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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

HERBAL-INFUSED EGYPTIAN BEE HONEY, A BOON OR A CURSE, ITS IMPACT ON SENSORIAL, PHYSICOCHEMICAL & ANTIBACTERIAL PROPERTIES

Bitkisel İçerikli Mısır Arı Balı, Nimet mi Lanet mi, Duyusal, Fizikokimyasal ve Antibakteriyal Özellikler Üzerindeki Etkisi

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ABSTRACT

Herbal honey mixture remedies are well known for their therapeutic benefits in traditional medicine. This research aspired to assess melissopalynological, sensorial, physicochemical, & antibacterial activity for three types of honey (clover, citrus, and cucurbits) and their mixtures with black seed, date palm pollen, & wheat germ at 1, 2.5, & 5%. The best mixtures were chosen according to overall acceptability. Consumer's preference was given to raw honey. However, some mixtures were as acceptable as raw honey. Melissopalynological analysis and lower glucose level compared to fructose are evidences that honeys are natural. Infusing herbs with different types of honey caused higher electrical conductivity, free acidity, ash, H_2O_2, HMF levels, and lower pH. Type of honey and herb may affect the physicochemical characteristics of honey in different ways. Honey whether used alone or in combination with the three herbs, demonstrated the same significant antibacterial effect for *Staphylococcus aureus* and MRSA. Inhibition zones of honey and its mixtures were lower than the control for *Pseudomonas aeruginosa*. Most undiluted samples created larger bacterial inhibition zones than their 50% diluted counterparts. Depending on the type of honey, the herb and additive concentration, infusing herbs with honey could alter its chemical, physical, and antibacterial qualities.

Keywords: Egyptian bee honey, Herbs, Sensory, Physicochemical & Antimicrobial

ÖZ

Bitkisel bal karışımı ilaçların geleneksel tıpta tedavi edici faydaları olduğu iyi bilinmektedir. Bu araştırma, üç bal türünün (yonca, narenciye ve kabakgiller) ve bunların çörek otu, hurma poleni ve buğday tohumu ile %1, 2,5 ve 5 oranında karışımlarının melissopalynolojik, duyusal, fizikokimyasal ve antibakteriyel aktivitelerini değerlendirmeyi amaçlamıştır. En iyi karışımlar genel kabul edilebilirliğe göre seçilmiştir. Tüketici tercihini ham baldan yana kullanmıştır. Ancak, bazı karışımlar ham bal kadar kabul edilebilirdi.Melissopalynolojik analiz ve fruktoza kıyasla daha düşük glikoz seviyesi balların doğal olduğunun kanıtıdır. Bitkilerin farklı bal türleriyle aşılanması daha yüksek elektrik iletkenliği, serbest asitlik, kül,$_{H2O2}$, HMF seviyeleri ve daha düşük pH'a neden olmuştur. Bal ve bitki türü balın fizikokimyasal özelliklerini farklı şekillerde etkileyebilir. Bal tek başına veya üç bitki ile birlikte kullanıldığında, Staphylococcus aureus ve MRSA için aynı önemli antibakteriyel etkiyi göstermiştir. Bal ve karışımlarının inhibisyon bölgeleri Pseudomonas aeruginosa için kontrolden daha düşüktür. Seyreltilmemiş numunelerin çoğu %50 seyreltilmiş muadillerine göre daha geniş bakteriyel inhibisyon bölgeleri oluşturmuştur. Balın türüne, bitkiye ve katkı maddesi konsantrasyonuna bağlı olarak, bitkilerin bal ile infüze edilmesi kimyasal, fiziksel ve antibakteriyel niteliklerini değiştirebilir.

Anahtar Kelimeler: Mısır arı balı, Bitkiler, Duyusal, Fizikokimyasal ve Antimikrobiyal

GENİŞLETİLMİŞ ÖZET

Amaç: Doğal bir ikili olan bal ve şifalı bitkiler geleneksel olarak yaraları tedavi etmek, astımı hafifletmek, hamile kadınlarda anemiyi önlemek, bağışıklığı artırmak ve sağlıklı bir yaşamı desteklemek için kullanılmaktadır. Bu araştırma, üç çeşit Mısır balı ve bunların bitkisel karışımlarının melissopalynolojik, duyusal, fizikokimyasal ve antibakteriyel aktivitelerini incelemeyi amaçlamıştır.

Gereç ve Yöntem: Üç farklı arı balı örneği, hasat mevsimlerinde Mısır'daki El-Gharbia Valiliği'nde bulunan çeşitli arı kovanlarından elde edilmiştir. Hammaddeler (siyah tohumlar, palmiye polen taneleri ve buğday tohumu) yerel pazardan açıkta satılan bitkiler olarak satın alınmıştır. Elde edilen sonuçlar, melissopalynolojik analizde bu türden gelen polenin baskın olduğunu, bu bitkinin arılar için birincil polen ve nektar tedarikçisi olduğunu ve potansiyel olarak fizikokimyasal ve granülasyon özelliklerini etkilediğini göstermiştir.

Bulgular: Fizikokimyasal özelliklere göre, yonca ve narenciye balı, istatistiksel analizde gösterildiği gibi, Cucurbitaceae balından önemli ölçüde farklı nem seviyelerine sahiptir. Bu oranlar normal aralıktadır (18.00-20.00g/100g). İstatistiksel olarak, glikoz ve fruktoz seviyelerinde bal türleri arasında önemli bir fark bulunmamıştır. Narenciye balı en yüksek şeker azalmasını (63,08±0,43g/100g) sergilemiş, bunu yonca balı (61,08±0,50g/100g) izlemiştir. Buna karşılık, en düşük değer Kabakgiller balında kaydedilmiştir (58.22±0.32g/100g). Kabakgiller balının DN değeri en yüksek (30,80±0,56) ve en düşüktür.

Bitkilerin bal ile karıştırılmasının pH üzerindeki etkisine ilişkin olarak, test edilen bal türleri ve karışımları için değerler 3,98±0,03 (1BS1) ile 5,75±0,11 (Kabakgiller balı) arasında değişmiştir. Yonca balına çörek otu eklenmesi pH'ı düşürürken, buğday tohumu eklenmesi pH'ı etkilememiştir. Bu çalışmada, bala bitki eklenmesi genel olarak serbest asitliği ve hidrojen peroksiti (H_2O_2) artırmıştır. Yonca ve narenciye balına buğday tohumu eklendiğinde elektriksel iletkenlik değerleri artmıştır. Bununla birlikte, kabakgil balına buğday tohumu eklendiğinde istatistiksel olarak anlamlı bir değişiklik olmamıştır. Kül içeriği balın botanik kökeni için bir kalite göstergesi olarak kabul edilmiştir. Yonca ve kabakgil balları bitkilerle birleştirildiğinde kül miktarı artmıştır. HMF, bu ilaveden etkilenmeyen narenciye balı hariç, yonca ve kabakgil ballarının bitkilerle karıştırılmasıyla artmaktadır. Test edilen tüm ballar kabul edilebilir sınırlar içinde kalmıştır. Hurma poleninin en yüksek toplam katı, kül ve karbonhidrat değerlerine (sırasıyla 96.80±0.21, 8.80±0.19 ve 68.11±0.09 g/100g) sahip olduğu tespit edilmiştir. En düşük toplam katı ve kül değerleri buğday tohumunda kaydedilirken (90.50±0.54 ve 3.50±0.06 g/100g), çörek otu karbonhidratlar açısından en düşük değere sahiptir.

Sonuç: Duyusal değerlendirmelerde, tüketiciler genellikle herhangi bir katkı maddesi içermeyen saf bal arısı ürünlerini tercih etmiştir. Bununla birlikte, bazı karışımlar da iyi karşılanmış ve faydalı özellikleri nedeniyle sağlık amacıyla kullanılabilmiştir. Bitkiler içeren veya içermeyen bal *Staphylococcus aureus* ve MRSA (Gram-pozitif bakteriler) üzerinde antibakteriyel etkiye sahip olup *Pseudomonas aeruginosa* (Gram-negatif bakteriler) üzerinde etkiye sahip değildir. Seyreltilmemiş örneklerin çoğunda seyreltilmiş örneklere kıyasla daha geniş bir bakteriyel inhibisyon bölgesi gözlenmiştir.

