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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INFLUENCE OF BOTANICAL COMPOSITION ON ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF HONEY

Balın Antioksidan ve Antibakteriyel Özellikleri Üzerinde Bitkisel Kökenin Etkisi

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ABSTRACT

A study conducted on 22 samples of honey harvested from different regions of Kyrgyzstan in 2023 confirmed that the quality and beneficial properties of honey directly depend on its botanical origin. This emphasizes the importance of considering these factors when evaluating honey and its potential use in the food industry, medicine, and other areas. The taste, aroma, color, and consistency of honey varied according to botanical origin. Honey collected from certain plants had a more pronounced aroma and intense flavor. Parameters such as moisture, free acidity, and diastase number were in line with international norms. These parameters also depended on the region of collection and the type of plants from which the nectar was collected. Notably, the diastase number was negatively correlated with the radical inhibition concentration (r = -0.464). For example, honey samples 1, 9, and 15 exhibit the highest antioxidant activity, which is attributed to their polyfloral composition, particularly when pollen of Lamiaceae (r=-0.527) and Asteraceae (r=-0.520) is present. The antioxidant properties of honey, which are essential in protecting the body from free radicals, vary according to the botanical composition. Some samples exhibited high activity, making them particularly valuable for health applications. Honey demonstrated the ability to inhibit the growth of certain bacteria, confirming its traditional use in folk medicine.

Keywords: Honey, Botanical origin, Geographical origin, Antioxidant activity, Antibacterial properties

ÖZ

2023 yılında Kırgızistan'ın farklı bölgelerinden toplanan 22 bal örneği üzerinde yapılan bir araştırma, balın kalitesinin ve faydalı özelliklerinin doğrudan botanik kökenine bağlı olduğunu doğrulamıştır. Bu, balı ve gıda endüstrisi, tıp ve diğer alanlarda potansiyel kullanımını değerlendirirken bu faktörleri dikkate almanın önemini vurgulamaktadır. Balın tadı, aroması, rengi ve kıvamı botanik kökenine göre değişiklik göstermektedir. Bazı bitkilerden toplanan balın aroması daha belirgin ve tadı daha yoğundur. Nem, serbest asitlik ve diastaz sayısı gibi parametreler uluslararası normlara uygundur. Bu parametreler ayrıca toplama bölgesine ve nektarın toplandığı bitki türlerine bağlıdır. Özellikle, diastaz sayısı radikal inhibisyon konsantrasyonu ile negatif korelasyon göstermektedir (r = -0,464). Örneğin, bal örnekleri 1, 9 ve 15, özellikle Lamiaceae (r=-0,527) ve Asteraceae (r=-0,520) polenlerinin bulunduğu durumlarda, polifloral bileşimlerine atfedilen en yüksek antioksidan aktiviteyi sergilemektedir. Vücudu serbest radikallerden korumada önemli olan balın antioksidan özellikleri, botanik bileşimine göre

değişiklik göstermektedir. Bazı numuneler yüksek aktivite göstererek sağlık uygulamaları için özellikle değerli hale gelmiştir. Bal, bazı bakterilerin büyümesini inhibe etme yeteneği göstererek, halk hekimliğinde geleneksel kullanımını doğrulamıştır.

Anahtar kelimeler: Bal, Botanik köken, Coğrafi köken, Antioksidan aktivite, Antibacterial özellikler

GENİŞLETİLMİŞ ÖZET

Amaç: Balın, maya, mantar ve bakteriler dahil olmak üzere geniş bir yelpazedeki patojenik ve patojenik olmayan mikroorganizmaları inhibe eden antimikrobiyal aktiviteye sahip olduğu gösterilmiştir. Balın antibakteriyel etkileri, çiçek kaynaklarındaki farklılıklar, iklim koşulları ve fenolik bileşikler ve hidrojen peroksit gibi biyoaktif bileşiklerin varlığına bağlanabilir. Bu araştırma, Kırgızistan'ın çeşitli bölgelerinden elde edilen balın antioksidan ve bakterisidal özellikleri üzerinde botanik bileşimin etkisini incelemek amacıyla yapılmıştır.

Malzeme ve yöntemler: Kırgızistan'ın farklı bölgelerinden toplam 22 bal örneği toplanmış ve incelenmiştir. Nem, serbest asitlik ve diastaz aktivitesi gibi kalite parametrelerinin analizi, Avrupa Bal Komisyonu'nun Uyumlaştırılmış Yöntemlerine karşılık gelen GOST 19792-2017 standardına göre gerçekleştirilmiştir. Polen analizi, Uluslararası Arı Botanik Komisyonu tarafından belirlenen bir yönteme göre yapılmıştır. Tanımlayıcı nitel yöntem, ISO standartlarına (ISO 5492, 2008; ISO 6658, 2005) uyarınca tanımlayıcı niteliksel yöntem uygulanmıştır. Bu yöntem, görünüm, kıvam, renk, koku, aroma ve tat gibi tüm tanımlayıcıları dikkate almaktadır.

Bulgular: Çalışma, balın diastaz sayısı ile botanik kökeni arasında yakın bir ilişki olduğunu ortaya koymuştur. Apiaceae familyasından elde edilen balın yüksek diastaz sayısı (r=0,565) ile karakterize olduğu belirtilmiştir. Aynı zamanda, Sainfoin sp. türünden üretilen balın diastaz sayısı genellikle düşüktür (r = -0,644). Ancak, çalışmanın sonuçları, ek bir nektar kaynağı mevcut olduğunda diastaz sayısının önemli ölçüde arttığını da göstermiştir. Diastaz sayısı, radikal inhibisyon konsantrasyonu ile negatif korelasyon göstermiştir (r = -0,464). Bal örnekleri 1, 9 ve 15, polifloral bileşimlerine bağlı olarak en yüksek antioksidan aktiviteyi sergilemektedir; bu, balda Lamiaceae (r=-0,527) ve Asteraceae (r=-0,520) polenlerinin bulunmasıyla ilişkilidir.

