



RESEARCH ARTICLE

Studying the uterine rest of the *Lagochilus inebrians* plant extract

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Abstract

Lagochilus inebrians is a medicinal plant valued for its hemostatic diterpenoid lagochilin and large-scale extraction of this compound generates substantial amounts of mother residue whose chemical composition remains insufficiently explored. Therefore, an investigation was carried out in 2025 using plant material cultivated in the foothill regions of Navoi region, Uzbekistan, to examine in detail the chemical constituents of the mother residue remaining after lagochilin extraction from *L. inebrians*. A comparative evaluation of wild and cultivated samples demonstrated that cultivated plants contain 20–22 % more lagochilin, confirming their higher biosynthetic productivity and supporting the relevance of controlled cultivation for sustainable raw material supply. During large-scale extraction, substantial amounts of dichloroethane mother residue are generated, yet their chemical profiles remain insufficiently explored. Therefore, this work focused on isolating and characterising the secondary metabolites present in these residues. Using column chromatography, a series of fractions enriched with biologically active constituents was obtained. Structural elucidation of the isolated compounds was performed using infrared (IR) and nuclear magnetic resonance spectroscopy (NMR) spectroscopy. Two previously undescribed natural products, 5-hydroxy-7,4'-dimethoxyflavanone and 5,7-dimethoxy-1,4-naphthoquinone, were identified for the first time in *L. inebrians*. Additionally, several acetylated derivatives of lagochilin were detected, indicating the diversity of diterpenoid transformations occurring during plant metabolism and extraction processes. The discovery of these compounds highlights the chemical richness of the mother residue, which is often considered a by-product. Overall, the findings demonstrate that the extraction residues of *L. inebrians* represent a valuable and previously underutilised source of phenolic and terpenoid constituents. These metabolites may possess promising pharmacological properties and warrant further biological evaluation, expanding the potential applications of this medicinally important plant.

Keywords: column chromatography; extractives; flavonoids; lagochiline; *Lagochilus inebrians*; medicinal plants; mother residue; naphthoquinones; phytochemicals; secondary metabolites

Introduction

Plants of the genus *Lagochilus* (family Lamiaceae) have been used in folk medicine since ancient times as effective hemostatic, sedative and anti-inflammatory agents. They are widely used in the countries of Central Asia, where the flora is distinguished by the greatest species of diversity. The distribution of *Lagochilus* encompasses the Tian Shan and Pamir-Alai regions and of the 40 known species, 20 are endemic. The most studied and widespread species is *Lagochilus inebrians* (*Lagochilus* intoxicating), which grows in the Samarkand, Jizzakh and Navoi regions of Uzbekistan, as well as in several regions of Central Asia (1, 2).

The main biologically active substance of *L. inebrians* is the diterpenoid lagochilin and its acetyl derivatives. These compounds form the basis of several preparations with hemostatic activity including Lagoden, Lagovin (intravenously), Inebrin and Glylagin

(oral), as well as external formulations such as gels, gauze wipes and collagen films (3, 4). Besides lagochilin, the plant contains a variety of other secondary metabolites that may also contribute to its pharmacological properties. Pharmacological studies have shown that *Lagochilus* extracts and compounds exhibit a wide range of activities, including hemostatic, antibacterial, anti-inflammatory, antispasmodic, antihypertensive and sedative effects (5, 6). Furthermore, 5-hydroxy-7,4'-dimethoxyflavanone isolated from *L. inebrians* was found to possess pronounced anticholinesterase activity (2.18 mg galantamine equivalent/g), exceeding that of the methanol extract (6).

The chemical study of mature *L. inebrians* seeds allowed, for the first time, the characterisation of the composition of free and polar lipids, which is significant from both the biochemical and taxonomic perspectives (7). The essential oil of another species,

Lagochilus macranthus, is characterised by a high content of oxygen-containing sesquiterpenes, including caryophyllene oxide (17.52 %), humulene epoxide II (11.02 %) and viridiflorol (9.32 %) (8).

