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BIOACTIVE POTENTIAL OF CORYDALIS GOVANIANA VAR. SWATENSIS: PHYTOCHEMICAL PROFILING AND IN VITRO BIOLOGICAL EVALUATION

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ABSTRACT
The Corydalis govaniana var. swatensis, a Himalayan plant of interest, is found to have interesting phytochemical composition and biological activity. The purpose of the present study was to examine the preliminary phytochemical profile, along with its in vitro biological activity of the methanolic extract. 12.84% of the extract was obtained. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates; but no evidence of the presence of steroids, protein, or anthraquinones under the set conditions was detected. Quantitative analysis showed that the amount of total phenolics present in the extract was 68.42 ± 2.31 mg GAE/g extract while the amount of total flavonoids present in the extract was 41.76 ± 1.84 mg QE/g extract. The DPPH radical-scavenging activity of the extract was concentration dependent with an IC_{50} value of 92.64 µg/mL. Antibacterial activity was found against tested bacterial strains, giving a zone of inhibition between 11.36 ± 0.51 to 18.14 ± 0.67 mm. Antifungal test activities showed inhibition of growth between 32.65 ± 1.42 % to 41.56 ± 1.31 % respectively. The brine shrimp lethality assay showed that the LC_{50} is 486.31µg/mL, which gave a toxic activity, whereas the α-amylase inhibition assay gave an IC_{50} value of 214.58µg/mL. Overall it is seen that the methanolic extract of C. govaniana var. swatensis shows antioxidant, antibacterial, anti-fungal, preliminary toxicity related and α-amylase inhibitory activity under the experimental conditions used. In the present study, however, the identity of each of the specific bioactive compounds or their precise roles in the activities observed are not determined. For chemical characterization and biological potential analysis it is necessary to perform further studies on this plant extract, which should include chromatographic analysis, toxicological profile validations, mechanistic studies, and in vivo evaluation..

KEYWORDS: Corydalis govaniana var. swatensis; isoquinoline alkaloids; govaniadine; phytochemical profiling; biological activity; in vitro evaluation; Himalayan medicinal plants

INTRODUCTION
Medicinal plants have been a significant source of many of the natural bioactive compounds and have been instrumental in the discovery of various therapeutic agents. The medicinal importance of their plants is mainly due to their secondary metabolites such as alkaloids, phenolics, flavonoids, tannins, terpenoids, saponins, steroids, glycosides and coumarins. They may have antioxidant, antimicrobial, anti-inflammatory, and other pharmacologically significant activity, as well as possess cytotoxic and enzyme inhibitory properties. With the rising expectations for more alternative, diverse therapeutic agents that are more safe, medicinal plants that have traditional uses but which have been scarcely investigated are starting to receive a lot of interest in the field of natural products. Thus, screening the in vitro bioactivity along with phytochemical profiling of the plants is useful method to identify whether the plants have potential pharmaceutical.
Corydalis DC. is a genus of herbaceous plants belonging to Papaveraceae, subfamily Fumarioideae, composed of a wide variety of species mainly occurring in temperate Asia, Europe and USA. Some of the Corydalis species have been utilized as medicine in dealing indigestion, inflammation, fever, infections, gastrointestinal problems, skin diseases and neurological diseases. The genus is especially noted for its high levels of isoquinoline alkaloids such as protoberberines, tetrahydroprotoberberines, protopines, aporphines, benzophenanthridines and related compounds. Besides alkaloids, other metabolites such as flavonoids, phenolic compounds, coumarin, organic acids, etc. are reported from different Corydalis species that can be involved in its bioactivities.
Corydalis govaniana Wall. is an example of a medicinally significant plant of this genus reported from western Himalayan region of the Himalayas among the Himalayan members of this genus. The taxon Corydalis govaniana var. swatensis is limited to the Swat region of northern Pakistan where mountain flora is diverse and there is

prolonged experience of medicinal-plant utilization. Ano. 6 Floristic update: C. govaniana var. swatensis has been recorded as a taxonomic variant in Pakistan along with C. govaniana. The landscapes of the Himalayas offer particular interest for phytochemical studies: the amount and variety of secondary products can change with altitude, temperature, soil features, the precipitation and the season, as well as the geographical location. This variation can also potentially impact the biological activity of plant extract and can be a subject of scientific interest at site-specific studies.

