Citation/Atıf: Abd Alla AE, Shehata MG, Masry SHD. Profiling and antimicrobial activity of Egyptian loofah honey as an unconventional bee honey: a comprehensive study. U. Bee J. / U. Arı D. 2025; 25 (1): 73-88. DOI: 10.31467/uluaricilik.1647070

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

PHYSICOCHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF EGYPTIAN LOOFAH HONEY AS AN UNCONVENTIONAL BEE HONEY: A COMPREHENSIVE STUDY

Geleneksel Olmayan Bir Arı Balı Olarak Mısır Lif Kabağı Balı'nın Fizikokimyasal Görünüşü ve Antimikrobiyal Aktivitesi: Kapsamlı Bir Çalışma

Asmaa E. ABD ALLA[1]*, Mohamed G. SHEHATA[2,3], Saad H. D. MASRY [4,5]

[1]Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, 12613 Giza, EGYPT, Corresponding author / Yazışma Yazarı E-mail: Supersemsema@gmail.com, ORCID No: 0000-0001-6140-8446

[2]Food Research Section, Applied Research and Capacity Building Division, Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), Abu Dhabi 52150, UNITED ARAB EMIRATES, E-mail: gamalsng@gmail.com, ORCID No: 0000-00030760-5945,

[3]Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), Alexandria 21934, EGYPT

[4]Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), Al Ain 52150, UNITED ARAB EMIRATES, E-mail: sahmasry72@gmail.com, ORCID No: 0000-0002-9741-0238

[5]Department of Plant Protection and Biomolecular Diagnosis, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications, New Borg El-Arab City P.O. Box 21934, EGYPT

Received / Geliş: 26.02.2025 Accepted / Kabul: 15.04.2025 DOI: 10.31467/uluaricilik.1647070

ABSTRACT

This investigation focuses on exploring the physicochemical characteristics and antimicrobial activity of loofah honey in the Egyptian governorates of Kafr El-Shaikh and El-Beheira. A novel variety of honey, designated as a supplementary resource, has been identified as a means of sustenance for bees during periods of scarcity. Pollen analysis of the examined honey samples revealed its natural origin from various plant sources in trace amounts. The physicochemical analysis produced noteworthy results, with estimated reducing sugars ranging from 61.10±0.20 to 69.29±0.12 g/100g and pH values varying between 3.53±0.01 and 3.74±0.01. There were notable variations amongst the samples in terms of free acidity, total lactone, and total acidity, while no significant distinctions were noted in ash content. The study further identified the highest recorded values for H_2O_2, DN, and HMF as 76.80±0.01 mg/kg, 12.50±0.06 U/kg, and 5.35±0.01 mg/kg, respectively. Additionally, the maximum levels of phenols, flavonoids, and DPPH were determined as 210.56±0.01 mg/kg, 52.84±0.01 mg/kg, and 83.33±0.01 %, respectively. In terms of antimicrobial activity, all samples exhibited efficacy against *Bacillus subtilis* and *Klebsiella pneumoniae*, except for one sample that demonstrated antimicrobial activity against all six tested microorganisms' types.

Keywords: Loofah honey, Physicochemical characteristics, Pollen analysis, Antimicrobial activity

ÖZ

Bu araştırma, Mısır'ın Kafr El-Shaikh ve El-Beheira vilayetlerinde lif kabağı balının fizikokimyasal özelliklerini ve antibakteriyel aktivitesini araştırmaya odaklanmaktadır. Ek bir kaynak olarak belirlenen yeni bir bal çeşidi, kıtlık dönemlerinde arılar için bir beslenme aracı olarak tanımlanmıştır. İncelenen

bal örneklerinin polen analizi, eser miktarda çeşitli bitki kaynaklarından gelen doğal kökenini ortaya koymuştur. Fizikokimyasal analizler, 61,10±0,20 ile 69,29±0,12 g/100g arasında değişen tahmini indirgen şekerler ve 3,53±0,01 ile 3,74±0,01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir. Örnekler arasında serbest asitlik, toplam lakton ve toplam asitlik açısından önemli farklılıklar bulunurken, kül içeriğinde önemli bir farklılık gözlenmemiştir. Çalışmada ayrıca H2O2, DN ve HMF için kaydedilen en yüksek değerler sırasıyla 76,80±0,01 mg/kg, 12,50±0,06 U/kg ve 5,35±0,01 mg/kg olarak belirlenmiştir. Ayrıca, maksimum fenol, flavonoid ve DPPH seviyeleri sırasıyla 210.56±0.01 mg/kg, 52.84±0.01 mg/kg ve %83.33±0.01 olarak belirlenmiştir. Antimikrobiyal aktivite açısından, test edilen altı mikroorganizma türünün tümüne karşı antimikrobiyal aktivite gösteren bir örnek dışında, tüm örnekler *Bacillus subtilis* ve *Klebsiella pneumoniae*'ya karşı etkinlik göstermiştir.

Anahtar Kelimeler: Lif kabağı balı, Fizikokimyasal özellikler, Polen analizi, Antimikrobiyal aktivite

GENİŞLETİLMİŞ ÖZET

Giriş: Oldukça besleyici bir gıda olan bal, çeşitli faktörlerden etkilenen fizikokimyasal özellikler sergiler. Bu çalışmanın amacı, Haziran'dan Ekim'e kadar üretilen yeni bir ikincil bal türü olarak potansiyeline odaklanarak lif kabağı balının özelliklerini araştırmaktır. Bileşimini, kalitesini ve potansiyel faydalarını inceleyerek, bu çalışma lif kabağı balının farklı özellikleri hakkında değerli bilgiler sağlamayı ve gıda ve ilaç endüstrilerinde uygulanabilirliğinin daha iyi anlaşılmasına katkıda bulunmayı amaçlamaktadır.

Gereç ve Yöntem: Bu araştırma, Mısır'ın Kafr El-Shaikh ve El-Beheira vilayetlerinde lif kabağı balının fizikokimyasal özelliklerini ve antibakteriyel aktivitesini araştırmaya odaklanmaktadır. Alışılmadık ve nispeten yeni bal türlerinden biri olan bu bal, arıların kıtlık zamanlarında kullandığı ek bir kaynak olarak bilinmektedir. Beş bal örneği, bitkilerin çiçeklenme döneminde Haziran ve Kasım 2021 tarihleri arasında iki ildeki farklı arılıklardan toplanmıştır. Üç örnek El-Beheira'dan, iki örnek ise Kafr El-Shaikh'ten alınmıştır. Her biri üç kopyadan oluşan örnekler, daha sonra kimyasal bileşim açısından analiz edilene kadar Kahire Üniversitesi Ziraat Fakültesi Deney İstasyonu'nun arı kovanı bahçesindeki laboratuvarında -28±2ºC'de saklanmıştır. Kimyasal analiz; şeker, nem içeriği, pH, serbest asitlik, hidroksimetilfurfural (HMF), toplam fenoller, toplam flavonoidler, 2,2-difenil-1-pikrilhidrazil (DPPH), C vitamini, diastaz aktivitesi, hidrojen peroksit (H_2O_2) ve iletkenliğin değerlendirilmesinin yanı sıra melissopalinoloji ve antimikrobiyal aktivitenin incelenmesini de içermektedir. Fizikokimyasal analiz, 61,10±0,20 ila 69,29±0,12 g/100g arasında değişen tahmini indirgen şekerler ve 3,53±0,01 ila 3,74±0,01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir.

