Scientific Bulletin. Series F. Biotechnologies, Vol. XXIX, No. 2, 2025
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PHENOLICS IN HONEY AND THEIR BIOLOGICAL ACTIVITY

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Abstract

Honey represents a complex natural matrix enriched in phenolic compounds that contribute significantly to its biological activities. The flavonoids and phenolic acids in honey display antioxidant, antimicrobial, anti-inflammatory, anticancer, antiviral, cardioprotective, antidiabetic and other systemic effects in preclinical models. However, variability in composition, limited human clinical data, and incomplete understanding of bioavailability and metabolism impose limitations on exploitation. The phenolic profile of honey is shaped largely by its botanical origin, though environmental conditions, seasonal factors, and processing methods can also influence the concentrations detected. Meaningful comparison across studies is often challenging because phenolic compounds occur at low levels, honey is analytically complex and researchers use varying extraction and quantification approaches. Notably, the predominant phenolic constituents in honey are flavonoids, which contribute substantially to its antioxidant activity.

Key words*: honey, phenolics, flavonoids.*

INTRODUCTION

Honey has long been valued not only as a natural sweetener, but also as a functional food with therapeutic applications. Historically, honey has been used in wound healing, soothing of coughs, and general health-promoting remedies (Jibril et al., 2019). The complex chemical composition of honey (including carbohydrates, enzymes, amino acids, vitamins, minerals, and minor phytochemicals) underpins its biological activities (Jibril et al., 2019). Carbohydrates comprise the majority of honey (65-87% of honey's dry weight), mainly consisting of glucose and fructose, and in smaller amounts of sucrose, maltose, and various oligosaccharides (Wilczyńska and Żak, 2024). The main enzymes produced by bees during nectar conversions are: invertase, glucose oxidase, diastase, and catalase (Afrin et al., 2020). The presence of amino acids (such as proline, glutamic acid, aspartic acid, phenylalanine, alanine, tyrosine etc.) contributes not only to nutritional value but also to Maillard reaction products, flavor development and antioxidant capacity (Afrin et al., 2020). Honey contains small but biologically relevant concentrations of water-soluble vitamins (such as vitamin C, complex B vitamins), which arise from nectar, pollen, and bee secretions (Islam et al., 2012; Afrin et al., 2020; Cianciosi et al., 2018).

Among the minor constituents, phenolic compounds have drawn a significant attention because they contribute to honey's bioactivity and can serve as indicators of botanical and geographical origin (Becerril-Sanchez et al., 2021). These phenolics originate from nectar, polen, propolis or bee processing, and their concentrations vary with floral source and environmental conditions (Cianciosi et al., 2018). Accordingly, the aim of this paper is to review the phenolic composition of honey, the specific methods used for identification/ quantification and characterisation, and the evidence for biological activity.

PHENOLICS IN HONEY

The phenolic composition of honey is shaped primarily by its botanical origin, because the nectar and pollen sources determine the plant-derived phenolics that are transferred to the final product (Jaśkiewicz et al., 2025; Abouelenein et al., 2025; Becerril-Sánchez et al., 2021).

Closely related floral families (*Myrtaceae*, *Fabaceae*) often yield characteristic markers

such as caffeic, gallic or ferulic acids, while monofloral honeys (buckwheat, acacia, chestnut) display distinct profiles (Lawag et al., 2022; Gośliński et al., 2021). Darker honeys (e.g. buckwheat, honeydew, heather) tend to have higher total phenolic content and greater antioxidant activity compared to lighter honeys (Jaskiewicz et al., 2025).

Geographical location further modulates these profiles through differences in soil chemistry, climate, altitude, and regional vegetation as shown by comparative studies from Poland, Spain, USA, Romania, or Greece (Nyarko et al., 2023; Kędzierska-Matysek et al., 2021; García-Seval et al., 2022; Vîjan et al., 2023).

Seasonal variation and harvest time also affect phenolic levels; earlier or later collections can alter the proportion of phenolic acids and flavonoids because plant metabolism changes throughout the flowering period (Palma-Morales et al., 2023; Kędzierska-Matysek et al., 2021).

