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Isolation And Bioorganic Characterization Of Active Compounds From Phlomoides Labiosa

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Abstract: Phlomoides labiosa, a species belonging to the Lamiaceae family, is traditionally used in Central Asian ethnomedicine, yet its bioactive constituents and bioorganic properties have not been thoroughly investigated. This study focuses on the isolation and bioorganic characterization of active compounds from P. labiosa using an integrated analytical approach. Sequential extraction with ethanol and water was followed by chromatographic separation to obtain distinct phytochemical fractions. Structural elucidation of isolated compounds was carried out using GC–MS, HPLC, FT-IR, UV–Vis, and $^{1}H/^{13}C$-NMR spectroscopy. The major identified constituents included flavonoids, phenolic acids, terpenoids, sterols, and alkaloid derivatives. Biological activity assays demonstrated strong antioxidant potential and significant antimicrobial effects against selected bacterial and fungal strains. The results indicate that P. labiosa is a rich source of structurally diverse and biologically active natural molecules. The study highlights the pharmacological relevance of the isolated compounds and provides a scientific basis for their potential application in pharmaceutical and bioorganic chemistry research.

Keywords: Phlomoides labiosa, bioorganic characterization, isolation of active compounds, phytochemistry, antioxidant activity, antimicrobial activity, natural products.

Introduction: Plants of the Lamiaceae family are well known for their rich secondary-metabolite profiles and long-standing use in traditional medicine. Many species within this family contain phenolic acids, flavonoids, terpenoids, sterols, and alkaloid derivatives that contribute to antioxidant, antimicrobial, and anti-inflammatory activities. As interest grows in discovering natural bioactive compounds suitable for pharmaceutical and bioorganic applications, the need for detailed chemical investigations of lesser-known species has become increasingly important.

Phlomoides labiosa is an understudied member of the Lamiaceae family, traditionally used in Central Asian ethnomedicine. Despite its cultural and medicinal relevance, scientific information regarding its chemical constituents and biological properties remains limited. Most existing studies on Phlomis/Phlomoides species focus on related taxa, leaving P. labiosa largely unexplored in terms of its bioorganic composition, structural diversity of its compounds, and potential therapeutic value.

Modern analytical techniques have made it possible to isolate and characterize complex natural molecules with high precision. Chromatographic methods such as column chromatography, HPLC, and GC–MS, combined with spectroscopic tools like FT-IR, UV–Vis, and NMR, provide powerful means of identifying structurally distinct phytochemicals. When supported by biological assays, these analytical approaches enable a comprehensive assessment of structure–activity relationships and the pharmacological relevance of isolated compounds.

Given the lack of systematic research on Phlomoides labiosa, there is a clear need to isolate its principal active constituents and evaluate their chemical structures and biological activities. Such work not only enhances the scientific understanding of this species but also contributes to the broader search for natural compounds with potential applications in bioorganic chemistry, drug development, and nutraceutical formulation.

The present study aims to isolate major active compounds from Phlomoides labiosa and characterize them using advanced chromatographic and spectroscopic techniques. Through an integrated

chemical and biological evaluation, this research seeks to reveal the plant's bioactive potential and provide a foundation for further pharmacological investigations.

LITERATURE REVIEW

Research on medicinal plants, particularly those belonging to the Lamiaceae family, has expanded significantly due to their diverse phytochemical composition and broad spectrum of biological activities. Foundational works on the flora of Uzbekistan by Abdullayev and Tojibaev (2012) provide essential taxonomic context for understanding regional plant diversity, including species of Phlomis and Phlomoides. Studies on medicinal plants published in Uzbekistan, such as those by Kamilov and Qodirov (2010) and Saidov and Tojibaev (2018), further emphasize the pharmacognostic value of native flora and highlight the high concentration of bioactive compounds in Lamiaceae species. Local investigations also confirm the relevance of phytochemical studies for species growing in Uzbekistan's climate, as demonstrated by Qodirov, To'xtayev, and Tursunov (2015) and Yusupov and To'layev (2016).

At the international level, the Lamiaceae family has been extensively studied for its rich secondary metabolites. Harborne's (1998) methodological framework provides the basis for modern phytochemical analysis, while Aripov and Mamadaliyev (2000) offer complementary regional perspectives on natural compound chemistry. Numerous studies indicate that species within Phlomis and Phlomoides are rich in phenolic acids, flavonoids, terpenoids, and sterols. Kahraman, Lakušić, and Doğan (2012) contributed important taxonomic clarifications for Turkish species of Phlomis and Phlomoides, establishing a foundation for comparative cytological and phytochemical investigations.

Comprehensive reviews by Ahmed, Mandal, and Ansari (2017) and Nabavi et al. (2015) outline the pharmacological relevance of Phlomis species, documenting antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities. These works support the hypothesis that species of the genus—and closely related Phlomoides—possess structurally diverse bioactive compounds with therapeutic potential.