İncelenen göstergeye göre, incelenen tüm bal türleri standardın gerekliliklerini karşılamaktadır. Balın inhibisyon konsantrasyonu 1 ila 16 mg/100 g aralığındadır. Kırgızistan'ın farklı bölgelerinden alınan bal örnekleri, botanik ve coğrafi çeşitliliklerini yansıtan geniş bir renk yelpazesi sergilemektedir.

Genel olarak, veriler Kırgız balının çeşitli derecelerde antibakteriyel aktiviteye sahip olduğunu göstermektedir. En önemli inhibisyon *Shigella flexneri* (6) karşısında gözlemlenmiştir, bunu *Staphylococcus aureus* (29213) ve *Escherichia coli* izlemektedir. Sonuçlar, farklı bölgelerden elde edilen balın çeşitli inhibe edici etkiler sergilediğini, bazı numunelerin (N4, 6, 14, 16) özellikle Shigella flexneri (6) üzerinde önemli antibakteriyel aktivite gösterdiğini ortaya koymaktadır. Farklı bal örnekleri arasında gözlemlenen antibakteriyel etkilerdeki farklılıklar, çiçek kaynakları, iklim koşulları ve fenolik bileşikler ve hidrojen peroksit gibi biyoaktif bileşiklerin varlığındaki farklılıklara bağlanabilir. Bal örnekleri 1 ve 15 en yüksek antioksidan aktiviteye sahiptir ve yüksek miktarda Asteraceae poleni içerir (Şekil 1). Örnek 9'un en yüksek antioksidan aktivitesi, Caryophyllaceae poleninden (13%) kaynaklanıyor olabilir.

Sonuç: Kırgız bal örneklerinin antibakteriyel aktivitesi, coğrafi kökenleri ve test edilen bakteri suşu ne olursa olsun tutarlı kalmıştır. Sonuçlar, Kırgızistan'ın tüm bölgelerinden alınan bal örneklerinin önemli antibakteriyel özellikler sergilediğini ve en yüksek inhibisyonun Shigella flexneri (6) karşısında gözlemlendiğini göstermiştir. Bu, Kırgızistan'dan gelen balın antimikrobiyal tedavilerde potansiyel uygulamaları olabileceğini düşündürmektedir.

INTRODUCTION

Natural honey is a product of beekeeping, which has a complex chemical composition. It contains more than 300 different substances and has antioxidant, antibacterial, and antimicrobial properties (Korzh 2021). Natural honey contains sugars (mainly fructose, glucose and sucrose), biologically active compounds including phenolic compounds (phenolic acids, flavonoids and polyphenols), amino acids (mainly proline), peptides, proteins, enzymes,

vitamins (especially vitamin B complex and vitamin C), minerals (Ca, Cu, Fe, K, Na, Mg, Zn, Mn, P, S), organic acids (gluconic acid formed by enzymatic oxidation of glucose), lipids (including waxes), aromatic compounds, carotenoid-like substances and pollen grains (Bogdanov et al. 2008, Maddocks & Jenkins 2013). Natural honey is a concentrated, high-calorie bee product with potential beneficial properties for the human body (George et al. 2025).

Scientific evidence suggests that honey can exert several health benefits, including antiulcer, hepatoprotective, reproductive, hypoglycemic, antioxidant, antibacterial, antifungal, and anti-inflammatory effects (Xie & Coghi 2025, El-Din et al. 2025, Ogwu & Izah 2025). It has the following advantages: it does not irritate the digestive glands, promotes wound healing, and has a health-improving and calming effect, facilitating rapid recovery from energy loss (Hanif & Ali 2024). Honey has been shown to exhibit antimicrobial activity, inhibiting a wide range of pathogenic and non-pathogenic microorganisms, including yeasts, fungi, and bacteria such as *Salmonella, Shigella, Escherichia coli, Staphylococcus aureus*, *Pseudomonas* spp., and *Helicobacter pylori* (Pham & Nguyen 2022). Alcoholic extracts of honey have an inhibitory effect on aerobic and anaerobic, Gram-positive and Gram-negative bacteria (Feknous & Boumendjel 2022). In a dilution of 1:80 with water, honey exhibits an antimicrobial effect against Staphylococci, Streptococci, and diphtheria bacilli (Alvarez-Suarez & Tulipani 2011, Jeffrey & Echazarreta 1996).

Honey contains various antioxidants, including phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids), flavonoids (such as apigenin, kaempferol, quercetin, galangin, chrysin and hesperetin), organic acids, and enzymes like catalase and glucose oxidase (Campone et al. 2014, Ranneh et al. 2018). Maillard reaction products and peptides, most of which interact with each other to provide a synergistic antioxidant effect (Rakha et al. 2008). The botanical origin of honey has been shown to have the greatest influence on its antioxidant activity. At the same time, processing, handling, treatment, and storage have only a minor effect on its antioxidant capacity (Sereia et al. 2011). In addition, a strong correlation was found between antioxidant activity and honey color. Many researchers have observed that dark honey has a higher total phenolic content and, consequently, a higher antioxidant capacity (Anand et al. 2018, Jeffrey & Echazarreta 1996). Dark-coloured honeys have been found to contain higher levels of phenolic acid compounds but lower levels of flavonoids compared to light-coloured ones (Smetanska & Salman 2021).

The chemical composition, physical properties, and bioactivity of honey depend on several factors, including nectar composition, botanical origin, geographical origin, bee breed, climate, environmental conditions, seasonal conditions, feeding methods and honey processing during extraction and storage (Molan 1992, Wang & Li 2011, Mazhitova & Smanalieva 2022). The purpose of this research is to investigate the quality of honey and the impact of botanical composition on its antioxidant activity and bactericidal properties, sourced from various regions of Kyrgyzstan.

