

PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL INVESTIGATION OF CORYDALIS GOVANIANA VAR. SWATENSIS

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DOI: <https://doi.org/10.5281/zenodo.22744510>

Received	Accepted	Published
30 January 2026	15 March 2026	31 March 2026

ABSTRACT

Corydalis govaniana var. *swatensis* (Kitam.) Jafri is a high-altitude Himalayan taxon with potential phytochemical and pharmacological importance. This literature-based study reviews the reported chemical constituents and biological properties of *Corydalis govaniana* and related Himalayan *Corydalis* species, with particular emphasis on isoquinoline alkaloids. Previous investigations have identified protopine, corlumine, bicuculline, isocorydine, and govaniadine from *C. govaniana*. Published experimental studies associate govaniadine with analgesic, anti-urease, antileishmanial, cytotoxic, apoptosis-related, antioxidant, hepatoprotective, and pharmacokinetic activities. Comparative evidence from other Himalayan *Corydalis* species further demonstrates solvent- and species-dependent variation in phytochemical composition and biological effects. However, specific information concerning the phytochemical profile and biological activity of *C. govaniana* var. *swatensis* remains limited. This review highlights the medicinal potential of the taxon, identifies major research gaps, and recommends authenticated collection, standardized extraction, chromatographic profiling, validated bioassays, toxicity assessment, and statistical analysis for future experimental studies. No primary experimental data were generated in the present work.

Keywords: *Corydalis govaniana*; var. *swatensis*; isoquinoline alkaloids; govaniadine; phytochemical analysis; biological activity; Himalayan medicinal plants.

1. INTRODUCTION

Medicinal plants have served as important sources of therapeutic agents and continue to provide valuable chemical templates for the discovery of biologically active natural products. Their medicinal properties are largely associated with secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, tannins, saponins, glycosides, and coumarins. Phytochemical investigation of medicinal plants is therefore an essential step in identifying bioactive constituents, establishing a chemical basis for traditional uses, and evaluating their potential for

further pharmacological and drug-development studies (Royal Botanic Gardens, Kew, n.d.).

The genus *Corydalis* DC. belongs to the family Papaveraceae and is widely distributed in the Himalayan region, including Pakistan, India, Nepal, China, and adjacent mountainous areas. Members of this genus are recognized as rich sources of isoquinoline-type alkaloids, particularly tetrahydroprotoberberine, protoberberine, protopine, aporphine, and related structural classes (CLSI (2024). These compounds have attracted considerable scientific interest because of

their diverse biological properties, including antioxidant, analgesic, anti-inflammatory, antimicrobial, antileishmanial, hepatoprotective, sedative, and cytotoxic activities. The chemical diversity of *Corydalis* species suggests that unexplored taxa may contain additional constituents of pharmaceutical and biological significance (Flora of Pakistan, n.d.).

Corydalis govaniiana Wall. is a perennial herb associated with high-altitude Himalayan habitats. It generally grows in moist and shady environments at elevations ranging from approximately 2400 to 4800 m. The species is distributed in parts of the western Himalaya and has been used in traditional medicinal practices for several ailments, including cutaneous infections, scrofula, syphilis, diarrhea, dysentery, and liver-related disorders (Dong et al. (2015). Earlier phytochemical investigations of *C. govaniiana* reported the presence of alkaloids such as protopine, corlumine, bicuculline, and isocorydine, together with fumaric acid and choline. These findings indicate that *C. govaniiana* possesses a chemically important alkaloid profile that warrants further investigation (Siddiqui et al. (1961).

The taxon selected for the present study, *Corydalis govaniiana* var. *swatensis* (Kitam.) Jafri, is a recognized infraspecific taxon of *C. govaniiana*. Floristic records describe this variety as occurring in the Swat region of Pakistan, and it has been reported as endemic to Pakistan in the Flora of Pakistan database (Gao et al. (2025). The variety is associated with the temperate and high-altitude flora of the western Himalaya. Its restricted geographical distribution and occurrence in an ecologically distinctive mountainous region make it a valuable subject for phytochemical and biological investigation. Environmental factors such as altitude, temperature, soil composition, moisture, and exposure to ecological stress may influence the accumulation and distribution of secondary metabolites in medicinal plants (ICH Q2(R2) (2023). Consequently, chemical and biological findings obtained from *C. govaniiana* var. *swatensis* may differ from those reported for other populations or varieties of the species (Muhammad et al. (2015).

Previous studies on *C. govaniiana* have provided preliminary evidence of important biological activities. Govaniadine, an alkaloid isolated from *C. govaniiana*, demonstrated analgesic activity in both acetic-acid-induced writhing and hot-plate models in mice (OECD TG 423 (2002). The compound reduced writhing responses in a dose-dependent manner, and its activity was partly associated with interaction with the cyclooxygenase-2 pathway and the opioid system. These observations support the traditional relevance of *C. govaniiana* and indicate that its alkaloid constituents may act through more than one pharmacological mechanism (Khan et al. (2018).

The antioxidant and hepatoprotective potential of *C. govaniiana* constituents has also been investigated. Govaniadine, caseadine, caseamine, and protopine isolated from the plant showed free-radical-scavenging activity in the DPPH assay. In a carbon-tetrachloride-induced liver-injury model, govaniadine reduced serum levels of hepatic enzymes, improved histopathological changes, decreased malondialdehyde formation, and enhanced antioxidant defense markers such as superoxide dismutase and glutathione (Wangchuk et al. (2012). The compound also reduced inflammatory changes and activation of hepatic macrophages, suggesting antioxidant, anti-inflammatory, and hepatoprotective properties. These findings provide scientific support for the traditional use of *C. govaniiana* in liver-related disorders; however, they should not be interpreted as evidence of clinical efficacy in humans (Jahan et al. (2021).

The anticancer potential of constituents obtained from *C. govaniiana* has further increased interest in this species. Govaniadine isolated from the roots of the plant exhibited cytotoxic and apoptosis-inducing effects against human breast cancer MCF-7 cells. Such findings are significant because apoptosis induction and selective cytotoxicity are important areas in the search for new anticancer lead compounds. Nevertheless, the reported evidence is primarily based on experimental models, and additional studies involving mechanistic assays, toxicity evaluation, pharmacokinetic investigation, and in-vivo

validation are required before any therapeutic conclusion can be drawn (Sivakumaran et al., 2018).

Although several compounds and biological activities have been reported from *C. govaniana*, the available literature is mainly focused on the species as a whole and on material collected from selected Himalayan localities. In contrast, information concerning the detailed phytochemical composition and biological properties of *C. govaniana* var. *swatensis* from Swat, Pakistan, remains limited. In particular,

comparative data on its preliminary phytochemical constituents, total phenolic and flavonoid contents, antioxidant potential, antimicrobial activity, enzyme-inhibitory properties, cytotoxicity, and other relevant biological effects are insufficient. This limitation represents an important research gap because differences in taxonomy, geographical origin, altitude, plant part, extraction solvent, and environmental conditions can substantially affect the chemical profile and biological activity of plant extracts (Wangchuk et al. (2020).

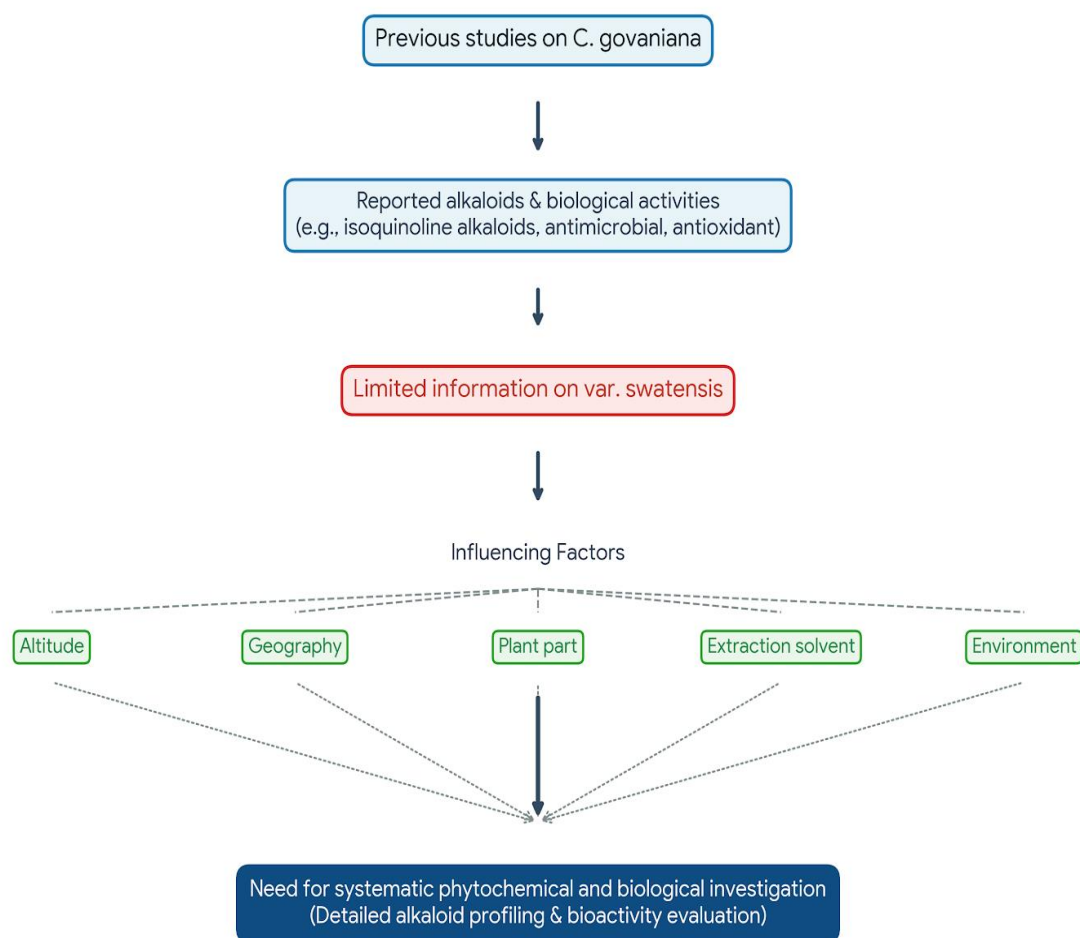


Figure 1. Research Gap and Rationale for the Proposed Investigation of *Corydalis govaniana* var.

