Original Article

PRELIMINARY STUDY ON POTENTIAL ANTIMICROBIAL ACTIVITY OF HONEY FROM EGRICAYIR PLATEAU, TURKEY

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Received: 12 November 2023; accepted: 07 November 2024

Abstract

Egricayir honey named after the Egricayir Plateau in Mersin, Turkey is rare and is said to have a healing effect by the local people. This preliminary and first-ever study on honey from the Plateau aims to investigate the claim through total antimicrobial activity (TA). Thirty honey samples were collected twice from the Plateau in early July and late August 2023, and then TA was determined through the use of the phenol equivalence test. Samples had a homogeneous TA distribution and each had a TA>10, lower limit for therapeutic potency. Of the samples, 28% had a therapeutically beneficial potency (10≤TA<20) and 72% had a therapeutically high potency (TA≥20). On average, the TA of the honey samples was 21 falling into the category of therapeutically high potency. The findings imply that the honey from the Egricayır Plateou is a good candidate for further therapeutic tests and geographical indication.

Keywords: antibacterial, antimicrobial, Egricayir, honey, Turkey

INTRODUCTION

Safety and authenticity are indispensable attributes of any honey. Antimicrobial activity (AMA) is attributed to some, and because of therapeutic effects these honeys are considered more valuable than regular ones. The AMA has emerged as a robust tool to add further value to honey as an indicator of therapeuticity, and determining it continues to gain significant consideration and mainly focuses on microbial effects, action mechanisms, clinical uses and honey sources (Combarros-Fuertes et al., 2019; Combarros-Fuertes et al., 2020a).

Approaches for determining the AMA of honeys and honey products have been extensively discussed. The phenol equivalence test has been largely adopted by the industry for the practical assessment and categorization of honey (Hossain et al., 2022). The test determines the AMA against *Staphylococcus aureus* ATCC 25923, due to the peroxide and non-peroxide components of honey and shortened as total activity (TA). It is a variant of the agar well diffusion method (Balouiri et al., 2016) and compares the inhibition zone formed by the honey sample and standard phenol solutions.

Honeys already have inherent basic AMA due to high sugar content, low water activity, acidic pH and bioactive compounds (Combarros-Fuertes et al., 2020b). However, besides the basic one, the therapeutic effect is mainly linked to the AMA coming from hydrogen peroxide as in 'peroxide honeys' or methylglyoxal (MGO) as in 'non-peroxide honeys' (Mandal & Mandal, 2011). Honeys with TA≥10, i.e. AMA of honey ≥AMA of 10% phenol solution, are considered therapeutically beneficial (Carter et al., 2010), and the more potent the honey the higher the TA.

Turkey, a leader in pine honey production, possesses a diverse and rich indigenous flora of more than 10,000 endemic flowers suitable for beekeeping and blossom honey production for (Arslan & Turhan, 2022). Such fertile land should have a considerable portfolio of honeys with therapeutical AMA. However, research on the AMA of Turkish honeys is scarce compared to its rich honey resources.

Some Turkish honeys were investigated for AMA (Cenet et al., 2015; Bayram et al., 2020; Kemal et al., 2023), which was reported through zone diameter, minimum inhibition concentration,

etc. which are expressive for the academic community. Most honeys from other geographies were similarly studied for their AMA (Junie et al., 2016; Mduda et al., 2023; Stagos et al., 2018), and their outcomes were not comparable due to the use of different methodologies. The therapeutic potencies of honeys were not assessed and not categorized with the use of a standard method. Honeys investigated this way are uncertain if they carry the criteria of being therapeutic (TA ≥ 10). Expressing AMA in terms of TA is regarded as more advantageous for both industry and academia.

Besides the vague rare evidence for AMA of Turkish honeys, some are colloquially alleged to have healing effects and are used folklorically, as in the case of the honey from the Egricayir Plateau, Mersin, Turkey. These anecdotes prompted this search to pursue supporting evidence for the honey from the Plateau. So, the objective of the current preliminary work is to determine the AMA of this rare honey on the scale of TA in order to assess and categorize its potential therapeutic potency with the use of the phenol equivalence test.