MATERIALS AND METHODS

Honey samples were obtained directly from beekeepers of the following regions of Kyrgyzstan: Osh region: Ozgon, Alai; Naryn region: At-Bashy, Kazarman; Jalal-Abad region: Kara-Alma district, Toktogul; Chui region: Suusamyr valley, Shamshy; Issyk-Kul region, Jeti Oguz district; Talas region, collected in 2023, (Table 1). The studied honey samples were stored in a refrigerator at 4 °C until analysis.

Palynological analysis

The botanical origin of the honey was determined using a generalised palynological technique (Von der Ohe 2004). Micro preparation from honey is made according to GOST 31769-2012. The essence of the method is that pollen grains are concentrated from the honey solution by centrifugation, a preparation for light microscopy, which enables the determination of a specific number of pollen grains and the percentage of pollen grains of a particular species among the total number of counted pollen grains. The determination of botanical origin is based on the calculation of the relative pollen frequency of honey-bearing plant species (Ishenbaeva et al. 2024). In most cases, honey is considered monofloral if the relative frequency of pollen from one species exceeds 45%, commonly represented by pollen (Louveaux et al. 1978).

Determination of physicochemical parameters of honey

The refractive index of honey samples was

measured using a refractometer (Abbe 2WAJ, Wincom Company Ltd., China) at 20 °C. Corresponding moisture content (%) was calculated from the relationship between the refractive index and water content. The free acid content was determined in a 10% (w/w) honey solution by acid-base titration with 0.1 M NaOH to pH 8.1, using a pH meter (AD8000, ADWA Instruments, Hungary). The results were presented in milliequivalents per kilogram (mEq/kg) (Bogdanov et al. 1997). Diastase (α-amylase) activity was measured using a spectrophotometer (Spectrophotometer UV-1800, China) and expressed as diastase number in Gothe units (GOST 34232-2017). Hydroxymethylfurfural (HMF) (qualitative) studies were carried out in line with GOST 31768-2012.

Determination of antioxidant activity

The antioxidant activity was measured using the method of Hangun-Balkir and McKenney. For extract preparation, a 5 g sample was mashed and added to a 20 mL solution of 80% ethanol, then stirred for approximately 20 minutes. This antioxidant solution was centrifuged at 5000 rpm for 3 minutes. The supernatant was retained as a liquid sample for further analysis. Individual antioxidant solutions were prepared at five concentrations (1, 2.5, 5, 7.5 and 10 µg/mL) in 80% ethanol. Two millilitres of 0.01% DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 80% ethanol were added to each of the respective 2 mL antioxidant solutions in vials. The solutions were shaken and incubated at room temperature for 30 minutes. Two millilitres of 80% ethanol combined with 2 mL of DPPH solution served as a control/blank. The absorbance of control and sample solutions was measured at 517 nm using a UV-VIS spectrophotometer (Specord 50, Analytic Jena, Germany). Values are expressed as IC50, the concentration of the samples that causes 50% scavenging of the DPPH radical (Hangun-Balkir & McKenney 2012).

Study of the antibacterial properties of honey samples

Each honey sample was diluted by adding 10 mL of sterile distilled water to 10 mL of honey, resulting in final concentrations of 50%, 60%, and 80%. The diluted honey (10 mL) was then transferred into a sterile container with a total volume of 50 mL. The test bacteria used in the food microbiology analysis included *Staphylococcus aureus* (29213), *Shigella flexneri* (6) from the Bishkek Department of Sanitary and Epidemiology, and *Escherichia coli* from the Medical Academy of Kyrgyzstan. The bacterial strains were maintained on nutrient agar at 4 °C. Before testing, bacterial cultures were grown in nutrient broth at 37 °C for 24 hours to obtain active cultures (CLSI 2018).

The antibacterial activity of the honey samples was evaluated using the agar well diffusion method. Plate Count agar plates were prepared and inoculated with standardized and 100 µL bacterial suspensions. Speared on the plate (Balouiri et al. 2016) of 5-7 mm in diameter, the aseptically punched wells were created using sterile pipettes. Then, 100 µL of each honey sample was introduced into the wells. The plates were incubated at 37 °C for 24 hours under aerobic conditions (CLSI 2018). After incubation, the antibacterial activity was determined by measuring the diameter of the inhibition zones around each well using a digital calliper. The experiment was conducted in triplicate, and the results, excluding the diameter of the well, were recorded as mean ± standard deviation (Mandal et al. 2010).

Sensory analysis of honey

In general, the following organoleptic parameters were analyzed in the work: appearance, physical state, colour, aroma intensity, persistence (aftertaste of aroma), and taste. The method of honey sensory analysis, widely recognized in European countries, enables the determination of a product's suitability for sale. If the product does not meet the sensory requirements, it raises doubts about its naturalness. Sensory (or organoleptic) analysis is an assessment of honey quality that utilizes the senses of sight, smell, taste, and touch.

Appearance, color, fluidity, transparency, homogeneity, and crystallization quality are perceived by the visual organs. This analysis was carried out in three stages. Firstly, it was analyzed visually. We evaluated its appearance and attractiveness, described its physical condition, and its color range. During the visual analysis, attention was paid to the purity and transparency of the honey, its homogeneity, and the presence or absence of any inclusions. Then it was sniffed, and then tasted for taste and tactile sensations (Piana et al. 2004). For the sensory analysis, we took a sample of 30-50 g of honey, which was stored in half-litre jars. We used glassware, as it excludes the possibility of contact

between the honey and unpleasant odors that plastic products can emit.

Color measurement

Color analysis was performed using a portable colorimeter (ColorTec-PCM, Accuracy Microsensors, Inc., USA). The device was calibrated using black and white reference standards and operated under D65 illuminant conditions. Measurements were recorded in the CIE L*a*b* color space, which corresponds to human visual perception of colour. The results are expressed as the mean of ten individual readings per sample (Nagai et al. 2018).

