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STRUCTURE–ACTIVITY RELATIONSHIPS AND BIOORGANIC ANALYSIS OF CONSTITUENTS FROM PHLOMOIDES LABIOSA

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ABSTRACT

Phlomoides labiosa is an understudied species of the Lamiaceae family known for its potential reservoir of biologically active secondary metabolites. This study investigates the chemical composition of P. labiosa and examines the structure–activity relationships (SAR) of its major constituents through an integrated bioorganic approach. Sequential extraction followed by chromatographic fractionation enabled the isolation of phenolic acids, flavonoids, terpenoids, sterols, and related metabolites. Structural identification was performed using GC–MS, HPLC, FT-IR, UV–Vis, and NMR spectroscopy. Biological activity assays demonstrated significant antioxidant and antimicrobial effects, with phenolic and terpenoid-rich fractions showing the highest functional potency. SAR analysis revealed a strong correlation between hydroxylated aromatic structures and radical-scavenging activity, while terpenoid skeletons were associated with antibacterial efficiency. The results indicate that Phlomoides labiosa contains structurally diverse natural compounds with promising pharmacological potential, supporting further targeted isolation and mechanistic studies for bioactive molecule development.

Keywords: Phlomoides labiosa, structure–activity relationships (SAR), bioorganic analysis, phytochemical constituents, flavonoids, terpenoids, antioxidant activity, antimicrobial activity.

INTRODUCTION

The investigation of natural products remains a central focus of contemporary bioorganic chemistry, particularly as interest grows in plant-derived compounds with therapeutic potential. The Lamiaceae family is widely recognized for its diverse range of secondary metabolites, including flavonoids, phenolic acids, terpenoids, and sterols, many of which exhibit notable antioxidant, antimicrobial, and anti-inflammatory activities. Species of the genera Phlomis and Phlomoides are well known in traditional medicine across Asia and the Mediterranean region; however, their phytochemical composition and structure–activity relationships (SAR) have only been partially studied.

Phlomoides labiosa is an understudied species within this group and has received limited scientific attention despite its ethnobotanical relevance. Current knowledge regarding its chemical constituents and biological potential is scarce, and systematic research using modern analytical techniques is lacking. This gap highlights the need for comprehensive studies aimed at identifying the plant's bioactive metabolites and understanding how their structural features influence biological activity.

Advances in chromatographic and spectroscopic technologies—such as GC–MS, HPLC, FT-IR, UV–Vis, and NMR—offer powerful tools for isolating and characterizing natural compounds with high precision. When combined with biological activity assays, these analytical approaches make it possible to establish meaningful SAR correlations that explain how specific functional groups, substitution patterns, and molecular frameworks contribute to pharmacological effects.

Given the promising bioactive profiles reported for related Phlomis and Phlomoides species, Phlomoides labiosa may represent an important yet unexplored source of valuable phytochemicals. Investigating its secondary metabolites and determining their SAR profiles is essential for revealing the plant's therapeutic potential and its relevance in pharmaceutical, nutraceutical, and bioorganic research.

The present study aims to isolate and identify the major constituents of Phlomoides labiosa, evaluate their antioxidant and antimicrobial activities, and explore structure–activity relationships among the isolated compounds. This work provides foundational knowledge on the bioorganic properties of P. labiosa and contributes to the broader understanding of natural product chemistry within the Lamiaceae family.

Literature Review

The study of natural products and plant-derived bioactive compounds has long been central to bioorganic and phytochemical research. Foundational analytical approaches for identifying secondary metabolites were established in the work of Harborne (1998), whose phytochemical methods continue to serve as the primary reference for chromatographic and spectroscopic analysis of plant constituents. Classical colorimetric assays for phenolic quantification and antioxidant evaluation, such as the Folin–Ciocalteu method described by Singleton, Orthofer, and Lamuela-Raventos (1999), the ABTS assay developed by Re, Pellegrini, Proteggente, Pannala, Yang, and Rice-Evans (1999), and the total phenol procedure of Slinkard and Singleton (1977), remain widely used and form the methodological basis for modern plant chemistry studies.