Bulgular: Fizikokimyasal analizler, 61.10±0.20 ile 69.29±0.12 g/100g arasında değişen tahmini indirgen şekerler ve 3.53±0.01 ile 3.74±0.01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir. Örnekler arasında serbest asitlik, toplam lakton ve toplam asitlik açısından önemli farklılıklar bulunurken, kül içeriğinde önemli bir farklılık gözlenmemiştir. Çalışmada ayrıca H2O2, DN ve HMF için kaydedilen en yüksek değerler sırasıyla 76,80±0,01 mg/kg, 12,50±0,06 U/kg ve 5,35±0,01 mg/kg olarak belirlenmiştir. Ayrıca, maksimum fenol, flavonoid ve DPPH seviyeleri sırasıyla 210.56±0.01 mg/kg, 52.84±0.01 mg/kg ve %83.33±0.01 olarak belirlenmiştir. Antimikrobiyal aktivite açısından, test edilen altı mikroorganizma türünün hepsine karşı antimikrobiyal aktivite gösteren bir örnek dışında, tüm örnekler *Bacillus subtilis* ve *Klebsiella pneumoniae'ya* karşı etkinlik göstermiştir. Geleneksel olmayan balın özelliklerinin incelenmesi, özellikle ana ürünlerin kıt olduğu zamanlarda hayati önem taşımaktadır. Geleneksel olmayan bal, arılar için önemli nektar kaynakları olan birkaç yeni bitkinin geliştirilmesinin bir sonucu olarak üretilmektedir. Son olarak, tüm yıl boyunca nektar bitkileri yetiştirmek önemlidir çünkü bu, arılara bal üretimini artıran sabit bir besin kaynağı sağlar.

Sonuç: Bu çalışma, son zamanlarda ortaya çıkan en yeni ve en tuhaf bal türlerinden biri olan lif kabağı balının özelliklerini daha iyi anlamak için yapılmıştır. Sonuçlar, lif kabağı poleninin test edilen tüm bal türlerinde ikincil bir kaynak olarak ortaya çıktığını vurgulamıştır. Bu türler, bitki kökeni, iklim koşulları, arı muameleleri ve depolama koşulları dahil olmak üzere çok çeşitli değişkenlere bağlı olarak yüksek nem içeriği, normal monosakkarit içeriği, sükroz

içeriği ve pH ile karakterize edilmiştir. Arı balının fizikokimyasal özellikleri bir bölgeden diğerine değişmektedir. Geleneksel olmayan balın özelliklerinin incelenmesi, özellikle ana ürünlerde kıtlık yaşandığında hayati önem taşımaktadır. Geleneksel olmayan bal, arılar için önemli nektar kaynakları olan birkaç yeni bitkinin geliştirilmesinin bir sonucu olarak üretilmektedir. Son olarak, tüm yıl boyunca nektar bitkileri yetiştirmek önemlidir çünkü bu, arılara bal üretimini artıran sabit bir besin kaynağı sağlar.

INTRODUCTION

Bees collect nectar from flowers or the secretions of sap-sucking insects, transform it through enzymatic processes, and store it in honeycombs, resulting in bee honey-a naturally sweet and flavourful product (Codex Alimentarius Commission 2001). It is well established that honey has been used by both ancient and modern civilisations for its therapeutic properties. It is a natural remedy for a variety of illnesses due to its antimicrobial, antioxidant and wound-healing abilities (Karabagias et al. 2014). Its extensive application spans both pharmaceutical and food industries, where it is valued not only as a functional food but also as a natural medicine. Furthermore, its pleasant taste and ease of digestion make it particularly beneficial for patients, the elderly and pregnant women, offering both nutritional and medicinal benefits (Bihonegn and Begna 2021). Honey is a supersaturated solution of sugars; it consists mainly of the sugar's fructose (~38%) and glucose (~31%), along with other 200 ingredients such as water, traces of organic acids, minerals, proteins, ashes, enzymes, amino acids, vitamins, antioxidants, phenol compounds, and flavonoids (Pasias et al. 2017, Da Silva et al. 2016, Ouchemoukh et al. 2006). Honey exhibits remarkable therapeutic properties and is widely used in traditional medicine due to its ability to combat pathogenic bacteria (Israili 2014). Its antimicrobial efficacy is primarily attributed to several mechanisms involving both enzymatic and non-enzymatic components. For instance, the high acidity of honey, with a typical pH range of 3.2 to 4.5, creates an inhospitable environment for many microorganisms. Additionally, hydrogen peroxide produced by the enzymatic action of glucose oxidase, acts as a potent antimicrobial agent by generating reactive oxygen species (ROS) that damage bacterial cells. Osmosis, resulting from the high sugar concentration in honey, dehydrates bacterial cells, leading to their inhibition (Combarros-Fuertes et al. 2020, Snowdon & Cliver 1996).

Nonperoxide compounds, such as phenolic acids and flavonoids, further enhance honey's antimicrobial activity through specific mechanisms. Phenolic acids and flavonoids disrupt bacterial cell membrane integrity, impairing vital processes such as nutrient transport and energy production. These bioactive compounds also induce oxidative stress by increasing ROS within bacterial cells, which damages proteins, lipids, and DNA. Furthermore, phenolics and flavonoids inhibit bacterial enzymes essential for replication and survival, such as those involved in quorum sensing and energy metabolism (Bucekova et al. 2018 and Israili 2014).

Beyond its antimicrobial properties, honey also demonstrates significant anti-inflammatory and antioxidant effects. It modulates inflammatory pathways by reducing pro-inflammatory cytokines and enhancing the expression of anti-inflammatory mediators, contributing to its use in wound healing. Honey's high phenolic and flavonoid content scavenges free radicals, mitigating oxidative stress and promoting tissue repair. In wound healing, honey accelerates tissue regeneration, stimulates the formation of granulation tissue, and reduces inflammation, leading to faster recovery and improved outcomes (Martinotti et al. 2019). The quality of honey is influenced by a range of factors, including its type, characteristics, composition, geographical and plant origins, the season of collection, local climate, improper beekeeping techniques, and storage conditions (García et al. 2020, El Sohaimy et al. 2015). These factors collectively shape the physicochemical properties, nutritional value, and therapeutic potential of honey, highlighting the importance of careful management and monitoring to ensure high-quality production. Since honeybees gather nectar from various blooms, honey can be either monofloral, derived primarily from one plant species with distinct characteristics, or polyfloral, sourced from multiple species, resulting in diverse flavours and bioactive compounds. These variations highlight the influence of plant diversity on honey's quality and properties. Due to increasing global trade and the higher economic values associated with specific types of honey, these products are especially vulnerable to adulteration, honey mixing, and misleading or dishonest labelling of honey of lower value. Honey's authenticity is assessed through methods like

melissopalynological analysis, chemical profiling (e.g., HMF levels and diastase activity), isotopic analysis for sugar adulteration, and advanced spectroscopic techniques (Soares et al. 2017). Melissopalynology, a subfield of palynology (the study of pollen and spores), is one of the best techniques for categorizing different kinds of honey because it focuses on microscopic studies of bee honey (Attia El-Sofany et al. 2020). By analysing the pollen content in honey, this method can identify the geographical origin and the plant species from which the nectar was collected. For example, specific pollen grains serve as markers for certain regions or floral sources, allowing precise tracing of honey's botanical and geographical origins, which is crucial for ensuring authenticity and understanding its unique properties (EL-Metwally 2015).

The three primary flowering honey crop seasons in Egypt—citrus fruits in March and April, Egyptian alfalfa in April to June, and cotton in July and August—are well-documented. However, loofah honey (Luffa Egyptiac), emerging as a secondary type of honey, serves as a critical resource during off-season periods of scarcity. Loofah blooms, which persist from June to October, provide an abundant and consistent nectar supply that supports the growth and sustenance of bee colonies. This is particularly beneficial for beekeepers in regions with limited alternative floral resources, allowing them to sustain production and maintain colony health. Additionally, loofah honey is gaining attention due to its distinct physicochemical properties and health benefits, making it a potential candidate for both local markets and international export (Taha et al. 2019). Therefore, the aim of this work is to investigate the characteristics of loofah honey, focusing on its potential as a new secondary type of honey produced from June to October. By examining its composition, quality, and potential benefits, this study seeks to provide valuable insights into the distinct properties of loofah honey, contributing to a better understanding of its applicability in the food and pharmaceutical industries.