From the ethanol extract of *Lagochilus platyacanthus*, 21 compounds were isolated, including 15 flavonoids, 3 lignans, 2 iridoids and 1 phenylethanoid glycoside. Most of these compounds were described for the first time (9). The essential oil of *Lagochilus diacanthophyllus* (71 components) exhibited pronounced antimicrobial activity against Gram-positive bacteria, with α -pinene (8.7 %) and dillapiole (3.1 %) being the main components (10–12).

As part of a comparative analysis of the essential oils of three *Lagochilus* species collected in Uzbekistan, it was found that *L. inebrians* contains significant amounts of trans-chrysanthemyl acetate (9.4 %) and eugenol (7.01 %). This essential oil exhibited the highest antioxidant activity (ferric reducing antioxidant power (FRAP): 2093.33 mg TE/g) and tyrosinase inhibitory activity (130.27 mg KAE/g) (11).

Additionally, *Lagochilus setulosus* exhibited moderate antioxidant activity (54.27 mg TE/g according to 2,2-diphenyl-1-picrylhydrazyl (DPPH)), although no antibacterial effect was observed (11). The drug Setulin, containing lagochilin, at a dose of 50 mg/kg in rabbits, eliminated the hypocoagulant effect of heparin and exhibited active hemostatic properties.

Setulin, a drug containing lagochilin, at a dose of 50 mg/kg in rabbits, eliminated the hypocoagulant effect of heparin by activating the phases of blood coagulation (13).

To increase the water solubility of lagochilin, supramolecular complexes with glycyrrhizic acid were obtained. The GC-AGL (4:1) complex reduced bleeding time by 12-fold and blood loss volume by 42-fold. Based on this complex, the gel formulation “Gliligel” was developed. At a concentration of 0.25–0.5 %, Gliligel reduced parenchymal bleeding time by 2.6–2.7-fold and blood loss by 7–9-fold compared with the control group ($p < 0.01$) (14–16).

However, due to the irrational collection of *L. inebrians* (4.5 tonnes in 1957, 15 tonnes in 1959 and only 200 kg in 1962), its natural reserves have sharply decreased and the species has been included in the Red Book of Uzbekistan. In this regard, research on *in vitro* microclan propagation is relevant, as it has been shown that cultivated plants contain 17–20 % more lagochilin than wild forms (17). Although *L. inebrians* has been extensively studied for its pharmacologically active diterpenoid lagochilin, the chemical composition of the mother residue remaining after extraction has not been sufficiently investigated. Since large-scale isolation generates substantial amounts of this by-product, elucidating its phytochemical profile is important for assessing its potential as an additional source of biologically active secondary metabolites.

Materials and Methods

Lagochilus inebrians Bunge plants were cultivated for several consecutive years in the foothill regions of the Navoi region, Uzbekistan. Mature aerial parts were collected during the flowering period. For comparison, wild-growing plants from nearby natural populations were collected under identical phenological conditions. All plant materials were air-dried in the shade at room temperature and ground to a uniform particle size before extraction. The lagochilin content of both cultivated and wild samples was assessed using standard extraction and gravimetric quantification procedures.

Comparative analysis demonstrated that cultivated plants contained 20–22 % more lagochilin than wild plants, supporting their suitability as a high-yielding raw material source. Large-scale extraction of lagochilin was performed using dichloroethane as the primary solvent. After the isolation of pure lagochilin and its acetylated derivatives, a significant amount of dichloroethane mother residue remained. This residue, which contained uncharacterised secondary metabolites, was collected, concentrated under reduced pressure and used for chromatographic separation. The concentrated mother residue was subjected to column chromatography on silica gel. The concentrated mother residue was subjected to column chromatography on silica gel (60–100 mesh) packed in a glass column with dimensions of 60 × 3 cm. Elution was initiated with hexane and then continued using the appropriate solvent system. If gradient elution was employed, a stepwise gradient of hexane-ethyl acetate was applied in the following ratios: 100:0, 95:5, 90:10, 80:20 and 70:30. Fractions of 20 mL each were collected and monitored by thin-layer chromatography (TLC). Fractions showing similar TLC profiles were pooled and subjected to further purification. The initial elution was carried out using hexane, resulting in the separation of 156 fractions, which were collected sequentially based on volume and changes in elution color or TLC profile. Fractions with similar chromatographic characteristics were combined for further purification.

