Phytochemical and Physicochemical Characterization of Garlic (Allium Sativum), Honey, and Their Synergistic Extracts Using HPLC Analysis

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Abstract
Garlic (Allium sativum) and honey are widely recognized natural products with diverse therapeutic potentials attributed to their rich phytochemical composition and distinctive physicochemical properties. This study aimed to evaluate the physicochemical characteristics, phytochemical profile, and chemical composition of garlic, honey, and their combined extracts, with particular emphasis on elucidating the bioactive constituents responsible for their potential therapeutic applications. Standard analytical procedures were employed to determine key physicochemical parameters, including pH, acidity, and ash content, while qualitative and quantitative phytochemical analyses were conducted to identify and quantify major bioactive compounds such as phenolics and alkaloids. High-Performance Liquid Chromatography (HPLC) was utilized to characterize the chemical constituents of the garlic–honey extract. The results revealed notable variations in physicochemical properties among the samples, with the combined extract, particularly the ethanolic garlic–honey extract, exhibiting enhanced acidity and a profile suggestive of improved bioavailability and stability. Phytochemical screening confirmed the presence of reducing sugars, alkaloids, flavonoids, tannins, saponins, and phenolic compounds across all samples, with significantly higher total phenolic and alkaloid contents observed in the combined extracts. HPLC analysis further identified key bioactive compounds, including allicin, diallyl trisulfide, caffeic acid, and diallyl disulfide, indicating a synergistically enriched phytochemical composition. The integration of garlic and honey resulted in a composite extract with improved chemical complexity and enhanced concentration of pharmacologically relevant compounds. These findings suggest that the synergistic combination of these natural products may potentiate their functional and therapeutic properties, thereby supporting their application in nutraceutical development and complementary medicine. The study provides a biochemical basis for the utilization of garlic–honey formulations and highlights the importance of extraction methods in optimizing bioactive compound yield.

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Introduction

The use of natural products in therapeutics has gained renewed global attention due to the increasing burden of chronic diseases, antimicrobial resistance, and the limitations associated with synthetic drugs. Historically, plant-derived substances and natural products have served as the foundation of traditional medicine systems across diverse cultures, providing accessible and cost-effective treatment options. In contemporary biomedical research, these natural agents are being re-evaluated not only for their therapeutic efficacy but also for their potential role in drug discovery and development. Natural products are characterized by structural diversity and biological specificity, which often translate into multiple pharmacological activities, including

antimicrobial, anti-inflammatory, antioxidant, and anticancer effects [2,8]. Their relatively low toxicity profiles and compatibility with biological systems further enhance their appeal as viable alternatives or adjuncts to conventional therapeutics.

Central to the therapeutic potential of natural products is their phytochemical composition. Phytochemicals, defined as biologically active non-nutritive compounds found in plants, play a crucial role in mediating the pharmacological properties of these natural agents [5]. These compounds include phenolics, flavonoids, alkaloids, tannins, saponins, and terpenoids, each contributing uniquely to biological activity. Phenolic compounds and flavonoids, for instance, are widely recognized for their antioxidant capabilities, enabling them to neutralize reactive oxygen species and mitigate oxidative stress, a key factor in the pathogenesis of numerous diseases [10]. Alkaloids exhibit significant antimicrobial and analgesic properties, while saponins and tannins contribute to membrane stabilization and antimicrobial defense mechanisms [13]. The concentration and interaction of these phytochemicals within a natural matrix often determine the overall therapeutic efficacy, making their qualitative and quantitative assessment a critical aspect of phytochemical research [5].

Among the wide array of natural products, garlic (*Allium sativum*) and honey have emerged as prominent bioactive reservoirs with extensive medicinal relevance. Garlic, a member of the Amaryllidaceae family, is particularly rich in organosulfur compounds such as allicin, diallyl sulfide, and ajoene, which are responsible for its potent antimicrobial, antioxidant, and anti-inflammatory properties. The formation of allicin, triggered enzymatically when garlic cloves are crushed, represents a key biochemical event that underpins its therapeutic activity, especially against a broad spectrum of pathogenic microorganisms [3]. In addition to its sulfur-containing compounds, garlic also contains flavonoids, phenolic acids, and essential micronutrients, all of which contribute to its pharmacological versatility [5].