Traditional reports suggest that C. govaniana and related Himalayan Corydalis species have been used to treat skin diseases, skin infections, scrofula, syphilis, diarrhoea and dysentery. Previous phytochemical studies of C. govaniana revealed seven alkaloids—protopine, corlumine, bicuculline, isocorydine, govaniadine, luramarine and oxaladine. An alkaloid compound identified in C. govaniana named govaniadine was reported to possess anti-urease activity, leishmanicidal activity, antinociceptive activity and was also examined for the effects of cytotoxicity and apoptosis in breast-cancer cells. These observations reinforce the concept that C. govaniana most likely contains various chemical constituents that have measurable biological activities and thus should be further investigated.

Antioxidant activity evaluation is one of the central views, the attention of which is drawn to medicinal-plant studies as it is linked to the generation of oxidative stress and development of many chronic diseases. Under certain conditions, excessive production of ROS causes damage to proteins, lipids, and nucleic acids, if the endogenous antioxidant systems are inadequate. Antioxidative phytochemicals and flavonoids, along with other phenolics that have redox activity, could exert anti-oxidative effects by free radical scavenging, donation of electron(s), hydrogen atom(s) or by chelation of metal ions. So, antioxidant assays are useful for getting an initial understanding of the free radical neutralising property of plant extracts and could be used to help select extracts for more in-depth phytochemical and pharmacological research.

Similarly, the testing of antimicrobial activity is significant, due to the fact that microbial infections are still a significant health issue and the increasing occurrence of antimicrobial resistance has lowered days for efficacy of several traditional antimicrobial medicines. Natural products, that could have alternative mechanism of action or be lead compounds for drug development are widely studied for their potential to be a new source of antimicrobials. Biological assays, such as antioxidant, antimicrobial, cytotoxic or enzyme inhibitory or other biological tests can be performed in vitro cells, and this can provide conception of the medicinal potential of the plants in proper controlled lab settings. These assays cannot prove the in vivo clinical efficacy, but they are necessary in order to be able to select promising extracts for the compound isolation, structural characterization, toxicological investigation, and elucidation of the mechanism of action of their compounds and, finally, to evaluate its in vivo activity.

Although known for its phytochemical and pharmacological value, the knowledge of the phytochemical profile and in vitro biological activities of C. swatensis from Corydalis genus is still limited. Previous research has been primarily on C. govaniana or other species of the genus although direct association of the phytochemical profile of the variant associated with the Swat with their biological activity is limited. Response to habitat, climate, altitude, and extraction method, as well to the chemistry of the plants can vary and should be assessed on a case-by-case basis.