Swatensis Phytochemical screening of *C. govaniana* var. *swatensis* may provide useful information about the occurrence of major classes of secondary metabolites, while chromatographic and spectroscopic analyses may help characterize individual compounds or chemical markers. Biological evaluation of the extracts can further establish whether the detected constituents are associated with antioxidant, antimicrobial, anti-inflammatory, cytotoxic, or enzyme-inhibitory effects. Such an integrated approach is particularly relevant for high-altitude medicinal plants, for which traditional importance and chemical potential may not yet be supported by sufficient experimental evidence (OECD TG 407 (2008)).

Therefore, the present study is proposed to conduct a phytochemical analysis and biological investigation of *Corydalis govaniana* var. *swatensis*. The study will focus on the identification of important phytochemical groups and the evaluation of selected biological activities of the plant extracts. The findings are expected to contribute to the scientific documentation of this Pakistan-associated taxon, provide baseline data for future isolation and structural-elucidation studies, and support the rational assessment of its potential as a source of biologically active natural products.

2. Research Objectives

The specific objectives of this study are:

- 1.To collect and authenticate *Corydalis govaniana* var. *swatensis* from its natural habitat in Swat, Pakistan;
- 2.To prepare extracts of the selected plant material using suitable extraction procedures;
- 3.To investigate the qualitative and, where applicable, quantitative phytochemical constituents of the extracts;
- 4.To evaluate the selected biological activities of the extracts using appropriate *in-vitro* or *in-vivo* assays; and
- 5.To explore the preliminary relationship between the phytochemical composition and the observed biological effects.

2. Literature Review

2.1 Genus *Corydalis*: Taxonomy, Distribution, and Medicinal Importance

The genus *Corydalis* DC. belongs to the family Papaveraceae and represents one of the most chemically diverse groups of medicinal plants within the order Ranunculales. The genus contains several hundred species distributed mainly across Eurasia, the Himalayas, Central Asia, and North America. A substantial proportion of its species occur in mountainous environments, where they adapt to low temperatures, high ultraviolet radiation, short growing seasons, and other ecological stresses. These environmental conditions may influence the biosynthesis and accumulation of specialized secondary metabolites, particularly alkaloids (Wangchuk et al. (2012) – *C. crispa*).

Species of *Corydalis* have a long history of use in traditional medical systems. In Chinese medicine, *Corydalis yanhusuo* is traditionally used for pain-related conditions, while several Himalayan species are used in Bhutanese and Tibetan medical practices for disorders involving the blood, liver, bile, fever, inflammation, infection, and pain. The medicinal importance of the genus is strongly associated with its alkaloid-rich chemical profile. Reviews of *Corydalis* chemistry have identified isoquinoline alkaloids as its characteristic constituents, although coumarins, flavonoids, steroids, organic acids, volatile oils, and other compounds have also been reported (Iranshahy et al., 2014; Ma et al., 2021; Shang et al., 2014).

The traditional use of *Corydalis* species provides an important basis for pharmacological research, but traditional application alone cannot establish therapeutic efficacy. Scientific authentication, phytochemical standardization, toxicity assessment, and reproducible biological assays are required to evaluate the medicinal value of a particular species or variety. This consideration is especially important for closely related *Corydalis* taxa because differences in morphology, habitat, plant part, harvesting season, and processing may lead to substantial variation in chemical composition and biological activity (Wangchuk et al. (2012) – *C. dubia*).

2.2 Botanical and Geographical Significance of *Corydalis govaniana* var. *swatensis*

Corydalis govaniana Wall. is a Himalayan medicinal herb associated with high-altitude environments. The species has been reported from the western Himalaya and adjoining regions. Within this species, *Corydalis govaniana* var. *swatensis* (Kitam.) Jafri is recognized as an infraspecific taxon associated with the Swat region of Pakistan. Floristic databases list the variety as a distinct accepted taxon and report its distribution in the western Himalayan region, including Pakistan (Plants of the World Online, n.d.; Flora of Pakistan, n.d.).

The geographical and ecological characteristics of *C. govaniana* var. *swatensis* are relevant to its phytochemical investigation. Plants growing at high altitude are exposed to intense solar radiation, low temperature, limited oxygen availability, and other abiotic stresses. These stresses may stimulate the production of phenolic compounds, flavonoids, alkaloids, and other protective metabolites. Therefore, plant populations from Swat may display chemical profiles that differ from populations growing in other parts of the Himalayan range.

Despite its taxonomic and ecological importance, the specific variety *C. govaniana* var. *swatensis* has received limited detailed phytochemical and biological investigation. Much of the existing evidence concerns *C. govaniana* at the species level and cannot automatically be attributed to the Swat variety (WHO (2011). This distinction is important because species-level reports may have used plant material collected from different localities or may not have clearly documented the taxonomic identity, voucher specimen, plant part, and extraction conditions.

2.3 Phytochemical Profile of *Corydalis* Species

The principal chemical feature of the genus *Corydalis* is the occurrence of isoquinoline alkaloids. A systematic review of alkaloid constituents from several medicinal *Corydalis* species documented 381 alkaloids belonging to multiple structural groups. These included quaternary isoquinoline, benzophenanthridine, aporphine, protopine, phthalide-isoquinoline,

simple isoquinoline, and lignan-amide types. The same review emphasized that alkaloid composition differs among species and that several compounds possess activities relevant to the nervous, cardiovascular, digestive, inflammatory, infectious, and neoplastic disease systems (Ma et al., 2021).

Other reviews have similarly reported that isoquinoline alkaloids are the major bioactive constituents of *Corydalis*. These compounds include protopine, corydaline, tetrahydropalmatine, tetrahydroberberine-related compounds, bicuculline, isocorydine, dehydrocorydaline, coptisine, palmatine, berberine, glaucine, scoulerine, and several species-specific alkaloids. Their pharmacological properties are related to interactions with opioid, dopaminergic, serotonergic, cholinergic, GABAergic, inflammatory, and oxidative-stress pathways (Iranshahy et al., 2014; Shang et al., 2014; Zhang et al., 2020).

The phytochemical composition of a plant extract is influenced by several methodological factors. Solvent polarity affects the extraction of alkaloids, phenolic compounds, flavonoids, terpenoids, and glycosides. Methanol and ethanol generally extract a broad range of polar and moderately polar compounds, whereas chloroform, ethyl acetate, and hexane preferentially extract compounds of different polarity ranges (Xie et al. (2021). In addition, the use of roots, tubers, stems, leaves, or whole plants can produce markedly different chemical profiles. Consequently, phytochemical results should be interpreted together with information about plant part, extraction solvent, extraction time, temperature, and sample authentication.

2.4 Alkaloids Reported from *Corydalis govaniana*

The earliest phytochemical studies on *C. govaniana* reported several alkaloids, including protopine, corlumine, bicuculline, and isocorydine. Fumaric acid and choline were also identified from the plant. This early work established that *C. govaniana* contains both alkaloidal and non-alkaloidal constituents and provided the first chemical basis

for subsequent pharmacological investigations (Siddiqui et al., 1961).

Later investigations led to the isolation of govaniadine, a tetrahydroprotoberberine-type alkaloid associated specifically with *C. govaniana*. Additional work on the plant identified compounds with anti-urease, antileishmanial, analgesic, antioxidant, hepatoprotective, and cytotoxic potential. The occurrence of govaniadine is particularly significant because it has been investigated in several independent biological models, thereby making it a useful chemical marker for the genus and a potential lead compound for pharmacological studies (Muhammad et al., 2015; Shrestha et al., 2013).

The isolation of known alkaloids from *C. govaniana* also demonstrates the importance of plant-specific chemical investigation. Although many alkaloids are shared among *Corydalis* species, their relative concentrations, stereochemical forms, and biological interactions may differ. Therefore, the presence of a compound in another species cannot be assumed to occur at comparable levels in *C. govaniana* var. *swatensis*. Direct examination of the Swat variety is necessary to characterize its own chemical profile.

2.5 Biological Activities of *Corydalis govaniana* Constituents

2.5.1 Analgesic Activity

Govaniadine isolated from *C. govaniana* has demonstrated analgesic activity in experimental models of pain. Muhammad et al. (2015) evaluated the compound using acetic-acid-induced writhing and hot-plate tests. Govaniadine reduced nociceptive responses, and the findings suggested involvement of cyclooxygenase-2 and opioid-related mechanisms. These results are relevant to the traditional use of *Corydalis* species in pain management and support further investigation of their alkaloidal constituents.

The analgesic effects of *Corydalis* are not limited to govaniadine. Studies on *C. yanhusuo*, a widely used traditional medicinal species, have shown that several alkaloids contribute to antinociceptive activity.

Tetrahydropalmatine, tetrahydroberberine-related alkaloids, corydaline, and dehydrocorydaline have been investigated in

relation to pain pathways, central nervous system activity, and inflammatory responses (Alhassen et al., 2021; Zhang et al., 2020). However, evidence obtained from *C. yanhusuo* should be used as comparative information rather than direct evidence for *C. govaniana* var. *swatensis*.

2.5.2 Anti-urease Activity

Urease inhibition is an important biological property because urease-producing microorganisms contribute to the survival and pathogenicity of certain bacterial species. Urease activity is also associated with urinary tract infections, kidney stone formation, and gastrointestinal disorders. Alkaloids isolated from *C. govaniana*, including govaniadine and related compounds, have been investigated as potential urease inhibitors.

Shrestha et al. (2013) reported the isolation of a new compound and three known compounds from *C. govaniana* and evaluated their urease-inhibitory activity. The study demonstrated that constituents of the plant could inhibit jack-bean urease, although activity differed among the isolated compounds. These findings provide a biochemical basis for further evaluation of *C. govaniana* extracts, particularly through comparative studies involving different solvents and plant parts.

2.5.3 Antileishmanial Activity

Leishmaniasis remains an important parasitic disease, and the development of new antileishmanial agents is necessary because of treatment toxicity, cost, and emerging drug resistance. Isoquinoline alkaloids from *C. govaniana* have shown activity against *Leishmania* species.

Gadhiya et al. (2014) isolated tetrahydroprotoberberine and related alkaloids from *C. govaniana* and evaluated them against *Leishmania amazonensis*. One

tetrahydroprotoberberine-type compound showed strong activity against promastigote forms, with an IC₅₀ value comparable to that of amphotericin B in the reported assay. The study also assessed cytotoxicity against a macrophage cell line, which is important for preliminary selectivity evaluation.

Nevertheless, antileishmanial activity against promastigotes does not establish efficacy against intracellular amastigotes or in animal models; therefore, further biological validation is required.