INTRODUCTION

Antimicrobial resistance (AMR), caused by bacteria evolving to resist antibiotics, is a pressing public health issue in the 21st century. By the year 2050, it is estimated that AMR bacteria could result in the loss of 10 million lives annually. (Kraker et al. 2016, O'Neill 2016). Prestinaci et al. (2015) agreed that the prevalence of antimicrobial resistance was a pressing issue that required a global response and a coordinated action plan to address it. The emergence of antibiotic-resistant bacteria worldwide has led to a lack of effective treatments for several ailments, lengthening treatment times and raising medical expenses (Albaridi, 2019). Ancient cultures recognized honey's therapeutic properties and used it to promote health and treat ailments. The human use of honey since 8,000 years, as reflected in certain Stone Age paintings (Kuropatnicki et al. 2018, Saikaly and Khachemoune 2017). Honey has a long history of use as a natural remedy for common infections. It continues to be a prominent component of many traditional medical regimens for maladies such as cancer, heart disease, cataracts, asthma, and infected wounds (Gündoğdu et al. 2019). Furthermore, the properties and structure of honey are affected by botanical origin, geography, season, environmental conditions, and beekeeper practices (Hossain et al. 2022, Young and Blundell 2023). In recent years, it has been discovered that many drugs employed in conventional medicine originate from organic sources such as honey and medicinal plants (Khan et al. 2018). Current scientific studies have disclosed that honey is rich in biologically active substances, frequently utilised in folk medicine. It contains antimicrobial, anti-inflammatory, antiproliferative, antimutagenic, anticancer, antidiabetic, antioxidant, antibacterial, and antifungal properties (Küçükaydın et al. 2023).

Honey and herbs, a natural duo, have been traditionally used to treat wounds, alleviate asthma, prevent anemia in pregnant women, boost immunity, and support a healthy life (Kumar et al. 2024). For centuries, *Nigella sativa* seeds and oil have been valued in Ayurvedic, Unani Tibb, and other traditional healing practices for their potential benefits in treating divergent illnesses (Khatoon et al. 2024, Shafodino et al. 2022). It belongs to the Ranunculaceae family (Yarnell and Abascal 2011**).** On the other hand, date palm pollen, or DPP (*Phoenix dactylifera* L.), is a member of the Aceraceae family; it was historically utilized as a medicinal substance by the ancient Chinese and Egyptian cultures. Its widespread utilization is prominent in the Middle East, due to its impressive nutrient profile, comprising proteins, vitamins, minerals, trace elements, carbohydrates, lipids, organic acids, sterols, nucleic acids, enzymes, and cofactors, date palm pollen is a valuable natural dietary supplement. The crucial role of bioactive volatile unsaturated fatty acids, phenolic acids, flavonoids, and other phenolic compounds lies in their powerful antioxidant properties and their anti-breast cancer abilities (El-Kholy et al., 2019).

Wheat germ is a valuable by product of wheat processing, comprising 2-3% of the whole wheat kernel (Yu et al. 2015). It is estimated that its global production reaches 25 million tons per year (Song et al. 2019). Its extract offers a natural and accessible source of antibacterial and antioxidant compounds, making it a potential candidate for use in food supplements or pharmaceutical applications (Mahmoud et al. 2015**).** This study represents the first attempt to evaluate the effects of combining several herbs with honey. No prior research has been done to determine the impact of combining herbs in this manner; however, studies have only examined the effects of combining black seed oil with honey, not whole black seeds like (Raimi et al. 2024). Even if there are studies on this topic, they focus on the impact of consuming the mixture on patients who have stomach bacteria (Abdullahi, 2023). Thus, understanding how mixing affects the properties, composition, efficacy, and quality of honey is so crucial.

This research sought to examine melissopalynological, sensorial, physicochemical, & antibacterial activity for three types of Egyptian bee honey and their herbal blends.

MATERIALS AND METHODS

Materials

Honey samples: Three different samples of bee honey were obtained from several apiaries located at El-Gharbia Governorate specifically in Tanta region, Egypt during their harvest seasons as follow clover honey (April-May), citrus honey (March) and cucurbitaceae honey (August-September) during 2023. At the laboratory of the apiary yard, Experimental Station, Faculty of Agriculture, Cairo University, all samples (three triplicates per each) were kept at -18ºC until analysis.

Herbs: Raw materials (black seeds, date palm pollen, & wheat germ) were purchased from Harraz Medicinal plant company, the most famous Egyptian herbalist located in Giza Governorate as openly sold herbs. Black seeds were ground before adding them to honey while the other two materials were used directly.

Chemicals: All chemicals used for analysis were purchased from El-Gomhouria Company for Trading Chemicals and Medical Supplies, Cairo, Egypt.

Bacterial Strains: Pathogenic bacteria used as indicators in this assay were obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25,923, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43,300, and *Pseudomonas aeruginosa* ATCC 35,032.

METHODS

Pollen analysis: As claimed by Louveaux et al (1978), pollen grains from all evaluated bee honey specimens were examined. A total of ten grams of honey were mixed in twenty millilitres of warm water and centrifuged at 3,500 rpm for ten minutes. After discarding the liquid filtrate, fresh water was added into the tube, and then the centrifuge ran for a further ten minutes. After the silt was completely spread out over a 20 x 20 mm area and dried slightly at 40 degrees, the glycerine gelatine was added, and a light microscope was used to examine the results. Each sample had a minimum of 100 pollen grains, which were counted and identified via light microscopy.

Physiochemical analysis: The honey samples were subjected to chemical analysis at the food safety and quality control laboratory of Cairo University's Faculty of Agriculture, Giza, Egypt as follow: -

Sugars (Fructose, Glucose, and Sucrose): These were analyzed by HPLC with a Phenomenex Luna NH_2 column (250×4.6 mm). The column temperature was maintained at 30 ºC, and the mobile phase consisted of Acetonitrile: HPLC grade water in an 80:20 (v:v) ratio. Detection was performed using an RI detector, and data integration was carried out using ClarityChrom software.

Hydroxymethylfurfural (HMF): It was determined by spectrophotometer UV/ V, Jenway, England.

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

Electrical conductivity: This was conducted via a conductivity meter on a 20% honey weight/volume solution in water at 20 ºC, with the 20% representing the honey's dry matter content. The instrument utilized was a conductivity meter, Five Easy, Mettler-Toledo, Switzerland.

pH: It was measured by pH meter, Boeco, Germany, calibrated with buffers having pH values of 4, 7, and 10.

Ash content: It was determined according to the methods of **AOAC, 1990**.

Free acidity: This was measured by equivalence point titration methods**.**

Hydrogen peroxide assay (H_2O_2): In the presence of peroxidase (HRP), H_2O_2 reacts with 3.5-dichloro-2-hydroxybenzensulphonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore (**Aebi 1984**).

Diastase activity: This was gauged after shade to get the DN (Gothe unit).

Sensory evaluation

Samples preparation: Honey samples were analyzed at room temperature. The honey samples were presented in clear containers to facilitate colour assessment. The samples were prepared one day prior to tasting in order to permit the honey's scent to develop within the headspace of the containers. Honey and additives were blended using wooden spoons. Samples were labeled as follows: 1, 2, and 3 for clover, citrus and cucurbit family honey, respectively, as well as black seed (BS), date palm pollen (DPP) and wheat germ (WG).

