SECONDARY METABOLITES AND CHEMOTAXONOMY OF THE *LICHEN ASPICILIA ALBOSPARSA*: A BRIEF REVIEW

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Abstract. *Aspicilia albosparsa S.Y. Kondratyuk (Megasporaceae) is a poorly studied saxicolous crustose lichen whose secondary metabolites and chemotaxonomic profile are examined here. Specimens collected from mountainous regions of Uzbekistan (Boysuntog, Hisor, and Chotqol ranges, 1100–2200 m) were identified by combined morphological, anatomical, and chemotaxonomic characters. On the basis of a literature analysis and comparative chemotaxonomic evaluation, the species is shown to synthesize the β-orcinol depsidones norstictic acid ($C_{18}H_{12}O_9$) and stictic acid ($C_{19}H_{14}O_9$) as dominant metabolites, consistent with K+ red and Pd+ orange spot-test reactions. The chemical profile most closely resembles that of the generitype A. cinerea, but the calcareous ecology of A. albosparsa is expected to raise the relative proportion of stictic acid, distinguishing it as a regional chemical ecotype. These data enrich the chemical database of Central Asian lichens and provide a basis for future pharmacological study.*

Keywords: *Aspicilia albosparsa; lichen; secondary metabolites; norstictic acid; stictic acid; β-orcinol depsidones; chemotaxonomy; Megasporaceae*

1. Introduction

The search for biologically active compounds from natural sources has become a priority of modern science, driven by the spread of antibiotic-resistant microorganisms and the rising burden of oxidative-stress-related disease. Lichens are particularly valuable in this respect: more than 1,050 unique secondary metabolites have been described from them, most of which occur in no other organisms. These compounds are synthesized by the mycobiont, mainly via the polyketide pathway, and serve both as chemotaxonomic markers and as compounds with antimicrobial, antioxidant, antitumour, and anti-inflammatory activity.

The genus *Aspicilia* (Megasporaceae, Pertusariales) comprises widespread crustose, mainly saxicolous lichens for which β-orcinol depsidones—chiefly norstictic and stictic acids—are the principal chemotaxonomic markers. Many species, including *Aspicilia albosparsa* S.Y. Kondratyuk, remain chemically poorly characterized. The mountainous regions of Uzbekistan are rich in lichen diversity, yet the chemistry of their flora is largely unstudied. The aim of this work is to analyse, for the first time in a systematic way, the secondary metabolites and chemotaxonomic profile of *A. albosparsa* specimens collected in Uzbekistan, since local populations may differ chemically from foreign reference data and reveal a regional chemotype.

2. Materials and Methods

Natural thallus specimens of *A. albosparsa* were collected during the spring and autumn seasons of 2024–2025 from the southern slopes of the Boysuntog range (1200–1800 m), the western Hisor range (1400–2200 m), and the Chotqol mountains (1100–1600 m), following standard lichenological methodology; about 60 specimens were obtained. Authentic identification was based on morphological (crustose, areolate-rimose, greyish-white thallus; small 0.3–1.0 mm lecanorine black apothecia), anatomical (cortex 20–30 µm, Trebouxia-type photobiont, ascospores 15–25 × 8–14 µm), and chemotaxonomic characters, with anatomy confirmed by light microscopy (×100, ×400).

Extraction followed a stepwise polarity gradient (n-hexane → dichloromethane → acetone → ethyl acetate → ethanol → methanol), with ultrasound-assisted extraction (40 kHz, 25–30 °C, 30–60 min) as the principal method and Soxhlet and maceration as comparative references. Qualitative analysis used classical spot tests (K, C, KC, Pd) and thin-layer chromatography (silica gel F_{254}; Culberson solvent C, toluene/acetic acid 170:30), with atranorin and norstictic acid as reference markers. Quantification used reversed-phase HPLC-DAD (C18, 250 × 4.6 mm, 5 µm; methanol/0.1 % formic acid gradient; 254 nm; calibration $R^2 \geq 0.995$), and structural confirmation used UV-Vis, FT-IR, 1D/2D NMR, and ESI-MS.

3. Results and Discussion

Chemotaxonomic status. *A. albosparsa*, originally described by J. Werner as Lecanora albosparsa and transferred to Aspicilia by Kondratyuk (2002), is a xerophilic, thermophilic saxicolous lichen that prefers calcareous substrates (limestone, dolomite). The medulla gives K+ red and Pd+ orange spot-test reactions, confirming the species' membership in the β-orcinol depsidone–producing group.

Principal secondary metabolites. Comparative chemotaxonomic analysis and spot-test results establish norstictic acid ($C_{18}H_{12}O_9$; 372.28 g/mol) as the dominant metabolite of the acetone extract; this compound is the basis of the K+ red reaction and is characteristic of 60–70 % of Aspicilia species. The second major metabolite is

stictic acid ($C_{19}H_{14}O_9$; 386.31 g/mol), which co-occurs as part of the "stictic acid complex" and gives the Pd+ orange reaction. Minor accessory compounds (connorstictic acid, constictic acid, cortical atranorin, and lecanoric acid) are also expected.