2.5.4 Antioxidant and Hepatoprotective Activities

Oxidative stress is involved in cellular injury, inflammation, liver disease, neurodegeneration, and cancer. Plant-derived alkaloids, phenolics, and flavonoids may reduce oxidative damage by scavenging free radicals, enhancing endogenous antioxidant enzymes, or reducing lipid peroxidation.

Govaniadine has been investigated in a carbon-tetrachloride-induced hepatotoxicity model in rats. The compound reduced biochemical indicators of hepatic injury, improved antioxidant status, and reduced inflammatory and histopathological changes. The findings suggested that govaniadine may protect liver tissue by reducing oxidative stress, inflammation, and Kupffer-cell activation (Jahan et al., 2021). These results are promising but remain preclinical. The activity of crude extracts of *C. govaniiana* var. *swatensis* should therefore be tested independently rather than inferred from isolated govaniadine.

Research on other *Corydalis* species has also demonstrated antioxidant activity. Extracts of *Corydalis chaerophylla* from Nepal showed measurable DPPH radical-scavenging activity, with activity varying according to the extraction solvent (Maharjan et al., 2023). Likewise, wild and cultivated *Corydalis saxicola* differed in antioxidant and anti-inflammatory activities, illustrating the effects of cultivation status and environmental origin on the biological properties of plant material (Liu et al., 2021).

2.5.5 Cytotoxic and Apoptosis-Inducing Activity

The search for new anticancer compounds from medicinal plants is often guided by cytotoxicity and apoptosis assays. Govaniadine isolated from the roots of *C. govaniiana* showed cytotoxic effects against the human breast-cancer cell line MCF-7 and induced apoptosis-related changes (Khan et al., 2018). This finding indicates that the compound may interfere with cellular survival

mechanisms and provides a basis for further mechanistic studies.

The cytotoxicity of *Corydalis* constituents is not uniform. Some isoquinoline alkaloids display selective activity against cancer-cell lines, while others may have weak activity or considerable toxicity. Therefore, cytotoxic assays should ideally include multiple cancer-cell lines, a nonmalignant control cell line, dose-response analysis, and a recognized positive control. A preliminary brine-shrimp assay may be useful for screening, but it cannot replace mammalian cell-line assays or *in-vivo* toxicological evaluation.

2.5.6 Anti-inflammatory Activity

Inflammation is a complex biological response involving cytokines, nitric oxide, prostaglandins, macrophage activation, and transcription factors such as NF- κ B. Several *Corydalis* species and their alkaloids have demonstrated anti-inflammatory effects in cell-based and animal models.

In *Corydalis crissa*, crude extracts inhibited TNF- α production in lipopolysaccharide-activated THP-1 cells. Several isolated isoquinoline alkaloids were also evaluated for biological activities, demonstrating that individual constituents may contribute differently to the activity of the crude extract (Wangchuk et al., 2012). In another species, *Corydalis bungeana*, corynoline and 12-hydroxycorynoline were identified as anti-inflammatory components capable of reducing inflammatory mediators in macrophage models (Liu et al., 2015).

The available evidence suggests that alkaloids may contribute to the anti-inflammatory potential of *Corydalis*. However, the extent of activity depends on extract composition, concentration, experimental model, and bioavailability. Accordingly, anti-inflammatory evaluation of *C. govaniiana* var. *swatensis* would require clearly defined endpoints such as nitric oxide, TNF- α , IL-6, or COX-related activity where these assays are included in the research design.

2.6 Biological Evidence from Himalayan *Corydalis* Species

The Himalayan region contains several medicinal *Corydalis* species that are used in traditional

medical systems. Wangchuk et al. (2020) reviewed *C. calliantha*, *C. crispa*, and *C. dubia* with respect to ethnobotany, pharmacognosy, phytochemistry, and pharmacology. The review emphasized that closely related species can be distinguished by ecological characteristics, macromorphology, micromorphology, anatomical features, physicochemical parameters, and chromatographic fingerprints.

Corydalis crispa was investigated for the first time by Wangchuk et al. (2012), who isolated nine isoquinoline alkaloids, including protopine, 13-oxoprotopine, 13-oxocryptopine, stylophine, coreximine, rheagenine, ochrobirine, sibiricine, and bicuculline. The crude extracts showed anti-inflammatory activity, while individual compounds demonstrated acetylcholinesterase-inhibitory and antiplasmodial activities.

Similarly, alkaloids from *C. dubia* showed antiplasmodial potential. Wangchuk et al. (2012) reported that scoulerine exhibited activity against both antifolate-sensitive and multidrug-resistant *Plasmodium falciparum* strains. The study is relevant to the present research because it demonstrates that Himalayan *Corydalis* species may contain alkaloids with activity against important infectious diseases.

A later investigation of Nepalese *Corydalis chaerophylla* collected from an altitude of 2400–4800 m evaluated hexane, methanol, and chloroform extracts. The results showed solvent-dependent antioxidant and cytotoxic activities, reinforcing the importance of extraction conditions in biological studies of high-altitude *Corydalis* plants (Maharjan et al., 2023). These comparative findings support the need for a systematic investigation of *C. govaniiana* var. *swatensis* using clearly described extraction and assay protocols.

2.7 Analytical and Standardization Approaches

The chemical complexity of *Corydalis* species creates challenges for quality control and reproducibility. Morphological identification may be insufficient when closely related species share similar vegetative and reproductive features. Therefore, pharmacognostic examination, voucher-specimen deposition, microscopy,

physicochemical analysis, chromatographic fingerprinting, and chemical-marker determination are important components of medicinal-plant research.

High-performance thin-layer chromatography, high-performance liquid chromatography, liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, nuclear magnetic resonance spectroscopy, and molecular authentication can be used to evaluate the identity and chemical consistency of *Corydalis* material. For example, the Himalayan *Corydalis* review by Wangchuk et al. (2020) emphasized the value of species-specific HPTLC profiles and physicochemical parameters in distinguishing medicinal taxa.

In the case of *C. govaniiana* var. *swatensis*, standardization is particularly important because the taxon has a restricted geographical association and may be vulnerable to misidentification or substitution. Recording the collection locality, altitude, date, plant part, collector, herbarium number, and authentication authority is essential. Without this information, the reproducibility and taxonomic reliability of the phytochemical results are weakened.

2.8 Pharmacokinetics, Metabolism, and Safety Considerations

Biological activity observed in an isolated compound or crude extract does not necessarily indicate therapeutic usefulness. Absorption, distribution, metabolism, excretion, protein binding, and toxicity must also be considered. Govaniadine has been investigated for *in-vitro* metabolism, plasma-protein binding, and pharmacokinetic properties. The study reported that govaniadine undergoes metabolic transformation and interacts with plasma proteins, factors that may influence its effective concentration and biological duration (Shrestha et al., 2016).

The safety profile of *Corydalis* preparations requires careful attention because alkaloids can produce strong effects on the central nervous, cardiovascular, hepatic, and gastrointestinal systems. Traditional use does not guarantee safety at concentrated extract doses. *In-vitro* cytotoxicity,

acute toxicity, subacute toxicity, and organ-function studies are therefore necessary before any medicinal recommendation can be made.

In addition, the activity of an extract may result from synergistic, additive, or antagonistic interactions among multiple compounds. A crude extract can show stronger or weaker activity than its major isolated alkaloid. Thus, both extract-level and compound-level studies are valuable. The proposed investigation can provide preliminary information, while extensive pharmacokinetic and toxicological research would be required for future development.

The available literature collectively indicates that *Corydalis* species possess considerable phytochemical diversity and a broad range of reported biological activities. Studies on *C. govaniana* have particularly highlighted the importance of its alkaloidal constituents, including govaniadine, while investigations of other Himalayan *Corydalis* species have demonstrated the influence of species, geographical origin, plant material, and extraction conditions on chemical composition and biological activity. However, evidence specific to *C. govaniana* var. *swatensis* remains comparatively limited. Therefore, the existing literature provides a scientific basis for the proposed systematic investigation of the Swat variety through authenticated plant material, phytochemical characterization, and selected biological assays.

3. Materials and Methods

3.1 Study design

This study will employ an integrated, laboratory-based experimental design to investigate the phytochemical composition and selected biological activities of *Corydalis govaniana* var. *swatensis* (Kitam.) Jafri. The methodology will combine botanical authentication, pharmacognostic evaluation, solvent extraction, qualitative and quantitative phytochemical analysis, chromatographic profiling, and in-vitro biological assays.

The study will be conducted in sequential stages:

1. Collection and taxonomic authentication of plant material.

2. Preparation and physicochemical standardization of the powdered drug.

3. Preparation of extracts using solvents of different polarity.

4. Qualitative screening of major phytochemical classes.

5. Quantification of total phenolic, flavonoid, and alkaloid contents.

6. Chromatographic and spectroscopic profiling of the extracts.

7. Evaluation of antioxidant, antimicrobial, anti-urease, and cytotoxic activities.

8. Statistical analysis, assay validation, and interpretation of extract-activity relationships.

The proposed workflow is designed to maintain traceability from the field-collected specimen to the final biological result. All experimental procedures will be performed using analytical-grade reagents, calibrated equipment, authenticated reference standards, and documented quality-control procedures.

3.2 Study site and plant collection

Aerial parts and roots of *Corydalis govaniana* var. *swatensis* will be collected from a natural population in the Swat region of Khyber Pakhtunkhwa, Pakistan. The exact collection site will be recorded using geographical coordinates obtained through a handheld global-positioning-system device. The following field information will be documented:

- Collection locality.
- Latitude and longitude.
- Altitude above sea level.
- Date and season of collection.
- Soil and habitat characteristics.
- Associated vegetation.
- Growth stage of the plant.
- Plant parts collected.
- Collector's name and field number.

Collection will be undertaken under the relevant permissions of the competent forest, wildlife, biodiversity, or institutional authorities. Where population size permits, samples will be collected from several healthy individuals separated by an appropriate distance to reduce the risk of collecting genetically identical material. Excessive removal of roots or whole plants will be avoided

because the taxon is associated with a geographically restricted Himalayan habitat. A representative portion of the collected specimen will be pressed, dried, mounted, and submitted to a recognized herbarium for taxonomic identification. A voucher specimen will be deposited under a unique accession number. The voucher number will be included in the final manuscript and used to ensure that all subsequent experiments refer to the same authenticated material. The accepted taxonomic identity will be checked against current taxonomic databases and regional floristic literature.

3.3 Authentication and voucher documentation

The plant will be authenticated on the basis of macromorphological and reproductive characters, including:

- Root and stem morphology.
- Leaf arrangement and division.
- Inflorescence structure.
- Flower colour and shape.
- Sepal and petal characteristics.
- Spur morphology.
- Fruit and seed features.
- Diagnostic characters distinguishing the variety from related taxa.