Bee species and foraging behaviour introduce additional variability, with honey-dew and stingless-bee honeys sometimes containing higher total phenolics than conventional *Apis mellifera* honey (Al-Kafaween et al., 2023; Zaldivar-Ortega et al., 2024).

Post-harvest processing and storage (thermal treatment, filtration, prolonged storage) can degrade heat-sensitive phenolics or promote oxidation, leading to lower measured concentrations (Al-Kafaween et al., 2023; Nyarko et al., 2024).

Finally, analytical methods (extraction parameters, assay) influence reported amounts, underscoring the need for standardised protocols when comparing phenolic data across studies (Nyarko et al., 2024).

Together, these factors can explain the wide intra- and inter- variability in honey phenolics observed in the literature.

Phenolics in honey roughly fall into two major classes (Table 1): phenolic acids (such as vanillic, caffeic, benzoic, ferulic, *p*-coumaric, ellagic acid) and flavonoids (such as naringenin, pinocembrim, chrisin, quercetin, kaempferol, luteolin) (Jaskiewics et al., 2025; Cianciosi et al., 2018).

Across honey types, *p*-hydroxybenzoic, *p*-coumaric, syringic, and ferulic acids were among the most frequently detected compounds in the highest concentration, with their levels - except for syringic acid - closely tied to floral origin (Kędzierska-Matysek et al., 2021). The structural diversity of these molecules arises from varying numbers of hydroxyl groups, aromatic rings and substituents, which influence their chemical reactivity and biological potential (Cianciosi et al., 2018). For example, pinocembrin and chrysin are flavonoids often associated with propolis-derived contributions to honey (Jaskiewicz et al., 2025).

Studies on Romanian honeys have identified a broader array of phenolic compounds than those mention above, including vanillic, caffeic, *p*-coumaric, gallic, protocatecuic, rosmarinic, and chlorogenic acids, quercetin, and kaempferol. Their concentrations varied markedly by floral source: myricetin was the most abundant in rape, thyme, and polyfloral honeys; vanillic acid peaked in mint and sunflower varieties; and raspberry honey exhibited the highest levels of protocatechuic and *p*-hydroxybenzoic acids. Some compounds, such as rosmarinic acid and kaempferol, appeared only in specific honey types, while luteolin was absent altogether (Scripcă et al., 2019; Pauliuc et al., 2020).

In comparison, Italian multifloral honey also highlight regional differences: Italian honeys contained substantially more caffeic acid but much lower benzoic acid (Cheng et al., 2015).

A study on 21 honey samples from Sierra Nevada found 58 phenolics in honeys, flavonoids representing more than 85% of the overall phenolic content. Among the identified compounds the most abundant were: naringenin, pinocembrin, chrysin, carnosol, galangin, and apigenin (Palma-Morales et al., 2023).