The three raw materials were incorporated into each type of bee honey at three discrete percentages (1%, 2.5%, and 5%). This resulted in a total of 27 samples as follows:

- 1BS (1%), 1BS (2.5%), &1BS (5%); 1DPP (1%), 1DPP (2.5%), & 1DPP (5%); 1WG (1%), 1WG (2.5%), & 1WG (5%).

- 2BS (1%), 2BS (2.5%), & 2BS (5%); 2DPP (1%), 2DPP (2.5%), & 2DPP (5%); 2WG (1%), 2WG (2.5%), & 2WG (5%).

- 3BS (1%), 3BS (2.5%), & 3BS (5%); 3DPP (1%), 3DPP (2.5%), & 3DPP (5%); 3WG (1%), 3WG (2.5%), & 3WG (5%).

The sensory attributes (odor, colour, taste, texture, and overall acceptability) of each sample were assessed using a 10-point unstructured descriptive evaluation scale, where 1 indicates strong dislike and 10 represents strong liking (Singh-Ackbarali & Maharaj 2014). Twenty panelists from the Faculty of Agriculture, Cairo University, were asked to rate the sensory appeal of honey samples and their mixtures. The sensory analysis took place in a controlled environment with standard lighting conditions. Instructions were written under sensory sheets as follows:

- Evaluate odor first.
- Mix before taste.
- Before and during the analysis sessions, rinse your mouth with water

Measurement of antimicrobial activity: The antimicrobial properties of honey samples and their mixtures were carried out by the agar-well diffusion assay, following the protocol described by Balouiri et al. (2016). All samples were diluted in absolute ethanol at a 1:1 ratio. In brief, bacterial inoculum was evenly spread across the entire Mueller-Hinton agar plate surface. An aseptic hole with a diameter of 8 mm was then created using a sterile cork borer. Subsequently, 100 µL of the extract solution was added to the well at the specified concentration. Negative control involved the absolute ethanol alone, while the positive control consisted of novobiocin and polymyxin B against Gram-positive and Gram-negative bacteria, separately. The agar plates underwent a 24-hour incubation period at 37 °C. The inhibition zone diameters (mm) around the wells were measured to evaluate the antimicrobial properties. The assay was conducted in triplicate.

Statistical analysis

Differences between samples were explored through one-way ANOVA, T-tests, and LSD tests at a significance level of $P \leq 0.05$.

RESULTS

Melissopalynological analysis

Figure (1) shows the pollen varieties in examined bee honey; there was a wide variability between bee honey samples according to the melissopalynological analysis. It could be concluded that the highest percentage of pollen grains was for clover (*Trifolium alexandrinum*) (56%) (Fig. 1A). The second place was Family: Umbelliferae (16%) followed by pollen of *Eucalyptus* spp. (12.26%) in clover honey. On the other side, citrus pollen had the highest percentage (30.43%) and followed by pollen of date palm (*Phoenix dactylifera*) (Fig. 1B). The main source of nectar and pollen comes from Cucurbitaceae (52.38%) for the third bee honey sample (Fig. 1C). Otherwise, *Zea mays* pollen grain is the second most frequent grain (19.04%).

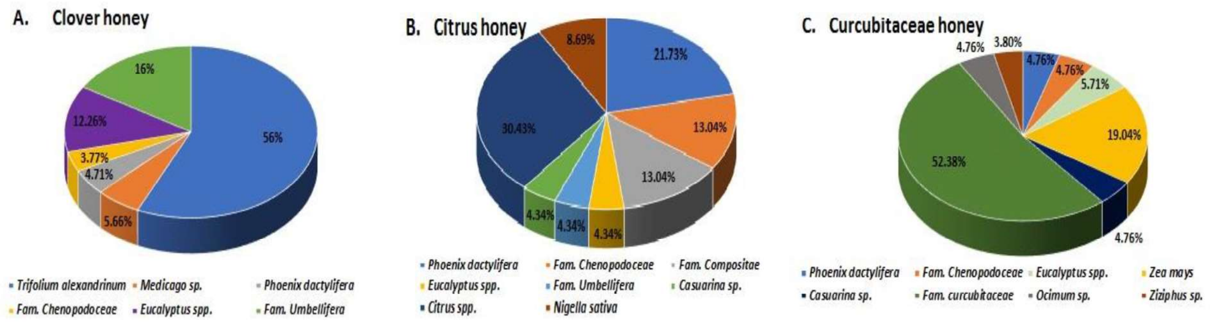


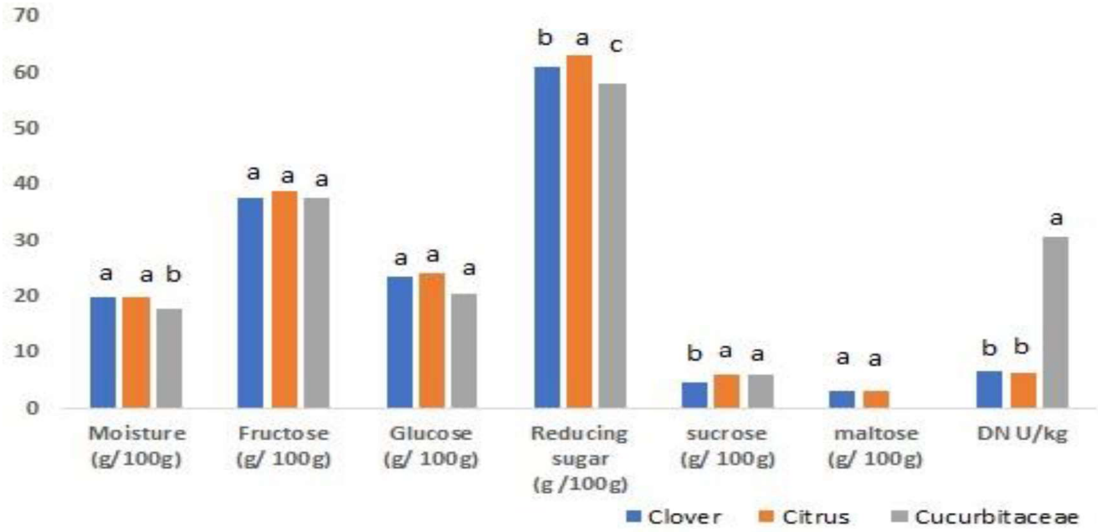
Figure 1. Pollen spectrum of the tested bee honey

Physicochemical characteristics

Bee honey: Clover and citrus honey had significantly distinct moisture levels than Cucurbitaceae honey, as displayed by statistical analysis. These percentages were within the normal range (18.00-20.00g/100g) when compared with Codex & the Egyptian Organization for Standardization and Quality Control (EOSC)**.**

Statistically, no significant differences were found between honey types in glucose and fructose levels (Fig. 2). However, clover honey had significantly lower sucrose levels ($P \leq 0.05$) than the other two types of honey. Sucrose content ranged from 4.70±0.07 to 6.33±0.15 g/100g. Except for clover honey (4.70±0.07g/100g), the examined honey samples did not meet international and national rules, which state that the concentration of sucrose content should not exceed 5g/100g. Furthermore, no significant differences were noticed between clover and citrus honey regarding maltose levels (3.12±0.14 & 3.28±0.19g/100g) in the previous respective order. The obtained data show that disaccharides, such as sucrose and maltose, were present at higher concentrations than those specified honey requirements published by the EOSC. Citrus honey (Fig. 2) exhibited the greatest reducing sugar (63.08±0.43g/100g) followed by clover honey (61.08±0.50g/100g). Conversely, the least value was recorded in Cucurbitaceae honey (58.22±0.32g/100g) this value is not accepted by both of Codex, 2001 and EOSC, 2005**.** The variations of reducing sugars among the three types were substantial ($P \leq 0.05$). DN, one of the honey quality indicators, is adopted to ascertain if honey has been exposed to heating. In Fig. 2, the DN of Cucurbitaceae honey had the highest value (30.80±0.56) and it was acceptable by Codex

Alimentations (2001) (DN>8). In contrast, the least value was recorded by citrus honey (6.50±0.26). Statistically, no significant differences were discovered between clover and citrus honey varieties. However, substantial differences were noted between both of these varieties and the third type.