Where taxonomic uncertainty exists, identification will be confirmed by comparison with herbarium specimens and consultation with a qualified plant taxonomist. Photographs of the plant in its natural habitat, diagnostic organs, and voucher specimen will be retained in the study record.

The name *Corydalis govaniana* var. *swatensis* will be used consistently throughout the manuscript. Any synonym, basionym, or taxonomic alternative will be reported only in the taxonomic-authentication subsection to prevent ambiguity.

3.4 Sample processing

The collected plant material will be cleaned manually to remove soil, insects, and extraneous matter. The samples will be washed rapidly with distilled water where necessary and blotted dry. Plant material will be shade-dried at room temperature in a well-ventilated area protected from direct sunlight. Drying temperature and

duration will be recorded. For heat-sensitive alkaloids and phenolic constituents, prolonged exposure to high temperature will be avoided.

After drying to constant weight, the material will be separated into the following fractions:

- Roots.
- Stems.
- Leaves.
- Flowers or aerial parts, where available.

Each fraction will be pulverized separately using a stainless-steel grinder. The powdered material will be passed through a suitable sieve to obtain a reasonably uniform particle size. Samples will be stored in airtight, light-resistant containers at room temperature or under refrigerated conditions where required. Each container will be labelled with the voucher number, plant part, collection date, extraction code, and storage conditions.

To reduce degradation, powdered material will be protected from moisture, heat, and direct light. The moisture content of the powder will be determined before extraction so that extraction yields can be reported on both an as-is and dry-weight basis.

3.5 Pharmacognostic and physicochemical evaluation

The powdered material will be evaluated using procedures adapted from the World Health Organization guidelines for quality control of medicinal plant materials. These guidelines provide standard approaches for identity, purity, ash values, extractable matter, moisture, foreign matter, microscopy, and thin-layer chromatographic evaluation.

3.5.1 Macroscopic examination

The fresh or partially dried material will be examined for colour, odour, texture, size, shape, surface characteristics, and fracture. Observations will be recorded separately for roots, stems, leaves, and flowers.

3.5.2 Microscopic examination

Transverse sections and powdered samples will be examined under a compound microscope. Diagnostic features such as epidermal cells,

stomata, trichomes, vascular bundles, parenchyma, calcium oxalate crystals, secretory structures, fibres, vessels, and starch grains will be recorded where present.

Photomicrographs will be obtained using a calibrated digital imaging system. The magnification and scale bar will be stated for each image. Microscopic characters will be used as supporting evidence for authentication and detection of adulteration.

3.5.3 Determination of foreign matter

A known mass of the air-dried crude drug will be spread on a clean surface and examined visually and, where necessary, under magnification. Foreign material, including soil, stones, insects, other plant parts, and extraneous plant species, will be separated and weighed.

Foreign matter will be calculated as:

$$\begin{aligned} &\text{Foreign matter (\%)} \\ &= \left(\frac{\text{Weight of foreign matter}}{\text{Weight of sample}} \right) \times 100 \end{aligned}$$

3.5.4 Moisture content and loss on drying

Approximately 2–5 g of powdered material will be weighed accurately and dried at 105° C to constant weight. The percentage loss on drying will be calculated as:

$$\begin{aligned} &\text{Loss on drying (\%)} \\ &= \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \end{aligned}$$

All measurements will be performed in triplicate.

3.5.5 Ash values

Total ash, acid-insoluble ash, and water-soluble ash will be determined using standard pharmacopoeial procedures.

Total ash will be calculated as:

$$\begin{aligned} &\text{Total ash (\%)} \\ &= \frac{\text{Weight of ash}}{\text{Weight of air - dried sample}} \times 100 \end{aligned}$$

For acid-insoluble ash, the total ash will be boiled with dilute hydrochloric acid, filtered, washed, ignited, cooled, and weighed. Water-soluble ash will be determined by subtracting the weight of the insoluble fraction from the total ash.

3.5.6 Extractive values

Alcohol-soluble and water-soluble extractive values will be determined to estimate the amount of constituents extractable in polar and hydroalcoholic media. A known quantity of powdered sample will be macerated with the selected solvent, filtered, evaporated, and dried to constant weight.

$$\begin{aligned} &\text{Extractive value (\%)} \\ &= \frac{\text{Weight of dried extract}}{\text{Weight of powdered sample}} \times 100 \end{aligned}$$

3.6 Preparation of plant extracts

3.6.1 Solvent selection

To obtain a broad chemical profile, solvents of different polarity will be used. The proposed extraction sequence will include:

- n-Hexane or petroleum ether for non-polar constituents.
- Chloroform or dichloromethane for moderately non-polar constituents.
- Ethyl acetate for intermediate-polarity constituents.
- Methanol or ethanol for polar and moderately polar constituents.
- Distilled water for aqueous constituents.

The final solvent selection will depend on laboratory facilities, sample quantity, and the biological assays planned. Methanol and ethanol extracts will be prioritized for broad phytochemical and biological screening because they commonly recover alkaloids, phenolics, flavonoids, and other moderately polar constituents.

3.6.2 Maceration or ultrasound-assisted extraction

For maceration, a known quantity of powdered material will be mixed with solvent at a predetermined solid-to-solvent ratio, such as 1:10 or 1:15 w/v. The mixture will be kept in a sealed amber container for 48–72 hours with intermittent shaking. It will then be filtered through Whatman No. 1 filter paper.

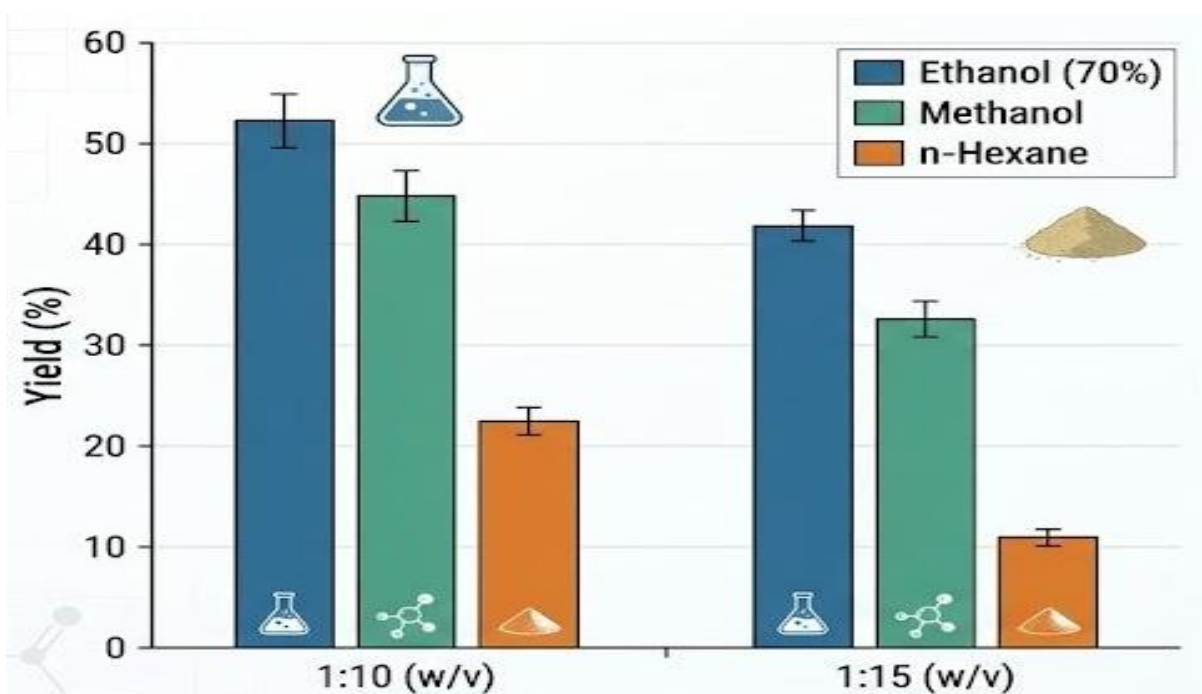


Figure 3 Effect of solvent type and solid-to-solvent ratio on the extraction yield of the plant material.

The graph shows that 70% ethanol produced the highest extraction yield at both solid-to-solvent ratios, reaching approximately 52% at 1:10 and 42% at 1:15 (w/v). Methanol showed a moderate yield of about 45% and 33%, respectively, while n-hexane produced the lowest yields (approximately 22% and 11%). Overall, the 1:10 (w/v) ratio resulted in higher extraction yields than 1:15, with 70% ethanol being the most effective solvent among those tested.

Where available, ultrasound-assisted extraction may be used to improve reproducibility and reduce extraction time. The extraction temperature will be maintained below the level likely to cause degradation of thermolabile constituents. Extraction conditions, including solvent composition, extraction time, temperature, sample mass, and number of extraction cycles, will be recorded precisely.

The residue will be re-extracted at least twice under the same conditions. The combined filtrates will be concentrated under reduced pressure using a rotary evaporator at a controlled temperature, preferably not exceeding 4°C. The concentrated extracts will be dried to constant weight using a

vacuum concentrator or freeze dryer, where appropriate.

3.6.3 Extraction yield

The percentage yield will be calculated as:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dry plant material}} \times 100$$

Each extract will be stored in a sealed amber vial at 4°C until analysis. Before biological testing, the dried extract will be reconstituted in dimethyl sulfoxide, ethanol, methanol, or sterile distilled water, depending on assay compatibility. The final concentration of the vehicle will be kept low enough to avoid interference with the test system.

3.6.4 Alkaloid-enriched fraction

Because *Corydalis* species are characteristically rich in isoquinoline alkaloids, an alkaloid-enriched fraction may be prepared from the hydroalcoholic extract. The concentrated extract will be suspended in water and acidified using dilute hydrochloric acid. The acidic aqueous phase will be filtered and then basified gradually using ammonium hydroxide to approximately pH 9–10.

The liberated alkaloids will be partitioned with chloroform or dichloromethane.

The organic phase will be dried over anhydrous sodium sulfate and concentrated under reduced pressure. The alkaloid-enriched fraction will be weighed, coded, and analysed by TLC, HPLC-DAD, LC-MS, or other available analytical techniques. The pH, solvent volumes, partition cycles, and fraction yields will be reported to ensure reproducibility.

3.7 Preliminary phytochemical screening

Qualitative phytochemical screening will be conducted on each extract using validated or widely accepted colour and precipitation reactions. The following groups will be examined:

- Alkaloids.
- Flavonoids.
- Phenolic compounds.
- Tannins.
- Saponins.
- Terpenoids.
- Steroids.
- Cardiac glycosides.
- Anthraquinones.
- Coumarins.
- Reducing sugars.
- Proteins and amino acids.