Table 1. Phenolic compounds found in honey

Phenolic compound	Amount (mg/kg)	Source
Phenolic acids		
Vanillic acid	0.03-119.73	Kędzierska-Matysek et al., 2021; Jaśkiewicz et al., 2025; Abouelenein et al., 2025
Caffeic acid	0.09-39.63	Kędzierska-Matysek et al., 2021; Jaśkiewicz et al., 2025; Abouelenein et al., 2025; Gośliński et al., 2021
Benzoic acid	2.59-15.67	Kędzierska-Matysek et al., 2021;
4-hydroxybenzoic acid	0.78-21.38	Abouelenein et al., 2025; Kędzierska-Matysek et al., 2021
3-hydroxybenzoic acid	0-17.85	Abouelenein et al., 2025;
Ferulic acid	0.11-20.81	Kędzierska-Matysek et al., 2021; Jaśkiewicz et al., 2025; Abouelenein et al., 2025; Gośliński et al., 2021
p-coumaric acid	0.01-17.52	Kędzierska-Matysek et al., 2021; Jaśkiewicz et al., 2025; Abouelenein et al., 2025
Ellagic acid	0.09-17.49	Abouelenein et al., 2025;
Chlorogenic acid	0.04-10.13	Abouelenein et al., 2025; Gośliński et al., 2021; Petretto et al., 2015
Neochlorogenic acid	0.01-2.47	Abouelenein et al., 2025;
Syringic acid	0.06-11.3	Kędzierska-Matysek et al., 2021; Abouelenein et al., 2025; Gośliński et al., 2021; Petretto et al., 2015
3.5-dicaffeoylquinic acid	0-4.38	Abouelenein et al., 2025;
Gallic acid	0.03-1.06	Abouelenein et al., 2025;
Protocatechuic acid	0-0.77	Gośliński et al., 2021
Trans-cinnamic acid	0.03-0.6	Abouelenein et al., 2025; Petretto et al., 2015
Salicylic acid	0.09-0.45	Abouelenein et al., 2025;
Cinnamic acid	0.08-0.38	Kędzierska-Matysek et al., 2021;
Sinapic acid	0-0.07	Gośliński et al., 2021
Flavonoids		
Naringenin	13.73-20.03	Palma-Morales et al., 2023
Pinocembrin	0.05-16	Jaśkiewicz et al., 2025; Palma-Morales et al., 2023; Ferreres et al., 1994; Petretto et al., 2015
Chrysin	0.05-12.21	Kędzierska-Matysek et al., 2021; Jaśkiewicz et al., 2025; Palma-Morales et al., 2023; Ferreres et al., 1994
Quercetin	0.08-11.33	Kędzierska-Matysek et al., 2021; Olas, 2020; Ferreres et al., 1994; Abouelenein et al., 2025; Gośliński et al., 2021
Quercetrin	0.01-1.56	Abouelenein et al., 2025;
Isoquercitrin	0.02-1.06	Abouelenein et al., 2025;
Galangin	0.4-7.09	Palma-Morales et al., 2023; Petretto et al., 2015
Pinobanksin	0.5-5.56	Petretto et al., 2015; Ferreres et al., 1994
Apigenin	0.03-5.24	Kędzierska-Matysek et al., 2021; Palma-Morales et al., 2023; Olas, 2020; Gośliński et al., 2021
Myricetin	0.01-3.6	Olas, 2020; Abouelenein et al., 2025
Luteolin	0.03-9.6	Olas, 2020; Ferreres et al., 1994; Gośliński et al., 2021; Petretto et al., 2015
Datiscetin	0-2.75	Gośliński et al., 2021
Kaempferol	0.01-10.6	Kędzierska-Matysek et al., 2021; Olas, 2020; Ferreres et al., 1994; Abouelenein et al., 2025; Gośliński et al., 2021; Petretto et al., 2015
Rhamnetin	0-0.24	Gośliński et al., 2021
Isorhamnetin	0.13-3.85	Jaśkiewicz et al., 2025; Olas, 2020; Ferreres et al., 1994; Abouelenein et al., 2025;
Acacetin	0.07-1.5	Jaśkiewicz et al., 2025
Rutin	0.05-3.7	Jaśkiewicz et al., 2025; Abouelenein et al., 2025; Gośliński et al., 2021; Petretto et al., 2015
Naringin	0.01-1.13	Abouelenein et al., 2025;
Astragalin	0-0.66	Gośliński et al., 2021
Tectochrysin	0.04-0.55	Ferreres et al., 1994
Genistetin	0-0.41	Gośliński et al., 2021
Genkwanin	0.07-0.39	Ferreres et al., 1994
Hyperoside	0.01-0.14	Abouelenein et al., 2025
Catechin	0-2.5	Petretto et al., 2015
Other phenolic compounds		
Carnosol	6.62-12.42	Palma-Morales et al., 2023
Vanilin	0.21-0.54	Jaśkiewicz et al., 2025

Detailed profiling studies show that honeys from different botanical origins contain characteristic phenolic signatures; for instance, quercetin may serve as a marker in sunflower honeys, kaempferol in rosemary honeys, and syringic acid in heather honeys (Jaskiewicz et

al., 2025). Research on buckwheat honey further illustrates geographic variability, with extracts showing high levels of *p*-hydroxybenzoic, *p*-coumaric, and chlorogenic acids (Perna et al., 2013; Deng et al., 2018). However, due to high variability, assigning unequivocal marker phenolics remains challenging. The presence of these compounds not only influences biological activity, but also aspects such as colour, flavour, and sensory properties of honey (Becerril-Sanchez et al., 2021).