Each bar represents the mean; means with the same letter are not significantly different ($P \leq 0.05$).

Figure 2. Moisture and sugar analysis of three tested types of honey

Bee honeys and their mixtures: Regarding the effect of mixing herbs with honey on pH, data in Table 1 clarified that pH values for tested types of honey and their mixtures ranged from 3.98±0.03 (1BS1) to 5.75±0.11 (Cucurbitaceae honey). Adding black seeds to clover honey reduced pH, while adding wheat germ did not affect it. No statistical differences were found between citrus honey and its samples. Conversely, mixing cucurbit honey with herbs reduced the pH. The order of the samples was as follows: cucurbit honey, cucurbit honey mixed with wheat germ, and then cucurbit honey mixed with

Table 1. Effect of infusing herbs in types of honey on physicochemical parameters

Parameters	Honeys and their mixtures (mean ±SD)									
	Clover	1BS1	1WG2.5	Citrus	2BS1	2DPP1	2WG2.5	Cucurbitaceae	3BS1	3WG1
pH	4.05 ± 0.09^{Bab}	3.98 ± 0.03^{b}	4.23 ± 0.11^{a}	4.12 ± 0.07^{B}	4.31±0.05	4.34±0.20	4.27±0.07	5.75 ± 0.11^{Aa}	5.34 ± 0.15^{c}	5.53 ± 0.06^{b}
Free acidity meq/kg	17.50 ± 0.35^{Ac}	22.00 ± 0.31^{b}	23.60 ± 0.04^{a}	14.00 ± 0.20^{Bd}	16.00 ± 0.17^{c}	18.00 ± 0.35^{b}	23.00 ± 1.00^{a}	18.00 ± 0.20^{Ac}	23.5 ± 0.62^{a}	20.50 ± 0.04^{b}
Ec (ms/cm)	0.241 ± 00.21^{Ac}	0.273 ± 0.0004^{b}	0.322 ± 0.002^{a}	0.02 ± 0.0017^{Bd}	0.246 ± 0.002^{c}	0.299 ± 0.001^{b}	0.304 ± 0.002^{a}	0.1316 ± 0.001^{Cb}	0.350 ± 0.003^{a}	0.140 ± 0.0002^{b}
Ash	0.03 ± 0.01^{Bb}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.06 ± 0.01^{Bb}	0.15 ± 0.01^{a}	0.15 ± 0.02^{a}	0.04 ± 0.01^{b}	0.79 ± 0.02^{Ac}	0.90 ± 0.03^{b}	1.30 ± 0.01^{a}
H_2O_2 (mM/100 g)	80.00 ± 2.60^{Ac}	217.00 ± 2.00^{a}	189.66 ± 5.03^{b}	67.00 ± 1.50^{Bd}	183.00 ± 1.31^{b}	205.00 ± 2.00^{a}	127.00 ± 2.00^{c}	60.84 ± 1.00^{Cc}	365.00 ± 10.00^{a}	328.00 ± 0.70^{b}
HMF (mg/kg)	16.20 ± 0.47^{Ac}	21.11 ± 0.05^{b}	23.10 ± 0.07^{a}	5.50 ± 0.18^{Ba}	1.00 ± 0.10^{b}	0.10 ± 0.01^{c}	5.50 ± 0.20^{a}	1.30 ± 0.05^{Cc}	2.20 ± 0.03^{b}	3.00 ± 0.07^{a}

Mean±SD followed by the capital different letters within rows denote significant differences between the three types of honey ($P\leq0.05$).

Mean±SD followed by the small different letters within rows stand for significant differences between the three types of honey and their mixtures ($P\leq0.05$).

Bee honeys and their mixtures: Regarding the effect of mixing herbs with honey on pH, data in Table 1 clarified that pH values for tested types of honey and their mixtures ranged from 3.98±0.03 (1BS1) to 5.75±0.11 (Cucurbitaceae honey). Adding black seeds to clover honey reduced pH, while adding wheat germ did not affect it. No statistical differences were found between citrus honey and its samples. Conversely, mixing cucurbit honey with herbs reduced the pH. The order of the samples was as follows: cucurbit honey, cucurbit honey mixed with wheat germ, and then cucurbit honey mixed with black seed. The pH values of three tested types of honey and their mixtures were acidic and within the standard limits of Codex, 2001.

In this study, adding herbs to honey generally increased free acidity and hydrogen peroxide (H_2O_2) levels. All samples did not exceed the total acidity than limit 50 med /kg as required by Codex Alimentations (2001).

For EC, all tested samples ranged from 0.02±0.0017 for citrus honey to 0.350±0.003 for cucurbitaceae honey with black seed. Electrical conductivity values increased when wheat germ was added to clover and citrus honey. Nonetheless, there was no statistically significant change when wheat germ was added to cucurbit honey. The electrical conductivity values increased when honey was combined with palm pollen or black seed at lower rates. The examined Egyptian types of honey and their mixtures were within the standard limits (≤ 0.8 ms/cm) of Codex Alimentations (2001). A honey's ash content can be used to evaluate its mineral content. It is regarded as an indicator of quality for the botanic origin of honey. The amount of ash increased when cucurbit and clover honeys were mixed with herbs. Herbal infused citrus honey had similar trend, except for the wheat germ-infused one. Cucurbit honey and it blends are within the acceptable range (0.6–1.2 g/100 g) as mentioned by Codex Alimentations (2001).

HMF value of tested honeys and their mixture ranged from 0.10± 0.01 (2DPP1) to 23.10±0.07 in (1WG2.5). HMF increases by mixing clover and cucurbit honeys with herbs, except citrus honey, which was different by this addition. All tested honeys fell within the acceptable limits, as mentioned by the EOSC (2005) (not exceeding 80 mg/kg).

Raw materials used in honey mixtures: The outcome of the chemical composition of black seeds, date palm pollen & wheat germ represented in Table 2. It was discovered that date palm pollen possessed the highest total solid, ash, and carbohydrate (96.80±0.21, 8.80±0.19, & 68.11±0.09 g/100 g), respectively. The lowest values of total solid and ash were recorded in the wheat germ (90.50±0.54 & 3.50±0.06 g/100 g) while the black seed had the lowest value regarding carbohydrates. The differences between the three herbs in the previous nutrients were significant (*p*≤0.05). Moreover, descendingly substantial variations were noticed between values of fat and fiber of black seed, wheat germ, & palm pollen.

Table 2. Raw materials used in honey mixtures

Raw	Total solid	Ash	Fat	Protein	Fiber	Carbohydrate
Black seed	95.00±0.37^{b}	4.00±0.04^{b}	20.00±0.10^{a}	25.00±0.12^{a}	8.00±0.08^{a}	38.00±0.23^{c}
Date palm pollen	96.80±0.21^{a}	8.80±0.19^{a}	1.70±0.05^{c}	20.74±0.31^{b}	0.65±0.02^{c}	68.11±0.09^{a}
Wheat germ	90.50±0.54^{c}	3.50±0.06^{c}	5.00±0.05^{b}	20.50±0.02^{b}	2.70±0.05^{b}	58.80±0.14^{b}

Mean ±SD followed by the small different letters within the same column denote signify significant differences between the three types of herbs (*P*≤0.05).

Sensory evaluation

In order to explore the impact of distinct additives (black seed, date palm pollen, and wheat germ) on the sensory properties of honey, a human sensory analysis was conducted. The best samples were chosen according to the total acceptability of the consumers.

Table (3) illustrates the sensory analysis of clover honey and its mixture samples. Clover honey, clover honey + black seed at 1%, clover honey + wheat germ at 1 & 2.5% are similar in total acceptability.