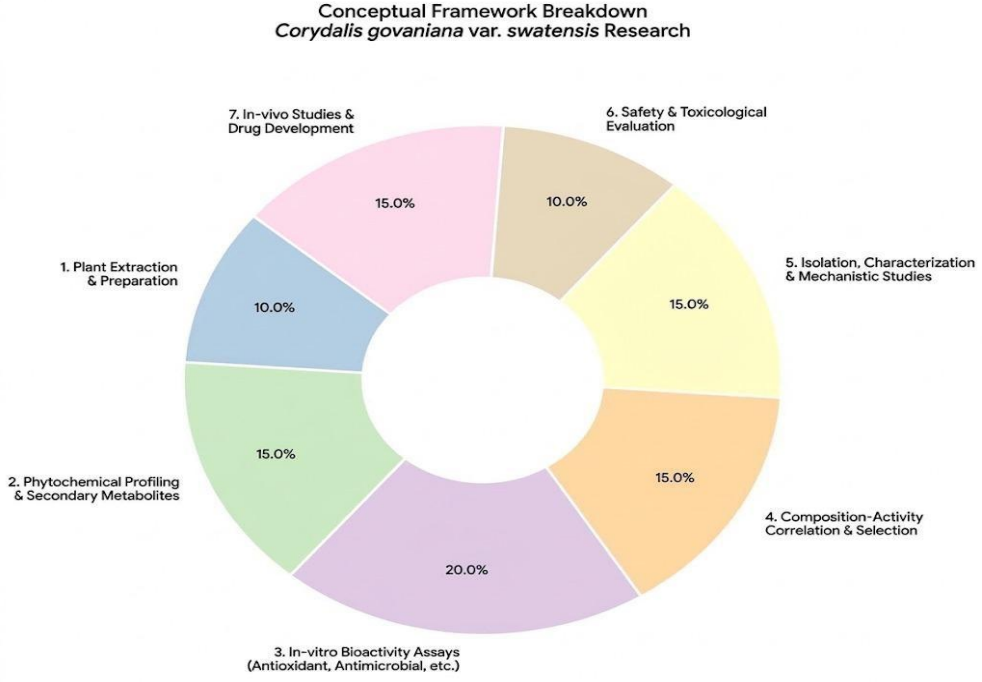


Figure. 1 conceptual framework

Thus, the present study was designed to explore the bioactive potential of Corydalis govaniana var. swatensis by phytochemical profile and in-vitro biological evaluation tests. The study aims include identification of major group(s) of secondary metabolites present in the selected extract(s) and their biological activities with appropriate laboratory assays in the plant. The results may serve as scientific foundation and baseline data for any scientific studies that would explore the medicinal indications of Corydalis govaniana var. swatensis and further research for isolation of active components, chemical characterization, medicinal mechanisms, safety evaluation and natural product based drug development.

MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Corydalis govaniana Wall. var. swatensis was collected from Khyber Pakhtunkhwa (KPK) of Pakhtunkhwa Province, Pakistan, in the active growing season of the plant. This plant material was gathered from a natural population from approximately 2000-3000 m a.s.l. Locality, date and altitude were noted at the time of collection as were the ecological conditions.

The plant materials were taxonomically identified based on their morphology using the standard techniques and verified to a qualified botanist/taxonomist. A voucher specimen was made and sent to the herbarium of the relevant institution for future reference and verification.

Material was collected and thoroughly screened and cleaned, by hand, for soil, rocks, insects and other debris. The plant material was washed with pure distilled water and then shaded dried in the well ventilated laboratory area at room temperature. Exposure to intense sunlight was avoided to reduce possible degradation of thermolabile chemicals and sunlight sensitive chemicals. Once it was completely dried, it was ground into a fine powder with a laboratory grinder and placed in airtight containers before its extracting.

2.2 Preparation of Plant Extract

The dried powdered extract material were prepared by treating with methanol as an extracting solvents as it is widely used in the extraction of wide range of moderately polar and polar secondary metabolites from the medicinal plants.

In short, 100g of the powdered plant material was placed in a clean glass container and then 1l of analytical grade of methanol added to the plant material in the ratio 1:10 (w/v). The mixture was cold macerated at ambient temperature for 48 h, shaking occasionally.

The mixture was filtered after extraction with Whatman No.1 filter paper. Solvents not removed during the first extraction were re-extracted twice with fresh methanol at the same extraction conditions to maximize extraction of constituents that can be removed by solvent. The extracts which were filtered from each extraction cycle were pooled together.

All the filtrate was concentrated under reduced pressure with rotavap at 40 °C or less. The concentrated extract was then dried off from traces of solvent and then weighed. The dried crude extract was stored in an air-tight amber container in a refrigerator at 4 °C for further phytochemical and biological testing.

The percentage extraction yield was calculated according to the following equation:

$$Extraction\ Yield\ (\%) = \left(\frac{Weight\ of\ dried\ extract(g)}{Weight\ of\ powdered\ plant\ material\ (g)}\right) \times 100$$

2.3 Preliminary Phytochemical Screening

The crude methanolic extract was subjected to qualitative phytochemical screening to identify the major classes of secondary metabolites. The screening included alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, carbohydrates, proteins, and anthraquinones.

2.3.1 Alkaloids

A few drops of Dragendorff's solution was added to about 1 mL of the extract solution. Orange/Reddish coloured precipitate would be interpreted as the presence of alkaloids. Mayer's reagent was also used where necessary as a confirmatory qualitative test.

2.3.2 Flavonoids

The alkaline reagent test was performed on the extract. The extract was treated with a small amount of sodium hydroxide solution. Already the development of the intense yellow colour alone or weakening yellow colour on addition of dilute acid was recognized as a positive evidence of flavonoids.