RESULTS

Sensory, physico-chemical parameters and botanical origin of Kyrgyz honey

Using the technique and methodology proposed by the Italian scientist and founder of sensory analysis, Michel Gonne, the taste and odor, aroma, color, consistency, mechanical impurities and signs of fermentation of the presented honeys were determined (Table 1). The color of the honey samples was from white to deep amber. The overall assessment of organoleptic properties corresponds to honey quality standards.

Table 1. Organoleptic analysis results for honey types

№	Geographical origin of samples	Visual analysis			Olfactory and odour analysis				Consis-tency
		appearance	physical condition	color	odor	aroma intensity	aroma aftertaste	flavor	
1	Osh region, Ozgon, Kurshab	homogeneous, clear	small crystal	deep amber	floral	quite strong	extra long	very strong, sweet	sticky
2	Naryn region , At-Bashy/Rahat	homogeneous, foam	medium crystal	white	floral	medium	long	medium-sweet	tight
3	Jalal-Abad region, Kara-Alma	homogeneous	medium crystal	amber	floral	quite strong	extra long	very strong, sweet, pleasant	tight
4	Naryn region, At-Bashy	homogeneous, clear	large-crystal	white	floral - rose scent	medium	short	medium, sweet, pleasant	tight
5	Naryn region, At-Bashy	homogeneous	large-crystal	white	floral	medium, short	short	medium-sweet	tight
6	Osh region, Chon Alai, Zhar Bashy	opaque	pasty	straw yellow	vegetal	medium	short	medium, sweet, pleasant	tight
7	Osh region, Chon Alai	homogeneous, foam	small crystal	deep yellow	floral	strong	short	medium, pleasant	soft, crystallised
8	Osh region, Chon Alai	pure, clear	pasty	light yellow	floral	weak	short	sweet, pleasant	soft, crystallised
9	Osh region, Chon Alai	pure, clear	small crystal	straw yellow	floral	very strong	long	strong, pleasant	tight
10	Osh region, Chon Alai	heterogeneous, fractional	large-crystal	light yellow	pet	weak	short	sweet, weak	dense mass
11	Osh region, Chon Alai	homogeneous	small crystal	straw yellow	pet	medium	extra long	strong, sweet	dense mass
12	Osh region, Chon Alai	heterogeneous, fractional	medium crystal	straw yellow	Chemi-cal	medium	average	sweet, weak	dense mass
13	Jalal-Abad region, Toktogul	opaque	large-crystal	deep amber	Chemi-cal	medium	average	strong, swee	tight
14	Naryn region, Kazarman	heterogeneous	medium crystal	amber	floral	strong	extra long	very strong, sweet, fragrant	tight
15	Chui region, Suusamyr	Heterogeneous	medium crystal	deep amber	fruity, flavour	very strong	extra long	very strong, sweet, fragrant	tight
16	Jalal-Abad region, Toktogul	Homogeneous, foam	large-crystal	red	floral	very strong	extra long	sweet, sweet, strong	tight
17	Issyk-Kul region, Zheti Oguz	Homogeneous, foam	pasty	yellow	floral rose fragrance	medium	average	pleasant, medium	sticky
18	Chui region, Shamshy	Heterogeneous	medium crystal	deep amber	chemi-cal	medium	long	pleasant, medium	tight
19	Talas region	Homogeneous, foam	medium crystal	light yellow	floral	medium	short	sweet, pleasant	sticky
20	Talas region	Heterogeneous, fractional	large-crystal	light yellow	floral	medium	short	sweet, pleasant	sticky
21	Jalal-Abad region, Toktogul	Heteroge-neousfractional	large-crystal	deep amber	floral	very strong	extra long	sweet, fragrant	tight
22	Issyk-Kul region, Tup	Homogeneous, foam	pasty	light yellow	floral	medium	long	sweet, pleasant	dense, hard

The results of the physicochemical parameters of honey are shown in Table 2. The average moisture content, diastase number and free acidity of the studied honey samples were 17.2 %, 20.0 Gothe unit, and 21.0 mEq/kg, respectively. This study found that diastase activity in samples ranged from 10.1 to 30.2 Gothe units. High diastase activity appeared in samples 1, 3, 15, 16, and 21 (25.8-30.2 Gothe). Samples 2, 5, 17, and 20 showed low activity (10.1-11.3 Gothe). In examining free acidity, the studied samples ranged from 13 to 33.3 mEq/kg. Specifically, light honey varieties had the lowest mean value of 13.1 ± 2.1 mEq/kg, while polyfloral mountain honey types had the highest values, from 24 to 30.3 mEq/kg. In terms of regulation, the maximum permitted level of free acid is 50 mg/kg. Turning to the qualitative analysis of the HMF study, results showed that the studied samples do not contain this substance. All studied species of honey, as indicated by the considered indicator, meet the requirements of the standard. Inhibition concentration of honey was in the range from 1 to 16 mg/ 100 g. The honey samples from different regions of Kyrgyzstan showed a wide range of color characteristics, reflecting their botanical and geographical diversity.

According to the palynological study, all samples corresponded to the names declared by Kyrgyz beekeepers. Of 22 honey samples, 11 samples are sainfoin *(Sainfoin* sp.*)* honey, 2- thyme *(Thyme* sp.), 1 - bilberry *(Echium vulgare*), and 8 - polyfloral. The share of sainfoin pollen *(Sainfoin sp.)* in the monofloral honeys ranged from 45 to 86 %; thyme *(Thyme* sp.) - from 23.5 to 24.8 %, bilberry *(Echium vulgare*) - 47.5 %. In polyfloral honey species (Table 4), the pollen of these plant families prevails: umbrella *(Apiaceae)* up to 40.0%, astroflowers (Asteraceae) up to 38.0%, spongiosa *(Lamiaceae)* 22.0%, legumes *(Fabaceae)* up to 18.0%, cruciferous *(Brassicaceae)* up to 17.2%, carnation *(Caryophyllaceae)* up to 13.0%, rosaceous *(Rosaceae)* up to 12.0%, lily *(Liliaceae)* up to 3%, and borage *(Boraginaceae) up to 2.9%. Mellifers with accompanying pollen in the honeys studied include* onion *(Allium* sp.*)* up to 14.0%, black-root *(Cynoglossum* sp.*)* up to 12.4%, tan eremurus *(Eremurus fuscus)* up to 12.0%, angelica *(Angelica* sp.*)* up to 12.0%, iceplant *(Lotus* sp.*)* up to 11.0%, lilac *(Linaria* sp.*)* up to 10.7%, and mugwort *(Fumaria* sp.*)* up to 7.7%. All honey samples contain pollen of common bruise *(Echium vulgare*) at 0.5-17.2%. Pollen-bearing plants also occur in honey samples: *Hypericum* sp. 0.5-44.0%, *Artemisia* sp. 0.5-20.0% *Poaceae* sp. 0.3-20.0%, Chenopodiaceae 3.3-14.4%, *Xanthium* sp. 7.7%, *Plantago* sp. 0.5-5.5%, *Sanguisorba* sp. 0.6-2.0%, and *Galium* sp. *0.3-0.7*%. Only honey from the Yssyk-Köl region contains *Tilia* sp.