The Lamiaceae family is recognized for its rich diversity of phenolic compounds, flavonoids, terpenoids, and essential oils. Barros, Dueñas, Dias, Sousa, Santos-Buelga, and Ferreira (2013) demonstrated substantial variability in phenolic profiles of Melissa officinalis, influenced by cultivation methods and processing conditions. Their findings underscore the importance of environmental and technical factors in determining phytochemical composition. Essential oils within this family also exhibit noteworthy biological activities. Burt (2004) provided a

comprehensive review showing strong antibacterial potential of essential oils, primarily attributed to terpenoid and phenolic constituents. Miguel (2010) further emphasized the antioxidant and anti-inflammatory mechanisms of essential oils, supporting their broad pharmacological relevance.

Extraction methodology significantly affects the chemical composition and bioactivity of plant extracts. Chemat, Abert Vian, and Cravotto (2012) introduced principles of green extraction, highlighting solvent efficiency, reduced thermal degradation, and ecological sustainability as key advantages. Such approaches are increasingly employed in modern phytochemical investigations to improve yield and preserve sensitive metabolites.

The genus Phlomis and its closely related genus Phlomoides have attracted extensive scientific attention due to their ethnomedicinal uses and phytochemical richness. Kahraman, Lakušić, and Doğan (2012) clarified the taxonomy of Turkish Phlomis and Phlomoides species, providing an essential framework for subsequent chemical and pharmacological studies. A broad review by Nabavi, Marchese, Izadi, Curti, Daglia, and Nabavi (2015) summarized the phytochemistry and biological activities of Phlomis species, identifying flavonoids, phenolic acids, terpenoids, and sterols as dominant constituents with antioxidant, antimicrobial, anti-inflammatory, and cytoprotective effects. Kumar and Pandey (2013) contributed a detailed overview of flavonoid chemistry and their biological functions, highlighting their importance as core secondary metabolites in Lamiaceae plants.

Several studies from Uzbekistan have focused on the chemical and biological characteristics of local medicinal plants. Mamatqulova, Dexqanov, and Abdullayev (2021) examined approaches for classifying and certifying biologically active compounds extracted from Helianthus tuberosus, contributing to regional methodologies for natural product standardization. Mamadjonova, Usmanova, and Abdullayev (2021) compared the ash content and elemental composition of Lamiaceae species (Nepeta, Lophanthus), emphasizing the ecological and biochemical variability of local flora. Yulbarsova, Khaydarova, Siddikov, and Abdullaev (2022) investigated Phlomoides nuda as a natural source of β-sitosterol, a valuable sterol with demonstrated pharmacological activity. These studies highlight the chemical diversity of plants growing in Uzbekistan and reflect the increasing interest in bioactive constituents of the Lamiaceae family.

Research on antimicrobial properties of plant extracts has also been significant. Parekh and Chanda (2007) reported strong antibacterial activity of methanolic extracts from Woodfordia fruticosa, demonstrating how solvent polarity influences the extraction of active compounds. These findings complement earlier evidence on essential oil activity presented by Burt (2004) and Miguel (2010), reinforcing the role of phenolic and terpenoid components in antimicrobial function. In parallel, the biochemical mechanisms of oxidative stress and antioxidant defense have been extensively analyzed by Halliwell and Gutteridge (2015), whose work provides the molecular basis for interpreting antioxidant assay outcomes in phytochemical studies.

Together, these references demonstrate that Lamiaceae species possess a high diversity of bioactive constituents with strong antioxidant and antimicrobial potential. Despite this, certain species—particularly Phlomoides labiosa—remain insufficiently studied in terms of phytochemical profiling, structural characterization, and structure–activity relationships. The existing literature therefore identifies a clear gap: the need for comprehensive bioorganic analysis and SAR evaluation of understudied Phlomoides species to better understand their pharmacological value.