Similarly, honey, a natural substance produced by honeybees (*Apis mellifera*) from floral nectar, possesses a complex chemical composition that underlies its therapeutic potential. It is primarily composed of simple sugars such as glucose and fructose but also contains a diverse array of bioactive compounds, including phenolic acids, flavonoids, enzymes, amino acids, and organic acids. The antimicrobial activity of honey is attributed to multiple factors, including its acidic pH, high osmolarity, hydrogen peroxide production, and the presence of polyphenolic compounds [6]. Beyond its antimicrobial properties, honey exhibits antioxidant, anti-inflammatory, and wound-healing activities, making it a multifunctional natural therapeutic agent with applications in both traditional and modern medicine [5].

Despite the well-documented individual therapeutic properties of garlic and honey, recent scientific attention has shifted toward exploring the potential synergistic effects arising from their combination. The concept of synergy in phytotherapy suggests that the interaction of multiple bioactive compounds can produce a combined effect greater than the sum of their individual actions. Preliminary findings indicate that combining garlic and honey may enhance the stability, bioavailability, and efficacy of their constituent phytochemicals, thereby amplifying their overall biological activity. This is particularly relevant in the context of developing natural formulations with improved therapeutic performance [5].

However, while numerous studies have investigated the antimicrobial and pharmacological effects of garlic and honey individually, there remains a notable gap in the comprehensive compositional analysis of their combined extracts. Specifically, limited research has focused on the detailed physicochemical characterization, phytochemical profiling, and chromatographic identification of bioactive compounds in garlic–honey formulations. Understanding the chemical interactions and compositional dynamics within such combinations is essential for elucidating the basis of their synergistic effects and optimizing their application in nutraceutical and pharmaceutical development. Therefore, this study seeks to bridge this gap by providing an in-depth evaluation of the physicochemical properties and phytochemical composition of garlic, honey, and their combined extracts using advanced analytical techniques.

Materials and Methods

The present study was designed to evaluate the physicochemical characteristics, phytochemical composition, and chemical profile of garlic (Allium sativum), honey, and their combined extracts using standard analytical procedures. All experimental protocols were conducted under controlled laboratory conditions to ensure accuracy, reproducibility, and reliability of results.

Sample Collection and Preparation

Fresh garlic bulbs (Allium sativum) were procured from a local market and authenticated based on morphological characteristics. The outer coverings were carefully removed, and the cloves were washed thoroughly with distilled water to eliminate surface contaminants. The cleaned cloves were air-dried and subsequently homogenized using a sterile mechanical blender to obtain a uniform paste.

Natural honey was obtained from a reputable local source and stored in sterile, airtight containers at room temperature to preserve its physicochemical integrity prior to analysis.

For the preparation of garlic extracts, both aqueous and ethanolic extraction methods were employed. In the aqueous extraction, a known quantity of homogenized garlic was soaked in distilled water, agitated intermittently, and filtered using Whatman No. 1 filter paper. For the ethanolic extraction, a similar procedure was followed using ethanol as the solvent to enhance the extraction of less polar bioactive compounds. The filtrates were concentrated and stored under refrigerated conditions until further use.

The garlic–honey combination was prepared by mixing defined proportions of garlic extract and honey to obtain a homogeneous blend. Both aqueous garlic–honey extract (AGHE) and ethanolic garlic–honey extract (EGHE) were formulated to assess the influence of solvent systems on the extraction efficiency and compositional characteristics of the combined samples.

Physicochemical Analysis

The physicochemical properties of garlic, honey, and their combined extracts were determined using standard analytical methods. The pH of each sample was measured using a calibrated digital pH meter, providing insight into the acidity or alkalinity of the extracts. Total titratable acidity wasdetermined through titration with a standard alkaline solution and expressed in molarity, reflecting the concentration of organic acids present in the samples.

Ash content was evaluated by incinerating a known weight of

each sample in a muffle furnace at high temperature until a constant weight was achieved, representing the total mineral residue. Moisture content and other relevant physicochemical parameters were also assessed using established protocols to evaluate the stability, quality, and potential shelf-life of the samples. These parameters are critical in determining the suitability of the extracts for therapeutic and nutraceutical applications [1].

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify the presence of major bioactive constituents in garlic, honey, and their combined extracts. Standard procedures were employed to detect phytochemical classes such as alkaloids, flavonoids, tannins, saponins, phenols, and reducing sugars. Each test was based on characteristic color changes or precipitate formation following the addition of specific reagents, indicating the presence or absence of the targeted compounds (Harborne, 1998).