MATERIALS AND METHODS

Bee honey samples

Five honey samples were collected from different apiaries in two governorates between June and November 2021, during the blooming period of the plants. Three samples were obtained from El-Beheira, and two samples were taken from Kafr El-Shaikh. The samples, each consisting of three duplicates, were stored at the apiary yard laboratory of Cairo University's Faculty of Agriculture, Experimental Station, at -28±2 ºC until they were later analysed for chemical composition.

Examination of melissopalynology

The pollen grains from each examined honey sample were analysed using the methodology described by Louveaux et al. (1978). Ten grams of honey were dissolved in 20 millilitres of warm water and centrifuged at 3500 revolutions per minute for 10 minutes. The liquid was then decanted, replaced with fresh water, and centrifuged again for an additional 10 minutes. The sediment was gently dried by heating it to 40°C, then placed on a microscope slide and spread evenly over an area of approximately 20 × 20 mm. Glycerin gelatin was applied to the sediment, which was subsequently examined under a light microscope. Pollen grain frequency was classified as follows: "Very frequent" for grains constituting more than 45%, "Frequent" for grains comprising 16–45%, "Rare" for grains ranging from 3 to 15%, and "Sporadic" for grains constituting less than 3% of the total grains, based on the criteria outlined by El Sohaimy et al. (2015).

Physiochemical analysis of loofah honey

Chemical analysis, which included the assessment of sugars, moisture content, pH, free acidity, hydroxymethylfurfural (HMF), total phenols, total flavonoids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), vitamin C, diastase activity, hydrogen peroxide (H_2O_2), and conductivity, was thoroughly performed at Cairo University's Faculty of Agriculture, Food Safety, and Quality Control Laboratory in Giza, Egypt. The quantification of sugars, specifically fructose, glucose, and sucrose, was accomplished through high-performance liquid chromatography (HPLC). The analysis employed a Phenomenex Luna NH2 column (250×4.6 mm), with the column temperature maintained at a constant 30º C. The mobile phase consisted of acetonitrile and HPLC-grade water in a ratio of 80:20 (v: v). Detection of the sugars was achieved using a refractive index (RI) detector, and data integration was performed through ClarityChrom software. Calibration of the system was carried out using standard sugar solutions, and the detection limits for the sugars were determined to ensure accurate quantification (El Sohaimy et al. 2015).

Measurement of Hydroxymethylfurfural (HMF): The determination of hydroxymethylfurfural (HMF) was carried out using a combination of UV/V spectrophotometry and a modified White technique. To begin, five grams of honey were weighed and homogenized with distilled water. HMF stabilization was achieved by adding 10 mL of 2% w/v sodium bisulfite solution, followed by a 15-minute incubation. Acid hydrolysis was performed by adding 10 mL of 4N hydrochloric acid, with a subsequent 30-minute incubation in a controlled-temperature water bath (60-70°C). After cooling, HMF was extracted using 10 mL of acetone, and the solution was filtered for clarity. UV/Vis spectrophotometric measurements were taken at 284 nm. HMF quantification was conducted using a calibration curve created with standard HMF solutions. The curve equation was derived from the linear relationship between known HMF concentrations and their absorbance. The method was validated using certified reference materials, ensuring precision in duplicate analyses and confirming its reliability for measuring HMF levels in honey samples (Pasias et al. 2017).

Moisture content: Water content was determined with a digital refractometer at 20 ºC according to AOAC 1990.

Electrical conductivity: The conductivity was measured using a conductivity meter for a 20% honey volumetric weight in a water-based solution at 200 ºC, where the honey dry matter was represented by 20% (FiveEasy, Mettler Toledo, Switzerland) (AOAC 1990).

pH value: The pH value of the honey samples was measured using a pH meter from Boeco, Germany. The meter was calibrated using buffer solutions with pH values of 4, 7, and 10, ensuring accurate readings according to international standards.

Free acidity: Free acidity was determined using the equivalence point titration method, as specified in the Codex Alimentarius (2001), ensuring that the procedure adheres to internationally recognized food quality standards.

Hydrogen peroxide assay (H_2O_2): The hydrogen peroxide (H_2O_2) assay was performed utilizing peroxidase (HRP). In the presence of 3.5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) and 4-aminophenazone (AAP), H_2O_2 reacts to produce a chromophore. The enzymatic reaction, catalysed by HRP, leads to the formation of a quinone imine dye along with the generation of four molecules of water. This method, as described by Lehmann et al. (2019), provides a reliable means of assessing hydrogen peroxide levels.

Diastase activity: This was assessed to obtain the diastatic number (DN) following a period of shading. The resulting measurement is expressed in Gothe units (Lehmann et al. 2019). The method was conducted in accordance with international standards, specifically the Codex Alimentarius and the AOAC (2010) guidelines.

Total phenols content: The total phenols content was quantified calorimetrically using the Folin-Ciocalteu reagent, following the method outlined by Singleton and Rossi (1965). For the analysis, 10 g of honey were dissolved in 50 mL of distilled water to prepare the sample extract. A standard curve was constructed using gallic acid solutions with concentrations ranging from 10 to 300 mg gallic acid equivalent per kilogram (mg GAE/kg). The regression equation of the standard plot ($y = 101.71x - 0.4181$, $R^2 = 0.9979$) was used to calculate the total phenolic content. The results were expressed as milligrams of gallic acid equivalents per kilogram of honey (mg GAE/kg). The analysis was performed using a UV/Vis spectrophotometer from Jenway, England.

Total flavonoid content: The total flavonoid content was determined using the aluminium chloride colorimetric technique. A solution was prepared by dissolving 10 g of honey in 50 mL of distilled water. The following mixture was then prepared: 1 mL of the prepared honey extract, 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate, and 5.6 mL of distilled water. After allowing the mixture to stand at room temperature for 30 minutes, absorbance was measured at 420 nm. Rutin was used as the standard, with concentrations ranging from 10 to 100 mg rutin equivalent per kilogram (mg RE/kg) to construct the standard curve. The regression equation of the standard plot ($y = 365.26x - 6.1589$, $R^2 = 0.9978$) was used to calculate the total flavonoid content. The results were expressed as milligrams of rutin equivalents per kilogram of honey (mg RE/kg). The analysis was conducted based on the method described by Abu Safe et al. (2023).

Antioxidant Activity by DPPH: The activity of DPPH radical scavenging was determined by preparing concentrations ranging from 1% to 5% with 50% methanol from each sample extract (100 µL). To this, 100 µl of DPPH radical solution (0.2

mM) dissolved in methanol was added. After stirring the mixture, it was left in the dark for fifteen minutes. Subsequently, the absorbance was measured using a UV/Vis spectrophotometer (Jenway, England) at 517 nm in comparison to a blank. The scavenging impact percentage was calculated using the formula: [(A0 - A1) / A0] × 100, where A0 represents the absorbance of the control (without sample), and A1 is the absorbance in the presence of the sample. (Shehata et al. 2023)

Examination of water-soluble vitamins: Water-soluble vitamins were analysed using an Agilent 1260 Infinity HPLC (Agilent, USA) equipped with a Quaternary pump and a HyperClone BDS C18, 100 mm × 4.6 mm, 3 µm column (Phenomenex, USA). The instrument was operated at a temperature of 35°C. Separation was achieved using a binary linear elution gradient with mobile phase A consisting of 25 mM NaH2PO4 at pH 2.5 and mobile phase B consisting of methanol. The gradient started with 95% A and 5% B, gradually transitioning to 75% A and 25% B over 20 minutes, and then to 50% A and 50% B for column equilibration. The flow rate was set at 1.0 mL/min, and the injected sample volume was 20 µL. Detection was performed using a VWD detector set at 270 nm, with humidity maintained at 38% RH and the temperature at 25°C. Samples were filtered through a 0.45 µm syringe filter prior to injection. The method was based on the work of Abd El-Aziz et al. (2021).