MATERIAL AND METHODS

Honey samples

Honey samples were collected from five hives in the main honey-foraging region of the Egricayir Plateau, Turkey, Mersin (Fig. 1). Hives were selected among the existing ones within the smallest imaginary rectangle circumambienting the terrain. One of the hives was almost in the geometrical center of the rectangle, and the others were accordingly on the lengthways through the center. Each hive was almost 1000 m away from the others (Fig. 1).

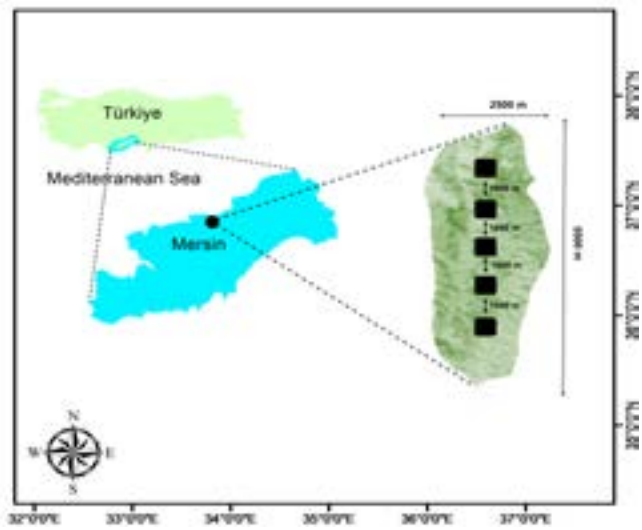

Fig. 1. Sketch of the main honey-foraging terrain on the Egricayir Plateau. Boxes show the hives used for the honey sampling.

Three different honeycombs (HCs) in a hive were arbitrarily selected for sampling at a collection time (CT). Samples were collected in early July and late August 2023. Half of each HC was cut out with the use of a sterile knife, put into separate sterile containers and kept dark and cold till analysis. Honey draining by itself from the HCs into the container was strained with the use of a sterile house-type strainer into sterile bottles. In total, thirty (2x3x5) TA analyses were performed for a CT. Furthermore, 5 g of honey used for each TA analysis was mixed (total 150 g) and analyzed by an accredited external laboratory (MRL Food Control Laboratory, Mersin, Turkey) (Tab. 1) to present the average physicochemical parameters of the mixture.

Inoculation

AMA of phenol solutions and honey samples were tested against *Staphylococcus aureus* ATCC 25923 with the use of the agar well diffusion method to determine the TA according to the phenol equivalence test by Allen et al. (1991). *S. aureus* culture was obtained from the stock culture collection of the Microbiology Laboratory, Department of Food Engineering, University of Mersin, Mersin, Turkey.

Mueller Hinton broth medium (Merck, Darmstadt, Germany) was prepared according to the manufacturer's instructions. It was autoclaved (15 min, 121°C), and allowed to cool to 50°C. *S. aureus* from the stock culture was inoculated into the broth with the use of a sterile loop. The *S. aureus* was grown in the broth for 18-24 h at 37°C and then adjusted to McFarland standard 0.5 (~10^8 cfu/ml) with the use of a spectrometer (Agilent, Cary 60 UV-Vis, Santa Clara, CA, USA). Mueller Hinton agar medium (Merck, Darmstadt, Germany) was prepared according to the manufacturer's instructions, autoclaved (15 min, 121°C), and allowed to cool to and kept at 50°C for 30 minutes. A 100 µl broth was seeded into 150 ml agar and mixed gently through swirling,

Table 1.

Physicochemical parameters of a mixture of honey samples collected in early July (CT1)[1] and late August 2023 (CT2)