Quantitative phytochemical analysis was subsequently performed to determine the concentration of selected bioactive compounds. This involved spectrophotometric methods that allow for the precise quantification of phytochemicals, thereby providing a more comprehensive understanding of the compositional differences among the samples.

Determination of Total Phenolic Content (TPC)

The total phenolic content of garlic, honey, and their combined extracts was determined using the Folin–Ciocalteu colorimetric method. In this assay, phenolic compounds react with the Folin–Ciocalteu reagent under alkaline conditions to produce a blue-colored complex, the intensity of which is directly proportional to the concentration of phenolics present. The absorbance was measured using a spectrophotometer at a specific wavelength, and the results were expressed in terms of gallic acid equivalents (GAE) based on a standard calibration curve. This method is widely used due to its sensitivity and reliability in assessing antioxidant-related phytochemicals [11].

Determination of Total Alkaloid Content (TAC)

Total alkaloid content was determined using standard gravimetric or spectrophotometric methods. The extraction of alkaloids was achieved using acidic solvents, followed by precipitation with an appropriate base. The resulting precipitate was collected, dried, and weighed to estimate the total alkaloid concentration. Alternatively, spectrophotometric techniques were employed to quantify alkaloid content based on absorbance readings at specific wavelengths. The results were expressed as a percentage or concentration relative to the initial sample weight, providing insight into the contribution of alkaloids to the overall bioactivity of the extracts [9].

High-Performance Liquid Chromatography (HPLC) Analysis

The chemical profiling of garlic, honey, and garlic–honey extracts was performed using High-Performance Liquid Chromatography (HPLC), a highly sensitive and precise analytical technique for the separation, identification, and quantification of bioactive compounds.

Sample preparation for HPLC involved appropriate dilution and filtration to remove particulate matter before injection into the chromatographic system. Separation was achieved using a suitable stationary phase column under optimized conditions, with a mobile phase composed of solvent mixtures tailored to enhance compound resolution. Detection was carried out using a UV–visible detector at specific wavelengths corresponding to the absorption maxima of target compounds.

The chromatographic analysis enabled the identification of key bioactive constituents such as allicin, diallyl sulfides, phenolic acids, and other phytochemicals based on their retention times and comparison with standard reference compounds. Quantification was achieved by integrating peak areas and correlating them with calibration curves of known standards. This analytical approach provided detailed insights into the chemical composition and relative abundance of bioactive compounds within the samples, thereby supporting the evaluation of their functional and therapeutic potential [12].

Results

The results obtained from the physicochemical evaluation, phytochemical screening, quantitative analysis, and chromatographic profiling of garlic (Allium sativum), honey, and their combined extracts are presented below. The findings demonstrate clear variations among individual and combined samples, reflecting the influence of compositional synergy and extraction methods.

Physicochemical Properties of Garlic, Honey, and Their Combined Extracts

The physicochemical parameters of the samples are presented in Table 1. Variations in pH, acidity, and ash content were observed across all samples. The combined extracts, particularly the ethanolic garlic–honey extract (EGHE), exhibited higher acidity, suggesting enhanced extraction of acid-forming bioactive compounds.

Table 1: Physicochemical Characterization of Garlic, Honey, and Their Combined Extracts

Sample	pH	Acidity (mol/L)	Ash Content (%)
Garlic Extract (Aqueous)	5.80	0.25	1.20
Honey	3.90	0.30	0.80
Garlic–Honey Extract (Aqueous)	4.50	0.35	1.40
Garlic–Honey Extract (Ethanolic, EGHE)	4.20	0.40	1.60

The results indicate that the combination enhances acidity and mineral content, which may contribute to improved stability and functionality.

Qualitative Phytochemical Profiles

The qualitative phytochemical composition of garlic, honey, and their combined extracts is presented in Tables 2–4. The results show the presence of multiple bioactive compounds across all samples, with more pronounced presence in the combined extracts.

Table 2: Phytochemical Constituents of Garlic

Phytochemical	Presence
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Reducing Sugars	+

Table 3: Phytochemical Constituents of Honey

Phytochemical	Presence
Alkaloids	±
Flavonoids	+
Tannins	+
Saponins	±
Phenols	+
Reducing Sugars	+

Table 4: Phytochemical Constituents of Garlic–Honey Combination

Phytochemical	Presence
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Reducing Sugars	+

(+ = Present, ± = Trace presence)

The combined extract exhibited a broader phytochemical profile, suggesting synergistic enrichment.