Antimicrobial activity

Using the agar well diffusion method, the honey extracts' antibacterial activity was assessed (Shehata et al. 2017). Nine species, regarded as harmful to humans, including *Escherichia coli* BA 12296, *Bacillus subtilis* DB 100 host, *Candida albicans* ATCC MYA-2876, *Klebsiella pneumoniae* ATCC12296, *Salmonella senftenberg* ATCC 8400, *Staphylococcus aureus* NCTC 10788, were used. Each microorganism's media was combined with 100 µL of the inoculum (1 x 10^8 CFU/mL) before being added to the Petri plate. The honey extracts were added to the well in a volume of 100 µL. After 48 hours of overnight incubation at 37 °C, the diameter (mm) of the resultant zone of inhibition was measured on the plates.

Statistical analysis

Data were analyzed using IBM SPSS software package version 16.0 (Kirkpatrick & Feeney 2013). Quantitative data were described using means and standard deviation. For normally distributed data, comparisons between the different studied inhibitors were performed using F-test (ANOVA). The significance of the obtained results was determined by p-value ($p < 0.05$) (Kotz et al. 2006).

RESULT

Sugar Content

The sugar profile of the honey samples from different regions exhibited significant variations ($P \leq 0.05$). Fructose, glucose, and sucrose concentrations were determined for all samples, as shown in Table 1. Fructose content ranged from 33.50±0.12 g/100 g to 39.90±0.06 g/100 g, with Sample 5 from Kafr El-Shaikh showing the highest fructose concentration, while Sample 2 from El-Beheira recorded the lowest.

Glucose concentrations varied significantly across the samples, with the highest value detected in Sample 1 from El-Beheira (32.29±0.12 g/100 g) and the lowest in Sample 5 from Kafr El-Shaikh (27.60±0.12 g/100 g). Sucrose content ranged from 0.81±0.12 g/100 g in Sample 5 to 5.03±0.01 g/100 g in Sample 1, revealing significant inter-sample differences.

Water Content and Sugar Ratios

The water content of the honey samples, as detailed in Table 1, showed significant differences ($P \leq 0.05$), ranging from 20.80±0.06% in Sample 1 to 22.40±0.06% in Sample 4, both from Kafr El-Shaikh. The glucose-to-water (G/W) ratio exhibited a significant difference ($P \leq 0.05$) across samples, with Sample 1 having the highest ratio of 1.55±0.01 and Sample 4 the lowest at 1.23±0.01. This ratio is important as it indicates the relative concentration of glucose to water, which influences the honey's viscosity and potential for fermentation. The fructose-to-glucose (F/G) ratio also varied significantly ($P \leq 0.05$), with Sample 5 from Kafr El-Shaikh recording the highest value of 1.46±0.01, while Sample 1 from El-Beheira had the lowest ratio at 1.15±0.01. The F/G ratio is significant because it reflects the sweetness and overall composition of the honey, which can affect its flavor profile and crystallization tendencies.

Table 1. Sugar spectrum, water content, and G/W and F/G ratios of examined honey samples

Honey samples	Fructose g/100 g	Glucose g/100 g	Estimated reducing sugars g/100 g	Sucrose g/100 g	Water content %	G/W ratio	F/G ratio
1	37.00± 0.12^{B}	32.29± 0.12^{A}	69.29± 0.12^{A}	0.81± 0.12^{E}	20.80±0.06^{D}	1.55±0.01^{A}	1.15± 0.01^{D}
2	33.50±0.12^{E}	27.60±0.12^{C}	61.10±0.20^{E}	1.07±0.01^{D}	21.00±0.05^{C}	1.31±0.01^{C}	1.21 ±0.01^{C}
3	35.75±0.12^{D}	29.26±0.12^{B}	65.01±0.20^{C}	5.03±0.01^{A}	21.20±0.06^{B}	1.38 ±0.01^{B}	1.22±0.01^{C}
4	36.28± 0.01	27.47±0.12^{DC}	63.75±0.12^{D}	2.50±0.12^{C}	22.40± 0.06^{A}	1.23±0.01^{D}	1.32±0.01^{B}
5	39.90±0.06^{A}	27.30±0.01^{D}	67.20± 0.06^{B}	3.10± 0.01^{B}	21.00±0.06^{C}	1.30±0.01^{C}	1.46±0.01^{A}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05). G/W: glucose/water and F/G: fructose/glucose.

Physicochemical Properties

The physicochemical properties of the honey samples showed significant variations (P ≤ 0.05) in pH, free acidity, total lactone, and total acidity, as summarized in Table 2. The pH values of the samples ranged from 3.53±0.11 in Sample 5 to 3.74±0.01 in Sample 3. Free acidity levels varied between 28.25±0.12 meq/kg in Sample 5 and 56.25±0.11 meq/kg in Sample 3. Total acidity values ranged from 40.75±0.01 meq/kg to 73.75±0.01 meq/kg, with two samples exceeding the Codex Alimentarius limit of 50 meq/kg for total acidity. These variations highlight the differences in the honey's acid content, which can influence its taste, preservation, and overall quality.

Table 2. The physicochemical characteristics of the tested honey samples

Honey samples	pH	Free acidity meq/kg	Total lactone meq/kg	Total acidity meq/kg	Ash %	Conductivity ms/cm
1	3.67±0.01^{ab}	56.25±0.11^{a}	17.50± 0.05^{d}	73.75±0.01^{a}	0.23±0.11^{a}	0.306±0.57^{b}
2	3.62± 0.01^{ab}	37.25±0.11^{c}	14.00± 0.06^{c}	51.25±0.11^{c}	0.08± 0.01^{a}	0.214± 0.57^{d}
3	3.74±0.01^{a}	28.25±0.12^{e}	12.50±0.12 ^{e}	40.75± 0.01^{e}	0.05±0.01^{a}	0.219±0.58^{c}
4	3.53±0.11^{b}	54.75± 0.01^{b}	15.00±0.06^{b}	69.75±0.01^{b}	0.05±0.01^{a}	0.363±0.58^{a}
5	3.70± 0.01^{ab}	32.25±0.02^{d}	13.00±0.11^{d}	45.25±0.01^{d}	0.11±0.01^{a}	0.214± 0.58^{d}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05).

Ash Content and Electrical Conductivity

The ash content and electrical conductivity values of the honey samples are summarized in Table 2. Ash content ranged from 0.05% in Sample 3 to 0.23% in Sample 4, with no significant differences (P > 0.05) between the samples. Electrical conductivity showed significant variation (P ≤ 0.05), with the highest value

recorded for Sample 4 (0.363±0.577 ms/cm) and the lowest for Samples 1 and 5 (0.214±0.577 ms/cm). According to the Codex Alimentarius, the maximum allowable ash content for honey is typically 0.6%, and the electrical conductivity should generally not exceed 0.8 ms/cm. Based on these standards, all honey samples in this study fall within the acceptable limits for both ash content and electrical conductivity.

Diastase Number and Enzyme Activity

The diastase number (DN), which reflects the enzyme activity in the honey samples, varied significantly ($P \leq 0.05$) across the samples, as shown in Figure 1. Sample 1 from El-Beheira had the highest DN (12.50±0.06 U/kg), while Sample 5 from Kafr El-Shaikh exhibited the lowest DN (3.33±0.01 U/kg). According to the Codex Alimentarius, the minimum allowable diastase number for honey is typically 8 U/kg, and honey with a DN lower than this may be considered substandard. Based on these criteria, Sample 5 falls below the acceptable limit, while all other samples meet the Codex standard for enzyme activity.

Hydrogen Peroxide and HMF Content

Hydrogen peroxide (H_2O_2) concentrations varied significantly ($P \leq 0.05$) across the samples, with Sample 5 showing the highest concentration (76.80±0.01 mg/kg) and Sample 3 exhibiting the lowest (35.20±0.01 mg/kg). HMF (hydroxymethylfurfural) content, which is an indicator of honey quality and freshness, ranged from 1.20±0.06 mg/kg to 5.35±0.01 mg/kg, as shown in figure 1. According to the Codex Alimentarius, the maximum allowable HMF content for honey is 40 mg/kg, beyond which honey may be considered of poor quality or adulterated. Based on this standard, all honey samples in this study fall well below the Codex limit for HMF.