Parameter	CT1	CT2	Method/Instrument
Moisture, %	16.9±0.4[a]	17.2±0.2[a]	IHC[2]/Refractometer
HMF, mg/kg	7.7±0.4[a]	5.7±0.4[b]	IHC/HPLC-DAD
pH	4.74±0.04[a]	4.81±0.07[b]	IHC/pH-meter
Proline, mg/kg	607±16[a]	453±23[b]	IHC/Spectrophotometer
Conductivity, mS/cm	0.616±0.014[a]	0.746±0.011[b]	IHC/Conductometer
Diastase number	27.3±0.4[a]	15.4±0.7[b]	IHC/Spectrophotometer
Fructose, %	35.6±0.7[a]	37.1±0.9[b]	AOAC 977.20/HPLC-RID[3]
Glucose, %	26.3±0.8[a]	31.2±01.0[b]	AOAC 977.20/HPLC-RID
Sucrose, %	ND	ND	AOAC 977.20/HPLC-RID
Maltose, %	ND	ND	AOAC 977.20/HPLC-RID
C13 Honey, δ C13 ‰	-26.92±1.91[a]	-28.55±1.73[b]	AOAC 998.12/IRMS[4]
C13 Protein, δ C13 ‰	-26.86±1.84[a]	-27.09±1.87[b]	AOAC 998.12/IRMS
C13 Difference, δ C13 ‰	0.33	1.46	AOAC 998.12/IRMS
C4 Sugar, %	0	0	AOAC 998.12/IRMS

Each parameter is a mean of 3 measurements and represents a mixture of 30 samples used for determining TA for a collection time (CT).
The same letter in a line shows a statistically significant difference.
[1]Conducted by an accredited external laboratory (MRL Food Control Laboratory, Mersin, Turkey).
[2]International Honey Commission (IHC) (Bogdanov et al. 2002).
[3]Official Methods of Analysis of AOAC INTERNATIONAL (2023a).
[4]Official Methods of Analysis of AOAC INTERNATIONAL (2023b).

and then the suspension was poured into sterile Petri dishes (20 ml). Petri dishes were left to cool to room temperature and kept at 4°C overnight. The thickness of the agar was almost 2.40 mm. Two wells were bored in each Petri dish with the use of a sterile cork borer with 8 mm inner diameter.

Phenol equivalence test

A stock solution of 10% (w/v) phenol was prepared in a sterile 100 ml volumetric flask. Phenol solutions from 1 to 8% (w/v) were prepared with sterile pure water in sterile 100 ml volumetric flasks. Three Petri dishes were used for each phenol solution. One of the wells in a petri dish was delivered 100 µL sterile water as the negative control and the other well was delivered 100 µL phenol solution. Dishes were incubated at 37°C for 24 h. After the incubation, the inhibition zone diameter (ZD) formed was measured with the use of a vernier caliper at least twice on different sites. The squared means of ZD values versus phenol concentration were drawn to obtain the calibration curve (Fig. 2).

Each honey sample was diluted to one-fourth to obtain a 25% w/v honey solution with the use of sterile pure water in a 100 ml sterile volumetric flask. Two Petri dishes were used for each honey sample. One of the wells in a Petri dish was delivered 100 µL sterile water as the negative control and the other well was delivered 100 µL honey solution. Dishes were incubated at 37°C for 24 h. After the incubation, the ZD was measured using a vernier caliper at least twice on different sites. The squared mean of ZD values was determined. The squared values for the honey solutions were compared with those of the phenol solutions with the use of the calibration curve (Fig. 2). The corresponding readings were multiplied by the dilution factor 4 to obtain the TA.

Colony forming units

The number of colony-forming units (cfu) on agar gels was counted before and after the incubation for arbitrarily selected five honey samples from each run and for every phenol solution. Plate count agar (Merck, Darmstadt, Germany) was prepared according to the manufacturer's instructions, autoclaved (12°C, 15 min), cooled to 50°C and poured (20 ml) into sterilized Petri dishes. Peptone water (Merck, Darmstadt, Germany) was prepared according to the manufacturer's instructions, autoclaved (121°C, 15 min) and cooled to room temperature. Approximately 1 g agar was drawn from the designated zones on gels with the use of sterile tweezers, put into a sterile 10 ml volumetric flask, completed to 10 ml, and diluted with the peptone water down to 10^{-7} in sterile 10 ml volumetric flasks. From the last dilution, 100 µL was homogeneously spread on the Petri dishes with the use of a sterile Drigalski spatula. They were incubated at 37°C for 24 h and cfu were counted under a magnifying glass.

Statistical analysis

The inferential and descriptive statistical analysis for ZD and TA values of honey samples was performed with the of use software (IBM, 2017). The inferential analysis was performed through ANOVA for $p < 0.05$.