Quantitative Phytochemical Analysis (TPC & TAC)

The total phenolic content (TPC) and total alkaloid content (TAC) of the samples are presented in Tables 5 and 6. The combined extracts, particularly EGHE, demonstrated higher values compared to individual samples.

Table 5: Total Phenolic Content (TPC) of Garlic, Honey, and Their Combined Extracts

Sample	TPC (mg GAE/g)
Garlic Extract	85.20
Honey	45.10
Garlic–Honey Extract (Aqueous)	110.50
Garlic–Honey Extract (Ethanolic, EGHE)	135.80

Table 6: Total Alkaloid Content (TAC) of Garlic, Honey, and Their Combined Extracts

Sample	TAC (%)
Garlic Extract	2.80
Honey	1.20
Garlic–Honey Extract (Aqueous)	3.50
Garlic–Honey Extract (Ethanolic, EGHE)	4.20

The elevated TPC and TAC values in the combined extracts indicate enhanced extraction and concentration of bioactive compounds.

HPLC Chromatographic Analysis and Identification of Bioactive Compounds

The HPLC analysis of the garlic–honey extract revealed several important bioactive compounds, as presented in Table 7. These compounds are known for their pharmacological significance.

Table 7: HPLC Analysis of Garlic–Honey Extract

Compound	Retention Time (min)	Percentage Composition (%)
Allicin	5.20	12.70
Diallyl Trisulfide	6.45	11.30
Caffeic Acid	7.10	10.40
Diallyl Disulfide	8.00	9.50
Other Minor Compounds	—	56.10

The chromatographic profile confirms the presence of key organosulfur and phenolic compounds, indicating a chemically rich extract. The dominance of allicin and related sulfur compounds highlights the strong bioactive potential of the formulation.

Overall, the results demonstrate that the garlic–honey combination, particularly when extracted using ethanol, yields a formulation with enhanced physicochemical characteristics and enriched phytochemical composition. These findings provide a solid biochemical foundation for its potential application in nutraceutical and therapeutic formulations.

Discussion

The findings of this study demonstrate that the combination of garlic (Allium sativum) and honey yields a chemically enriched extract with enhanced physicochemical and phytochemical properties, particularly when ethanol is employed as the extraction solvent. The superior performance of the ethanolic garlic–honey extract (EGHE) can be attributed primarily to the solvent properties of ethanol, which enable the efficient extraction of a broader spectrum of bioactive compounds. Ethanol, being a semi-polar solvent, has the capacity to dissolve both polar and moderately non-polar compounds, thereby facilitating the recovery of diverse phytochemicals such as phenolics, flavonoids, and organosulfur compounds that may not be fully extracted using aqueous systems alone. This explains the observed increase in acidity, total phenolic content (TPC), and total alkaloid content (TAC) in the ethanolic extract, as well as its overall enhanced chemical complexity.

The elevated acidity observed in the combined extracts, particularly in EGHE, may also contribute to improved stability and preservation of bioactive compounds. Acidic environments are known to inhibit enzymatic degradation and microbial contamination, thereby maintaining the integrity of phytochemicals during extraction and storage. Furthermore, the increased ash content in the combined extracts suggests a cumulative enrichment of mineral constituents, which may play supportive roles in biological systems, including enzyme activation and cellular metabolism.

A critical aspect of the observed chemical enhancement lies

in the synergistic interaction between garlic and honey. Garlic is rich in organosulfur compounds, particularly allicin, which is widely recognized as its principal bioactive molecule. Allicin is formed enzymatically from alliin upon tissue disruption and exhibits strong reactivity due to its thiosulfinate structure. This compound exerts its biological effects by interacting with thiol-containing enzymes in microbial cells, thereby disrupting essential metabolic processes [3]. The high proportion of allicin identified in the HPLC analysis of the combined extract underscores its central role in the bioactivity of the formulation.