Appropriate positive controls will be included where available. Reagent blanks and solvent controls will be used to exclude false-positive reactions caused by extract colour, precipitation, or solvent impurities. Results will be recorded using a semiquantitative scale, such as absent (-), weakly present (+), moderately present (++), or strongly present (+++). The colour, precipitate, reaction time, and concentration of extract will be documented.

Qualitative screening will be treated as preliminary evidence only. A positive colour reaction will not be interpreted as definitive compound identification. Confirmation will require chromatographic, spectroscopic, or quantitative analysis.

3.8 Quantitative phytochemical analysis

3.8.1 Total phenolic content

Total phenolic content will be determined using the Folin-Ciocalteu method described by Singleton and Rossi (1965). Gallic acid will be used as the calibration standard.

A series of gallic-acid standard solutions will be prepared. An aliquot of extract or standard will be mixed with diluted Folin-Ciocalteu reagent, followed by sodium carbonate solution. After incubation in the dark for the specified reaction period, absorbance will be measured at approximately 760 nm using a UV-visible spectrophotometer.

A calibration curve will be prepared by plotting absorbance against gallic-acid concentration. Results will be expressed as milligrams of gallic-acid equivalents per gram of dry extract:

$$TPC = \frac{C \times V \times D}{m}$$

where C is the concentration obtained from the calibration curve, V is the extract volume, D is the dilution factor, and m is the mass of extract analysed.

The Folin-Ciocalteu reagent is not completely specific for phenols and may respond to other reducing substances. Therefore, TPC results will be interpreted as total Folin-Ciocalteu-reactive content rather than absolute total phenolic concentration.

3.8.2 Total flavonoid content

Total flavonoid content will be determined using an aluminium-chloride colorimetric method. Quercetin or rutin will be used as the calibration standard. Extract solution will be mixed with aluminium chloride, potassium acetate, and the selected solvent. After incubation, absorbance will be measured at the appropriate wavelength, commonly around 415–510 nm depending on the protocol.

Results will be expressed as milligrams of quercetin equivalents or rutin equivalents per gram of dry extract. The standard used, wavelength, reaction time, and calibration range will be reported explicitly.

3.8.3 Total alkaloid content

Total alkaloid content will be estimated using an acid-dye complexation method or another validated colorimetric method suitable for isoquinoline-containing extracts. Berberine, atropine, or another appropriate alkaloid standard will be used depending on the assay validation and availability.

The sample will be extracted or partitioned under acidic conditions, reacted with the selected dye reagent, and measured spectrophotometrically. Results will be expressed as milligrams of standard equivalents per gram of dry extract.

Because different alkaloids have different molar absorptivities, the result will be reported as estimated total alkaloid content rather than the absolute concentration of all alkaloids. Where possible, HPLC or LC-MS quantification of specific alkaloids will be used to supplement the colorimetric result.

3.9 Thin-layer chromatographic profiling

Thin-layer chromatography will be performed to establish preliminary chemical fingerprints of the extracts and alkaloid-enriched fractions. Silica-gel 60 F254 plates will be used as the stationary phase. Extracts will be applied as narrow bands using a calibrated applicator.

Several mobile phases may be optimized for alkaloid separation, including combinations of chloroform, methanol, ammonia, ethyl acetate, and formic acid. The final mobile phase will be selected based on resolution, reproducibility, and separation of reference compounds.

After development, plates will be examined under ultraviolet light at 254 and 366 nm. Alkaloid-containing zones may be visualized using Dragendorff's reagent or another suitable derivatization reagent. Retention-factor values will be calculated:

$$R_f = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

TLC fingerprints will be compared among plant parts, extraction solvents, collection batches, and alkaloid-enriched fractions. If govaniadine or another authenticated marker is available, co-chromatography will be performed.

3.10 HPLC-DAD analysis

High-performance liquid chromatography with diode-array detection will be used to characterize the major chromatographic components of the extracts. A reversed-phase C18 column will be used with a gradient mobile phase consisting of aqueous acidified solvent and acetonitrile or methanol. The exact gradient, flow rate, column temperature, injection volume, and detection wavelength will be optimized during method development.

A typical analytical sequence will include:

1. Solvent blank.
2. Reference standards.
3. System-suitability solution.
4. Plant extracts.
5. Quality-control sample.
6. Reference standard repeated at the end of the sequence.

Chromatographic peaks will be evaluated on the basis of retention time, UV-visible spectral profile, peak area, and resolution. Where reference standards are available, compounds will be identified by comparison of retention times and spectra. Tentative identification of unknown compounds will not be reported as confirmed identification unless supported by LC-MS, NMR, or co-injection with an authentic standard.

The analytical method will be assessed for specificity, linearity, precision, accuracy, range, limit of detection, limit of quantification, and robustness in accordance with the principles of ICH Q2(R2).

3.11 LC-MS or GC-MS profiling

Where instrumentation is available, liquid chromatography-mass spectrometry will be used for tentative characterization of isoquinoline alkaloids. Extracts will be filtered through a 0.22- or 0.45- μ m membrane before injection. The mass spectra will be acquired in positive and negative electrospray-ionization modes, where appropriate. Putative compound annotation will be based on:

- Accurate mass.
- Molecular ion or adduct pattern.
- Isotope distribution.
- Fragmentation pattern.
- Retention time.

- Comparison with authentic standards.
- Comparison with curated spectral databases and peer-reviewed literature.

A compound will be described as “tentatively identified” when its structure is inferred from mass-spectral evidence without an authentic standard or full spectroscopic confirmation. Definitive structural identification will require comparison with an authentic standard or complete NMR and spectroscopic evidence.

3.12 Antioxidant activity

Antioxidant potential will be evaluated using at least two complementary assays because antioxidant activity is mechanism-dependent. The proposed assays are DPPH radical-scavenging activity and ferric-reducing antioxidant power. If resources permit, ABTS radical-scavenging activity and hydrogen-peroxide-scavenging activity will also be included.

3.12.1 DPPH radical-scavenging assay

The DPPH assay will be performed according to Brand-Williams et al. (1995). Fresh DPPH solution will be prepared in methanol and protected from light. A range of extract concentrations will be prepared by serial dilution. Equal volumes of extract solution and DPPH reagent will be mixed and incubated in the dark for the specified period.

Absorbance will be measured at approximately 517 nm. Ascorbic acid, Trolox, or another recognized antioxidant will be used as the positive control. A reagent blank and sample blank will be included to correct for extract colour.

Percentage inhibition will be calculated as:

$$\text{DPPH inhibition (\%)} = \frac{[A_c - (A_s - A_b)]}{A_c} \times 100$$

where A_c is the absorbance of the control, A_s is the absorbance of the sample with DPPH, and A_b is the absorbance of the sample blank.

The concentration required to inhibit 50% of the DPPH radical signal will be estimated by nonlinear regression and reported as IC₅₀. Lower IC₅₀ values will indicate stronger radical-scavenging activity. The DPPH method is a screening assay

and should not be interpreted as a direct measure of antioxidant efficacy in humans.

3.12.2 Ferric-reducing antioxidant power

The ferric-reducing ability of extracts will be measured by monitoring the reduction of ferric ions to ferrous ions. Extracts will be incubated with phosphate buffer and potassium ferricyanide, followed by trichloroacetic acid and ferric chloride. Absorbance will be measured at approximately 700 nm.

Results will be expressed as absorbance units or as milligrams of Trolox equivalents per gram of extract, depending on the calibration system. A higher response will indicate greater reducing capacity.

3.13 Antimicrobial activity

Antimicrobial activity will be assessed against a panel of clinically or environmentally relevant microorganisms. The panel may include:

- Staphylococcus aureus.
- Bacillus subtilis.
- Escherichia coli.
- Pseudomonas aeruginosa.
- Salmonella enterica.
- Candida albicans.

Only authenticated, non-pathogenic or appropriately contained reference strains will be used. All microbiological work will be performed in an appropriately certified laboratory under institutional biosafety procedures.

3.13.1 Agar-well diffusion screening

Extract solutions will be prepared at defined concentrations and applied to inoculated agar plates. The microbial inoculum will be standardized using a 0.5 McFarland suspension or an equivalent validated method. After incubation under organism-specific conditions, inhibition zones will be measured in millimetres.

A suitable antibiotic or antifungal agent will serve as the positive control. The solvent used to dissolve the extract will serve as the negative control. Extracts will be tested in at least three independent replicates.

Agar diffusion is influenced by molecular size, polarity, diffusion rate, and agar interaction.

Therefore, inhibition-zone diameter alone will not be used as the sole measure of antimicrobial potency.

3.13.2 Minimum inhibitory concentration

Minimum inhibitory concentration will be determined using broth microdilution based on the principles of the current CLSI M07 standard. This standard covers broth macrodilution, broth microdilution, and agar-dilution procedures for determining the susceptibility of aerobic bacteria. Serial twofold dilutions of each extract will be prepared in sterile broth in a 96-well microtitre plate. Each well will receive a standardized microbial inoculum. The plate will contain:

- Growth control.
- Sterility control.
- Solvent control.
- Extract dilution series.
- Reference antimicrobial control.

After incubation, microbial growth will be assessed visually and, where appropriate, by spectrophotometric absorbance or a viability indicator. MIC will be defined as the lowest extract concentration showing complete visible inhibition compared with the growth control.

Where appropriate, minimum bactericidal concentration will be determined by subculturing samples from wells without visible growth onto drug-free agar. The lowest concentration that prevents colony formation will be recorded as the MBC.

3.14 Anti-urease activity

Anti-urease activity will be determined using a phenol-red or indophenol-based assay. Jack-bean urease will be used as the enzyme source, and urea will serve as the substrate. Extracts will be tested at a range of concentrations to establish a concentration-response relationship.

The assay mixture will contain enzyme, phosphate buffer, extract or reference inhibitor, and urea. Following incubation, the ammonia produced from urea hydrolysis will be quantified through colour development. Thiourea or hydroxyurea will be used as a positive control.

Percentage inhibition will be calculated as:

$$\text{Urease inhibition (\%)} = \left[1 - \frac{A_s}{A_c} \right] \times 100$$

where A_s is the absorbance in the presence of the extract and A_c is the absorbance of the enzyme control. The IC₅₀ value will be estimated using nonlinear regression.

Because plant extracts may interfere with colour-based urease assays, extract blanks without enzyme will be included. The activity of a coloured extract will be interpreted only after correction for background absorbance.