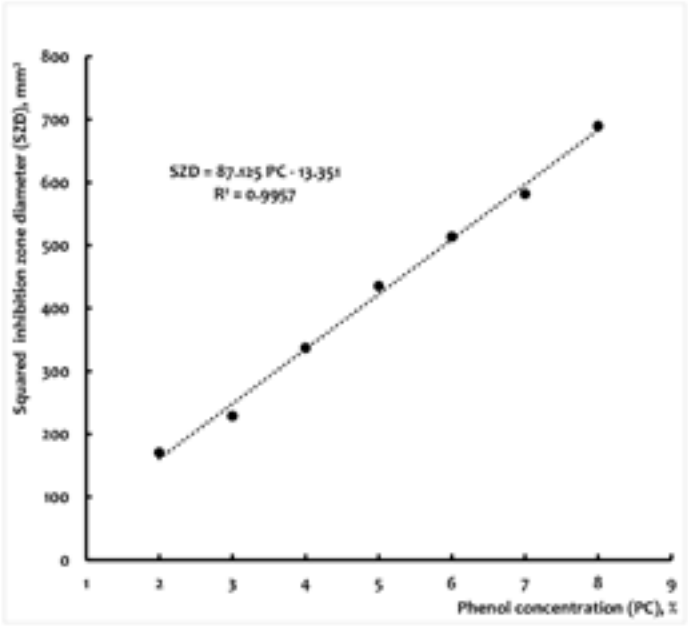

Fig. 2. Calibration curve used for the phenol equivalence test to determine TA of honey samples.

RESULTS

Egricayir Plateau

No trustworthy information on the Egricayır plateau is available in the literature but a rumor exists that its honey has a healing effect. The information on the geography of the Plateau was obtained from satellite imagery (Google Earth, 2024) and the beekeeping practices were compiled from the beekeepers operating on it (unpubl. data).

The area within the 15 km radius of the coordinates on the 36° 58' 25" N and 33° 49' 31" E on the Taurus Mountains is recognized as the Egricayir Plateau, Mersin, Turkey (Fig. 1). The honey named after the Plateau is foraged effectively over almost 14000 acres of the landscape at an altitude of around 2000 meters. The vegetation is mostly prairie plants, and the main nectar sources are thyme, keven, cornflower, wild clover, thistle, heather, phacelia, chaste berry, and unknown plants. The nectar flow occurs between mid-May and August, and the honey harvested is polyfloral. The harvesting lasts from the beginning of July to the end of August. The composition of the flora and weather show variation between the periods before and through harvesting. The weather shifts from fall-spring to spring-summer on the Plateau. Almost 2000 hives stand on the Plateau at a time with a total yield of around 30,000 kg in a season. The honey production capacity of the Plateau (30 tons/year) constitutes a tiny part (0.03%) of Turkey (~100,000 tons/year) (Burucu, 2022).

Experimental

Physicochemical parameters

The average physicochemical parameters of honey samples collected at the beginning (CT1) and end of the harvesting times (CT2) were statistically different except for the moisture content (Tab. 1). They were also determined to be free from naphthalene, pesticides and antibiotics. All parameters complied with the Turkish Food Codex (2020) and Council Directive (2001). The compliance reveals that the samples carry the traits of safe and ordinary organic honey. The honey from the Plateou is already marketed on the Turkish market and exported as organic honey.

Colony forming units

The number of *S. aureus* colonies was $6.4x10^3$ cfu/g on agar gels before the incubation. After the incubation, circular zones formed around the wells on the agar gels for all phenol and honey samples, and not for negative control samples. The phenol samples underwent a ZD enlarging from 13.06 mm to 26.26 mm with increasing phenol concentration from 2% to 8%. The colony formation was not observed in the zones of phenol samples and their number was $2.18x10^{10}$ cfu/g beyond the zones on average. Zones free from the colony were attributed to the microbicidal effect of the phenol solutions, and they were taken as the inhibition zone for the phenol solutions; the calibration curve (Fig.2) was drawn using them. The calibration curve exhibited a strong correlation with a coefficient of determination (R^2) of 0.9957. Zones of honey samples were in the form of two concentric circles, a wide inner zone and an enclosing thin halo zone (Fig. 3). Honey samples exhibited an inner zone diameter ranging from 17.01 mm to 23.89 mm (Tab. 2). The number of colonies was zero within the inner zones. On average, it was 15 cfu/g in the halo zones and $2.18x10^{10}$ cfu/per g beyond the zones. Zones free from colony formation were attributed to the microbicidal effect of the honey samples. The diameter of the inner zones was adopted as the ZD and the corresponding TA values were obtained from the calibration curve (Tab. 2).