Previous studies have shown that the main honey species produced in Kyrgyzstan contain pollen from sainfoin (*Onobrychis* sp.), thyme (*Thymus* sp.), sage (*Salvia* sp.), Anthriscus (a common plant genus in the family Apiaceae), and blueweed (*Eichium vulgare*) (Smanalieva 2008). Honey from the Sary-Chelek Biosphere Reserve mainly derives from plants that produce nectar, including those from the families Apiaceae, Asteraceae, Campanulaceae, Hypericaceae, Rosaceae, Lamiaceae, Liliaceae, Fabaceae, Boraginaceae, and Plantaginaceae. Most of these plants grow on warm slopes, along streams, and on farmland. Three monofloral honeydew types — thyme, sage, and eremurus — were identified, each with distinct organoleptic and physicochemical properties (Ishenbaeva et al. 2024).

Antibacterial activity of honey

The inhibition zone diameters (mean ± standard deviation) for each sample are presented in Table 3. Concentrations of honey did not significantly affect the antimicrobial properties. Overall, the data suggest that Kyrgyz honey possesses varying degrees of antibacterial activity, with the most significant inhibition observed against *Shigella flexneri* (6), followed by *Staphylococcus aureus* (29213) and *Escherichia coli*. The results indicate that honey from different regions exhibited diverse inhibitory effects, with some samples (N4, 6, 14, 16) showing significant antibacterial activity, particularly against *Shigella flexneri* (6).

Table 2. Physicochemical parameters and antioxidant capacity of honey

№	Botanical origin	Dominant pollen, %	Diastase number, Gothe	Moisture content, %	Free acidity, mEq/kg	IC 50%	Color Parameters		
							L*	a*	b*
1	Polyfloral	*Asteraceae* - 37.0 *Lamiaceae* - 22.0 *Apiaceae* - 17	27.7±1.9	17.0±0.7	33.3±2.3	1.1	42.07	-0.80	29.11
2	Monofloral sainfoin	*Sainfoin* sp.– 75.0 *Trifolium mediums* - 6.1 *Fumaria* sp. - 3,1	11.1 ± 1.2	17.8 ± 0.7	15.6 ± 2.3	>10	63.87	-3.26	11.74
3	Polyfloral	*Apiaceae* – 32.8 *Eremurus fuscus* – 12.0 *Apiaceae: Angelica* sp. - 12	29.6±2.2	16.6±0.7	29.6±4.4	>10	59.70	-2.14	22.62
4	Monofloral sainfoin	*Sainfoin* sp. – 86.0 *Rosaceae* - 3.5	16.8 ± 1.0	18.2 ± 0.7	16.4 ± 2.5	6.1	42.12	-3.42	13.65
5	Monofloral sainfoin	*Sainfoin* sp.– 79.0, *Brassicaceae* - 4.4 *Fabaceae* - 4.4 *Linaria vulgaris* - 4.4	11.7 ± 1.3	17.4 ± 0.7	18.7 ± 2.8	>10	47.06	-3.50	18.34
6	Polyfloral	*Apiaceae* – 33.0 *Sainfoin* sp.– 18.0 *Echium vulgare* - 12	23.5±1.6	18.2±0.7	21.4±1.5	6.2	57.31	0.22	28.16
7	Monofloral sainfoin	*Sainfoin* sp. – 50 *Echium vulgare* – 29.0 *Artem isia* sp. – 20* *Poaceae* – 20*	24.9±1.7	16.6±0.7	16.4±2.5	11	46.85	-3.32	23.51
8	Monofloral sainfoin	Sainfoin – 74.0, *Rosaceae* - 2.8 *Apiaceae* - 2.5	16.7±1.8	17.8±0.8	18.8±2.8	>10	63.27	-0.60	24.59
9	Polyfloral	*Sainfoin* sp. – 23.0 *Caryophyllaceae* - 13 *Echium vulgare* - 8	20.7±1.4	16.6±0.7	14.0±2.1	1	63.36	-0.89	18.61
10	Monofloral Echium vulgare	*Echium vulgare* – 47.5 *Onobrychis* sp. - 5.6 *Trifolium mediums* - 5.6 *Thalictrum* sp. - 5.6	17.4±1.4	16.0±0.7	13.0±2.1	>10	35.18	-1.93	22.74
11	Monofloral sainfoin	*Sainfoin* sp. – 60.0 *Lamiaceae* - 5.9 *Lotus* sp. - 5.9	22.3±1.6	17.0±0.7	14.6±2.2	10	32.95	-1.56	22.88
12	Monofloral sainfoin	*Sainfoin* sp. – 78.0 *Brassicaceae* - 2.8	21.8±1.5	17.6±0.7	24.7±1.7	>10	58.97	-2.79	21.35
13	Monofloral thyme	*Thyme* sp. – 24.8 *Echium vulgare* – 28.9 *Artemisia* sp. - 11.7*	20.5±1.4	18.2±0.7	16.5±2.5	>10	63.56	-1.47	25.10
14	Monofloral sainfoin	*Sainfoin* sp. – 45.0 *Echium vulgare* - 4.7 *Potentilla* sp. - 3.2*	24.9±1.7	18.8±0.6	16.4±2.5	6.2	58.89	-1.88	25.84
15	Polyfloral	*Sainfoin* sp. – 21.8 *Brassicaceae* – 11.4 *Fabaceae* - 8.3	30.2±2.1	18.6±0.7	21.3±1.5	3	nd	ndnd	
16	Polyfloral	*Apiaceae* – 37.0 *Echium vulgare* – 27.0 *Hypericum* sp. - 10*	27.9±2.0	17.0±0.7	25.2±1.8	5.5	57.94	-3.12	19.09
17	Monofloral sainfoin	*Sainfoin* sp. – 60.4 *Linaria vulgaris* - 10.7 *Fumaria* sp. - 7.7	11.3±1.2	17.4±0.7	18.9±2.8	>10	47.45	2.75	15.59
18	Monofloral thyme	*Thyme* sp. – 23.5 *Lotus sp.* - 21.0 *Hypericum* sp. - 19*	17.0 ±1.9	17.8 ± 0.7	13.3 ±2.0	10	Nd	nd	nd
19	Monofloral sainfoin	*Sainfoin* sp. – 49 *Chenopodiaceae* - 14.4* *Xanthium* sp. - 7.6* *Lamium* sp. - 5	12.2 ±1.3	15.8 ± 0.6	14.5 ± 2.1	>10	Nd	nd	nd
20	Monofloral sainfoin	*Sainfoin* sp. – 58 *Fabaceae* - 18 *Rosaceae* - 12	10.1± 1.1	15.4 ± 0.6	13.9 ± 2.1	>10	Nd	nd	nd
21	Polyfloral	*Apiaceae* – 40.0 *Hypericum* sp. - 44* *Allium* sp.-14.0 *Rubus idaeus* - 7	25.8± 1.8	18 ± 0.6	24.8 ± 1.7	>10	52.30	-0.80	22.14
22	Polyfloral	*Sainfoin* sp. – 18.9 *Echium vulgare* – 17.2 *Brassicaceae* - 17.2	16.1± 1.8	15.8 ± 0.6	14.9 ± 2.2	>10	43.69	-2.61	22.21