Phenolic compounds in Lamiaceae plants have been studied extensively due to their biological properties. Barros et al. (2013) demonstrated that phenolic profiles vary depending on growth conditions and processing methods, which underscores the importance of standardized extraction and analysis. Flavonoids, a core group of plant polyphenols, have been widely recognized for their health benefits, with Kumar and Pandey (2013) providing an in-depth overview of their chemical diversity and biological activities. Similarly, Miguel (2010) highlighted the antioxidant and anti-inflammatory effects of essential oils from Lamiaceae plants, noting their role in mitigating oxidative stress.

Analytical studies on phytochemical components reveal that Lamiaceae plants are often rich in essential oils and phenolic acids. Ghasemi and Yazdanpanah (2019) identified essential oil components with strong antioxidant activity in several Lamiaceae species, reinforcing the medicinal potential of this family. The evaluation of antibacterial properties of plant extracts, as conducted by Burt (2004) and Parekh and Chanda (2007), supports the traditional use of Lamiaceae species for treating infections.

Antioxidant activity remains one of the most investigated properties of Lamiaceae-derived compounds. Foundational studies by Re et al. (1999) and Slinkard and Singleton (1977) developed widely used analytical assays (ABTS, Folin–Ciocalteu), which continue to serve as benchmarks in natural product research. Singleton, Orthofer, and Lamuela-Raventos (1999) further refined phenolic quantification methods, allowing for accurate comparison of polyphenol levels across different plant species and extraction protocols. These analytical approaches are essential for examining plants such as Phlomoides labiosa, whose bioactive potential has not yet been fully characterized.

Oxidative processes and free radical biology are also central to understanding the significance of antioxidant compounds. Halliwell and Gutteridge (2015) provide the biological framework for interpreting antioxidant assays and highlight the importance of natural antioxidant sources in preventing cellular damage. Research by Zengin, Aktumsek, and Guler (2011) on Phlomis pungens demonstrated strong antioxidant activity and significant fatty acid content, supporting the likelihood that related species such as Phlomoides labiosa also possess valuable bioactive constituents.

Extraction methods significantly influence the chemical profile and biological activity of plant extracts. Chemat, Abert Vian, and Cravotto (2012) introduced green extraction principles that promote efficient, environmentally friendly isolation of natural products—methods increasingly used in phytochemical studies worldwide.

Taken together, the existing literature highlights the substantial phytochemical richness and biological significance of the Lamiaceae family, including species of Phlomis and Phlomoides. However, despite the substantial research on related taxa, Phlomoides labiosa itself remains largely unstudied. The current

study addresses this gap by focusing on the isolation and bioorganic characterization of active compounds from P. labiosa, thereby contributing new scientific knowledge to both regional and global phytochemistry.

METHODS

Plant material of Phlomoides labiosa was collected during its flowering stage from natural habitats in Central Asia. Species authentication was performed by botanists at a regional herbarium, where a voucher specimen was prepared and deposited for reference. The collected material was washed, shade-dried at room temperature, and ground into a uniform powder for analysis.

Sequential extraction was carried out using solvents of increasing polarity. The powdered plant material was first subjected to maceration in 95% ethanol for 72 hours with intermittent shaking. The extract was filtered and concentrated under reduced pressure at 45 °C. The remaining plant residue was subsequently extracted with distilled water by boiling for 30 minutes, followed by filtration and freeze-drying. All extracts were stored at 4 °C until further processing.

Isolation of active compounds was performed using chromatographic techniques. Crude extracts were fractionated through column chromatography using silica gel as the stationary phase and solvent gradients of hexane, ethyl acetate, methanol, and water as the mobile phases. Fractions showing similar TLC patterns were pooled and concentrated. Further purification was carried out using preparative HPLC when necessary.

Chemical profiling of isolated fractions was conducted using GC–MS to identify volatile and semi-volatile molecules. Analyses were performed with an HP-5MS capillary column, using helium as the carrier gas and a programmed temperature gradient. Mass spectra were compared with reference libraries for identification. HPLC analyses were performed using a C18 reverse-phase column and a water–methanol gradient containing 0.1% formic acid, with monitoring wavelengths of 254 nm and 280 nm. Quantification of selected compounds was carried out using external standards.

Spectroscopic characterization was used to confirm structural features of the isolated compounds. FT-IR spectra were recorded between 4000 and 400 cm^{-1} to identify major functional groups. UV–Vis absorbance was measured between 200 and 800 nm to detect chromophores associated with phenolic and flavonoid compounds. Purified fractions were further analyzed using ^{1}H-NMR and ^{13}C-NMR spectroscopy for detailed molecular structure determination.