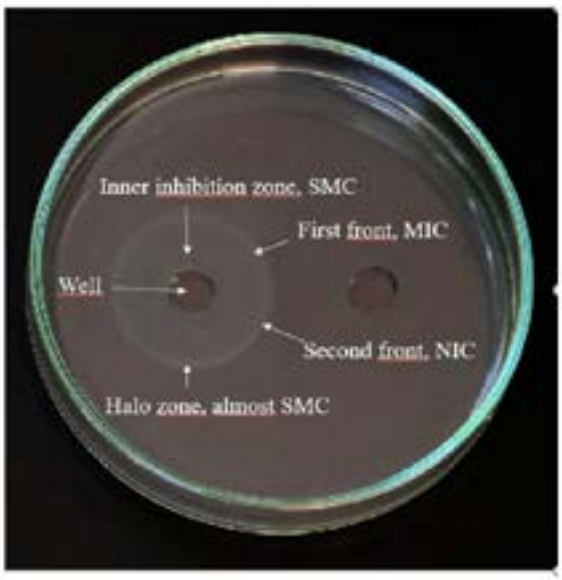

Fig. 3. An agar medium for honey samples after the incubation. The well on the right is for the negative control sample.

Statistics

Multidirectional statistical comparisons were performed for significant differences between groups of ZD values and the corresponding groups of TA values (Tab. 2). The difference between three HCs (two values per HC) was insignificant in terms of ZD and TA values in all five hives at both CTs. Then, six values in three HCs were combined in all hives. The difference between five hives (six values per hive) was insignificant for ZD and TA values at both CTs. The difference between the same hives (six values per hive) at two CTs was insignificant for ZD and TA values. Based on the inferential statistical analysis, thirty values at a CT were combined. The difference between the two CTs was insignificant for ZD and TA values. The inferential statistical analyses indicated that values from two CTs (60) could be gathered under one group. Then, the population of ZD and TA values were subjected to descriptive statistical analysis and their summaries are given in Fig. 4.

The experimental distribution of ZD and TA values showed qualitative and quantitative similarities (Fig. 4). Both had a mean, median and mode in a slightly increasing order. Their skewness was between -0.5 and -1.0. Their kurtosis was greater than +1. They had a Levene statistic greater than 0.05.

DISCUSSION

Physicochemical parameters of honey samples

The honey mixtures exhibited statistically significant differences almost in terms of all physicochemical parameters at CT1 and CT2 (Tab. 1). The samples collected at CT1 consist of honey made between the period of mid-May and the beginning of July, and the samples collected at CT2 consist of honey made almost at the end of the nectar-flow period. The flora composition and weather are different between these periods on the Plateou as mentioned above. Insignificant statistical differences between the parameters are normally not expected under such conditions in ordinary beekeeping practices. The insignificant difference between the moisture contents at CT1

Table 2.

Inhibition zone diameter (ZD, mm) and TA of honey samples at two collection times (CT1, early July and CT2, late August 2023)[1]

Hive	Parameter	Time	Honeycombs					
			HC1	HC2	HC3	HC4	HC5	HC6
1	ZD	CT1	21.97	22.92	19.60	18.45	22.34	22.07
		CT2	22.03	21.65	18.41	19.23	20.67	20.99
	TA	CT1	23	25	18	16	24	23
		CT2	23	22	16	18	20	21
2	ZD	CT1	18.40	22.78	20.61	17.67	21.46	22.38
		CT2	19.50	20.45	21.87	17.36	21.05	22.81
	TA	CT1	16	24	20	15	22	24
		CT2	18	20	23	14	21	25
3	ZD	CT1	17.01	21.01	18.87	19.34	21.89	22.12
		CT2	18.90	21.93	19.76	17.99	22.63	21.03
	TA	CT1	14	21	17	18	23	23
		CT2	17	23	19	15	24	21
4	ZD	CT1	21.80	20.87	21.31	23.89	22.34	20.46
		CT2	22.56	21.65	21.65	22.87	21.03	19.89
	TA	CT1	22	21	17	27	24	20
		CT2	24	23	19	25	21	19
5	ZD	CT1	22.51	21.78	22.01	18.56	21.95	20.15
		CT2	22.78	23.45	21.78	18.18	21.16	22.34
	TA	CT1	24	22	23	16	23	20
		CT2	24	26	22	16	21	24