Materials and Methods

Plant material of Phlomoides labiosa was collected during the flowering season from natural habitats in southern Central Asia. The species was taxonomically verified by botanists at a regional herbarium, and a voucher specimen was prepared and deposited for future reference. The collected plant material was washed, shade-dried at room temperature, powdered using a mechanical grinder, and stored in airtight containers until analysis.

Sequential extraction was performed using solvents of increasing polarity. The powdered material was first extracted with 95% ethanol by maceration for 72 hours with intermittent shaking. The extract was filtered and concentrated under reduced pressure at 45 °C using a rotary evaporator. The remaining plant residue was subsequently extracted with distilled water by boiling for 30 minutes, followed by filtration and lyophilization. Both ethanolic and aqueous extracts were stored at 4 °C until further processing.

Purification of constituents was carried out through column chromatography using silica gel as the stationary phase. A gradient solvent system consisting of hexane, ethyl acetate, methanol, and water was applied to elute fractions with varying polarity. Fractions displaying similar profiles on thin-layer chromatography (TLC) were pooled together. Selected fractions were further purified using preparative high-performance liquid chromatography (HPLC).

Gas chromatography–mass spectrometry (GC–MS) analysis was employed to identify volatile and semi-volatile constituents in the crude extracts and fractions. Analyses were performed on an HP-5MS capillary column with helium as the carrier gas under a programmed temperature gradient. Mass spectra were compared with reference databases for compound identification. Quantitative and qualitative profiling of phenolic and flavonoid components was performed using analytical HPLC with a C18 reverse-phase column and a water–methanol gradient containing 0.1% formic acid. Detection wavelengths were set at 254 nm and 280 nm, and standard compounds such as gallic acid, quercetin, and rosmarinic acid were used for calibration.

Spectroscopic analyses were conducted to confirm the structural features of the isolated constituents. Fourier transform infrared (FT-IR) spectroscopy was performed in the range of 4000–400 cm^{-1} to identify major functional groups. Ultraviolet–visible (UV–Vis) spectra were recorded between 200 and 800 nm to examine chromophoric characteristics of phenolic and flavonoid compounds. Structural elucidation of purified fractions was further achieved through proton and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy.

Antioxidant activity was assessed using the DPPH and ABTS radical scavenging assays. Different concentrations of extracts and fractions were mixed with standard radical solutions, and the reduction in absorbance was measured at 517 nm (DPPH) and 734 nm (ABTS). Ascorbic acid and Trolox were used as positive controls. The antioxidant potential was expressed in terms of IC_{50} and Trolox equivalent antioxidant capacity (TEAC) values.

Antimicrobial activity was evaluated using the disk diffusion method and minimum inhibitory concentration (MIC) assay against Staphylococcus aureus, Escherichia coli, and Candida albicans. Mueller–Hinton agar was used for bacterial strains and Sabouraud dextrose agar for fungal testing. Extracts and fractions of varying concentrations were applied to sterile disks placed on inoculated agar plates, and inhibition zones were measured after incubation.

All experiments were conducted in triplicate. Results were reported as mean ± standard deviation, and statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was accepted at $p < 0.05$.

Results and Discussion

Phytochemical screening of Phlomoides labiosa revealed the presence of a broad spectrum of secondary metabolites. Both ethanolic and aqueous extracts contained phenolic acids,

flavonoids, terpenoids, sterols, and saponins, while alkaloid-like components were detected primarily in the ethanolic extract. This composition aligns with typical chemical profiles reported for species of the Lamiaceae family, which are widely known for their rich phenolic and terpenoid content.

Column chromatography and subsequent fractionation produced several pooled fractions based on TLC patterns. Gas chromatography–mass spectrometry (GC–MS) analysis identified multiple volatile and semi-volatile compounds, including phytol, hexadecanoic acid, linoleic acid derivatives, β-sitosterol, and other terpenoid-based molecules. These constituents have been frequently reported in related Phlomis and Phlomoides species and are recognized for their antioxidant, antimicrobial, and anti-inflammatory properties. The presence of such compounds in P. labiosa provides evidence of its pharmacological potential.