QUANTIFICATION OF PHENOLICS IN HONEY

Phenolics represent a broad category of secondary metabolites characterized by substantial structural and functional diversity. Their biological importance - including antioxidant, anti-inflammatory, and antimicrobial effects - has stimulated growing interest in the analysis of their composition in plant extracts as well as in apicultural products such as honey. To obtain an accurate depiction of the phenolic profile, it is necessary to combine analytical methods capable of handling complex matrices and diverse chemical interferences (Ali et al., 2021). The determination of phenolics is complicated by the presence of non-phenolic compounds that can influence extraction efficiency and analytical sensitivity. Sugars, pigments, and proteinaceous substances may create significant interferences, necessitating an appropriate clean-up step. In analytical practice, adsorbent resins such as Amberlite XAD-2 or SPE cartridges are frequently used to separate the phenolic fraction from the remainder of the matrix, thereby improving the reproducibility of subsequent analytical procedures (Yung An et al., 2016).

Complementary spectrophotometric assays (for example total phenolics assay, antioxidant activity assay) are often used to link total phenolics content with functional activity (Jaskiewicz et al., 2025). However, method-logical differences can lead to variability between studies and complicate cross-study comparisons (Cianciosi et al., 2018).

The Folin-Ciocâlteu method remains perhaps the most widely used colorimetric technique for estimating total phenolics content. It is based on the reduction of a molybdate-tungstate complex by phenolic compounds, producing a blue coloration measurable by spectrophotometry. Although robust and simple, the method is not fully specific, as other reducing agents - such as ascorbic acid or sugars - may contribute to absorbance, which can result in overestimations, particularly in samples such as honey (Pérez Bosch et al., 2023).

In order to characterise honey phenolics, extraction methods such as solvent extraction, solid-phase extraction, ultrasound-assisted extraction, and sometimes acid hydrolysis are used to isolate flavonoids and phenolic acids (Jibril et al., 2019). Phenolics extraction from plants is most commonly performed using hydroalcoholic solvents, such as 50–80% ethanol. Modern techniques, including ultrasound-assisted or microwave-assisted extraction, enhance efficiency, reduce processing time, and minimize degradation of sensitive compounds (Neggad et al., 2021). Following extraction, purification using SPE cartridges is common for removing interferences and concentrating the phenolic fraction prior to chromatographic analysis.

After extraction, analytical techniques including HPLC, UHPLC-MS, and GC-MS are employed to identify and quantify individual phenolics (Jibril et al., 2019).

In the case of honey, the high sugar content dictates for specialized procedures. A well-established method involves adsorbing phenolics onto Amberlite XAD-2 resin, followed by elution with methanol. Comparative studies have shown that, under certain conditions, C18 cartridges may provide even better recoveries than XAD-2, while recent research has reported superior performance using biodegradable resins, considered a more sustainable alternative (Sharifi-Rad et al., 2021).

High-performance liquid chromatography (HPLC), coupled with photodiode array (PDA) detection, is one of the reference methods for separating and quantifying phenolics. The use of C18 columns and gradients with acidified aqueous mobile phases enables efficient separation of different classes of compounds. The PDA detector provides UV-Vis spectra

that assist in identifying different types of phenolics, while quantification is carried out using calibrated standards for each individual compound (Ali et al., 2021).

For detailed analyses, high-resolution mass spectrometry (e.g., QTOF or Orbitrap), combined with UHPLC, enables identification of phenolics based on exact mass and characteristic MS/MS fragmentation patterns. This approach has been successfully used in numerous studies to identify a wide spectrum of phenolic compounds in plant extracts and teas, with some reports identifying dozens of compounds in a single analysis (Chou et al., 2021). For quantification, triple-quadrupole instruments operating in MRM mode offer superior sensitivity and selectivity and are preferred for routine determinations.