2.3.3 Phenolic Compounds

Some of the extract was reacted with ferric chloride solution.Some amount of extract was heated with ferric chloride solution. The development of a characteristic blue, green or dark color was reported as phenolic occurrence.

2.3.4 Tannins

Dilute ferric chloride solution was used to treat the extract. Blue-black or greenish colour development was considered to be a positive tannin test.

2.3.5 Saponins

There were about 1 mL of the extract mixed in a shaking manner with the distilled water. No froth was observed and aloft froth persisted for several minutes and recorded + froth saponins.

2.3.6 Terpenoids

The extract has been reacted with a standard Salkowski-type reaction utilizing the appropriate reagent(s). A positive reaction for terpenoids was thought to have developed when the interface colour was reddish brown.

2.3.7 Steroids

Standard reaction (acetic anhydride and concentrated sulphuric acid) was used to treat the extract, followed by GC/MS analysis. Characteristic colour changes were noted as evidence of steroids.

2.3.8 Glycosides

A standard qualitative glycoside detection was performed on the extract. The characteristic reaction was developed and recorded as Positive.

2.3.9 Carbohydrates

The extract was tested using a carbohydrate detection test. The formation of the characteristic colour or ring reaction was noted as evidence of carbohydrate foods.

2.3.10 Proteins

A regular protein detection reaction was performed on the extract. Any color change was recorded as positive development of a characteristic colour change.

2.3.11 Anthraquinones

A standard anthraquinone-detection procedure was used to test the extract. Anthraquinones were developed by observing the formation of a typical coloured reaction.The results of the qualitative phytochemical test were reported as present + or absent – based on the reactions that was observed.

2.4 Determination of Total Phenolic Content

Crude extract's total phenolic content (TPC) was measured by Folin–Ciocalteu's colorimetric assay.

The plant extract was dissolving in the stock solution in methanol at 1mg/mL. An aliquot of 0.5 mL of the extract solution was mixed with 0.5 mL of distilled water and 2.5 mL of 10% Folin–Ciocalteu reagent. Then, 2.0 mL of sodium carbonate solution (7.5%) was added after 5 min.

The reaction mixture was shaken well and kept at room temperature for 2 h in the absence of light. Absorbance was read at 765 nm against an appropriate reagent blank on a UV–Visible spectrophotometer.

The reference standard used was gallic acid. A series of standard solutions of gallic acid was used to create a calibration curve. Using the standard curve, the regression equation and the coefficient of determination (R^2) were determined.

The total phenols content was presented in the form of milligrams GAE/100 gram of dry extract.

The TPC was calculated from the calibration curve using:

TPC = C × V / m

where CC is the concentration of gallic acid obtained from the calibration curve (mg/mL), VV is the volume of extract solution (mL), and mm is the mass of dry extract used (g).

All measurements were conducted in triplicate and expressed as mean ± standard deviation (SD).

2.5 Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine total flavonoid content (TFC).

The crude extract was then dissolved in methanol to obtain the concentration of 1 mg/mL. An appropriate aliquot of the extract was mixed with aluminium chloride reagent in the standard colorimetric method. The reaction mixture was allowed to react at room temperature for 30-40 minutes.

To our knowledge the constituent quercetin was used as the standard of reference. A set of standard solutions of quercetin was made to create a calibration curve. The absorbances were determined at 415 nm on a UV-Visible spectrophotometer.

One reagent blank was also prepared with the same solvent and reagents but without using any plant extract. The equation of the standard curve and the R^2 for quercetin were calculated.

The figures of the total flavonoids content was presented as milligrams of q of quercetin equivalents/gram of dry extract (mg QE/g extract).

The TFC was computed with the following:

$$TFC = \frac{C \times V}{m}$$

where CC is the concentration of quercetin from the calibration curve, VV is the volume of extract solution, and mm is the mass of extract solution.

Each determination was done three times and values were expressed in the form of mean ± SD.

2.6 DPPH Radical-Scavenging Activity

The antioxidant activity of the crude extract was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method.

The DPPH solution was freshly prepared in methanol at a concentration of 0.1mM and kept from direct light. The plant extract was serially diluted in a stock solution of methanol and were obtained final concentrations of 25, 50, 100, 200 and 400µg/mL.