High-performance liquid chromatography (HPLC) profiling showed that the extracts contained substantial amounts of gallic acid, quercetin, and rosmarinic acid. These well-known phenolic constituents have strong antioxidant capacities, and their abundance suggests that P. labiosa may serve as a promising natural source of phenolic antioxidants. Phenolic-rich fractions demonstrated higher UV absorbance peaks at 254 and 280 nm, confirming their enrichment during chromatographic separation.

Spectroscopic analyses supported the outcomes of the chromatographic evaluations. Fourier transform infrared (FT-IR) spectroscopy revealed characteristic absorption bands corresponding to hydroxyl, carbonyl, aromatic ring, and aliphatic chain functional groups—structural elements typically associated with phenolic acids, flavonoids, and terpenoids. UV–Vis spectra exhibited peaks within the 250–350 nm region, consistent with conjugated aromatic systems, while ^{1}H-NMR and ^{13}C-NMR analyses showed chemical shifts characteristic of flavonoid backbones and terpenoid carbon frameworks. These findings confirmed the structural identities of several key metabolites.

Antioxidant assays demonstrated that both crude extracts and purified fractions possessed strong radical-scavenging activity. The ethanolic extract exhibited the greatest activity in both DPPH and ABTS assays, with IC_{50} values approaching those of reference antioxidants. Fractions rich in flavonoids and phenolic acids showed the highest scavenging efficiency, indicating that hydroxylated aromatic structures significantly contribute to antioxidant potential. Structure–activity relationship (SAR) evaluation suggested that the number and position of hydroxyl groups on aromatic rings are major determinants of radical neutralization ability.

Antimicrobial activity testing revealed that P. labiosa displays broad-spectrum inhibitory effects. Staphylococcus aureus showed the highest sensitivity, followed by Escherichia coli, whereas Candida albicans exhibited moderate susceptibility. Fractions enriched with terpenoids and sterols displayed stronger antimicrobial effects than crude extracts, indicating that purification increased the biological potency of certain compounds. SAR analysis also suggested that lipophilic terpenoid frameworks enhance the interaction of bioactive molecules with microbial membranes, contributing to antibacterial efficiency.

Overall, the combination of chemical profiling, spectroscopic identification, biological assays, and SAR interpretation demonstrates that Phlomoides labiosa contains structurally diverse natural products with promising pharmacological activity. The strong antioxidant and antimicrobial results support the likelihood of synergistic interactions among phenolic acids, flavonoids, and terpenoids. These findings highlight P. labiosa as a valuable source of bioactive molecules and provide a foundation for further mechanistic and pharmacological investigations.

Conclusion

This study provides the first comprehensive bioorganic analysis and structure–activity evaluation of constituents isolated from Phlomoides labiosa. The combined chromatographic and spectroscopic investigations revealed that the plant contains a diverse array of secondary metabolites, including phenolic acids, flavonoids, terpenoids, sterols, and related natural compounds. These constituents exhibited notable antioxidant and antimicrobial activities, with phenolic- and terpenoid-rich fractions demonstrating the highest functional potency.

Structure–activity relationship (SAR) analysis indicated that hydroxylated aromatic structures strongly contribute to radical-scavenging capacity, while lipophilic terpenoid frameworks enhance antimicrobial effects through interactions with microbial membranes. The synergistic contributions of these compound classes explain the broad biological activity observed in the extracts and purified fractions.

Overall, the findings highlight Phlomoides labiosa as a promising yet previously underexplored source of bioactive natural products with potential pharmaceutical and nutraceutical applications. Further studies focusing on the isolation of individual compounds, mechanistic evaluations, toxicity assessments, and in vivo analyses are recommended to advance the therapeutic prospects of this species.

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