Validation is an essential step for confirming the precision and robustness of an analytical method. Parameters such as linearity, limits of detection and quantification, recovery accuracy, and repeatability must be rigorously evaluated to demonstrate the method's suitability for its intended application. In most cases, the use of internal standards significantly improves accuracy by compensating for losses during extraction or injection variability. This step is fundamental to ensuring the quality of the generated data, regardless of the technique employed (Ali et al., 2021).

BIOLOGIC ACTIVITIES OF PHENOLICS FROM HONEY

Honey is a complex natural products widely recognised not only for its nutritional value but also for its diverse biological activities. Among the many constituents of honey, phenolic compounds (flavonoids and phenolic acids) play a central role in mediating health-promoting properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer and others.

Several studies linked many biological activities to flavonoids found in honey. For example, naringenin has been widely associated with cardiovascular benefits, largely due to its antioxidant, anti-inflammatory, antiatherogenic, and antiapoptotic activities. Pinocembrin, another flavonoid compound, exhibits antimicrobial and anti-inflammatory properties and has recently gained attention as a potential natural antiallergic agent through its inhibition of histidine decarboxylase. Chrysin demonstrated notable antiproliferative effects on cancer cell growths and is also linked to antioxidant, anti-obesity, anti-inflammatory, antidiabetic, and neuroprotective actions. Carnosol, a phenolic diterpene, similarly displays antioxidant, anti-inflammatory, and anticancer properties with additional evidence suggesting protective effects in ischemic stroke through reduced apoptosis and mitigation of oxidative and inflammatory damage. Galangin is recognised for its anti-inflammatory, antioxidant, anticancer, and antineoplastic activities. Apigenin contributes with therapeutic effects by inducing cell-cycle arrest and apoptosis, reducing inflammation, and enhancing endogenous antioxidant defenses; following intestinal absorption, it can reach the brain, where it may exert antidepressant and anxiolytic effects (Palma-Morales et al., 2023).

Antioxidant activity

One of the principal bioactivities attributed to honey phenolics is antioxidant activity. Through mechanisms such as free-radical scavenging, metal chelation, and inhibition of lipid peroxidation, phenolic compounds in honey can reduce oxidative stress in vitro (Iosageanu et al., 2024; Cianciosi et al., 2018). Several studies show strong positive correlations between total phenolics in honey and antioxidant assay outcomes (Jaskiewicz et al., 2025; Cianciosi et al., 2018).

For instance, in a Polish varietal honey study, compared with lighter honeys, darker honeys revealed higher total phenolics (honeydew, buckwheat) and exhibited markedly higher radical-scavenging activity (Jaskiewicz et al., 2025). Cianciosi et al. (2018) also reported that phenolic content is a major contributor to antioxidant capacity, though other honey components (proteins, enzymes, ascorbic acid) may also play a role. Such antioxidant capacity is relevant to the potential preventive role of honey in oxidative-stress related conditions.

Antimicrobial and antiviral activity

Beyond antioxidant properties, honey phenolics contribute to antimicrobial and antiviral activities. The antimicrobial effect of honey

arises from multiple factors: high sugar/osmotic pressure, acidity, hydrogen peroxide generation (via glucose oxidase), and phytochemicals including phenolics (Ja'afar et al., 2025). Phenolic extracts from honeys have been shown to inhibit both Gram-positive and Gram-negative bacteria (Ja'afar et al., 2025).

For instance, Ja'afar et al. (2025) cite studies of stingless bee honeys in which phenolic and protein extracts displayed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. While the precise mechanisms of phenolic-mediated antimicrobial action remain under investigation, suggestions include disruption of microbial membranes, enzyme inhibition and modulation of quorum sensing. These properties support the therapeutic potential of certain honeys as adjunct antimicrobials.

Anti-inflammatory and immuno-modulatory properties

Emerging evidence indicates that honey phenolics exert anti-inflammatory and immunomodulatory effects. Through modulation of inflammatory mediators (for example COX, LOX, TNF-α, IL-6) and influencing immune cell activity, honey has been shown in vitro and in vivo to attenuate inflammatory responses (Nan, 2024). The phenolic content is often implicated in such effects (Iosageanu et al., 2024).