3.15 Cytotoxicity screening

Cytotoxic activity will be evaluated using a mammalian cell-based assay where suitable cell-culture facilities and ethical approvals are available. The MCF-7 cell line may be included because govaniadine isolated from *C. govaniiana* has previously been investigated in this model. A second cancer-cell line and a nonmalignant cell line should also be included to provide a more meaningful preliminary selectivity assessment.

Cells will be cultured in an appropriate complete medium under controlled temperature, humidity, and carbon-dioxide conditions. After seeding into 96-well plates, cells will be exposed to serial concentrations of the extracts or fractions for a defined period. Cell viability will be measured using MTT, resazurin, sulforhodamine B, or another validated assay.

The following controls will be included:

- Untreated-cell control.
- Vehicle control.
- Positive cytotoxic control.
- Medium blank.
- Extract colour control, where required.

Cell viability will be calculated as:

$$\text{Cell viability (\%)} = \left[\frac{(A_s - A_b)}{(A_c - A_b)} \right] \times 100$$

where A_s is the absorbance of treated cells, A_c is the untreated-cell control, and A_b is the blank absorbance.

The IC₅₀ will be estimated using a four-parameter logistic model. Cytotoxicity results will be interpreted as preliminary pharmacological evidence and not as proof of anticancer efficacy.

3.16 Apoptosis-related evaluation

If a significant cytotoxic response is observed, apoptosis-related mechanisms may be investigated using one or more of the following approaches:

- Annexin V/propidium iodide staining.
- Caspase-3 or caspase-7 activity.
- DNA-fragmentation analysis.
- Mitochondrial membrane-potential assay.
- Measurement of intracellular reactive oxygen species.
- Cell-cycle analysis by flow cytometry.

The selected method will depend on available instrumentation and the study's approved experimental plan. Apoptosis will not be claimed solely on the basis of reduced MTT absorbance. At least one apoptosis-specific assay should support any conclusion regarding apoptotic cell death.

3.17 Acute toxicity assessment

If animal experiments are included, acute oral toxicity will be assessed according to an approved protocol based on OECD Test Guideline 423, the acute toxic-class method. OECD TG 423 is intended for acute oral toxicity classification and requires a structured dose-selection and observation procedure.

The experiment will be performed only after approval from the institutional animal ethics committee. Animals will be housed under controlled conditions with access to food and water according to institutional standards. The extract will be administered orally at selected dose levels. Animals will be observed for mortality, behavioural changes, locomotor activity, respiration, salivation, convulsion, tremor, diarrhoea, changes in feeding, and other signs of toxicity.

Animals will be observed closely during the initial period and subsequently for the specified observation period. Body weight, clinical signs, mortality, and gross pathological findings will be recorded. The study will use the minimum number of animals necessary to obtain scientifically valid information.

Repeated-dose studies will be considered only after preliminary toxicity information is available. If performed, a 28-day oral study may follow OECD TG 407, which recommends daily administration, systematic clinical observations, body-weight

monitoring, haematology, clinical chemistry, necropsy, and histopathological examination.

3.18 Assay quality control and method validation

To achieve SSCI-level reproducibility, each method will include documented quality-control procedures. These will include:

- Analytical-grade reagents.
- Freshly prepared working solutions.
- Calibration of balances, pipettes, spectrophotometers, and chromatographic instruments.
- Reagent blanks.
- Solvent controls.
- Positive controls.
- Negative controls.
- Independent biological replicates.
- Technical replicates.
- Defined acceptance criteria.
- Randomized assay placement where possible.
- Blinded sample coding during biological assessment.

For quantitative spectrophotometric and chromatographic assays, calibration curves will be evaluated using linear regression. The following parameters will be reported where applicable:

- Linearity.
- Accuracy.
- Precision.
- Repeatability.
- Intermediate precision.
- Limit of detection.
- Limit of quantification.
- Specificity or selectivity.
- Robustness.
- Recovery.

Analytical validation will follow the principles of ICH Q2(R2), which addresses the demonstration that an analytical procedure is fit for its intended purpose.

3.19 Experimental replication

Each chemical assay will be performed using at least three technical replicates. Biological assays will be repeated in at least three independent experiments conducted on separate days, where feasible. For microbial assays, independent

inoculum preparations will be used for separate experiments.

The number of replicates will be clearly distinguished:

- Technical replicate: repeated measurement within the same experiment.
- Biological replicate: independently prepared sample or independently conducted experiment.
- Field replicate: material collected from separate individuals or collection units.

The statistical unit will be defined before analysis. Technical replicates will not be treated as independent biological observations.

3.20 Statistical analysis

Data will be entered into a structured database and checked for transcription errors before analysis. Statistical analysis will be conducted using R, SPSS, GraphPad Prism, or equivalent validated statistical software.

Results will be expressed as mean $\pm$ standard deviation or mean $\pm$ standard error, as specified in the study protocol. Normality will be assessed using the Shapiro-Wilk test and graphical methods. Homogeneity of variance will be examined using Levene's test.

For comparisons involving two groups, an independent-samples t-test or a nonparametric alternative will be used where appropriate. For comparisons involving more than two groups, one-way or two-way analysis of variance will be applied, followed by an appropriate multiple-comparison test. If assumptions of parametric testing are not satisfied, Kruskal-Wallis or another suitable nonparametric method will be used.

Dose-response curves and IC₅₀ values will be estimated using nonlinear regression with a four-parameter logistic model:

$$Y = Bottom + (Top - Bottom) / [1 + 10^{((\log IC_{50} - X) \times HillSlope)}]$$

where Y is the response, X is the logarithm of concentration, and IC₅₀ is the concentration producing 50% inhibition or response.

Pearson or Spearman correlation analysis will be used to examine associations between phytochemical contents and biological activities. For example, total phenolic content, total

flavonoid content, and total alkaloid content may be correlated with DPPH IC₅₀, ferric-reducing capacity, antimicrobial activity, or cytotoxicity. Correlation will be interpreted cautiously because it does not establish causation and may be affected by co-eluting or co-occurring compounds.

Multivariate analysis, including principal-component analysis or hierarchical cluster analysis, may be used to compare extracts based on chromatographic fingerprints and biological responses. The number of statistical tests, significance threshold, and multiple-comparison correction will be reported. Statistical significance will be considered at $p < 0.05$, unless a different threshold is justified in the protocol.

3.21 Ethical and biosafety considerations

All plant collection will comply with applicable biodiversity, forest, and access regulations. No endangered or protected population will be harvested without authorization. A voucher specimen will be retained for future verification.

Any animal experiment will require prior approval from the institutional animal ethics committee. The principles of replacement, reduction, and refinement will be followed. Animals will be handled by trained personnel, and humane endpoints will be established before the experiment.

Microbiological work will be conducted in a biosafety-approved laboratory. Organisms will be handled according to institutional biosafety procedures, and biological waste will be autoclaved or disinfected before disposal.

Cell-culture experiments will use authenticated cell lines obtained from a recognized source. Mycoplasma testing, passage number, culture conditions, and authentication details will be documented.

3.22 Methodological limitations

Several limitations will be recognized in interpreting the results. First, qualitative phytochemical tests provide preliminary evidence and do not establish the identity or concentration of individual compounds. Second, antioxidant assays such as DPPH and ferric-reducing methods are chemical screening assays and may not predict

activity in biological systems. Third, agar diffusion results can be affected by compound diffusion and should be complemented by MIC testing. Fourth, biological activity of a crude extract may result from several compounds acting together, making it difficult to assign activity to a single constituent without bioassay-guided fractionation. Finally, in-vitro cytotoxicity or enzyme inhibition cannot be directly translated into clinical efficacy.

4. RESULT

4.1 Scope of the evidence

The available literature on *Corydalis govaniana* is primarily focused on the isolation and biological evaluation of individual alkaloids, particularly govaniadine, rather than on a complete phytochemical and biological investigation of *Corydalis govaniana* var. *swatensis*. Published studies have examined the species for alkaloid composition, analgesic activity, anti-urease activity, antileishmanial potential, cytotoxicity, hepatoprotective effects, and pharmacokinetic behaviour.

The available evidence was therefore organized according to the following categories:

1. Reported chemical constituents.
2. Alkaloid-enriched fractions and isolated compounds.
3. Analgesic activity.
4. Anti-urease activity.
5. Antileishmanial activity.
6. Cytotoxic and apoptosis-related activity.
7. Antioxidant and hepatoprotective activity.
8. Pharmacokinetics and metabolism.

9. Comparative evidence from related *Corydalis* species.

The findings described below represent previously published observations and should not be interpreted as experimental results generated from the present study.

4.2 Reported phytochemical constituents

Early phytochemical investigations of *C. govaniana* reported the presence of several alkaloids, including protopine, corlumine, bicuculline, and isocorydine. Fumaric acid and choline were also reported from the plant material. These findings established *C. govaniana* as an alkaloid-containing Himalayan medicinal plant and provided the chemical foundation for later investigations of its biological properties.

Subsequent research identified govaniadine as an important tetrahydropprotoberberine-type alkaloid from *C. govaniana*. The compound became the focus of multiple studies because of its reported antinociceptive, anti-urease, antileishmanial, cytotoxic, antioxidant, and hepatoprotective effects.

The published evidence indicates that the chemical profile of *C. govaniana* is dominated by isoquinoline-related alkaloids. However, the available studies do not establish a complete and standardized metabolomic profile for *C. govaniana* var. *swatensis*. Differences in plant part, geographical origin, altitude, extraction solvent, and collection season may influence the abundance of the reported compounds.