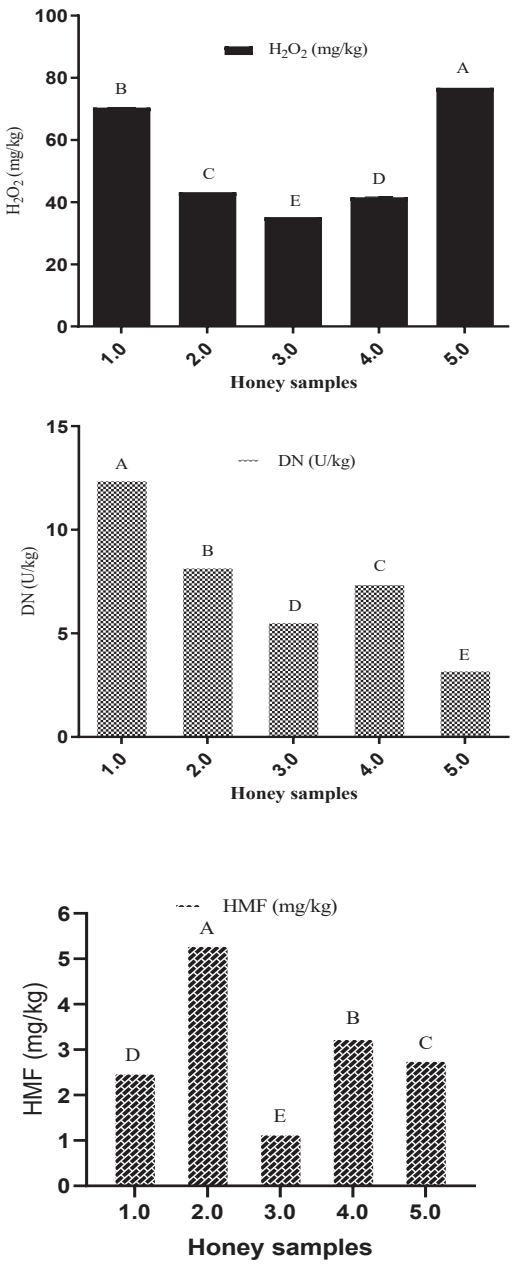

Figure 1: The Parameters of H_2O_2, DN, and HMF in the tested honey samples. The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different ($P < 0.05$). H_2O_2: Hydrogen Peroxide; DN: diastatic number; HMF: hydroxymethylfurfural

Phenolic, flavonoid Content and Antioxidant Activity

The total phenolic content of the honey samples showed significant differences (P ≤ 0.05), with Sample 4 from Kafr El-Shaikh having the highest value (210.56±0.01 mg/kg) and Sample 5 the lowest (18.61±0.01 mg/kg). Flavonoid content ranged from 32.76±0.01 mg/kg to 52.84±0.01 mg/kg, with Sample 2 from El-Beheira recording the highest flavonoid concentration, as shown in Table 3.

Antioxidant activity, as measured by the DPPH radical scavenging assay, ranged from 61.46±0.01% in Sample 3 to 83.33±0.01% in Sample 4. Vitamin C content showed significant variations (P ≤ 0.05), with values ranging from 10.12±0.01 to 22.56±0.01 mg/100 g.

Table 3. Phenols, flavonoids, DPPH, and V.C content in the tested honey samples.

Honey samples	polyphenols		DPPH (%)	Vitamin C (mg/kg)
	Total Phenolic content (mg gallic acid equivalent/100g)	Total Flavonoids content (mg rutin equivalent /100g)		
1	28.21±0.01^{c}	50.53±0.01b^{B}	80.21±0.01^{b}	1.72±0.01^{bc}
2	19.66± 0.01^{d}	52.84± 0.01^{a}	68.06± 0.01^{d}	1.73± 0.01^{ab}
3	169.01±0.01^{b}	32.76±0.01^{e}	61.46±0.01^{e}	1.71±0.01^{c}
4	210.56±0.01^{a}	33.11±0.01^{d}	83.33±0.01^{a}	1.73±0.01^{ab}
5	18.61±0.01^{e}	46.78±0.01^{c}	75.35±0.01^{c}	1.74±0.01^{a}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05).

Melissopalynological Analysis

The melissopalynological analysis revealed that Eucalyptus was the dominant pollen type in the examined sample, along with the presence of other pollen types in lower concentrations, as shown in Table 4. Sample 1 from El-Beheira contained 23.18% loofah pollen (*Luffa aegyptiaca*), while Sample 4 from Kafr El-Shaikh had 31.25% clover pollen (*Trifolium alexandrinum*). However, it should be noted that only Eucalyptus sp. qualifies as a dominant pollen type based on its percentage, while *Luffa aegyptiaca* and *Trifolium alexandrinum* are better described as secondary or minor pollen contributors. This clarification ensures consistency with the frequency-based classification of pollen types.

Table 4. The melissopalynological examination of pollen grains in the tested honey samples.

Melissopalynological analysis	Pollen types %				
	1	2	3	4	5
Family: Fabaceae ***Trifolium alexandrinum***	8.34 ± 0.04^{d}	26.31 ± 0.04^{b}	20.27 ± 0.05^{c}	31.35 ± 0.02^{a}	0
Family: Arecaceae ***Phoenix dactylifera***	21.08 ± 0.03^{b}	5.26 ± 0.06^{d}	16.27 ± 0.03^{c}	30.15 ± 0.02^{a}	4.50 ± 0.04^{e}
Family: Chenopodiaceae ***Chenopodium sp.***	17.04 ± 0.01^{c}	26.31 ± 0.05^{b}	37.01 ± 0.06^{a}	0	0
Family: Compositae ***Helianthus annuus***	4.50 ± 0.01^{a}	0	4.05 ± 0.02^{b}	0	0
Family: Myrtaceae ***Eucalyptus spp.***	8.38 ± 0.04^{c}	1.09 ± 0.04^{d}	0	10.47 ± 0.03^{b}	60.53 ± 0.02^{a}
Family: Umbelliferae	7.34 ± 0.05^{b}	0	4.00 ± 0.02^{c}	3.12 ± 0.03^{d}	7.58 ± 0.02^{a}
Family: Casuarinaceae ***Casuarina sp.***	10.14 ± 0.05^{a}	4.20 ± 0.55^{b}	2.70 ± 0.01^{c}	0	10.39 ± 0.03^{a}
Family: Cucurbitaceae ***Luffa aegyptiaca***	23.18 ± 0.05^{b}	23.15 ± 0.03^{b}	15.70 ± 0.02^{d}	25.00 ± 0.04^{a}	17.00 ± 0.03^{c}
Family: Cucurbitaceae ***Cucurbita sp.***	0	13.68 ± 0.03^{a}	0	0	0

Antimicrobial Activity

The antimicrobial activity of the honey samples was evaluated against six bacterial strains, as detailed in Table 5. Sample 2 from El-Beheira exhibited the strongest antibacterial activity, particularly against *Bacillus subtilis* (12.76±1.35 mm inhibition zone) and *Escherichia coli* (12.53±1.23 mm inhibition zone). Moderate antibacterial effects were also observed against *Salmonella senftenberg* and *Klebsiella pneumoniae*, with significant differences ($P \leq 0.05$) noted between the samples.

Table 5. Inhibition zone of six pathogen's microorganisms for the examined honey samples.