Thin-layer chromatography was performed on silica gel plates. Spots were visualised under UV light (254 and 365 nm) and by spraying with anisaldehyde-sulfuric acid reagent. R_f values were recorded to guide fraction pooling. Purified compounds were analysed using standard physicochemical and spectral methods, including infrared (IR) spectroscopy, proton nuclear magnetic resonance (¹H NMR) and carbon-13 nuclear magnetic resonance (¹³C NMR) spectra. Using a combination of IR and NMR spectroscopy, two previously undescribed compounds, 5-hydroxy-7,4'-dimethoxyflavanone and 5,7-dimethoxy-1,4-naphthoquinone, were identified. In addition, several acetylated derivatives of lagochilin were detected among the fractions. ¹H and ¹³C NMR spectra were recorded in CDCl₃ on a 500 MHz spectrometer using standard pulse sequences.

Results

Column chromatography of the dichloroethane mother residue obtained after lagochilin isolation yielded 156 fractions during hexane elution. Fractions with similar TLC profiles were combined into major groups and subjected to further purification. Three combined fraction groups (21–23, 29–35 and 36–40) contained pure or enriched compounds suitable for structural analysis. Fractions 21–23 yielded an oily substance with an R_f = 0.90, characterised by the presence of a strong absorption band at 1720 cm⁻¹ in the IR spectrum, corresponding to carbonyl stretching vibrations. A broad band at 3500–3100 cm⁻¹ indicated the presence of hydroxyl groups. The chemical structures of 5-hydroxy-7,4'-dimethoxyflavanone and 5,7-dimethoxy-1,4-naphthoquinone are presented in Fig. 1. The ¹H NMR spectrum exhibited multiplet signals at 3.4–3.8 ppm corresponding to protons attached to oxygenated methylene groups, a singlet at 1.8–2.2 ppm attributed to CH₂ groups; and a singlet at 1.22 ppm characteristic of the methyl groups of lagochiline (Fig. 2). Taking together, these spectral features indicated that the isolated substance was a diacetylated derivative of lagochilin. Fractions 29–35 were combined and recrystallised from petroleum ether. The resulting compound had an R_f = 0.84

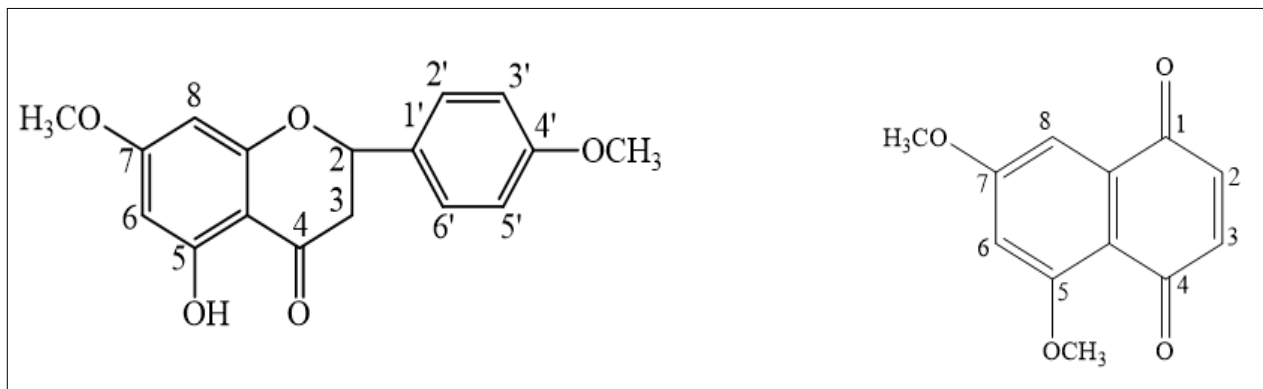


Fig. 1. Chemical structures of 5-hydroxy-7,4'-dimethoxyflavanone and 5,7-dimethoxy-1,4-naphthoquinone.

(ethyl acetate:heptane = 5:1) and a melting point of 174–175 °C. The ^1H NMR spectrum exhibited a characteristic singlet corresponding to the methoxy substituent and a proton signal at 1.0–1.4 ppm consistent with the CH_2 group of a flavonoid skeleton (Fig. 3).