Table 3. Sensory analysis of clover honey and its mixture samples

Samples	Odour (10)	Colour (10)	Taste (10)	Texture (10)	Total acceptability (10)
Clover honey (1)	7.85±1.59^{a}	8.30±1.45^{a}	8.05±1.20^{a}	8.10±1.61^{a}	8.50±1.24^{a}
1BS (1%)	6.65±1.96^{bc}	5.95±1.75^{e}	7.15±1.86^{abcd}	6.80±2.06^{bc}	8.10±1.14^{ab}
1BS (2.5%)	6.30±2.19^{cd}	4.90±1.37^{f}	6.45±2.04^{d}	6.65±1.93^{bc}	6.80±1.78^{de}
1BS (5%)	6.80±2.42^{abc}	4.70±1.87^{f}	6.20±2.32^{de}	6.10±2.39^{c}	6.20±2.11^{ef}
1DPP (1%)	6.45±2.13^{bcd}	7.00±1.84^{cd}	6.95±2.06^{bcd}	7.05±1.88^{bc}	7.45±1.88^{bcd}
1DPP (2.5%)	6.05±2.40^{cd}	6.10±1.69^{de}	6.20±1.99^{de}	7.15±1.35^{ab}	6.50±2.20^{def}
1DPP (5%)	5.35±2.48^{d}	4.90±2.47^{f}	5.25±2.34^{e}	6.45±1.83^{bc}	5.50±2.20^{f}
1WG (1%)	7.45±1.99^{ab}	8.00±1.18^{ab}	7.55±1.69^{abc}	8.05±1.32^{a}	8.25±1.64^{ab}
1WG (2.5%)	7.05±1.73^{abc}	7.25±1.37^{bc}	7.70±1.49^{ab}	7.35±1.53^{ab}	7.90±1.26^{abc}
1WG (5%)	6.70±1.68^{bc}	6.55±1.86^{cde}	6.55±1.56^{cd}	7.00±2.12^{bc}	6.85±1.56^{de}

Mean ±SD followed by the small different letters within the same column denote significant differences at ($P≤0.05$) BS: black seed, DPP: date palm pollen, and WG: wheat germ.

Regarding the sensory analysis of citrus honey and its mixtures, Table (4) displays that none of the blends are quite like raw citrus honey in their sensory properties. Next in preference in total acceptability are black seed (1%), date palm pollen (1%), and wheat germ with concentrations of 1 & 2.5%.

Table 4. Sensory analysis of citrus honey and its mixture samples

Samples	Odour (10)	Colour (10)	Taste (10)	Texture (10)	Total acceptability (10)
Citrus honey (2)	8.85±1.01^{a}	9.20±0.81^{a}	8.75±0.98^{a}	8.80±0.98^{a}	9.00±1.00^{a}
2BS (1%)	7.05±1.83^{bc}	6.30±2.15^{de}	7.30±1.73^{b}	6.65±1.71^{bc}	7.05±1.63^{bcd}
2BS (2.5%)	6.60±1.50^{bcd}	6.35±1.90^{de}	6.95±1.77^{bc}	6.60±1.74^{c}	6.75±1.70^{cd}
2BS (5%)	5.80±1.78^{def}	5.15±1.77^{fg}	6.30±2.18^{c}	6.05±2.09^{cd}	5.75±2.12^{e}
2DPP (1%)	6.35±1.80^{cde}	6.60±1.85^{cd}	6.90±1.90^{bc}	6.85±1.49^{bc}	7.10±1.7^{bc}
2DPP (2.5%)	5.65±1.82^{ef}	5.60±1.59^{ef}	6.15±1.10^{c}	6.35±1.74^{c}	6.20±1.86^{de}
2DPP (5%)	4.95±2.01^{f}	4.60±1.77^{g}	5.20±2.08^{d}	5.35±1.96^{d}	5.40±1.91^{e}
2WG (1%)	7.40±1.43^{b}	7.70±1.35^{b}	7.60±1.50^{b}	7.50±1.36^{b}	7.80±1.29^{b}
2WG (2.5%)	7.00±1.73^{bc}	7.25±1.69^{bc}	7.25±1.74^{b}	6.80±2.01^{bc}	7.30±1.55^{bc}
2WG (5%)	6.55±1.80^{bcd}	6.65±2.06^{cd}	6.85±1.80^{bc}	6.55±2.04^{c}	6.75±2.05^{cd}

Mean ±SD followed by the small different letters within the same column denote significant differences at ($P≤0.05$). BS: black seed, DPP: date palm pollen, and WG: wheat germ.

Table (5) illustrates the sensory analysis of cucurbit family honey and its mixture samples. The most aacceptable samples in table 5 are cucurbit honey, cucurbit honey+ black seed (1%) and wheat germ (1%).

In general, it could be concluded from the three sensory tables that the consumer's preference was given to honey without additives in all sensory characteristics. The most acceptable additive was for both black seed & wheat germ, while the least acceptability was for palm pollen.

Statistical analysis revealed that the most acceptable mixtures were for seven samples (1BS 1%, 2BS 1%, 3BS1%, 2DPP 1%, 1WG2.5%, 2WG2.5%, & 3WG1%). Depending on the obtained results, physicochemical analysis and microbiological tests were performed for previous mixtures.

Table 5. Sensory analysis of cucurbit family honey and its mixture samples

Samples	Odour (10)	Colour (10)	Taste (10)	Texture (10)	Total acceptability (10)
Cucurbit family honey (3)	7.35±1.87^{a}	7.85±1.71^{a}	7.75±1.10^{a}	7.65±1.81^{a}	7.75±1.89^{a}
3BS (1%)	7.15±1.46^{ab}	6.65±1.42^{b}	6.70±1.8^{ab}	6.45±1.47^{b}	6.85±1.93^{abc}
3BS (2.5%)	6.65±1.50^{abc}	6.05±1.57^{bc}	6.50±2.12^{b}	6.15±1.59^{bc}	6.45±1.96^{bc}
3BS (5%)	5.90±1.86^{cd}	4.40±1.31^{e}	5.05±2.06^{de}	5.50±1.82^{bc}	5.15±1.87^{e}
3DPP (1%)	5.80±1.54^{cd}	6.05±1.39^{bc}	5.85±1.50^{bcde}	6.35±0.88^{bc}	6.00±1.30^{bcde}
3DPP (2.5%)	5.65±1.69^{cd}	5.35±1.50^{cd}	5.35±1.69^{cde}	5.90±1.02^{bc}	5.40±1.43^{de}
3DPP (5%)	5.00±1.81^{d}	4.55±1.47^{de}	4.80±1.91^{e}	5.85±1.84^{bc}	5.05±1.90^{e}
3WG (1%)	6.50±2.09^{abc}	6.90±1.21^{b}	6.65±1.84^{b}	6.60±1.14^{b}	7.00±1.49^{ab}
3WG (2.5%)	6.30±1.69^{bc}	6.50±1.79^{b}	6.10±2.22^{bcd}	6.50±1.61^{b}	6.25±1.86^{bcd}
3WG (5%)	6.50±1.70^{abc}	6.20±1.64^{bc}	6.15±2.18^{bc}	6.05±1.79^{bc}	5.85±2.21^{cde}

Mean ±SD followed by the small different letters within the same column denote significant differences at (P≤0.05). BS: black seed, DPP: date palm pollen, and WG: wheat germ.

Antimicrobial activity

The antimicrobial activity of honeys and their mixtures against three different pathogenic bacteria is depicted in Figure 3. Honey, whether used alone or combined with black seeds, had the same effect on *Staphylococcus aureus* as control (novobiocin antibiotic).

The impact of mixtures '' honey with wheat germ and palm pollen'' was weak, as the inhibition zone ranged from 20.3 to 26.7 mm.

For MRSA bacteria, wheat germ and a mixture of clover honey with wheat germ 2.5% had no significant effect on bacteria. The remaining samples, entailing honey alone and various honey mixtures, exhibited inhibitory effects on MRSA growth. Date palm pollen (b) being the most effective in suppressing its development.

