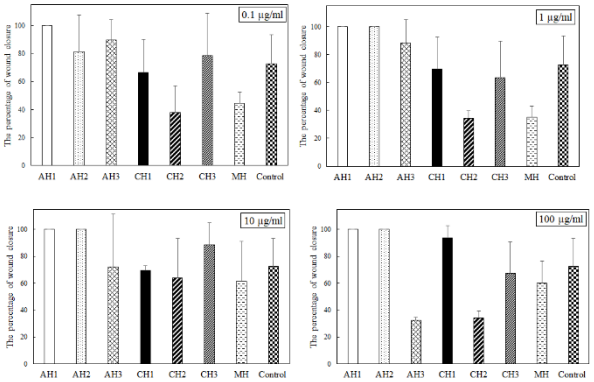


Figure 7. Wound healing activity of different honey samples on ASC cells at different concentrations

On the other hand, AH2 was found to have no effect on the cell migration, since it showed the same percent of wound closure with control group. Only 100 µg/mL concentration of AH1 was observed to enhance wound closure. 0.1 µg/mL of AH3 provided higher closure and the percentage of wound closure decreased as the honey concentration increased. For the other honey samples, the concentration was found to be negligible in terms of the results of the scratch assay.

When primary ASCs was used in the scratch analysis, AH1 showed up providing 100% of wound closure for all concentrations tested (Figure 6). AH2 also provided 100% of wound closure except for 0.1 µg/mL concentration. AH3 may also be regarded as effective on the wound closure providing values in the range of 72-90%. On the other hand, CH2 and MH were found to inhibit the migration of the cells for all concentrations tested (0.1-100 µg/mL), however, only 10 µg/mL of CH3 supported wound closure (Figure 7).

Discussion

Different physical-chemical properties of the seven honey samples were measured such as moisture, protein content, °Brix, pH, total acidity and electrical conductivity. The results showed that physicochemical properties of Anzer honey samples were compatible with the literature (Hepsağ, 2019). Acidity values of the honey samples were all lower than 50 meq/kg as required in EU (EU Council, 2002). As these properties directly affect the bacterial growth and aid wound healing, Turkish honey showed the potential to be used as an alternative to Manuka honey by these initial experiments and therefore further analysis have been performed.

Hydrogen peroxide contents of Chestnut honey samples were found to be higher than all the samples investigated. This result is compatible with the literature (Strelec et al., 2018). The change in the hydrogen peroxide content of the honey samples is basically due to the botanical origin but dilution factor is also presented as one of the reasons. However, the optimum dilution factors of the samples were provided in the analysis of this study to eliminate this factor. Considering the presented values, Manuka and Anzer honey (AH2 and AH3) can be concluded to have no cytotoxic damage.

The phenolic content of honey has a considerable contribution to its antioxidant activity. The range of the total phenolic content of the samples were close to the results reported by Tezcan et al. (2011) (Tezcan et al., 2011), which were 0.95 mg/g - 1.13 mg/g. The phenolic content of the Chestnut honey samples, especially CH1, are very promising since they have similar phenolic content with Manuka honey. Anzer honey samples possessed lower total phenolic content than other samples, but they were found to be compatible with the literature (Hepsağ, 2019; Malkoç et al., 2019). Contrary to the low phenolic content, two samples of Anzer honey contained higher flavonoids than the other samples. Flavonoid contents of the Anzer honey samples were found to be higher than a

previous report (Malkoç et al., 2019). When the flavonoid contents of the honey samples were compared AH1, AH3, CH1 and CH3 may be regarded as good candidates to compete with MH.

Antioxidant activity values of AH and especially CH were considerably higher than that of Manuka honey. When the antioxidant activity values are evaluated together with total phenol and total flavonoid contents, the Chestnut honey sample, namely CH1 stood out. Together with CH1, the other Chestnut honey sample, CH3, can also be regarded as a good candidate to compete with Manuka Honey. On the other hand, all honey samples were found to inhibit skin pathogens. Especially AH3 and CH1 showed up *S. aureus*. Similar to our results, it has been shown that undiluted and diluted honey samples at 75, 50, 30, and 10% were effective in inhibiting *S. aureus and S. epidermidis* (Basualdo et al., 2007). It has been demonstrated in different studies that honey exhibited a broad range of antibacterial activity against numerous bacteria (Matzen et al., 2018; Wadi, 2022), which testified that natural components of different kinds of honey have various activities against different pathogens. *In vitro* scratch analysis of both ASC cells and L929 cells showed that Turkish honey promoted wound closure.

Conclusion

Manuka honey is a well-known honey that has precious properties and considered almost a golden standard among the honey samples for medical purposes. In this study, different honey types from Black Sea Region of Turkey were screened to find out a honey type having biochemical and wound-healing properties close to Manuka honey.

The results revealed that Chestnut honey group (CH1, CH2, CH3) showed similar total phenol content to Manuka honey but higher flavonoid content and higher antioxidant activity values. CH1 and CH2 showed up in the scratch analysis of L929 cells, while AH1 and AH2 provided better closure for ASC cells. Besides, CH1 has shown a broad spectrum of antibacterial activity against all skin pathogens tested.

The properties of honey are known to change seasonally and according to the origin therefore both higher and lower values (total phenolic content, flavonoid content and antioxidant activity) than the values found in this study can be found in the literature for Manuka honey. However, the significant properties of Chestnut honey presented in this study are comparable with Manuka Honey.

Evaluating all analysis performed, we present CH1 as a new candidate to be used in different pharmaceutical applications such as wound dressing, supplements, etc.

Declarations

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Funding

The authors gratefully acknowledge the financial support provided by The Scientific and Technical Research Council of Turkey (TUBITAK 2209-B 2020/1 Term, Project No. 1139B412000512)

The Declaration of Conflict of Interest

The authors declare that there are no competing financial and non-financial interest.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

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