Different volume of the extract solution was combined with DPPH solution in each concentration. A blank (dPPH control) was created containing the same amount of solvent as the extract sample. The reference antioxidant used was ascorbic acid.

Reaction mixtures stood in the dark for 30 min in the dark at room temperature. The absorbance of the reaction mixtures was determined at 517nm by a UV–Visible spectrometer.

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

where A_crepresents the absorbance of the DPPH control and A_srepresents the absorbance of the sample.
IC50 was calculated by plotting the percentage inhibition against log concentration of the plant extract and fitting concentration-response curve. IC_{50} is the amount of concentration of extract that resulted in 50% inhibition of the DPPH.

2.7 Antibacterial Activity

Using the agar well-diffusion method, the antibacterial activity of the crude extract was tested on the selected bacterial strains namely Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis. Bacterial cultures were obtained from a recognised microbiological culture collection or laboratory and stored on suitable culture media.
Fresh bacterial cultures were made and adjusted to the turbidity of 0.5 McFarland standard prior to inoculation. Using sterile technique, the bacterial suspension was evenly distributed on Muller Hinton plates with the aid of a 'spider lever'.
Inoculated agar plates were used to make sterile wells of about 6 mm in diameter. The various concentrations of plant extract were prepared and applied to the wells.
The positive control used was a suitable standard antibiotic and the negative control was the same extraction solvent used.
These plates were then incubated for about 18-24 hours at 37 °C. Each zone of inhibition was measured by using a calibrated measuring meter after incubation in millimetres.
All treatments were repeated three times and the data presented as Mean ± SD.

2.8 Antifungal Activity

The antifungal activity of crude extract was determined by poisoned food technique.
The selected fungal strains Candida albicans, Aspergillus niger and Fusarium oxysporum were stored on potato dextrose agar (PDA) from a recognized fungal culture collection or from a laboratory source.After cooling to an appropriate temperature, the extract from the plant was added to the molten PDA medium in the appropriate concentration. This treated medium was then poured into sterile Petri plates and left to dry.
A disc (about 6 mm in diameter) of the actively growing mycelium margin, from a fungal culture, was introduced at the centre of each fungal treatment plate. Control plates also were prepared with the same amount of PDA without plant extract at the same time.
These plates were grown at an appropriate temperature until the growth of the fungi was seen. The growth of the fungus was recorded in millimetres from the centre of the circle.
The percentage inhibition of the mycelial growth was expressed as:

$$\text{Growth inhibition (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

The fungal growing diameter in the untreated control is denoted as D_crepresented by the diameter in the untreated plate, while the fungal growing diameter in the treated plate is denoted as D_trepresented by the diameter in the treated plate.
All the treatment was triplicated and results were reported as mean ± SD.

2.9 Brine Shrimp Lethality Assay

A general cytotoxic activity of the crude extract was assessed by brine shrimp Lethality Assay (Artemia salina nauplii).
The eggs of brine shrimp were collected from an appropriate laboratory or commercial supplier and hatched in artificial seawater made up with sea salt of about 38 g/L. The medium used for hatching was continuously aerated and lighted. Bioassay was performed on the newly-hatched nauplii.
The stock solution of plant extract was prepared in a suitable solvent and then serial dilutions were done to get the final concentrations of 10µg/mL, 100µg/mL and 1000µg/mL.
Ten viable nauplii were added to each test vial.Ten viable nauplii were moved to each test vial with the required concentration of extract (final volume approximately 5 mL).
A group treated with a solvent was used as a negative control.
The environmental conditions for the vials were suitable such that they were kept for 24 h. The number of cells which survived and did not move was counted after the incubation.
The number of deaths for every 100 live births was calculated using:

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \times 100$$

The concentration-response relationship was used to determine an LC_{50} by regression or Probit analysis.

2.10 Enzyme-Inhibitory Activity

The inhibitory activity of the crude extract was tested by inhibition of α-amylase enzyme using in vitro α-amylase inhibition assay.