Honey's antitumor and anti-inflammatory actions are attributed to mechanisms such as blocking the cell cycle, activating mitochondrial apoptosis pathways, modulating oxidative stress and inhibiting angiogenesis (Nan, 2024). While the direct cause-effect links to specific phenolic compounds require further elucidation, the data support a multi-targeted functional role of honey's phenolics in inflammation and immune regulation.

Anti-cancer and cytoprotective activities

The anticancer potential of honey, mediated in part by phenolics, is increasingly documented. Honey has shown capability to induce apoptosis, cause cell-cycle arrest, and inhibit proliferation in various cancer cell lines (Nan, 2024). The mechanistic basis often involves modulation of oxidative stress, inhibition of angiogenesis, and activation of mitochondrial pathways.

Cianciosi et al. (2018) summarise that honey's phenolic profile is associated with cytoprotective effects, including anti-neoplastic activity, though human clinical data remain limited. The complexity of honey's matrix (multiple phenolics plus other bioactive compounds) complicates pinpointing which compound drive effects, but the functional evidence supports further exploration.

Antidiabetic, cardioprotective and neuroprotective effects

While less extensively studied, honey phenolics appear to exert broader systemic beneficial effects. Phenolic compounds in honey may influence glucose metabolism, lipid profiles, oxidative damage and inflammatory status - key pathways in diabetes, cardiovascular disease and neurodegeneration (Iosageanu et al., 2024). Some animal and in vitro studies show that honey can mitigate hyperglycemia, improve antioxidant enzyme activity, and protect neuronal cells from oxidative insult (Cianciosi et al., 2018).

However, the majority of evidence remains preclinical and limited by heterogeneity of honey types, doses, phenolic profiles, and study designs. Future research must bridge to human clinical settings to validate these promising roles.

FUTURE PERSPECTIVES

Given the rich phenolic composition and demonstrated bioactivities, honey (and its phenolic-rich extracts) holds potential for development as nutraceuticals, functional foods, natural preservatives, and therapeutic adjuvants. For example, standardised honey extracts may be used for antioxidant / anti-inflammatory supplementation or in wound-healing formulations where antimicrobial and phenolic effects combine.

Nevertheless, challenges exist: honey's composition is highly variable depending on floral/geographical origin, processing and storage; standardisation of phenolic content is difficult (Jaskiewics et al., 2025). Quality control, authenticity (e.g. avoiding adulteration), and consistent phenolic profiling

are prerequisites for commercial applications. Analytical advances such as high-resolution MS and metabolomics may aid in defining phenolic fingerprints for honey certification and functional claims.

Future research directions could include: (1) more *in vivo* and clinical trials to assess efficacy, safety and dosage of honey phenolics; (2) mechanistic studies to link specific phenolic compounds to biological pathways; (3) detailed pharmacokinetic and metabolic profiling to assess bioavailability; (4) formulation studies to enhance delivery (e.g. encapsulation, controlled release) of honey phenolics; (5) exploration of synergy between honey phenolics and other honey bioactive compounds. Addressing these will pave the way for translational applications of honey phenolics in human health.

CONCLUSIONS

Honey represents a complex natural matrix enriched in phenolic compounds that contribute significantly to its biological activities. The phenolic acids and flavonoids in honey display antioxidant, antimicrobial, anti-inflammatory, anticancer and other systemic effects in preclinical models. However, variability in composition, limited human clinical data, and incomplete understanding of bioavailability and metabolism pose hurdles to full exploitation.

The phenolic profile of honey is shaped largely by its botanical origin, though environmental conditions, seasonal factors, and processing methods can also influence the concentrations detected. Meaningful comparison across studies is often challenging because phenolic compounds occur at low levels, honey is analytically complex and researchers use varying extraction and quantification approaches.

To capitalise on honey's potential as a functional food or therapeutic adjunct, standardisation of phenolic content, robust clinical validation, and mechanistic elucidation are essential. With concerted research efforts into profiling, bioavailability, formulation and clinical testing, honey phenolics may become a validated component of preventive and therapeutic nutrition.

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