Clear significant differences between the control sample (polymyxin B) and the rest samples (types of honey or mixtures) were noticed; none of them reached the same degree of inhibition of *Pseudomonas aeruginosa* bacterial growth as the control (C).

Figure 4 shows the antimicrobial activity of samples compared with the same diluted samples (50%). For *Staphylococcus aureus*, there are no significant differences between the diluted and nondiluted samples in the microbial growth at clover honey, palm pollen, citrus honey with black seeds 1%, cucurbit honey with black seeds 1% or wheat germ 1%, and clover honey with wheat germ 2.5%. Nonetheless, nondiluted samples (citrus, cucurbit honeys, clover honey+ black seeds 1%, and citrus honey with palm pollen 1% or wheat germ 2.5%) inhibited bacterial growth more than themselves in diluted cases. In contrast, the diluted black seeds & wheat germ had a greater effect than the nondiluted samples, as the inhibitory zone was larger.

The inhibitory zones of clover, citrus, and cucurbit honeys, along with citrus honey containing 1% palm pollen or 2.5% wheat germ, and cucurbit honey with 1% black seeds or 1% wheat germ, disclose statistically greater antibacterial activity against MRSA than the same samples in diluted form. However, there are no notable variances in bacterial proliferation among the samples (black seeds, palm pollen, 1% black seeds in clover honey, and citrus honey with 1% black seeds), regardless of whether they are diluted or not Conversely, the administration of wheat germ and a mixture of clover honey with wheat germ (2.5%) has been observed to have no effect on the activity of MRSA. The tested honeys, black seeds, and cucurbit honey with black seeds 1% or wheat germ 1% showed antibacterial activity against *Pseudomonas aeruginosa* without dilution. The results demonstrated no notable differences in bacterial growth between the diluted and undiluted palm pollen, clover honey with black seeds (1%), citrus with black seeds (1%), and citrus honey with palm pollen samples. Nevertheless, wheat germ powder or its mixture (2.5%) with clover or citrus honeys had no inhibition effect on *Pseudomonas aeruginosa* growth.

From Figure 4, undiluted samples consistently demonstrated significantly greater antibacterial activity than diluted samples against all types of bacteria investigated.

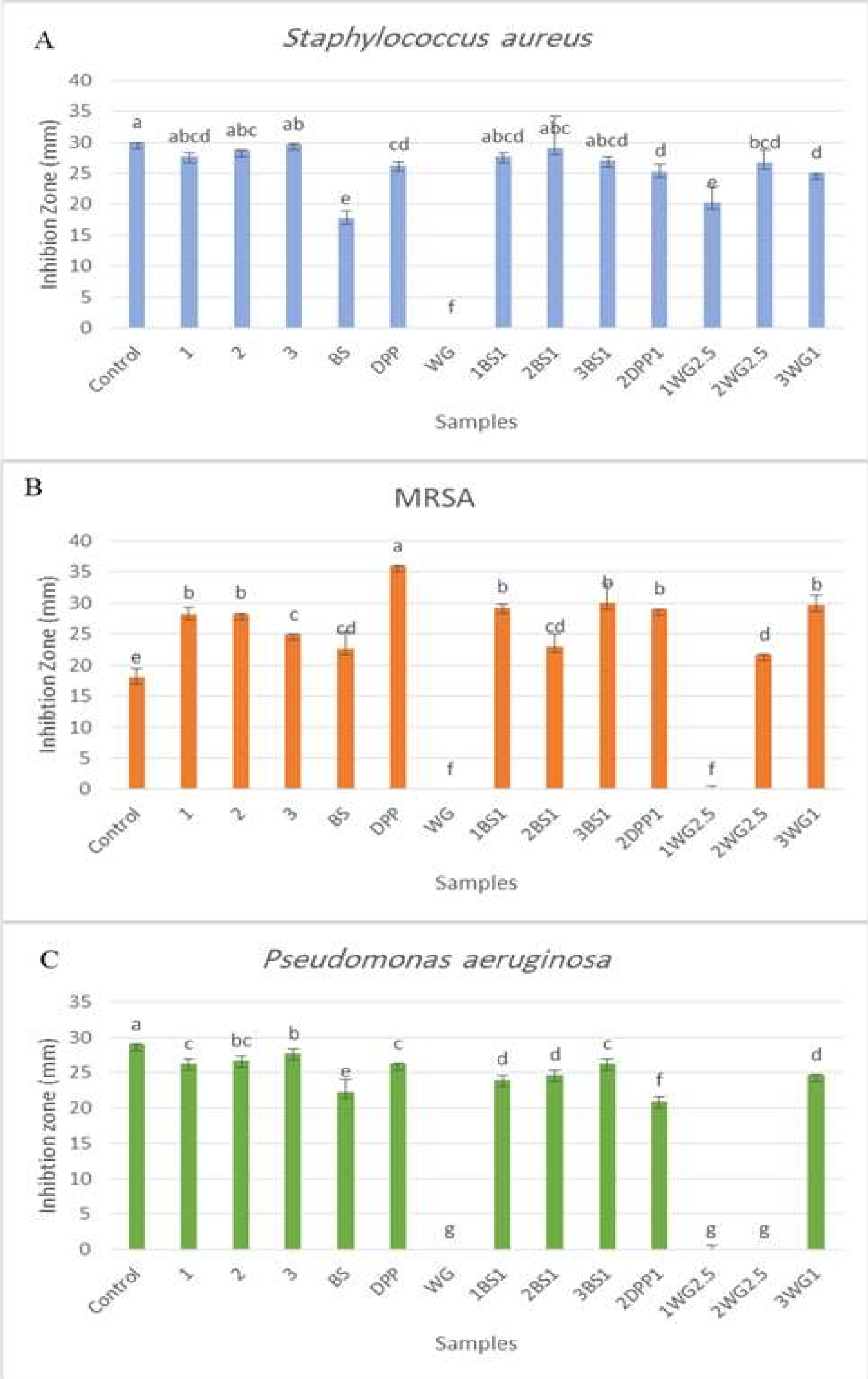


Figure 3. Inhibition zones (mm) of honeys (clover (1), citrus (2), and Cucurbit (3)), black seeds (Bs), Date palm pollen (Dpp), wheat germ (WG), and mixture samples. Nondiluted samples against *Staphylococcus aureus* (A), MRSA, methicillin-resistant *Staphylococcus aureus* (B), and *Pseudomonas aeruginosa* (C). Each bar represents mean ± standard deviation. Means with the same letter are not significantly different ($P < 0.05$).