Pathogenic microorganisms	Diameter of inhibition zone (mm)				
	Honeybee samples				
	El-Beheira 1	El-Beheira 2	El-Beheira 3	Kafr El-Shaikh 4	Kafr El-Shaikh 5
***Escherichia coli* BA 12296**	7.09±0.49^{b}	12.53±1.23^{a}	7.46±1.22^{b}	ND	ND
***Bacillus subtilis* DB 100 host**	7.37±0.91^{c}	12.76±1.35^{a}	8.73±0.32^{bc}	9.20±0.70^{a}	8.63±0.96^{bc}
***Candida albicans* ATCC MYA-2876**	ND	8.13± 0.21^{a}	ND	ND	ND
***Klebsiella pneumoniae* ATCC12296**	9.21±0.35^{ab}	10.13±0.65^{a}	8.45±0.93^{b}	7.23±0.70^{c}	6.80±0.46^{c}
***Salmonella senftenberg* ATCC 8400**	5.63±0.61^{b}	10.53±0.94^{a}	ND	6.50±0.88^{b}	ND
***Staphylococcus aureus* NCTC 10788**	ND	5.87±0.24^{a}	ND	ND	ND

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed to examine the relationship between antimicrobial activity, total phenols, flavonoids, and DPPH activity (Fig. 2). The first two principal components (PC1 and PC2) accounted for a significant proportion of the total variance, with PC1 explaining X% and PC2 explaining Y% of the variance, collectively accounting for Z%. The PCA plot revealed a clear clustering of antimicrobial activity with total phenols and flavonoids, suggesting a positive correlation between these variables. Samples with higher phenolic and flavonoid contents showed stronger antimicrobial properties. However, DPPH activity, which measures antioxidant potential, displayed a weaker correlation with antimicrobial activity, though it was still somewhat associated with phenols and flavonoids. This indicates that antioxidant activity, while not directly contributing to antimicrobial activity, shares some overlap with phenolic compounds in the dataset.

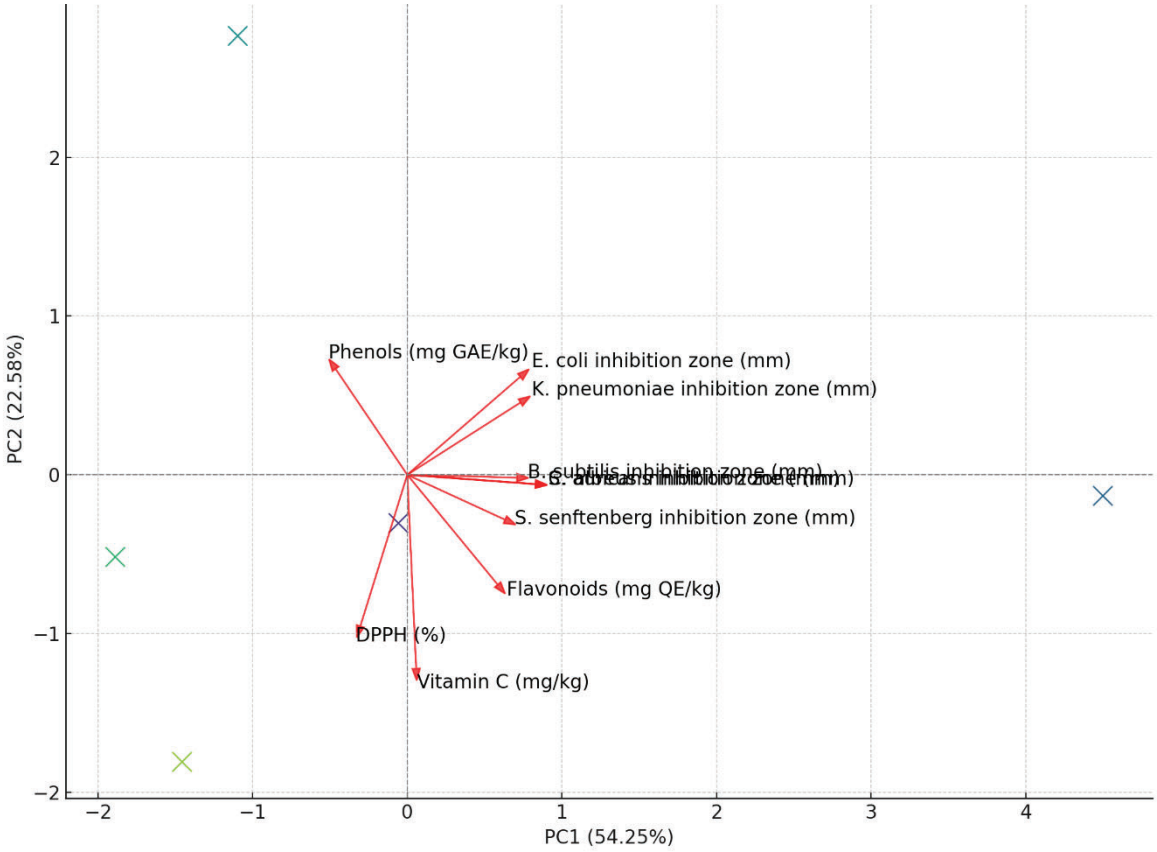

Fig. 2. Principal Component Analysis (PCA) Biplot showing the relationships between antimicrobial activity, total phenols, flavonoids, and DPPH activity. The first two principal components (PC1 and PC2) account for X% and Y% of the total variance, respectively.

DISCUSSION

The current study focused on the physicochemical properties, sugar content, and bioactive compounds in honey samples from different regions of Egypt, including El-Beheira and Kafr El-Shaikh governorates. The results provide valuable insights into the variations in honey composition depending on geographical origin, and these findings align with the established literature while also contributing new data to the field.

The observed differences in fructose and glucose contents among the honey samples are consistent with previous studies that have reported similar variations based on geographical and botanical origins. For instance, Persano Oddo et al. (2004) highlighted that the fructose/glucose ratio (F/G) is a critical determinant in honey crystallization. Our findings, which show a higher fructose content in Kafr El-Shaikh samples compared to El-Beheira, corroborate earlier studies by White (1975) and Siddiqui (1970), which indicated that honey with higher fructose levels tends to crystallize more slowly. This is supported by Draiaia et al. (2015), who found that honey samples with an F/G ratio greater than 1.0 crystallize more slowly, which is consistent with the slow crystallization observed in our samples.

The glucose-to-water (G/W) ratio is another significant factor influencing honey crystallization. The highest G/W ratio observed in El-Beheira sample 1 aligns with findings from Escuredo et al. (2014), who noted that higher G/W ratios increase the tendency of honey to crystallize. However, our data suggest that the honey samples from Kafr El-Shaikh, with lower G/W ratios, may have a reduced crystallization tendency. This observation is crucial for honey producers and consumers, as

crystallization affects the texture and marketability of honey.

The pH values of the tested honey samples fell within the range specified by Codex Alimentarius (2001), indicating freshness and quality. The slight variations observed in pH values between samples are in line with the results reported by Borawska, & Socha (2020) and El Sohaimy et al. (2015), who found that honey pH can vary depending on the floral source and environmental conditions. The moderate acidity levels detected in the honey samples are attributed to the presence of organic acids, which are known to influence honey's flavor and preservation qualities. This is consistent with the findings of Karabagias et al. (2014), who suggested that the fermentation of sugars into organic acids by bee enzymes plays a significant role in honey's acidity.

The variation in total acidity across different samples, with some exceeding the Codex Alimentarius limits, suggests that certain environmental factors or floral sources may contribute to higher acid levels. This observation is supported by Ndife et al. (2013) and Borawska, & Socha (2020), who reported that total acidity in honey could vary widely based on botanical and geographical factors. The higher total acidity observed in some samples may be indicative of the fermentation process or the presence of specific organic acids, as discussed by Diafat et al. (2017).

The ash content, which is indicative of the mineral content in honey, demonstrated no significant differences among the samples, aligning with the findings of previous studies by Živkov Baloš et al. (2018). These researchers suggested that ash content reflects the inorganic mineral composition and is a marker for the botanical and geographic origin of honey. The electrical conductivity values, however, varied significantly between samples, with Kafr El-Shaikh samples showing higher conductivity. This finding is consistent with the research by Rysha et al. (2021), who noted that higher ash and acid contents in honey are directly related to increased electrical conductivity. The higher conductivity in Kafr El-Shaikh samples suggests a richer mineral content, potentially due to the specific floral sources in that region.

The significant variations in hydrogen peroxide (H_2O_2) levels among the samples align with studies by Martinotti et al. (2019) and Bucekova et al. (2018), who reported that H_2O_2 is a major factor in the antimicrobial properties of honey. The highest H_2O_2 levels observed in Kafr El-Shaikh sample 5 could be attributed to the specific floral sources in that region, which may enhance the production of glucose oxidase, the enzyme responsible for H_2O_2 generation in honey.