In the IR spectrum, a broad absorption band at 3500–3100 cm^{-1} indicated phenolic hydroxyl groups (Fig. 4). These data confirmed the structure of the compound as 5-hydroxy-7,4'-dimethoxyflavanone, which was identified for the first time in

L. inebrians. Fractions 36–40 yielded crystalline material with $R_f = 0.44$ (benzene:ether = 3:1) and a melting point of 108–110 °C. The IR spectrum exhibited carbonyl stretching bands at 1700–1680 cm^{-1} , typical of quinones, along with aromatic C=C stretching bands at 1520–1515 cm^{-1} and deformation vibrations of aromatic C–H at 890–600 cm^{-1} (Fig. 5). In the ^1H NMR spectrum, aromatic protons appeared as a quartet between 7.0 and 7.6 ppm, while a singlet at 3.6 ppm corresponded to methoxy protons at position 5.

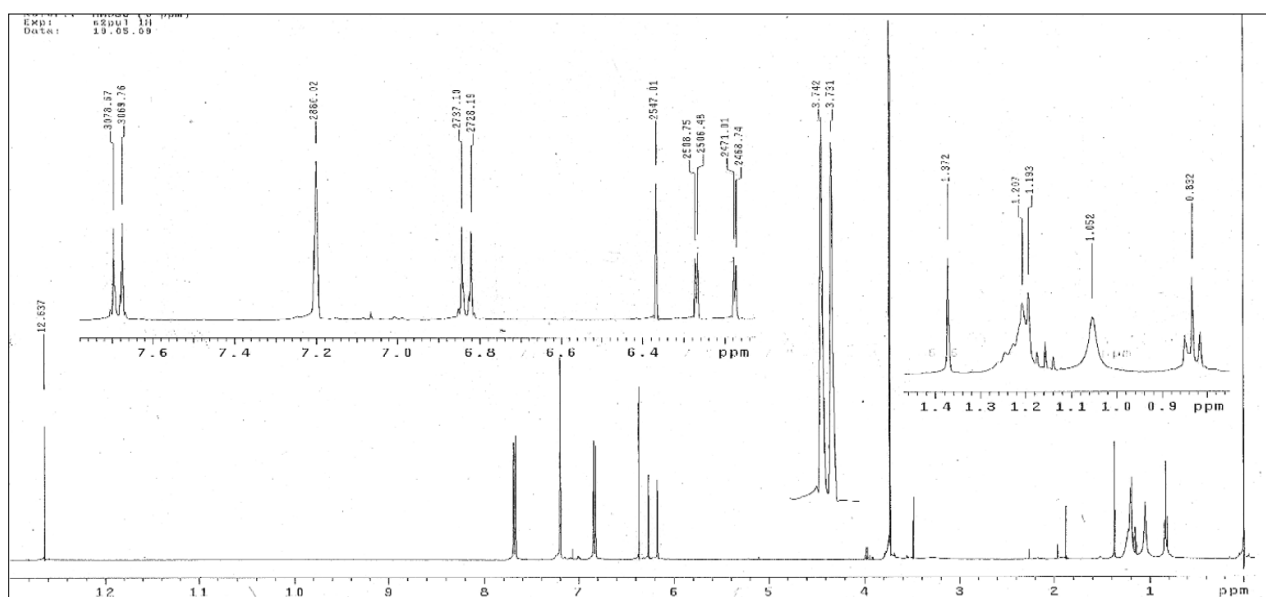


Fig. 2. NMR spectrum of the substance from fractions 21–23.