nd- not determined, *main pollen sources

Table 3. Inhibition zone diameters (mean ± standard deviation) of honey solutions against *E.coli*, *Shigella flexneri (6)* and *Staphylococcus aureus* (29213)

No	Geographical origin	Activity against *E.coli D=0.5 cm*			Activity against *Shigella flexneri (6) D=0.7cm*			Activity against *Staphylococcus aureus (29213) D=0.6 cm*		
		50%	60%	80%	50%	60%	80%	50%	60%	80%
1	Osh region, Ozgon, Kurshab				0.27± 0.06	0.13± 0.06	0.23± 0.15	0.57± 0.06	0.167±0.06	0.80± 0.10
2	Naryn region, At-Bashy Rahat				0.58± 0.03	0.09± 0.03		1.90± 0.10	1.67± 0.25	0.40± 0.10
3	Jalal-Abad region, Kara-Alma							3.07± 0.15	3.30± 0.10	1.30± 0.10
4	Naryn region, At-Bashy				3.70± 0.10	3.37± 0.15	0.97± 0.15	3.70± 0.10	3.37± 0.15	0.97± 0.15
5	Naryn region, At-Bashy				0.77± 0.06	1.10± 0.10	0.53± 0.12	0.57± 0.06	0.73± 0.12	0.25± 0.05
6	Osh region, Chon Alai, Zhar Bashy	0.80± 0.10	0.20± 0 .10	0.57± 0.06	1.20± 0.10	0.90± 0.10	0.63± 0.15	0.70± 0.10	0.80± 0.10	0.97± 0.12
7	Osh region, Chon Alai	0.50± 0.10	0.57± 0.12	1.00±0.36	1.03± 0.06	1.23± 0.21	1.17± 0.25	0.93± 0.06	1.17± 0.12	0.98± 0.10
8	Osh region, Chon Alai	0.50± 0.10	0.70± 0.10	1.07±0.40	0.53± 0.15	1.03± 0.21	1.07± 0.12	0.08± 0.10	0.97± 0.40	1.30± 0.46
9	Osh region, Chon Alai	0.63± 0.12	0.93± 0.21	1.37±0.10	0.30± 0.10	1.40± 0.10	1.27± 0.06	0.90± 0.10	1.07±0.15	1.40± 0.36
10	Osh region, Chon Alai	0.20± 0.10	0.45± 0.05	0.20±0.10	0.43± 0.12	0.70± 0.10	0.83± 0.06	0.32± 0.10	0.43± 0.06	0.37± 015
11	Osh region, Chon Alai	1.67± 0.58	1.07± 0.32	0.83±0.06	0.40± 0.10	0.57± 0.06	0.58± 0.62	0.53± 0.12	0.53± 0.06	0.65± 0.05
12	Osh region, Chon Alai				0.95±0.13	0.73± 0.12	0.60± 0.26	0.85± 0.05	0.32± 0.08	0.80± 0.10
13	Jalal-Abad region, Toktogul				0.20± 0.10			0.55± 0.05	0.35± 0.05	0.67± 0.06
14	Naryn region, Kazarman				1.30± 0.10	1.20± 0.10	0.97± 0.06	0.87± 0.06	1.38± 0.13	1.20± 0.10
15	Chui region, Suusamyr					0.33± 0.21			0.20±0.10	
16	Jalal-Abad region, Toktogul				1.08± 0.08	0.40± 0.10	0.67± 0.15	0.87± 0.06	1.02± 0.08	0.92± 0.08
17	Issyk-Kul region, Zheti Oguz				0.40± 0.10	0.43± 0.12	0.22± 0.08	0.60± 0.17	1.90± 050	1.63± 0.25
18	Chui region, Shamshy	0.40± 0.36	0.50± 0.10	0.30± 0.10	0.47± 0.15	0.17± 0.15	0.25± 0.05	0.10± 0.10	0.23± 0.32	0.33± 0.21
21	Jalal-Abad region, Toktogul	0.57± 0.15	0.73± 0.06	0.63± 0.06	0.47± 0.06	0.62± 0.08	0.85± 0.05	0.50± 0.10	0.55± 0.13	1.03± 0.15
22	Issyk-Kul region, Tup	0.45± 0.05	1.03± 0.06	0.65± 0.05	0.20± 0.10	0.52± 0.03	0.77± 0.06	0.55± 0.05	1.20± 0.26	0.60± 0.10