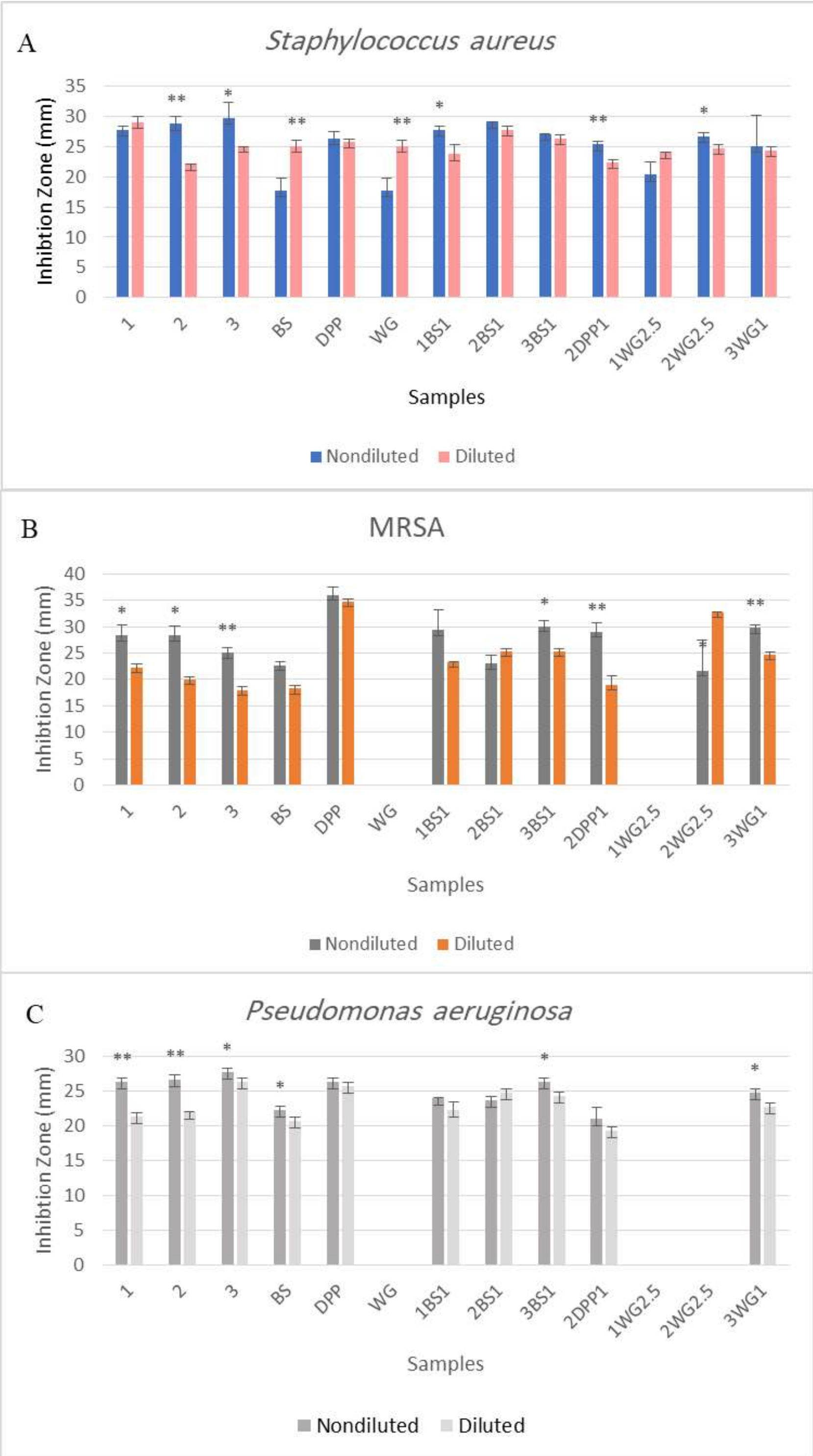


Figure 4. Inhibition zones (mm) of honeys (clover (1), citrus (2), and Cucurbit (3)), black seeds (BS), Date palm pollen (DPP), wheat germ (WG), mixtures, and their dilutions (50%). Samples against *Staphylococcus aureus* (A), MRSA, methicillin-resistant *Staphylococcus aureus* (B), and *Pseudomonas aeruginosa* (C). Each bar represents mean ± standard deviation. Bars with the * are significantly different ($P < 0.05$); bars with ** are significantly different.

DISCUSSION

Public interest in functional foods made from natural ingredients has skyrocketed, driven by a desire for healthier dietary choices (Abd Elmontaleb et al. 2023). There was a wide variability between bee honey samples according to the melissopalynological analysis. Our results are consistent with Louveaux et al. 1978 who explained that dominant pollen is defined as more than 45 percent of the pollen in honey with the exception of citrus honey must include 10%–20% citrus pollen in order to be considered monofloral (Fig 1). Furthermore, findings unveil that the pollens from these species are predominant, making this plant the primary supplier of pollen and nectar for bees, potentially impacting the physicochemical and granulation traits. Our results concur with those attained by Abd El-Dayem et al. (2024) and Seraglio et al. (2021), it was established that melissopalynological analysis represents a fundamental approach for the botanical and/or geographic identification of honey.

Concerning the physical-chemical characteristics, clover and citrus honey had significantly distinct moisture levels than Cucurbitaceae honey, as displayed by statistical analysis (Fig 2). These percentages were within the normal range (18.00-20.00g/100g) when compared with Codex Alimentations (2001) & the Egyptian Organization for Standardization and Quality Control (EOSC) (2005) which state that a honey's moisture content cannot exceed 20%. The moisture composition stands as a vital quality parameter crucial for the longevity, stability, resistance to microbial growth, and overall quality of honey. It can be affected by multiple factors such as harvest timing, seasons, nectar conditions, hive humidity levels, laboratory testing methods, storage and extraction practices (Gela et al. 2021, Singh and Singh 2018).

A higher fructose-to-glucose ratio generally indicates better honey quality and natural bee feeding. Therefore, these findings ascertained the previous studies on different types of Egyptian honey (Abd-Alla & Abd El-Wahab 2019, Abdel-Hameed 2020). The obtained data show that disaccharides, such as sucrose and maltose, were present at higher concentrations than those specified in the 2005 honey requirements published by the Egyptian Organization for Standardization and Quality, (EOSC). Based on the EOSC rules, honey's overall apparent sucrose content (sucrose plus maltose) should not exceed 5%. On the other hand, clover and citrus types of honey were accepted in reducing sugar by both Codex (2001) & EOSC (2005). However, Cucurbitaceae honey reflected lower levels than the standard limits (Not less than 60%). Diastase activity ranged from 6.50±0.26 to 30.80±0.56, which is similar to that reported by El-Metwally (2015) which mentioned that the diastase number ranges from 3.00 to 100.00 U/Kg After evaluating 184 samples of Egyptian honey, the average DN value 18.32 U/Kg. In addition to its floral source, diastase is an essential enzyme that bees use to transform nectar into honey. The activity of the diastase, a measure of honey freshness, is greatly affected by factors such as flower type, climate, inappropriate storage, and heating (Raweh et al. 2023).

Regarding the effect of mixing herbs with honey on pH, values of three tested types of honey and their mixtures were acidic and within the standard limits of (3.40 to 6.10) of Codex Alimentations (2001). Also, all of the mixtures fell within the acceptable range, although their acidity values were higher than those of the pure honey samples. Saeed& Jayashankar (2020) revealed that honey's pH ranged from 3.28 to 5.60. It could be due to the fermentation of the honey's carbohydrates into organic acid or the concentration of minerals (El-Metwally 2015). In this study, adding herbs to honey generally increased free acidity and hydrogen peroxide (H_2O_2) levels. All samples did not exceed the limit of free acidity than 50 meq/kg as required by Codex Alimentations (2001) for acidity. Increased acidity could be an indicator that sugars are fermenting into organic acids (Sancho et al 2013). Honey's sour taste makes it less acceptable, on the other hand, low acidity values indicated freshness (da Silva et al. 2016). Although H_2O_2 is thought to be a significant antibacterial component in diluted honey, some research has shown that its concentration in various types of honey does not match antibacterial action. Additionally, Bucekova et al (2019) and Farkasovska et al (2019) concluded that most significantly the total polyphenol content and the amount of H_2O_2 already present in ripened honeys were connected to the overall antibacterial activity of honeys. Furthermore, the electrical conductivity values increased when honey was combined with palm pollen or black seed at lower rates. The examined Egyptian types of honey and their mixtures were within the standard limits (≤ 0.8 ms/cm) of Codex Alimentations (2001). The electrical conductivity of honey fluctuates significantly based on the floral origin of the honey, mineral content, overall ash content, salts, organic acids, and protein levels (El-Sohaimy et al. 2015, Leo´n-Ruiz et al. 2011). When clover and cucurbit types of honey were combined with herbs, the amount of ash rose. Herbal infused citrus honey had similar trend, except for the wheat germ-infused one. Multiple factors such as plant type and physiology, soil diversity, weather patterns, and foraging materials collected by bees may affect the mineral (ash) content of honey (Mesele 2021). Concerning hydroxymethylfurfural (HMF), the results showed that the increases by mixing clover and cucurbit types of honey with herbs, except citrus honey, which was not affected by this addition. HMF is a critical component to consider while monitoring beekeeping procedures, honey exposure to high temperatures, and storage circumstances. HMF naturally exists in minimal quantities in fresh honey, but as it is stored and heated longer, it becomes more concentrated (Tafinine et al. 2018).