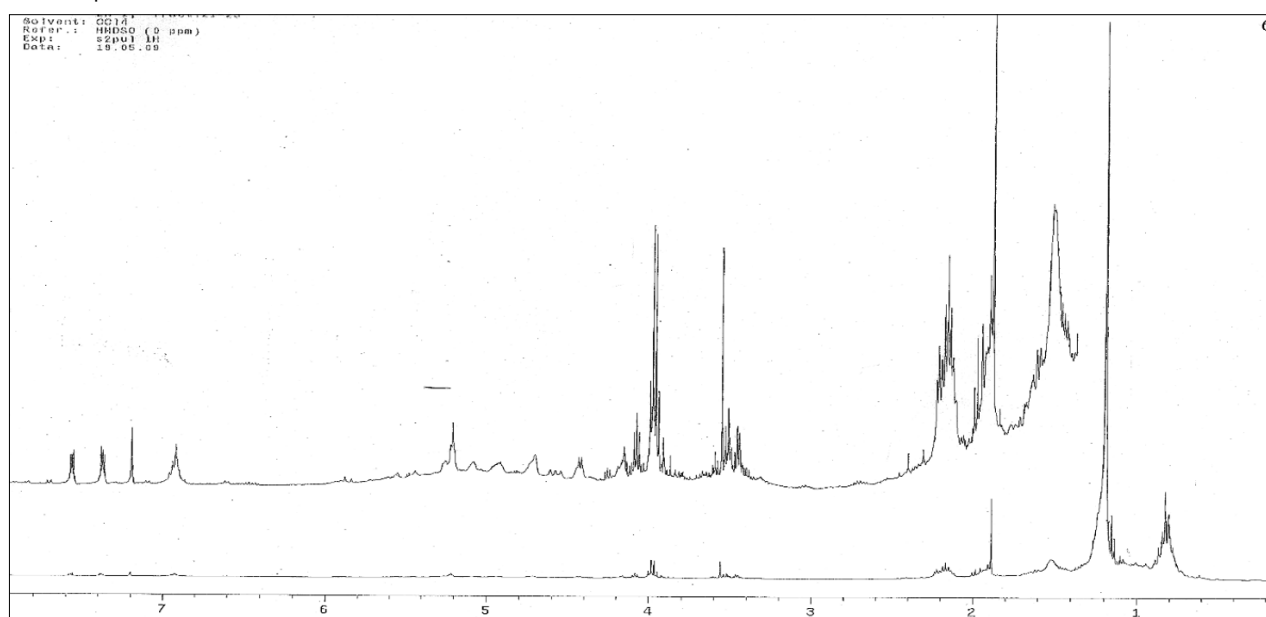


Fig. 3. NMR spectrum of the substance from fractions 29–35.