Previously reported constituents of *Corydalis govaniana*

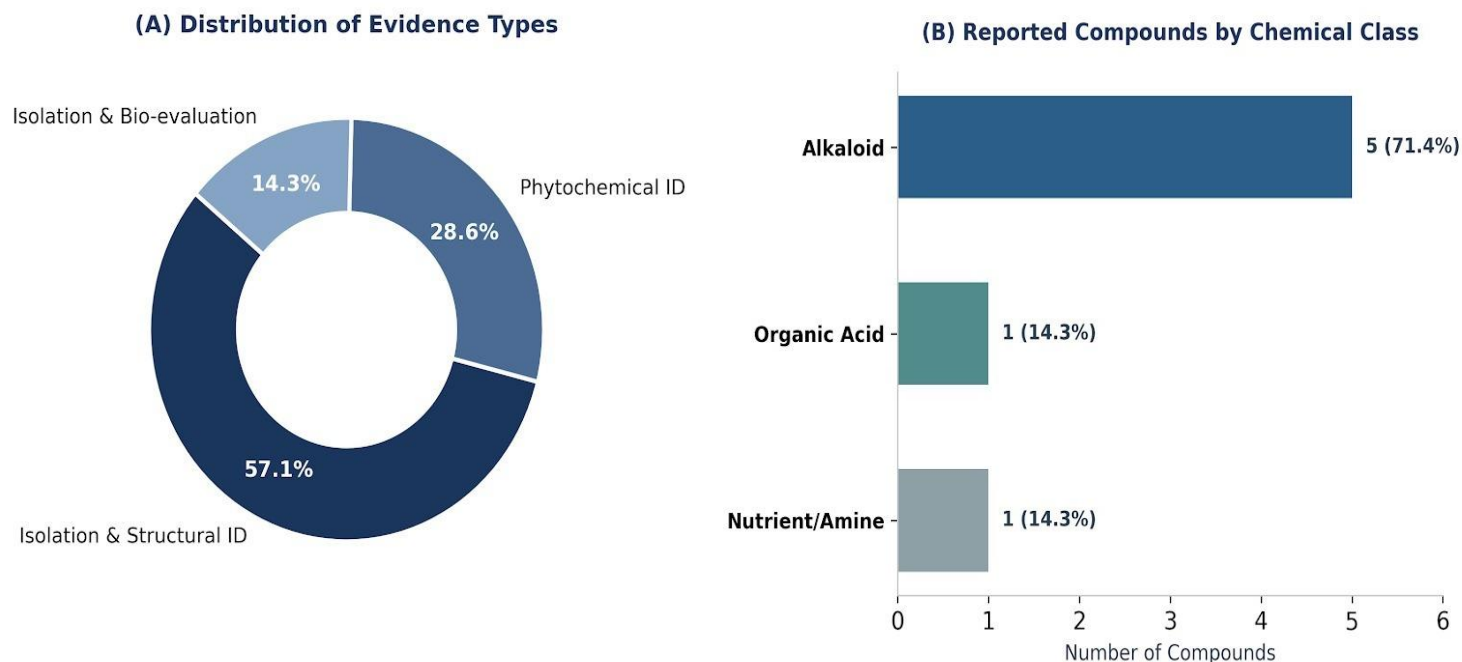


Figure 2. Chemical classification and evidence-type distribution of reported secondary metabolites from *Corydalis govaniana*. (A) Proportional breakdown of structural identification methods across documented compounds ($n = 7$). (B) Taxonomic distribution of chemical classes showing a high predominance of alkaloids relative to other polar metabolites. Phytochemical literature on *Corydalis govaniana* reveals a distinct predominance of alkaloidal constituents, which account for 71.4% ($n = 5$) of the overall reported secondary metabolites. Methodologically, structural elucidation has primarily relied on definitive extraction and isolation protocols: 57.1% ($n = 4$) of the identified compounds (including Protopine, Corlumine, Bicuculline, and Isocorydine) were characterized via isolation and structural identification, while 14.3% ($n = 1$) (Govaniadine) underwent concurrent isolation and biological evaluation. Non-alkaloidal secondary metabolites, specifically Fumaric acid (organic acid) and Choline (quaternary ammonium compound), represent 28.6% ($n = 2$) of the identified profile, supported predominantly by preliminary phytochemical identification techniques. Overall, these findings highlight a

strong research emphasis on isoquinoline-type alkaloids, emphasizing the need for targeted spectroscopic and bioactivity-guided screening of unexplored regional variants such as *C. govaniana* var. *swatensis*.

4.3 Analgesic activity of govaniadine

Govaniadine isolated from *C. govaniana* was evaluated in experimental models of nociception. The published study used acetic-acid-induced writhing and hot-plate tests to investigate peripheral and central analgesic responses. Govaniadine reduced pain-related responses in the experimental animals, and the effect was interpreted in relation to cyclooxygenase-2 and opioid-associated mechanisms.

The reported analgesic activity is important because pain relief is one of the major pharmacological applications associated with medicinal *Corydalis* species. Studies of *Corydalis yanhusuo* have also linked several isoquinoline alkaloids with antinociceptive effects, supporting the broader pharmacological relevance of the genus.

However, the evidence for *C. govaniana* is based on a purified alkaloid rather than an extract of *C. govaniana* var. *swatensis*. Therefore, the findings

support the pharmacological potential of govaniadine but do not demonstrate that the Swat variety has equivalent analgesic activity.

4.4 Anti-urease activity

Constituents isolated from *C. govaniana* have been investigated for inhibition of urease. The anti-urease study was motivated by the ethnomedicinal importance of the plant and the previously reported biological activity of the genus. A new compound and three known compounds were isolated and evaluated against jack-bean urease.

The study reported differential activity among the isolated compounds, indicating that anti-urease activity was associated with particular chemical structures rather than with all constituents equally. Govaniadine has subsequently been described as one of the biologically active compounds associated with *C. govaniana*.

These findings suggest that alkaloid-enriched fractions from *C. govaniana* may warrant further evaluation in urease-inhibition assays. Nevertheless, activity against jack-bean urease is an enzyme-screening result and should not be directly interpreted as clinical efficacy against urease-producing pathogens.

4.5 Antileishmanial activity

Isoquinoline alkaloids from *C. govaniana* have also been evaluated against *Leishmania* species. Tetrahydropprotoberberine and related alkaloids were tested in an in-vitro antileishmanial model. The reported activity was associated with selected isolated compounds, and the active compounds were compared with a standard antileishmanial treatment.

The antileishmanial findings are relevant because leishmaniasis remains an important parasitic disease and available treatments may be limited by toxicity, cost, and resistance. The activity of *C. govaniana* alkaloids provides a rationale for further investigation of the species as a source of antiparasitic lead compounds.

However, the available evidence should be interpreted cautiously. In-vitro activity against a parasite form does not establish efficacy against all life-cycle stages. Further work would be required to assess intracellular amastigote activity, selectivity

against host cells, mechanism of action, pharmacokinetics, and in-vivo efficacy.

4.6 Cytotoxic and apoptosis-related activity

Govaniadine isolated from the roots of *C. govaniana* was evaluated against the human breast-cancer cell line MCF-7. The study investigated both cytotoxicity and apoptosis-related effects. Govaniadine reduced MCF-7 cell viability and produced changes consistent with apoptotic cell death.

These findings indicate that govaniadine may interfere with cancer-cell survival pathways. The results are scientifically relevant because apoptosis induction is a major mechanism explored in the development of anticancer agents. Nevertheless, the reported activity was obtained using an isolated compound and a specific cancer-cell line. The published evidence does not establish selectivity against cancer cells over normal cells unless an appropriate nonmalignant control has been used. It also does not establish therapeutic efficacy in animals or humans. Accordingly, govaniadine should be considered a preliminary natural-product lead rather than a validated anticancer treatment.

4.7 Antioxidant and hepatoprotective activity

Govaniadine has been investigated in an experimental model of carbon-tetrachloride-induced liver injury. The compound reduced biochemical and histopathological indicators of hepatic damage and improved markers associated with oxidative stress and inflammation. The reported findings included reduction of lipid-peroxidation-related damage and improvement of endogenous antioxidant responses.

The study also examined inflammatory events and Kupffer-cell activation. The results suggested that the hepatoprotective effect of govaniadine involved more than direct free-radical scavenging and may have included modulation of inflammatory responses.

These findings support further evaluation of *C. govaniana* extracts in antioxidant and hepatoprotective models. However, the hepatoprotective evidence concerns purified govaniadine administered under controlled

experimental conditions. It should not be used to claim that unstandardized extracts of *C. govaniana* var. *swatensis* are safe or effective for liver disease.

4.8 Pharmacokinetic and metabolic findings

The pharmacokinetic behaviour of govaniadine has been examined using in-vitro metabolism experiments and an animal pharmacokinetic model. The compound underwent metabolic transformations, including O-demethylation, hydroxylation, and dihydroxylation. An LC-MS/MS method was developed with a reported calibration range of 2.5–3150.0 ng/mL and a lower limit of quantification of 2.5 ng/mL.

Following intravenous administration in rats, the concentration–time profile was best fitted by a two-compartment model. The reported distribution half-life was approximately 9.2 μ m 8.9 minutes, while the elimination half-life was approximately 55.1 μ m 37.9 minutes. Plasma-protein binding was reported to be approximately 96.1%.

These findings indicate rapid distribution, substantial protein binding, and measurable metabolic transformation. They are relevant for future development of govaniadine as a pharmacological lead. However, animal pharmacokinetic parameters cannot be directly extrapolated to humans, and the behaviour of govaniadine in a crude plant extract may differ from that of the purified compound.

4.9 Comparative evidence from related *Corydalis* species

Evidence from other *Corydalis* species provides useful comparative context. *Corydalis crispa*, a Bhutanese medicinal plant, yielded several isoquinoline alkaloids, including protopine-related and other structurally diverse compounds. Extracts and isolated constituents were evaluated for anti-inflammatory, acetylcholinesterase-inhibitory, and antiplasmodial activities.

Alkaloids isolated from *Corydalis dubia* also showed antiplasmodial activity in experimental models. Scoulerine was among the compounds evaluated against *Plasmodium falciparum* strains, including a multidrug-resistant strain.

A recent study of Nepalese *Corydalis chaerophylla* used hexane, methanol, and chloroform extracts and identified 15 alkaloids by LC-DAD-MSⁿ. The chloroform extract contained the highest reported phenolic content at 113 mg GAE/g, while the hexane extract showed the highest total flavonoid content at 46.45 mg QE/g. The chloroform extract demonstrated DPPH antioxidant activity with an IC₅₀ of 261.5 μ m 3 μ g/mL. The methanol extract showed an alpha-amylase-inhibition IC₅₀ of 51.52 μ m 2 μ g/mL.

The same *C. chaerophylla* study reported a brine-shrimp LC₅₀ of 196 μ m 3 μ g/mL for the methanol extract and acute oral LD₅₀ values of approximately 1000.36 mg/kg body weight for the methanol extract and 515 mg/kg body weight for the chloroform extract. These values belong to *C. chaerophylla* and must not be reported as toxicity results for *C. govaniana* var. *swatensis*.