[1]In a hive, honey samples were from different honeycombs (HC1…HC6).
For an HC, 2 ZD, and then 2 TA values were determined. Six ZD and TA values were obtained for a hive for a CT. For a CT, 30 ZD and 30 TA values were obtained for all hives totaling 60 values for both CTs.

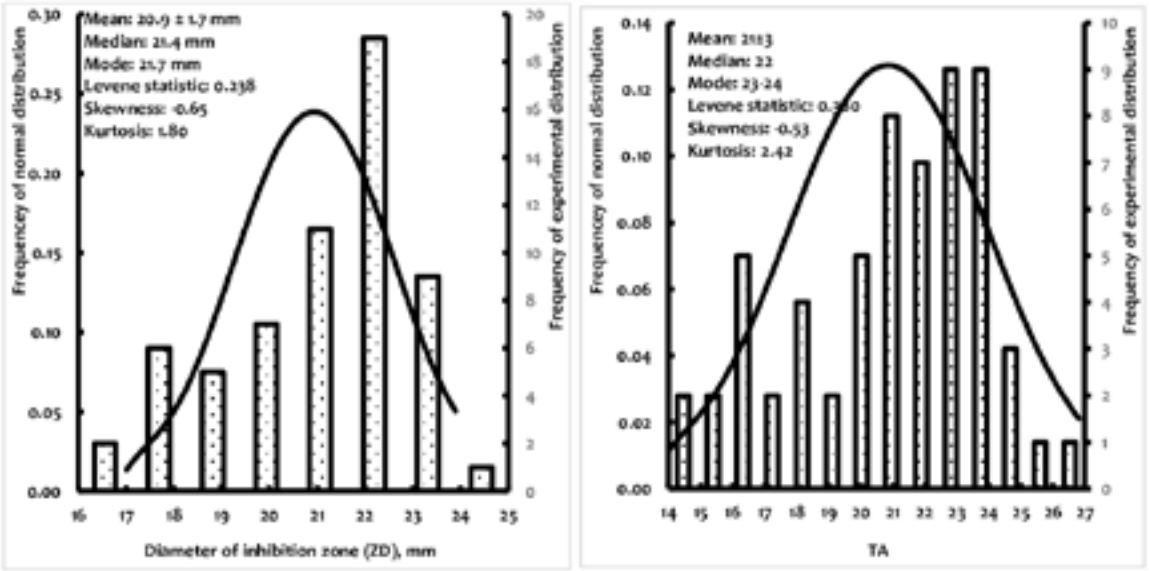

Fig. 4. Distribution of inhibition zone diameter (ZD) (a) and TA (b) values. Smooth curves and boxes are for the normal and experimental distributions, respectively.

and CT2 is considerable, which points out that the honey-making process is completed and samples mature at the beginning and end of the harvesting period. Every parameter of both honey mixtures complies with related regulations for honey (Turkish Food Codex, 2020; Council Directive, 2001). The compliance reveals that the samples carry the traits of safe and ordinary organic honey. The inferential statistical analysis above indicated that the honey samples collected at CT1 and CT2 were from the same population in terms of ZD and TA and shows that the statistical difference between given physicochemical parameters practically did not lead to a significant difference in terms of ZD and TA.

Inhibition zones, and ZD and TA values of honey samples

Various agents are credited effective in varying degrees for the AMA of honey (Szweda, 2017). Among them, H_2O_2 is the dominant one for most honeys, forming through the catalyzes of glucose by glucose oxidase, and its concentration exhibits a strong correlation with TA (Guttentag et al., 2021). The well diffusion method used in this study depends on how agents from the well are able to radially diffuse through the agar gel; concentration and effect decrease with an increased diffusion path. Their diffusivity in the agar is inherently different and the distance they travel differs which would the subject of a detailed work.