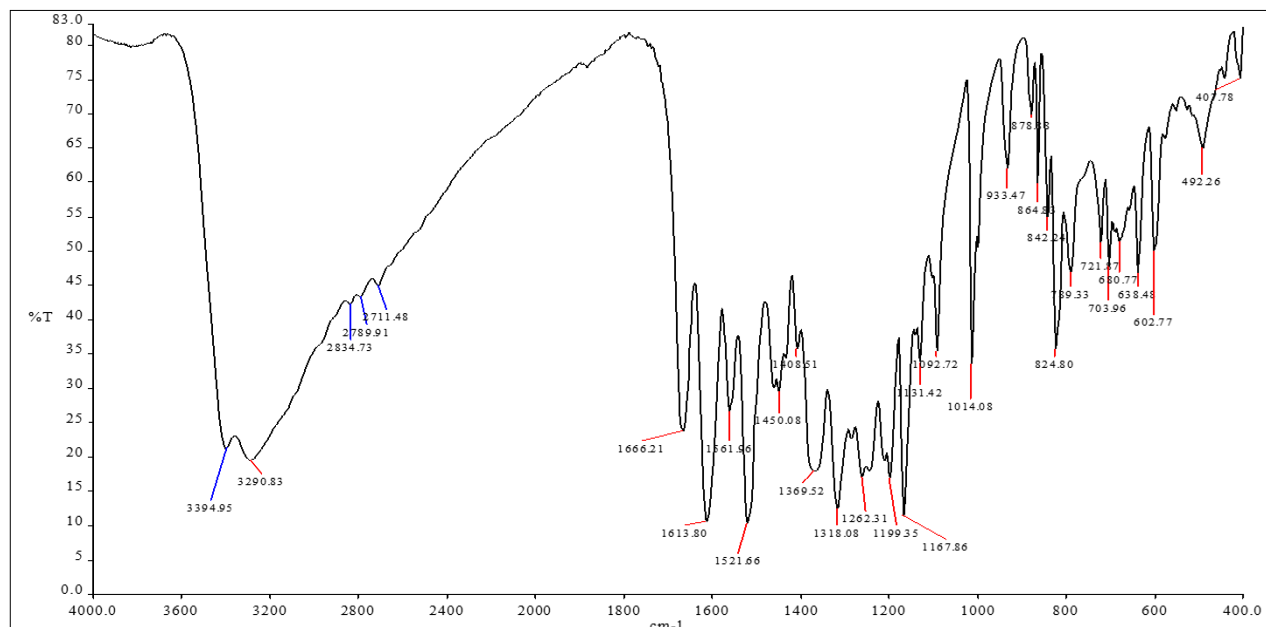


Fig. 4. IR spectrum of fraction 29–35, 5-hydroxy-7,4'-dimethoxyflavanone.

This compound was identified as 5,7-dimethoxy-1,4-naphthoquinone, which has not previously been reported in *L. inebrians*. In addition to these two identified compounds, several fractions contained acetylated derivatives of lagochilin with varying degrees of substitution. These findings indicate that the mother residue is a chemically rich by-product containing structurally diverse diterpenoids, flavonoids and naphthoquinones.

Discussion

The present study provides the first comprehensive chemical characterisation of the dichloroethane mother residue obtained after lagochilin extraction from cultivated *L. inebrians*. Although this residue is typically considered a low-value by-product, our findings demonstrate that it contains a diverse array of biologically relevant secondary metabolites. This significantly expands the known phytochemical profile of *L. inebrians* and contributes new insights into its metabolic pathways. The higher lagochilin content observed in cultivated plants compared with wild specimens (20–22% increase) is consistent with earlier reports showing that controlled cultivation improves the accumulation of diterpenoids (17). Such results support the idea that agricultural propagation of *L. inebrians* may reduce pressure

on natural populations, which have declined sharply due to historical overharvesting. At the same time, the increased biomass obtained from cultivated sources generates larger quantities of extraction residues, emphasising the need to valorise these materials. Chromatographic analysis revealed that the mother residue contains not only acetylated derivatives of lagochilin but also previously undescribed phenolic and quinone-type compounds. The identification of 5-hydroxy-7,4'-dimethoxyflavone is consistent with earlier findings that members of the genus *Lagochilus* are rich in flavonoids, which contribute to anticholinesterase, anti-inflammatory and antioxidant activities (5, 18). However, the limited reports of this flavone in *L. inebrians* may indicate chemical variation among populations or differences in biosynthetic expression and it may also be overlooked when present at low levels (16). More notably, the discovery of 5,7-dimethoxy-1,4-naphthoquinone represents a significant addition to the known chemistry of *L. inebrians*. Naphthoquinones are recognised for their broad pharmacological potential, including antimicrobial, cytotoxic and antioxidant properties (14, 19). Their presence in the extraction residue indicates that standard lagochilin preparation processes may remove or transform other active metabolites that have not yet been studied. The detection of multiple acetylated lagochilin derivatives with varying degrees of