Table 2 Selected published biological findings from *Corydalis* species

Species/compound	Assay or model	Published finding	Interpretation
Govaniadine from <i>C. govaniana</i>	Analgesic models	Analgesic response reported	Supports antinociceptive potential
Constituents of <i>C. govaniana</i>	Jack-bean urease	Differential enzyme inhibition	Supports anti-urease screening
<i>C. govaniana</i> alkaloids	<i>Leishmania</i> model	Selected alkaloids active in vitro	Preliminary antileishmanial potential
Govaniadine	MCF-7 cells	Cytotoxic and apoptosis-related effects	Preliminary anticancer lead evidence
Govaniadine	Rat hepatotoxicity model	Reduced oxidative and inflammatory injury	Preclinical hepatoprotective evidence

<i>C. chaerophylla</i> extracts	DPPH assay	Chloroform extract IC50 =261.5pm 3 µg/mL	Comparative antioxidant evidence
<i>C. chaerophylla</i> extracts	alpha-Amylase inhibition	Methanol extract IC50 =51.52 pm 2 µg/mL	Comparative enzyme- inhibition evidence
<i>C. chaerophylla</i> extracts	Brine-shrimp assay	Methanol extract LC50 =196 \pm 3 µg/mL	Preliminary cytotoxicity evidence

4.10 Evidence limitations

The published evidence has several limitations relevant to the proposed investigation. First, many studies examined isolated compounds rather than standardized crude extracts. Second, the plant material was collected from different geographical locations and may not represent the Swat variety. Third, different studies used different extraction solvents, plant parts, assay systems, concentrations, and control treatments, which limits direct comparison.

Fourth, numerical values reported for related *Corydalis* species cannot be transferred to *C. govaniana* var. *swatensis*. Fifth, positive in-vitro results do not establish clinical efficacy or safety. Finally, the available evidence does not provide a complete validated phytochemical fingerprint or a comprehensive biological profile specifically for *C. govaniana* var. *swatensis*.

5. Discussion

5.1 Scope and interpretation of the evidence

Because no primary experimental data were generated for the present study, this chapter discusses the **published evidence** concerning *Corydalis govaniana* and related *Corydalis* species. The reported findings cannot be interpreted as original results from *Corydalis govaniana* var. *swatensis*. Instead, they provide the scientific basis, comparative context, and rationale for conducting a future experimental investigation of the Swat variety.

The available literature consistently identifies *Corydalis* as an important source of isoquinoline alkaloids with diverse pharmacological properties. These alkaloids have been associated with analgesic, anti-inflammatory, antioxidant, antimicrobial, antiparasitic, cytotoxic, hepatoprotective, and neuroactive effects. Nevertheless, activity varies considerably according to species, plant part, extraction

method, compound concentration, and experimental model.

5.2 Phytochemical significance

Early investigations of *C. govaniana* reported protopine, corlumine, bicuculline, isocorydine, fumaric acid, and choline. The occurrence of these compounds supports the classification of the species as an alkaloid-containing medicinal plant. The identification of govaniadine further increased the phytochemical importance of *C. govaniana*. Govaniadine belongs to the tetrahydroprotoberberine-related group of isoquinoline alkaloids, a chemical class widely represented in the genus. The biological properties of isoquinoline alkaloids are often influenced by substitution pattern, oxidation state, stereochemistry, and interaction with molecular targets. Therefore, the medicinal potential of *C. govaniana* cannot be explained simply by total alkaloid content; the identity and relative abundance of individual compounds are also important.

A major implication of the existing literature is that species-level information should not automatically be transferred to *C. govaniana* var. *swatensis*. The variety is associated with the Swat region and may experience distinct altitude, soil, climate, and ecological conditions. Such factors can influence secondary-metabolite biosynthesis. Therefore, future work should include authenticated plant material, voucher deposition, chromatographic fingerprinting, and quantitative analysis of chemical markers.

5.3 Pharmacological relevance of govaniadine

Published studies on govaniadine provide the strongest evidence for the pharmacological relevance of *C. govaniana*. The compound demonstrated analgesic activity in experimental models of nociception, including writhing and

hot-plate tests. The reported findings suggested involvement of cyclooxygenase-2 and opioid-associated mechanisms.

This observation is consistent with the long-standing use of several *Corydalis* species in traditional pain management. However, the analgesic evidence concerns purified govaniadine rather than an unstandardized extract or the Swat variety. The effect of a crude extract may differ because of synergistic, additive, or antagonistic interactions among alkaloids and non-alkaloidal constituents.

Govaniadine has also been investigated for hepatoprotective effects in a carbon-tetrachloride-induced liver-injury model. The reported reduction in oxidative, inflammatory, and histopathological damage suggests that the compound may act through multiple mechanisms rather than through simple radical scavenging alone. These results are promising for natural-product research but remain preclinical and cannot support therapeutic recommendations without additional safety, dose-response, and clinical evidence.

5.4 Anti-urease and antileishmanial potential

The anti-urease activity reported for constituents of *C. govaniana* supports the traditional and pharmacological relevance of the species. Urease inhibition may be useful in the search for agents against urease-producing microorganisms and related pathological processes. However, inhibition of jack-bean urease is a preliminary biochemical result and does not establish antimicrobial efficacy in a biological system.

Similarly, selected alkaloids from *C. govaniana* showed antileishmanial activity in in-vitro assays. This is significant because natural products remain an important source of antiparasitic lead structures. However, activity against cultured parasite forms must be followed by selectivity studies, intracellular assays, mechanism-of-action investigations, pharmacokinetics, and in-vivo testing.

The combined anti-urease and antileishmanial evidence indicates that the alkaloid fraction may be a particularly appropriate target for future

bioassay-guided fractionation of *C. govaniana* var. *swatensis*.

5.5 Cytotoxicity and therapeutic relevance

Govaniadine isolated from *C. govaniana* roots produced cytotoxic and apoptosis-related effects in MCF-7 breast-cancer cells. This finding demonstrates the potential of the species as a source of anticancer lead compounds and is consistent with the broader literature on biologically active isoquinoline alkaloids.

However, several limitations should be recognized. Activity against a single cancer-cell line cannot establish broad anticancer efficacy. A meaningful evaluation should include multiple cancer models, nonmalignant control cells, dose-response analysis, apoptosis-specific assays, and mechanistic endpoints. In addition, in-vitro cytotoxicity does not establish therapeutic index, bioavailability, or clinical safety.

The most appropriate interpretation is therefore that govaniadine has preliminary cytotoxic and apoptosis-inducing potential, not that *C. govaniana* is an established anticancer treatment.

5.6 Comparison with related Himalayan species

Studies of other Himalayan *Corydalis* species support the view that environmental origin and taxonomic identity influence chemical and biological profiles. *C. crispa* yielded several isoquinoline alkaloids and showed anti-inflammatory, acetylcholinesterase-inhibitory, and antiplasmodial activities. *C. dubia* also produced alkaloids with antiplasmodial potential.

Research on *C. chaerophylla* collected from Nepal demonstrated solvent-dependent differences in phenolic content, flavonoid content, antioxidant activity, enzyme inhibition, and toxicity. These observations are important because they show that the most active extract is not necessarily the extract with the highest yield. They also indicate that biological activity may be associated with a particular chemical fraction rather than with the crude extract as a whole.

Accordingly, future research on *C. govaniana* var. *swatensis* should compare plant parts and extraction solvents rather than relying on a single extract. A combination of total-content assays,

TLC or HPLC fingerprinting, and targeted biological testing would provide stronger evidence than preliminary phytochemical screening alone.

5.7 Pharmacokinetic and safety implications

The reported pharmacokinetic investigation of govaniadine indicates that the compound undergoes metabolism, shows high plasma-protein binding, and has a relatively short distribution and elimination profile in the studied animal model. These findings are important because biological activity in an assay does not necessarily translate into adequate exposure in vivo.

High protein binding may influence the free concentration available to tissues, while rapid metabolism may limit duration of action. Conversely, metabolites may contribute to biological activity or toxicity. Future studies should therefore evaluate not only the parent alkaloid but also its major metabolites.

Safety is another important consideration. The presence of potent alkaloids means that concentrated extracts should not automatically be considered safe because the plant has a history of traditional use. Acute and repeated-dose toxicity, hepatic and renal biochemical parameters, genotoxicity, and drug-herb interactions should be assessed before any therapeutic application is proposed.

5.8 Research gap and implications

The literature establishes a clear rationale for investigating *C. govaniiana* var. *swatensis*, but it also reveals important gaps. Most published studies concern *C. govaniiana* at species level or focus on purified govaniadine. There is limited publicly documented information about the complete phytochemical profile, quantitative marker content, antioxidant capacity, antimicrobial activity, anti-urease activity, cytotoxicity, and safety of the Swat variety.

The lack of variety-specific data has three implications:

1. Existing findings provide a rationale but cannot substitute for direct investigation.
2. Taxonomic authentication and voucher documentation are essential.

3. Any future biological claim should be based on standardized extracts and appropriate controls.

A future study should therefore use a bioassay-guided design in which extracts are chemically profiled first, followed by fractionation of the most active extract. This would make it possible to determine whether activity is associated mainly with govaniadine, other isoquinoline alkaloids, phenolic compounds, or interactions among multiple constituents.

5.9 Limitations of the present literature-based chapter

The present discussion is limited by the absence of primary experimental data. No original extraction yield, phytochemical concentration, IC₅₀, MIC, inhibition zone, chromatographic peak, toxicity value, or statistical comparison can be attributed to the present study.

In addition, published studies differ in:

- Plant species or variety.
- Geographical origin.
- Plant part.
- Extraction solvent.
- Assay conditions.
- Reference standards.
- Biological model.
- Concentration range.
- Statistical methods.

These differences prevent direct pooling or meta-analysis of most findings. Published numerical values should therefore be presented as study-specific observations rather than as standardized values for *C. govaniiana* var. *swatensis*.

6. Conclusion

The published literature indicates that *Corydalis govaniiana* is a chemically important Himalayan medicinal plant containing several isoquinoline alkaloids, including protopine, corlumine, bicuculline, isocorydine, and govaniadine. Govaniadine has been investigated in experimental models of analgesia, urease inhibition, leishmaniasis, cytotoxicity, apoptosis, oxidative stress, hepatotoxicity, and pharmacokinetics.

The available evidence supports the medicinal and pharmacological importance of the species, but it

does not provide sufficient variety-specific information for *Corydalis govaniensis* var. *swatensis*. In particular, a standardized phytochemical profile and direct biological evaluation of material collected from Swat remain insufficiently documented.

Therefore, *C. govaniensis* var. *swatensis* represents a promising candidate for future phytochemical and biological investigation. However, its potential should be evaluated through authenticated plant collection, controlled extraction, chromatographic characterization, validated biological assays, toxicity assessment, and appropriate statistical analysis. The present chapter should be regarded as a **literature-based discussion and conclusion**, not as a report of original experimental findings.

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