More than one zone could be observed in the well diffusion method in the determination of the TA of honey (Szweda, 2017). Two circular zones were observed for honey samples in the current work (Fig. 3). The agents radially diffusing from wells reached a minimum inhibition concentration (MIC) at some point in the agar gels. The inner inhibition zone, the first front, formed between the well and where MIC was reached. The agents had a sufficient microbiocidal concentration (SMC) in the inner zone to decrease the number of colonies from $6.4x10^3$ cfu/g before the incubation to null after the incubation. The agents diffused beyond the first front and reached the null inhibition concentration (NIC) at some point. The halo zone formed between the first front and the second front, where NIC was reached. The agents almost had the SMC in the halo zone, and a negligible number of colonies formed there according to the decrease from $6.4x10^3$ before the incubation to 15 cfu/g after the incubation. NIC prevailed beyond the second front according to the increasing number of colonies from $6.4x10^3$ before the incubation to $2.18x10^{10}$ cfu/g after the incubation. TA values for honey samples were determined from the inner zone diameter used as the ZD (Fig. 3, Tab. 2). The similar inferential statistics for ZD and TA values mentioned above imply that both groups are qualitatively similar regarding the insignificant differences between the designated groups. Their close descriptive statistics results (Fig. 4) confirm that their distributions are also in agreement qualitatively and quantitatively. The statistical similarities point out that the experimentation for obtaining ZD values and processing them into TA values was performed appropriately and the possible erroneous TA outcomes were minimal under the given working conditions.

Statistical assessment of TA values of honey samples

TA values of honey samples ranged between 14 and 27 with a range of 13, standard deviation of 3, mean of 21, median of 22, mode of 23-24, and Levene statics of 0.380 (Fig. 4). Almost 95% of TA values were within the range of ±2 standard deviations of the mean ($15 \leq 21 \leq 27$), which implies a normal distribution. Albeit, the increasing order of the mean (21), median (22), and mode (23-24) point to the tilted distribution curve of TA values to the right. The relatively small differences in their magnitudes indicate a slight tilting. The negative sign and the magnitude of the skewness (-0.525) are in line with the increasing ordering of the mean, median, and mode. If the skewness is between -0.5 and -1.0, the distribution is considered to be moderately tilted to the right concerning the normal distribution (Hatem et al., 2022). The positive sign and the magnitude of the kurtosis (2.417) show a relatively low number of TA values on the tails of the distribution curve. If kurtosis is greater than +1, the distribution is leptokurtic, which means that it has thinner tails than those of the normal distribution (Hatem et al., 2022). The less-tailedness of the distribution

curve of TA values concerning the normal curve shows that TA values have relatively fewer outliers. The Levene statistics being greater than 0.05 shows that the difference between TA values is insignificant and that they are all most probably from the same population.

Physical assessment of TA values of honey samples

The total AMA of honey is categorized as undetectable for TA<5, low for 5≤TA<10, therapeutically beneficial for TA≥10, and therapeutically high for TA≥20 (Carter et al., 2010). The non-peroxide antibacterial activity of mānuka honey is assessed by the Universal Mānuka Factor (UMF) which is a well-established instituted method. It is categorized as undetectable for UMF≤4, not recommended for therapeutic use for 5≤UMF≤9, therapeutically useful for 10≤UMF≤15, and therapeutically high for 16≤UMF≤30 (Tsang et al., 2017). The lowest limit of 10 for therapeutic use is the same for TA and UMF assessing, and TA and UMF values within the same limits are categorized by almost the same potency.

All honey samples from the Egricayır Plateau had a TA>10 and the minimum TA was 14. Then, all samples can be categorized to be therapeutic. Most of the TA values (43 out of 60, 72%) were ≥20, which points to a high therapeutic potency according to both TA and UMF categorizing. The distribution curve of TA values tilting to the right (Fig. 4) indicated relatively more TA values greater than the mean TA value (21). More than half of the TA values (38 out of 60, 63%) were ≥21 which verifies the skewness of the distribution curve (tilting to the right). A very small portion of the values (2 out of 60, 3%) could be assessed outliers considering the range of ±2 standard deviations (6) around the mean TA value (21). In the case of ±3 standard deviations, none of the TA values emerges as an outlier, which is in line with the kurtosis of the distribution curve. Levene statistics supports the existence of a small number of outliers or their nonexistence, and TA values represent a homogeneous population. The distribution of TA values shows that the Egricayir Plateau is statistically and practically a homogeneous honey source in terms of TA for the season of 2023.