Samples N4 from the Naryn region, At-Bashy (Sainfoin 86.0%), showed the highest inhibition against *Shigella flexneri* (6), with zones reaching up to 3.70 ± 0.10 cm. In contrast, honey N3 from the Jalal-Abad region, Kara-Alma, which contains Apiaceae at 32.8% and *Eremurus fuscus* at 12.0%, exhibited potent antibacterial activity against *Staphylococcus aureus* (29213), reaching inhibition zones of 1.30±0.10 cm. The lowest inhibition against *Staphylococcus aureus* (29213) was observed in the honey sample N15 collected from the Chui region, Suusamyr, and N5 Naryn region, At-Bashy (*Sainfoin* sp. 79.0 %). The activity against *Escherichia coli* was relatively high in honey N11 (Sainfoin, 60%; Eichum vulgare, 2.3%) collected from the Osh region, Chon Alai, with inhibition zones ranging from 1.67 ± 0.58 cm in some samples. However, certain samples from Osh still demonstrated moderate antibacterial effects against *Shigella flexneri* (6), with inhibition zones exceeding 1.20 cm.

DISCUSSION

Notably, the diastase number of monofloral and polyfloral honey samples was statistically significant between different botanical origins ($p \leq 0.01$). It was noted that honey obtained from plants of the Apiaceae family had a particularly high diastase number ($r=0.565$). At the same time, honey produced from *Sainfoin* sp. usually has a low diastase number ($r = -0.644$) (Table 4). Apiaceae or

Umbelliferae is a family of mostly aromatic flowering plants named after the type genus *Apium*, and commonly known as the celery, carrot, or parsley family, or simply as umbelliferae (Stevens 2021). The free acidity of monofloral and polyfloral honey samples did not show statistically significant differences between samples of different geographical origins; however, significant differences were observed depending on the botanical origin. A positive correlation was found between free acidity and the percentage content of Apiaceae (r = 0.671), Lamiaceae (r = 0.544), and Asteraceae (r = 0.645).

The variation in antibacterial effects observed among the different honey samples may be attributed to differences in floral sources, climatic conditions, and the presence of bioactive compounds such as phenolic compounds and hydrogen peroxide. A similar study on Saudi honeys by Ghramh et al. (2019) confirmed that honeys with diverse floral origins possess varying antibacterial potentials. Further research is recommended to analyse these compounds and assess their efficacy against a broader range of bacterial pathogens. Honey has also shown promising results when used in combination with other antimicrobial agents. In one study, the combined effect of bacteriophages and 13 types of Portuguese honey was tested against *E. coli*, and it was concluded that the combined treatment enhanced antibacterial activity, especially for chronic wound infections (Henriques et al. 2005). Another investigation explored the use of honey, bee venom, and pomegranate peel extract embedded in polyvinyl alcohol to create a wound dressing, which demonstrated considerable antibacterial efficacy against *S. aureus* and *E. coli*. (Oliveira et al. 2017).

Table 4. Correlation coefficients between botanical sources (main pollen) and free acid content, IC50 and diastase number

Parameters		Apiaceae	Lamiaceae	Asteraceae	Saifon	Freeacids	IC50
Apiaceae	Pearson Correlation	1					
	Sig. (2-tailed)						
Lamiaceae	Pearson Correlation	.123	1				
	Sig. (2-tailed)	.617					
Asteraceae	Pearson Correlation	.153	.918**	1			
	Sig. (2-tailed)	.532	.000				
Saifon	Pearson Correlation	**-.660****	-.286	-.332	1		
	Sig. (2-tailed)	.003	.250	.178			
Free acids	Pearson Correlation	**.671****	**.544***	**.645****	-.433	1	
	Sig. (2-tailed)	.002	.016	.003	.073		
IC50	Pearson Correlation	-.143	**-.527***	**-.520***	.384	-.256	1
	Sig. (2-tailed)	.559	.020	.022	.115	.290	
Diastase	Pearson Correlation	**.565***	.342	.369	**-.644****	**.584****	**-.464***
	Sig. (2-tailed)	.012	.151	.120	.004	.009	.045

*p < 0.05, **p < 0.01

While many studies have confirmed honey's antimicrobial activity through in vitro testing, relatively few have addressed its effects in vivo, especially at sublethal concentrations or against biofilms. Further in vivo research is needed to explore these effects and better understand how honey modulates bacterial pathogenicity. Notably, despite growing global concerns over antimicrobial resistance, no microbial resistance to honey has been reported, making it a valuable component in the search for alternative or complementary treatments (Liu et al. 2018, Skadins et al. 2023).

The results indicate that honey from different regions exhibited diverse inhibitory effects, with some samples (N4, 6, 14, 16) showing significant antibacterial activity, particularly against *Shigella flexneri* (6). Samples N4 from the Naryn region, At-Bashy (Sainfoin 86.0%), showed the highest inhibition against *Shigella flexneri* (6), with zones reaching up to 3.70 ± 0.10 cm. In contrast, honey N3 from the Jalal-Abad region, Kara-Alma, which contains Apiaceae at 32.8% and *Eremurus fuscus* at 12.0%, exhibited potent antibacterial activity against *Staphylococcus aureus* (29213), reaching inhibition zones of 1.30±0.10 cm. The lowest inhibition against

Staphylococcus aureus (29213) was observed in the honey sample N15 collected from the Chui region, Suusamyr, and N5 Naryn region, At-Bashy (Sainfoin 79.0%). The activity against *Escherichia coli* was relatively high in honey N11 (Sainfoin 60%; *Eichum vulgare*, 2.3%) collected from the Osh region, Chon Alai, with inhibition zones ranging from 1.67 ± 0.58 cm in some samples.