The diastase number (DN), which serves as an indicator of honey freshness and enzymatic activity, showed significant differences among the samples. These findings are consistent with Tadesse et al. (2021) and El Sohaimy et al. (2015), who reported that DN can vary based on storage conditions, floral sources, and the physiological state of the bee colony. The lower DN observed in some samples may suggest longer storage periods or exposure to higher temperatures, which could degrade the enzymatic activity, as noted by Da Silva et al. (2016).

The hydroxymethylfurfural (HMF) content in all tested samples was within the acceptable limits set by Codex Alimentarius, indicating that the honey samples were fresh and had not undergone significant heat treatment. This is consistent with findings by Pasias et al. (2017), who stated that HMF levels increase during storage and heat treatment due to the Maillard reaction. The low HMF content in our samples suggests minimal processing and good storage conditions.

The significant variations in phenolic content among the honey samples are consistent with the findings of Saeed et al. (2021) and Al-Mamary et al. (2002), who reported that the phenolic content in honey is highly dependent on its botanical source, color, and geographical origin. The higher phenolic content observed in Kafr El-Shaikh samples may be attributed to the specific floral sources in that region, which are known to be rich in phenolic compounds. The variations in flavonoid content among the samples further support this, as flavonoids are also influenced by the floral origin of honey.

The antioxidant activity, as measured by DPPH, varied significantly among the samples, with Kafr El-Shaikh samples showing higher antioxidant activity. This finding aligns with the literature, which suggests that honey's antioxidant properties are primarily due to its phenolic and flavonoid content (Saeed et al. 2021). The higher antioxidant activity in Kafr El-Shaikh samples may be indicative of the region's richer floral diversity, which contributes to the higher levels of bioactive compounds in honey.

The melissopalynological analysis revealed significant differences in the pollen content among

the honey samples, indicating diverse floral sources. The dominance of loofah pollen in the El-Beheira samples is consistent with the findings of Taha et al. (2019), who reported that certain regions have characteristic pollen profiles that reflect the local flora. The presence of eucalyptus pollen in Kafr El-Shaikh samples suggests that these honey samples may have unique antimicrobial properties, as noted by Cortopassi-Laurino and Gelli, (1991) in their study on the antibacterial properties of eucalyptus honey.

The antimicrobial activity of the honey samples against various pathogens is a critical finding, particularly the higher activity observed in El-Beheira sample 2. This aligns with studies by Stefanis et al. (2023) and Kwakman and Zaat (2012), who reported that the antimicrobial properties of honey are influenced by its phenolic and flavonoid content, as well as the presence of hydrogen peroxide, methylglyoxal, and other bioactive compounds. The higher antimicrobial activity in El-Beheira sample 2 suggests that the floral sources in this region contribute to the production of honey with enhanced bioactive properties.

The PCA results indicate that total phenols and flavonoids are key contributors to antimicrobial activity, supporting previous research highlighting their bioactive roles in inhibiting microbial growth (Boy et al. 2021). The positive correlation observed between these compounds and antimicrobial activity suggests that phenolic compounds, through mechanisms such as enzyme inhibition and membrane disruption, may enhance the antimicrobial potential of the samples. On the other hand, the relatively weak association between DPPH activity and antimicrobial properties suggests that antioxidants like phenolics may protect against oxidative stress in microbial cells but are not the primary agents behind antimicrobial effects. This aligns with existing studies which have shown that while antioxidants and antimicrobial properties both provide protective benefits, they operate through distinct mechanisms (Rammali et al. 2024, Gupta et al. 2022). The variability in DPPH activity could also stem from differences in the antioxidant capacity of individual phenolic compounds, further complicating the direct link between antioxidant and antimicrobial functions.

Conclusion: This study was done to gain a better understanding of the properties of loofah honey, one of the newest and most peculiar types of honey to appear recently. The results highlighted that the loofah pollen came as a secondary source in all tested honey types. These types were characterized by high moisture content, normal content of monosaccharides, sucrose content, and pH depending on a wide range of variables, including the plant origin, climatic conditions, bee treatments, and storage conditions. The physicochemical properties of bee honey vary from one region to another. Studying the properties of nontraditional honey is vital, particularly when the main crops are in shortage. Nontraditional honey is produced as a result of the development of several new plants, which are significant sources of nectar for bees. Finally, it is important to grow nectar crops all year round because it guarantees bees a steady supply of food, which boosts the production of honey.

Acknowledgement: The authors sincerely thank Dr. El-Kazafy A. Tahaa, Professor at the Department of Economic Entomology, Faculty of Agriculture, Kafr El-Sheikh University, Egypt, for his assistance in sample selection from Kafr El-Sheikh governorate.

Authors' Contributions: AEA, MGSH, and SHD: planning, data collection, methodological planning, experimenting, and setting up the study. All authors read and approved the final manuscript.

Consent to participate: Not applicable**.**

Consent for publication: Not applicable**.**

Competing interests: The authors declare no competing interests.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

REFERENCES

Abd El-Aziz N M, Eldin Awad O M, Shehata M G & El-Sohaimy S A, Antioxidant and anti-acetylcholinesterase potential of artichoke phenolic compounds. Food Biosci. 2021;41, 101006. https://doi.org/10.1016/j.fbio.2021.101006.

Abu Safe F A, Badr A N, Shehata M G & El-Sayyad G S. Antimicrobial and anti-aflatoxigenic

activities of nanoemulsions based on *Achillea millefolium* and Crocus sativus flower extracts as green promising agents for food preservatives. BMC Microbiol. 2023;23, 289. https://doi.org/10.1186/s12866-023-03033-2.

Al-Mamary M, Al-Meeri A & Al-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr. Res. 2002; 22(9): 1041-1047. https://doi.org/10.1016/S0271-5317(02)00406-2.

AOAC. Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, 2010; Washington, DC.

AOAC. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, 1990; Inc., Arlington.

Attia El-Sofany A, Al Naggar Y, Naiem E, Giesy J P & Seif A. Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods. J. Apic. Res. 2020; 946-955. https://doi.org/10.1080/00218839.2020.1720950.

Bihonegn A & Begna D. Beekeeping Production System, Challenges, and Opportunities in Selected Districts of South Wollo Zone, Amhara, Ethiopia. Adv. Agric. 2021;(1): 2278396. https://doi.org/10.1155/2021/2278396.

Borawska MH & Socha K. Modern methods for assessing the quality of bee honey and botanical origin identification. Foods 2020; 9(8): 1028. https://doi.org/10.3390/foods9081028.

Boy FR, Casquete R, Martínez A, Córdoba MG, Ruíz-Moyano S & Benito MJ. Antioxidant Antihypertensive and Antimicrobial Properties of Phenolic Compounds Obtained from Native Plants by Different Extraction Methods. *Int. J. Environ. Res. Public Health,* 2021;18(5): 2475. https://doi.org/10.3390/ijerph18052475.

Bucekova M, Buriova M, Pekarik L, Majtan V & Majtan J. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. Sci. Rep., 2018: 8:9061. https://doi.org/10.1038/s41598-018-27449-3.

Codex Alimentarius Commission. Revised Codex standard for honey, Codex STAN 12-Rev.2 (2001). Food and Agriculture Organization of the United Nations (FAO).

Combarros-Fuertes P, Fresno JM, Estevinho, MM, Sousa-Pimenta M, Tornadijo ME & Estevinho LM, Another alternative in the fight against antibiotic resistant bacteria? Antibiotics 2020; 9:774. https://doi.org/10.3390/antibiotics9110774.

Cortopassi-Laurino M & Gelli DS. Analyse pollinique, propriétés physico-chimiques et action antibactérienne des miels d'abeilles africanisées *Apis mellifera* et de Méliponinés du Brésil. Apidologie 1991; 22: 61-73. https://doi.org/10.1051/apido:19910108.

da Silva PM, Gauche C, Gonzaga LV, Costa AC & Fett R. Honey: Chemical composition, stability and authenticity. Food Chem. 2016; 196: 309-323. https://doi.org/10.1016/j.foodchem.2015.09.051.