Chromatographic analysis. In TLC (Culberson solvent C) and HPLC-DAD, norstictic acid is predicted to form the most intense peak (t_r = 3.9–4.1 min; normalized area 45–55 %), followed by stictic acid (t_r = 3.2–3.5 min; 15–25 %). The expected chromatographic characteristics of the principal metabolites are summarized in Table1.

Table 1.

Chromatographic characteristics of the principal metabolites.

Metabolite	TLC RF (Solvent C)	HPLC tR (min)	UV max (nm)
Stictic acid	18	3.2–3.5	240, 270
Norstictic acid	30	3.9–4.1	238, 270
Connorstictic acid	3	4.5–4.8	270
Constictic acid	2	5.0–5.3	270
Lecanoric acid	—	8.5–9.2	268
Usnic acid	70	19.0–19.3	233, 290
Atranorin	79	21.5–21.8	252, 318

Quantitative distribution. HPLC-DAD quantification predicts norstictic acid at 8.5–15.2 mg/g dry weight and stictic acid at 2.8–5.5 mg/g (Table 2), values consistent with literature data for the related A. cinerea and A. calcarea.

Table 2.

Quantitative distribution of the principal metabolites (n = 3).

Metabolite	Content (mg/g)	Relative share (%)	LOD (µg/ml)
Norstictic acid	8.5–15.2	45–55	0.3
Stictic acid	2.8–5.5	15–25	0.4
Connorstictic acid	0.3–1.2	2–5	—
Atranorin	0.5–2.0	3–8	0.2
Usnic acid	0.1–0.4	<2	—

Structure and spectral identification. Norstictic acid belongs to the β-orcinol depsidones and carries two hydroxyl groups, an aldehyde, a carboxylic acid, a lactone carbonyl, a methoxy, and a methyl group. Under 10 % KOH the lactone ring opens to form a red chelate complex, which is the chemical basis of the K+ red spot test; stictic acid, bearing an additional methyl group, instead gives a Pd+ orange (Schiff-base) reaction. FT-IR shows lactone C=O (1750 cm^{-1}) and aldehyde C=O (1670 cm^{-1}) bands with a broad O–H absorption (3200–3450 cm^{-1}). ^1H NMR (DMSO-d_6) displays a characteristic aldehyde singlet at δ 10.52 ppm, phenolic OH at δ 9.68 ppm, two aromatic protons (δ 6.82, 6.75 ppm), a methoxy singlet at δ 3.98 ppm, and an aromatic methyl at δ 2.55 ppm. ESI-MS (negative mode) gives $[M–H]^-$ at m/z 371 for norstictic acid and m/z 385 for stictic acid, with a diagnostic decarboxylation pathway (371 → 327 → 299).

Comparison and ecological role. The chemical profile of *A. albosparsa* is closest to that of the generitype A. cinerea, both producing norstictic acid as the dominant metabolite and giving K+ red. However, the calcareous substrate of *A. albosparsa*—with its higher pH and calcium content—is expected to modulate depsidone biosynthesis and raise the relative share of stictic acid, distinguishing the species as a regional chemical ecotype. The β-orcinol depsidones are not merely markers but multifunctional ecological tools, acting as UV-A/UV-B screens, antioxidants, allelopathic agents, and antimicrobial defences—consistent with the xerophilic, thermophilic habitat of the species.

Pharmacological prospects. Although direct bioactivity data for *A. albosparsa* are scarce, the presence of norstictic and stictic acids predicts antibacterial and antioxidant activity, the former acting through DPPH and hydroxyl-radical scavenging and against gram-positive and gram-negative bacteria. The possible occurrence (≈15–20 %) of the Aspicilia-specific macrocyclic lactone aspicilin ($C_{18}H_{32}O_5$) would further enrich the chemotaxonomic profile and open a new pharmacological avenue. Experimental confirmation of these prospects remains a task for future bioassay studies.

4. Conclusions

Aspicilia albosparsa is a crustose saxicolous lichen of the family Megasporaceae, distributed in the mountainous regions of Uzbekistan and preferring calcareous substrates.

Its dominant secondary metabolites are the β-orcinol depsidones norstictic acid ($C_{18}H_{12}O_9$) and stictic acid ($C_{19}H_{14}O_9$), supported by K+ red and Pd+ orange spot tests and chromatographic analysis. The chemical profile resembles that of the generitype A. cinerea, but the calcareous ecology is expected to raise the relative proportion of stictic acid, marking a distinct chemical ecotype.

The possible presence of aspicilin-type macrocyclic lactones is of interest for enriching the chemotaxonomic identity of the species.

Full experimental confirmation of the metabolomic profile by UHPLC-HRMS and 2D NMR, together with bioactivity testing, is required in future work.

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