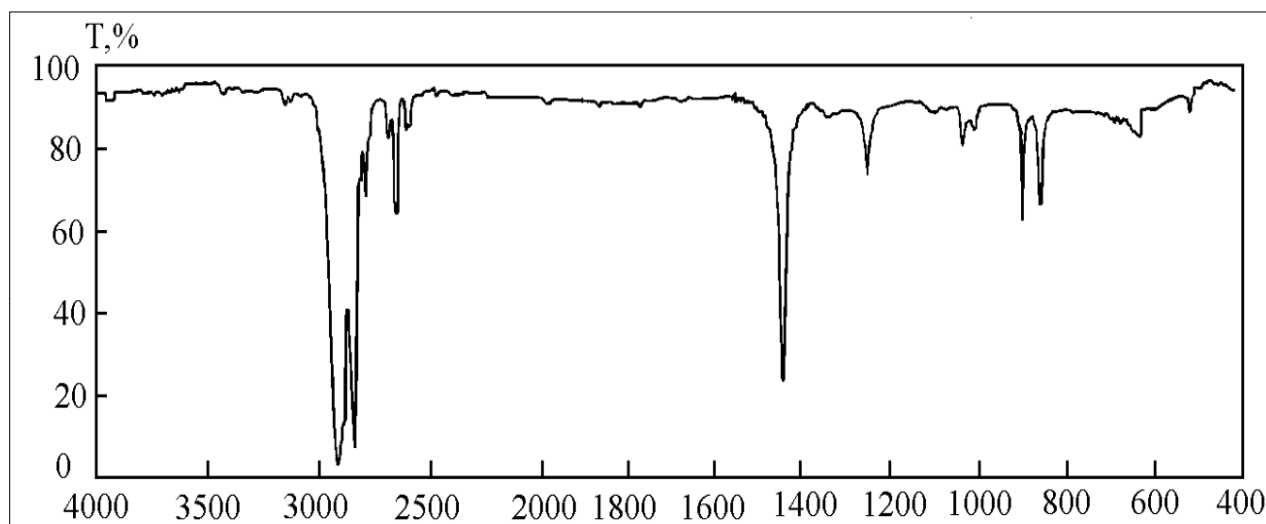


Fig. 5. IR spectrum of fraction 36–40, 5,7-dimethoxy-1,4-naphthoquinone.

substitution further highlights the structural diversity of diterpenoids in *L. inebrians*. These derivatives may arise from natural plant biosynthesis or may partially form during extraction. Regardless of their origin, their presence supports the idea that the mother residue is a valuable source of semi-synthetic or naturally occurring diterpenoids that could be explored for biological activity.

Beyond its phytochemical relevance, the valorisation of extraction by-products from *L. inebrians* has important ecological and industrial implications. Plant extraction residues are often treated as low-value waste, despite retaining structurally diverse secondary metabolites. Their further investigation and utilisation may reduce the environmental burden associated with solvent-based extraction processes and improve the overall efficiency of medicinal plant processing. From an industrial perspective, the identification of additional flavonoids, quinones and diterpenoid derivatives in the mother residue suggests that this by-product may serve as a supplementary source of biologically active compounds for future pharmacological, cosmetic, or nutraceutical applications. Thus, the rational use of extraction residues supports both sustainable resource management and the development of value-added products from medicinal plant raw materials.

Overall, the results demonstrate that the extraction by-products of *L. inebrians* contain both known diterpenoids and newly identified phenolic and quinone-type compounds. These findings emphasise the potential of mother residues as a previously overlooked resource for discovering of novel bioactive molecules. Further pharmacological evaluation of the identified compounds is warranted, as they may contribute to or even enhance the medicinal properties traditionally attributed to *L. inebrians*.

Conclusion

This study provides the first detailed chemical characterisation of the dichloroethane mother residue remaining after lagochilin extraction from cultivated *Lagochilus inebrians*. Comparative analysis confirmed that cultivated plants contain 20–22 % more lagochilin than wild-growing specimens, supporting the value of controlled cultivation as a sustainable source of high-quality raw material. Chromatographic separation of the mother residue followed by IR and NMR analyses, enabled the identification of two previously undescribed natural compounds, 5-hydroxy-7,4'-dimethoxyflavone and 5,7-dimethoxy-1,4-naphthoquinone, along with several acetylated lagochilin derivatives. The discovery of these flavonoid and naphthoquinone structures significantly expands the known phytochemical diversity of *L. inebrians* and demonstrates that extraction by-products represent an underutilised reservoir of bioactive secondary metabolites. These findings underscore the potential of mother residues as a source of new pharmacologically relevant compounds and highlight the importance of further biological evaluation of the isolated constituents. Future research should focus on assessing their hemostatic, antioxidant, antimicrobial and enzyme-modulating activities, which may contribute to the development of new therapeutics based on the chemistry of *L. inebrians*.

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Authors' contributions

ADM conceptualised the study and supervised the extraction and chromatographic procedures. EYB conducted sample preparation and data acquisition. FNT participated in spectral analyses. RSE assisted with IR and NMR data interpretation and structural elucidation. DSA supported the experimental work and contributed to the literature analysis. FAS assisted in methodology development and chemical characterisation. NSU contributed to data acquisition. URT contributed to data analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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