Pointed to raw materials used, herbs are consumed for their medicinal properties and a wide range of biological activities, such as antiviral, antibacterial, antifungal, anticoccidiosis, anti-parasitic, and antioxidant effects (Idowu et al. 2024). The differences between the three herbs in the previous nutrients were significant ($p \leq 0.05$). Moreover, descendingly substantial variations were noticed between values of fat and fiber of black seed, wheat germ, & palm pollen. These findings were in agreement with El-Rahman and Al-Mulhem (2017) and Salem (2001).

According to sensory evaluation, Piana et al. (2004) stated that the conventional sensory assessment of honey, which was extensively employed in all honey-producing regions of the world, has been shown to be a useful tool for quality improvement and control. Derndorfer et al. (2015) mentioned that Eastern cultures tended to consume honey more frequently than Western ones. The composition and sensory qualities of honey can vary depending on factors like the geographical and plant source of the flora, bee type and behaviour, the extraction methods used, and the storage conditions (Eleazu et al. 2013). In this respect, a study by Ndife et al. (2014) found that the top ratings for general acceptability were observed in honey samples from the northern region of Nigeria. Hashem-Dabaghian *et al.* (2016) discovered that *Nigella sativa* and honey worked well together to eradicate gastric *H. pylori* infections because they both contain anti-*H. pylorus* and anti-dyspeptic properties. Using a combined metabolomic and sensory study, Kang et al. (2023) scrutinized the relationships between the chemical constituents and sensory traits of honey from divergent sources. Analysis of the honey with the senses disclosed that manuka and coffee were comparatively less accepted than sugar-fed, multiflower and acacia honey.

Results in Tables 3, 4 and 5 about mixing honey with the three different herbs, Hassan (2011) stated that palm pollen grains were a promising economic resource, providing essential nutrients that could be used to enhance human nutrition. Also, Altamimi et al. (2020) enhanced the nutritional and taste profile of date palm spathes beverages by adding pollen grains. In an investigation by Dotimas et al. (2024), it was concluded that the wheat germ was packed with beneficial substances that may help improve the health problems associated with obesity. Moreover, the results of an experiment by Tahoon et al. (2024) indicated that honeybee and wheat germ might be beneficial in managing high cholesterol levels in diabetic patients, offering a functional food approach.

There is an urgent need to find substances that have an antimicrobial effect because of the proliferation of resistant harmful bacterial varieties (Kunat-Budzy´nska et al. 2023). Honey, a time-tested remedy, has been valued for its medicinal properties, particularly in treating burns, cataracts, ulcers, and wounds (Alvarez-Suarez et al. 2014). It has diverse health benefits, such as anti-inflammatory, antioxidant, antibacterial, and blood sugar-reducing properties (Erejuwa et al. 2014), make it an ideal candidate for treating gastrointestinal and ophthalmic disorders (Khan et al. 2007). Therefore, honey, with unique properties and botanical additives, increases its benefits, including antimicrobial (Kunat-Budzy´nska et al. 2023) and antioxidant activity (Miłek et al. 2023).

In our study honey, whether used alone or combined with black seeds (Fig.3), had the same effect on *Staphylococcus aureus* as novobiocin antibiotic, due to honey antimicrobial properties, which stated by Kunat-Budzy´nska et al. (2023). In addition, the black seeds (blessing seeds in Arabic countries) obtain aromatic plants (Kolayli et al. 2023), used in healing medicine (Aljawezjjah 2001). Thymoquinone is a key compound in the *N. sativa* seeds. Their extracts contain functional groups, which have antimicrobial properties (Khatoon et al. 2024, Shafodino et al. 2022,). Due to its antibacterial properties, thymoquinone from *N. sativa* seeds could be explored as a viable treatment for wound infections caused by *S. aureus* (Babu et al. 2023).

Our findings, mixtures of honey with wheat germ and palm pollen had a weak effect as anti-bacterial, agree with those conducted by Ashour et al. (2022) on the varieties cultivated in Libya. Despite palm pollen's abundance of phytochemicals, they discovered that it did not significantly reduce bacterial activity. However, the uniqueness of palm pollen lies in its great effect on the microbial activity of MRSA. It is partly similar to Bentrad et al. (2017) considering its effect on the *S. aureus*, MRSA, and *Listeria monocytogenes* (Tamma et al. 2020). Palm pollen demonstrated a greater efficacy against Gram-positive bacteria than Gram-negative bacteria (Daoud et al. 2019). Given the increasing prevalence of contagious and noncontagious illnesses, *P. dactylifera* should be explored as a promising medicinal plant with preventive potential (Mahomoodally et al. 2023).

In our investigation, wheat germ did not affect bacteria (A), although it was a rich source of nutrients (Brandolini & Hidalgo 2012) and antioxidants, with antimicrobial effects (Hozyen & El-Tohamy 2024), especially for *S. aureus* (Mahmoud et al. 2015).

In general, honey without any additives has an antibacterial effect on *Staphylococcus aureus* and MRSA not *Pseudomonas aeruginosa*. The inhibitory effect of honey on bacteria depends on the following: high viscosity and acidity, high concentration of sugar, low water activity, and the existence of hydrogen peroxide, nonperoxidase components, phenolic acids, flavonoids, proteins, peptides, and nonperoxidase glycopeptides (Luca et al. 2024), in addition to the botanical honey origin, seasonal variations, climate conditions, geographical origin, and applied techniques (Jia et al. 2020, Martinello & Mutinelli 2021).

Samples have an inhibitory effect on *Staphylococcus aureus* and MRSA (Gram-positive bacteria), but not on *Pseudomonas aeruginosa* (Gram-negative bacteria). Resistance can result from any modification of the outer membrane of Gram-negative bacteria, including changes to its hydrophobic abilities and mutations in porins. Therefore, Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria, which lack this important layer (Breijyeh et al. 2020).

Undiluted samples demonstrated significantly greater antibacterial activity than diluted samples against all types of bacteria investigated (figure 4). These results differ from those of previous studies, such as Hegazi research (2011) which used diluted honey at 20.3% and noticed antibacterial activity against *Staphylococcus aureus* & *Pseudomonas aeruginosa*. Moreover, Basualdo et al. (2007) found that honey at a concentration of 50% and undiluted honey had an inhibitory effect on the growth of *Staphylococcus aureus*. In 2005, the studies of Estrada et al. and Iurlina & Fritz differed on the concentration of honey that could inhibit microbial growth. Estrada et al. reported that the effective concentration was 25%, while Iurlina & Fritz stated that it was 75% or less.

Conclusion: Generally, infusing herbs with different types of honey increased electrical conductivity, free acidity, ash, H_2O_2, and HMF, while reducing pH. The type of honey and herb may affect the physicochemical characteristics of honey in different ways.

In sensory evaluation, consumers generally preferred pure honeybee products without any additives. However, some mixtures were also well received and could be used for health purposes due to their beneficial properties. Bee honey with or without herbs has an antibacterial effect on *Staphylococcus aureus* and MRSA (Gram-positive bacteria), not *Pseudomonas aeruginosa* (Gram-negative bacteria). A larger bacterial inhibition zone was observed in the majority of the undiluted samples than in the diluted samples. Researchers could significantly advance the field by undertaking subsequent studies that evaluate the antioxidant properties and anti-inflammatory effects of honey and herb combinations.

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Author contributions: GME, MIH, and AEA**:** planning, data collection, methodological planning, experimenting, and setting up the study. All authors read and approved the final manuscript.

Conflict of interest: The authors have no conflict of interest to declare.

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

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