Specific samples from Osh still demonstrated moderate antibacterial effects against *Shigella flexneri* (6), with inhibition zones exceeding 1.20 cm. This suggests that different floral compositions in these regions may contribute to their antibacterial properties; however, ANOVA revealed no statistically significant difference between samples from different botanical origins. Enzymatic activity (mainly glucose oxidase) generates hydrogen peroxide from glucose, a potent antimicrobial factor in many honeys (Almasaudi 2020). The presence of glucose oxidase in honey is attributable to the synthesis by honeybees, which is subsequently deposited into the honey. In this manner, the enzyme functions as a natural preservative (Feknous & Boumendjel 2022).

Honey is acidic, with a pH between 2 and 5, and the acidity is attributed mainly to the organic gluconic acid that glucose breaks down under the action of oxidative enzymes (Nikhat & Fazil 2022). This acidic environment not only gives honey its unique flavour but also has a pH lower than that of a condition at which most microorganisms can survive, forming a natural antimicrobial barrier against *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, and yeasts (Szweda 2017). In our study, however, the inhibition zone against *Shigella flexneri* (6) was negatively correlated with free acidity (r = -0.602), a correlation that was significant at the 0.05 level (2-tailed).

Antioxidant capacity

The body produces several active free radicals during normal oxidative processes, usually found in respiration and enzymatic reactions. The external environment can also contribute to the formation of free radicals, such as exposure to ultraviolet light and heavy metal chemicals, as well as air pollutants (Chandimali et al. 2025). Honey samples 1 and 15 have the highest antioxidant activity and contain a high amount of Asteraceae pollen (Fig. 1). The highest antioxidant activity of sample 9 may be due to Caryophyllaceae pollen (13%).

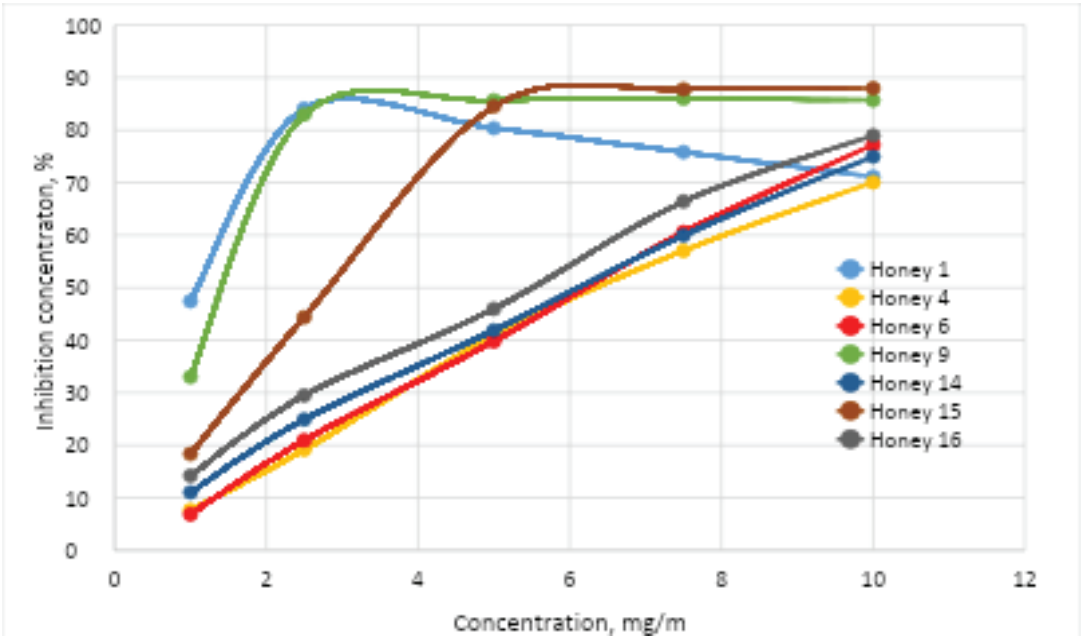

Fig. 1. Radical inhibition concentrations (50 %) of honey samples by the DPPH Assay

The antioxidant activity in honey primarily originates from phenolic compounds and was statistically significant across different botanical origins (ANOVA). Paired sample tests have shown that the antioxidant properties of honey vary according to the botanical composition. The percentage of Lamiaceae (r = -0.527) and Asteraceae (r = -0.520), as well as the IC50 parameter, show a good correlation.

Conclusion

The study revealed a close relationship between the diastase number of honey and its botanical origin. It was noted that honey obtained from plants of the family Apiaceae is characterised by a high diastase number (r=0.565). At the same time, honey produced from *Sainfoin* sp. usually has a low diastase number (r = -0.644). However, the study's results also showed that the diastase number increases significantly when an additional source of nectar is present. The diastase number was negatively correlated with the radical inhibition concentration (r = -0.464). Honey samples 1, 9, and 15 exhibit the highest antioxidant activity, which is attributed to their polyfloral composition, primarily when pollen of Lamiaceae (r=-0.527) and Asteraceae (r=-0.520) exist in honey. The antibacterial activity of Kyrgyz honey samples remained consistent regardless of their geographical origin and the bacterial strain tested. The results demonstrated that honey samples from all regions of Kyrgyzstan exhibited significant antibacterial properties, with the highest inhibition observed against *Shigella flexneri* (6). This suggests that honey from Kyrgyzstan could have potential applications in antimicrobial therapies.

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