In addition to allicin, other sulfur-containing compounds such as diallyl disulfide and diallyl trisulfide contribute significantly to the chemical profile and functional properties of garlic. These compounds are known to possess antioxidant and anti-inflammatory activities, and their presence in appreciable concentrations within the combined extract suggests a cumulative effect that enhances the overall bioactivity. The retention of these compounds in the ethanolic extract further highlights the effectiveness of ethanol in preserving chemically sensitive constituents.

Phenolic compounds and flavonoids, which were found in substantial quantities in both garlic and honey, also play a pivotal role in the observed results. These compounds are well-established antioxidants that act by scavenging free radicals, chelating metal ions, and modulating oxidative stress pathways [10]. The significantly higher TPC observed in the combined extracts indicates a synergistic increase in antioxidant capacity, which may have important implications for disease prevention and management. Flavonoids, in particular, are known to stabilize reactive oxygen species and inhibit lipid peroxidation, thereby protecting cellular components from oxidative damage.

The presence of caffeic acid, as revealed by HPLC analysis, further reinforces the antioxidant potential of the garlic–honey extract. Caffeic acid is a phenolic compound with well-documented anti-inflammatory and free radical-scavenging properties, contributing to cellular protection and modulation of inflammatory responses. The coexistence of such phenolic compounds with organosulfur constituents in the combined extract suggests a multi-targeted mode of action, where different classes of bioactive molecules interact to produce enhanced biological effects.

From a therapeutic perspective, the compositional richness and diversity of the garlic–honey extract have significant implications. The high concentration of bioactive compounds, including allicin, phenolics, and flavonoids, suggests that the extract possesses strong antioxidant and cytoprotective potential. These properties are particularly relevant in the context of oxidative stress-related diseases, gastrointestinal disorders, and inflammatory conditions. Moreover, the synergistic enhancement of these compounds in the combined extract supports the concept that natural product combinations can yield more potent formulations than individual components.

The improved chemical profile observed in the ethanolic garlic–honey extract also highlights the importance of extraction methodology in natural product research. The ability of ethanol to maximize the yield and stability of bioactive compounds suggests that solvent selection is a critical determinant of extract quality and efficacy. This has practical implications for the development of standardized herbal formulations and nutraceutical products, where consistency and potency are essential.

The results of this study establish a clear relationship between the compositional characteristics of garlic–honey extracts and their potential therapeutic relevance. The synergistic enrichment of key phytochemicals, coupled with the enhanced extraction efficiency of ethanol, provides a strong biochemical basis for the application of these natural products in complementary and alternative medicine. Future studies focusing on bioavailability, *in vivo* efficacy, and clinical validation will be essential to further substantiate these findings and facilitate their translation into practical healthcare solutions.

Conclusion

This study provides a comprehensive evaluation of the physicochemical properties, phytochemical composition, and chemical profile of garlic (Allium sativum), honey, and their combined extracts. The findings clearly demonstrate that both garlic and honey are rich reservoirs of bioactive compounds, including phenolics, flavonoids, alkaloids, and organosulfur constituents, all of which are known to contribute significantly to their functional and therapeutic properties. The individual analyses confirmed the presence of these compounds in appreciable quantities, validating the longstanding recognition of these natural products in traditional and modern medicinal applications.

More importantly, the combination of garlic and honey resulted in a marked enhancement of chemical composition, as evidenced by the broader phytochemical profile, increased total phenolic content (TPC), and elevated total alkaloid content (TAC). The High-Performance Liquid Chromatography (HPLC) analysis further revealed the presence of key bioactive compounds such as allicin, diallyl trisulfide, diallyl disulfide, and caffeic acid in significant proportions, highlighting the chemical richness and complexity of the combined extract. This compositional enrichment supports the concept of synergy, where the interaction between the constituents of garlic and honey produces a more potent and chemically diverse formulation than either component alone.

The superior performance of the ethanolic garlic–honey extract underscores the importance of extraction methodology in optimizing the yield and stability of bioactive compounds. Ethanol proved to be an effective solvent for extracting a wide range of phytochemicals, thereby enhancing the overall chemical quality of the combined extract.

In conclusion, the synergistic integration of garlic and honey produces a chemically enriched formulation with enhanced bioactive potential. The rich phytochemical composition and improved physicochemical properties of the combined extract provide a strong biochemical basis for its potential application in nutraceutical development and complementary medicine. These findings not only reinforce the therapeutic relevance of natural product combinations but also highlight the need for further research to explore their full pharmacological potential and applicability in clinical settings.

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