The consistent values do not necessarily mean that all honey samples from the Plateau will always have the same TA values. Though all TA values were >10, the TA value and the potency can notably differ even in the same hive. For example, the TA value was between 14 (beneficial potency) and 23 (high potency) in hive 3 at CT1, and between 14 (beneficial potency) and 25 (high potency) in hive 2 at CT2 (Tab. 2). The discrepancy could be more dramatic and, the distribution of TA values can vary year by year as TA could be affected by the weather conditions even for the same flora (Almasaudi, 2021). So, as much as possible honey from the Plateau is supposed to be blended, and every single blend should be analyzed for TA to ensure a consistent and trustworthy TA value and potency category. The antimicrobial attribute of honey from the Egricayır Plateau makes it a good candidate for further testing for therapeutic use and trading at higher values.

Comparing TA of honey samples with the literature

TA of honey samples from the Egricayir Plateau was comparable with the literature. Green et al. (2022) determined the TA of thirty mānuka honey from Australia and New Zealand. Some honey samples (47%) had an undetectable or therapeutically low potency with a TA<10. Other samples had a therapeutically beneficial potency with a 10≤TA<20 (23%) and a therapeutically high potency with a TA≥20 (30%). The incidence of therapeutic samples was 53% with a maximum TA of 31. They had an average TA of 19 which corresponds to the beneficial potency. Irish et al. (2011) worked TA of 477 honey samples throughout Australia. Their result showed the potency of honey samples to be undetectable or therapeutically low (43%), beneficial (40%), and high (17%). More than half of the samples (57%) were therapeutic with a maximum TA of 31. They had a median average TA of 16 indicating the beneficial potency. Allen et al. (1991) examined the TA of 345 honey samples throughout New Zealand. Honey samples had therapeutically low (53%), beneficial (37%), and high (10%) potency. Almost

half of the samples (47%) were therapeutic with a maximum TA of 42. They had an average TA of 19 falling into the category of beneficial potency. Honey samples from the Plateau are in the same category as high-potency honey (Green et al., 2023). Honey samples from the Plateau have a more homogeneous TA distribution and higher incidence of therapeutic AMA compared to the literature. They are all active without exception, e.g. 100% incidence of the therapeutic AMA. The small forage territory should probably help with such a high incidence. In the literature, honey samples were from various large territories, and the incidence of the therapeutic AMA from such territories is expected to be lower compared to small territories. The variation of the flora between different and within large lands is naturally much more than the flora within small lands. Also, the variation is supposed to be more between different and large lands from season to season. It would be plausible not to expect all honey from a large geography to be antimicrobially active. For a consistent AMA and solid geographical indication, small territories seem more manageable than large ones at the expense of the tonnage. In conclusion, the Egricayir Plateau is a small virgin piece of land accommodating honey potentially with high TA. Any honey sample from any spot of the Plateau has a high potency at least for the 2023 season. The current preliminary work is the first, and more detailed follow-up works will strengthen the position of the Plateau for its honey. The honey from the Plateau is a good candidate for further therapeutic tests and geographical indication and has the potential for positioning in the market with names evoking health.

Acknowledgment

The authors gratefully acknowledge the support of the University of Mersin under Project 2020-1-TP2-4007 and 2021-TP2-4373, and the great support, patience, care and respect of the beekeepers on the Egricayir Plateau, Mersin, Turkey.

Nomenclature

AMA	Antimicrobial activity
cfu	Colony forming units
CT	Collection time
HC	Honeycomb
MIC	Minimum inhibition concentration
n	Number of samples
NIC	Null inhibition concentration
PC	Phenol concentration, %
R^2	Coefficient of determination
SMC	Sufficient microbiocidal concentration
SZD	Squared zone diameter, mm^2
TA	Total antimicrobial activity (AMA)
UMF	Universal Mānuka Factor
ZD	Inhibition zone diameter, mm

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