Biological activity of the isolated compounds and crude extracts was assessed through antioxidant and antimicrobial assays. Antioxidant activity was measured using DPPH and ABTS radical-scavenging assays. Absorbance values were recorded at 517 nm for DPPH and 734 nm for ABTS, and activities were expressed as IC_{50} or TEAC values. Antimicrobial activity was evaluated against selected bacterial and fungal strains using disk diffusion and minimum inhibitory concentration methods. Zones of inhibition were measured after incubation on appropriate agar media.

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

RESULTS AND DISCUSSION

Phytochemical screening of Phlomoides labiosa extracts revealed the presence of several major groups of secondary metabolites. Flavonoids, phenolic acids, terpenoids, sterols, and saponins were clearly detected in both ethanolic and aqueous extracts, while alkaloid-type compounds appeared predominantly in the ethanolic fraction. This qualitative profile is consistent with previously documented chemical patterns of Lamiaceae plants, which are often rich in polyphenolic and terpenoid constituents.

Chromatographic fractionation yielded multiple pooled fractions based on TLC similarities. GC–MS analysis of volatile and semi-volatile components identified several biologically relevant chemical classes. Major constituents included phytol, hexadecanoic acid, linoleic acid derivatives, β-sitosterol, and terpenoid-based molecules known for antioxidant and antimicrobial activities. These compounds, particularly fatty acids and terpenoids, aligned with chemical profiles reported for related Phlomis/Phlomoides species.

HPLC analysis of the purified fractions revealed high concentrations of gallic acid, quercetin, and rosmarinic acid, which are well-established antioxidant and anti-inflammatory agents. The presence of these phenolic compounds strongly supports the bioactivity potential of P. labiosa. Fractions containing these molecules demonstrated higher absorbance values in the phenolic and flavonoid quantification assays, indicating a strong enrichment of polyphenolic compounds during purification.

Spectroscopic characterizations provided additional confirmation of compound structures. FT-IR spectra showed absorption bands corresponding to hydroxyl, carbonyl, aromatic ring, and aliphatic chain functional groups, which are typical of flavonoids, phenolic acids,

and terpenoids. UV–Vis spectra demonstrated characteristic peaks associated with conjugated aromatic systems, confirming the presence of polyphenolic cores. Results from ^{1}H-NMR and ^{13}C-NMR further verified structural assignments of the major isolated compounds, including flavonoid backbones and terpenoid skeletons.

Antioxidant assessments showed that both crude extracts and isolated active fractions exhibited strong radical-scavenging activity. The ethanolic extract revealed the highest activity in both DPPH and ABTS assays, with IC_{50} values approaching those of standard antioxidants such as ascorbic acid. This superior activity is attributed to the higher solubility of flavonoids and phenolic acids in ethanol, which concentrates these compounds during extraction. Fractions rich in quercetin and rosmarinic acid displayed the strongest responses, demonstrating clear structure–activity relationships.

Antimicrobial results showed broad-spectrum activity against tested microorganisms. Staphylococcus aureus exhibited the greatest sensitivity, followed by Escherichia coli and Candida albicans. Fractions enriched in terpenoids and phenolic acids produced larger inhibition zones than the crude extracts, indicating that isolation increased biological potency. These findings align with prior research on Lamiaceae species where terpenoids and polyphenols contribute significantly to antibacterial and antifungal mechanisms.

Overall, the integration of chromatographic and spectroscopic data with biological assays demonstrates that Phlomoides labiosa contains structurally diverse molecules with notable biological activities. The combination of phenolic acids, flavonoids, and terpenoids appears to play a synergistic role in antioxidant and antimicrobial effects. The successful isolation and structural characterization of active compounds provide a scientific foundation for further pharmacological exploration and support the potential application of P. labiosa in natural product development and bioorganic chemistry.

CONCLUSION

The present study successfully isolated and bioorganically characterized the major active compounds from Phlomoides labiosa, providing the first comprehensive chemical and biological assessment of this underexplored species. Sequential extraction, chromatographic fractionation, and advanced spectroscopic analyses revealed that the plant contains a diverse range of bioactive molecules, including flavonoids, phenolic acids, terpenoids, sterols, and fatty acid derivatives. The structural confirmation of these compounds through FT-IR, UV–Vis, GC–MS, and NMR validates the reliability of the analytical approach.

Biological assays demonstrated that both crude extracts and isolated fractions possess notable antioxidant and antimicrobial activities. The ethanolic extracts and phenolic-rich fractions exhibited the strongest radical-scavenging effects, while terpenoid- and phenolic-containing fractions showed clear inhibitory action against both bacterial and fungal strains. These findings highlight the synergistic interaction of multiple compound classes, which collectively contribute to the pharmacological potential of P. labiosa.

Overall, the study establishes Phlomoides labiosa as a promising natural source of structurally diverse and biologically active compounds relevant to pharmaceutical and bioorganic chemistry applications. Future research should focus on the purification of individual constituents, detailed mechanism-of-action studies, and evaluation of in vivo bioactivity to further clarify the therapeutic potential of this species.

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