Diafat AEO, Benouadah A, Bahloul A, Meribai A & Mekhalfi H. Physicochemical properties and pollen analyzes of some Algerian honeys. Int. Food Res. J. 2017; 24: 1453-1459. http://www.ifrj.upm.edu.my/24%20(04)%202017/(15).pdf.

Draiaia R, Chefrour A, Dainese N, Borin A, Manzinello C, Gallina A & Mutinelli F. Physicochemical parameters and antibiotics residuals in Algerian honey. Afr. J. Biotechnol. 2015;14(14):1242-1251. https://doi.org/10.5897/AJB2015.14456.

EL-Metwally, A.A.E. (2015). Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis, Faculty of Agriculture, Cairo University, Egypt. 320p. **DOI:**10.13140/RG.2.2.22564.30086

El Sohaimy S, Masry S & Shehata M. Physicochemical characteristics of honey from different origins. Ann. Agric. Sci. 2015; 60(2): 279-287. https://doi.org/10.1016/j.aoas.2015.10.015.

Escuredo O, Dobre I, Fernández-González M. & Seijo M C. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 2014; 149: 84-90.

https://doi.org/10.1016/j.foodchem.2013.10.097.

García S, Troncoso JM & Rondanelli-Reyes M. Study of honey according to botanical origin and physicochemical parameters in the Biobío Region, Chile. Chil. J. Agric. Res. 2020; 80: 675–685. https://doi.org/10.4067/S0718-58392020000400675.

Gupta T, Kataria R. & Sardana SA. Comprehensive Review on Current Perspectives of Flavonoids as Antimicrobial Agents. *Curr. Top. Med. Chem.,* 2022.;*22*(6): 425–434. https://doi.org/10.2174/1568026622666220117104709.

Israili, ZH. Antimicrobial properties of honey. Am. J. Ther. 2014;21(4): 304–323. https://doi.org/10.1097/MJT.0b013e318293b09b.

Karabagias IK, Badeka A, Kontakos S, Karabournioti S. & Kontominas MG. Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem. 2014; 146: 548–557. https://doi.org/10.1016/j.foodchem.2013.09.105.

Kirkpatrick LA & Feeney BCI, Belmont M. A Simple Guide to IBM SPSS Statistics for Version 20.0, 2013; Wadsworth, Cengage Learning.

Kotz S, Balakrishnan N, Read CB & Vidakovic B. In Encyclopedia of Statistical Sciences (2nd ed.). 2006; Wiley-Interscience.

Kwakman PH & Zaat SA. Antibacterial components of honey. IUBMB Life 2012; 64(1): 48–55. https://doi.org/10.1002/iub.578.

Lehmann DM, Krishnakumar K, Batres MA, Hakola-Parry A, Cokcetin N, Harry E & Carter DA. A cost-effective colourimetric assay for quantifying hydrogen peroxide in honey. Access Microbiology 2019;1(10). https://doi.org/10.1099/acmi.0.000065.

Louveaux J, Maurizio A & Vorwohl G. Methods of Melissopalynology. Bee World 1978; 59: 139-154. http://dx.doi.org/10.1080/0005772X.1978.11097714.

Martinotti S, Laforenza U, Patrone M, Moccia F & Ranzato E. Honey-Mediated Wound Healing: H2O2 Entry through AQP3 Determines Extracellular Ca2+ Influx. Int. J. Mol. Sci. 2019;20(3). https://doi.org/10.3390/ijms20030764.

Ndife J, Abioye L & Dandago M. Quality Assessment of Nigerian Honey Sourced from Different Floral Locations. Niger. Food J. 2013;32(2): 48-55. https://doi.org/10.1016/S0189-7241(15)30117-X.

Ouchemoukh S, Louaileche H & Schweitzer P. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control 2006;18(1): 52-58. https://doi.org/10.1016/j.foodcont.2005.08.007.

Pasias IN, Kiriakou IK & Proestos C. HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chem. 2017; 229: 425-431. https://doi.org/10.1016/j.foodchem.2017.02.084.

Persano Oddo L, Piana L, Bogdanov S, Bentabol A, Gotsiou P, Kerkvliet J, Martin P, Morlot, M, Ortiz Valbuena A, Ruoff K & Von Der Ohe K. Botanical species giving unifloral honey in Europe. Apidologie 2004; 35: S82-S93. https://doi.org/10.1051/apido:2004045.

Rammali S, Ciobică A, El Aalaoui M, Rahim A, Kamal FZ, Dari K, Khattabi A, Romila L Novac B, Petroaie A & Bencharki B. Exploring the antimicrobial and antioxidant properties of *Lentzea flaviverrucosa* strain E25-2 isolated from Moroccan forest soil. *Front. Microbiol.,* 2024;*15*: 1429035. https://doi.org/10.3389/fmicb.2024.1429035.

Rysha A, Kastrati G, Biber L, Sadiku V, Rysha A & Zogaj F. Evaluating the Physicochemical Properties of Some Kosovo's and Imported Honey Samples. Appl. Sci. 2021;12(2): 629. https://doi.org/10.3390/app12020629.

Saeed MA, Jayashankar M & Tejaswini HK. Evaluation of biochemical and antioxidant properties of Indian and Yemeni honey. J. Apic. Res. 2021; 62(4): 836–849.

https://doi.org/10.1080/00218839.2021.1898835.

Shehata MG, Abd El-Aziz NM, Mehany T & Simal-Gandara J. Taro leaves extract and probiotic lactic acid bacteria: A synergistic approach to improve antioxidant capacity and bioaccessibility in fermented milk beverages. LWT 2023;187: 115280. https://doi.org/10.1016/j.lwt.2023.115280.

Shehata MG, Badr AN, Abdel-Razek AG, Hassanein MM & Hassan A. Oil-bioactive films as antifungal application to save post-harvest food crops. Ann. Res. Rev. Biol. 2017; 16: 1-16.

Siddiqui IR. The sugars of honey. Adv. Carbohydr. Chem. Biochem. 1970; 285-309. https://doi.org/10.1016/s0065-2318(08)60430-8.

Singleton VL & Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viti c. 1965; 16: 144-158. https://doi.org/10.5344/ajev.1965.16.3.144.

Snowdon JA & Cliver DO. Microorganisms in honey. Int. J. Food Microbiol. 1996; 31, (1-3): 1–26. https://doi.org/10.1016/0168-1605(96)00970-1.

Soares S, Amaral JS, Oliveira MB PP & Mafra I. A comprehensive review on the main honey authentication issues: Production and origin. Compr. Rev. Food Sci. Food Saf. 2017;16(5):1072–1100. https://doi.org/10.1111/1541-4337.12278.

Stefanis C, Stavropoulou E, Giorgi E, Constantinidis TC, Vrioni G & Tsakris A. Honey's Antioxidant and Antimicrobial Properties: A Bibliometric Study. Antioxidants 2023;12(2). https://doi.org/10.3390/antiox12020414.

Tadesse B, Tilahun Y, Woyamo W, Bayu M & Adimasu Z. Factors influencing organic honey production level and marketing: Evidence from southwest Ethiopia. Heliyon 2021;7(9): e07975. https://doi.org/10.1016/j.heliyon.2021.e07975.

Taha EA, Taha RA & AL-Kahtani SN. Nectar and pollen sources for honeybees in Kafrelsheikh province of northern Egypt. Saudi J. Biol. Sci. 2019;26(5): 890-896. https://doi.org/10.1016/j.sjbs.2017.12.010.

White JW. Composition of honey. In: Crane, E. (Ed.), Honey: A Comprehensive Survey. Heinemann, 1975; pp.157-206.

Živkov Baloš M, Popov N, Vidakovic Knezevic S & Jakšić S. Electrical conductivity and acidity of honey. Arch. Vet. Med. 2018;11(1): 91-101. https://doi.org/10.46784/e-avm.v11i1.20.