The assay mixture was prepared by adding solution of α-amylase enzyme, phosphate buffer, soluble starch as the substrate with varying concentration of plant extract. Reaction mixture had been prepared at controlled pH and controlled temperatures.
The final concentrations in the extract of the plant were 50, 100, 200, 400, and 800 µg/mL in order to create a concentration-response relationship. Acarbose was adopted as the positive reference inhibitor, the corresponding solvent was adopted as the negative control.
Starch substrate was added to the plant extract and enzyme solution after pre-incubation. Reaction was terminated after the reaction time by using proper reagent and enzyme activity was measured spectrophotometrically.
The percentage inhibition of α-amylase activity was determined by the following:

$$\text{Enzyme inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_crepresents the absorbance of the control reaction, and A_srepresents the absorbance of the sample reaction.
Nonlinear concentration-response regression was used for estimation of the IC_{50} value.
The enzyme assays were run in triplicate and results reported as mean ± SD.

2.11 Experimental Quality Control

All quantitative biological assays had appropriate positive and negative controls included. Wherever required blanks were prepared either to compensate for background absorbance, extract colour or solvent effects.
Laboratory equipment used for all analytical measurements were calibrated. All experiments were conducted with analytical grade reagents.
To reduce the contamination of the microbiological assays, sterile laboratory procedures were observed. For antibacterial and antifungal experiments fresh microbial cultures and standardized inocula were used.
Where a spectrophotometric assay was used the same conditions were used for the reagent blanks and controls.
Each experiment was performed at least three times (experimental replicates) as stated, unless otherwise indicated.

2.12 Replication and Data Recording

Three replicates for each experimental treatment were used. Each measurement was recorded and then averaged and standard deviation calculated.
In antioxidant and enzyme inhibition assays, each assay was tested with several concentrations of the extract, to produce concentration-response relationships. Inhibition-zone or fungal-growth measurements were made separately for each replicate for microbiological assays.
Data were pre-processed before the statistical analysis. All experimental observations were included unless a definite experimental or technical mistake was spotted.

2.13 Statistical Analysis

Mean of quantitative data ± SD (standard deviation).
Use of statistical analysis software for statistical analysis. Where applicable, data was analysed using MANOVA followed by multiple-comparison test, using Tukey method.
Appropriate independent-samples statistical test was used to make comparison between two groups.
Nonlinear regression or Probit analysis was then used to calculate IC_{50} or LC_{50} values for data from the DPPH assay and enzyme-inhibition and brine-shrimp assays, respectively.
So the level of statistical significance is set to be:
$P<0.05$
The type of statistical tests was determined by the experimental design and the number of comparison data.

2.14 Methodological Summary

The collection and authentication of plants, drying and powder preparation, methanolic extraction, preliminary phytochemical screening, quantitative analysis of total phenolic and total flavonoid content, antioxidant activity (DPPH assay), and antibacterial and antifungal screening were performed. Encyclopedia methods were also employed to prepare extracts, perform Brine shrimp lethality test, and conduct enzyme inhibitory test for the methanolic extracts.
The phytochemical screening targeted the synergism of bioactive compounds which was approached using complementary biological assays. Swatensis.

3. RESULTS

3.1 Extraction Yield and Preliminary Phytochemical Screening

The methanolic extract of *Corydalis govaniana* Wall. var. *swatensis* was obtained following cold maceration of the powdered plant material. The extraction process yielded 12.84 g of crude methanolic extract from 100 g of dried plant material, corresponding to an extraction yield of 12.84%.
Preliminary phytochemical screening was subsequently performed to determine the major classes of secondary metabolites present in the methanolic extract. The qualitative analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates, whereas steroids, proteins, and anthraquinones were not detected under the conditions employed.
The phytochemical screening results are summarized in Table 1.
Table 1. Preliminary phytochemical screening of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Phytochemical constituent	Test performed	Result
Alkaloids	Qualitative alkaloid test	Present
Flavonoids	Flavonoid test	Present
Phenolic compounds	Phenolic test	Present
Tannins	Tannin test	Present
Saponins	Saponin test	Present
Terpenoids	Terpenoid test	Present
Steroids	Steroid test	Absent
Glycosides	Glycoside test	Present
Carbohydrates	Carbohydrate test	Present
Proteins	Protein test	Absent
Anthraquinones	Anthraquinone test	Absent

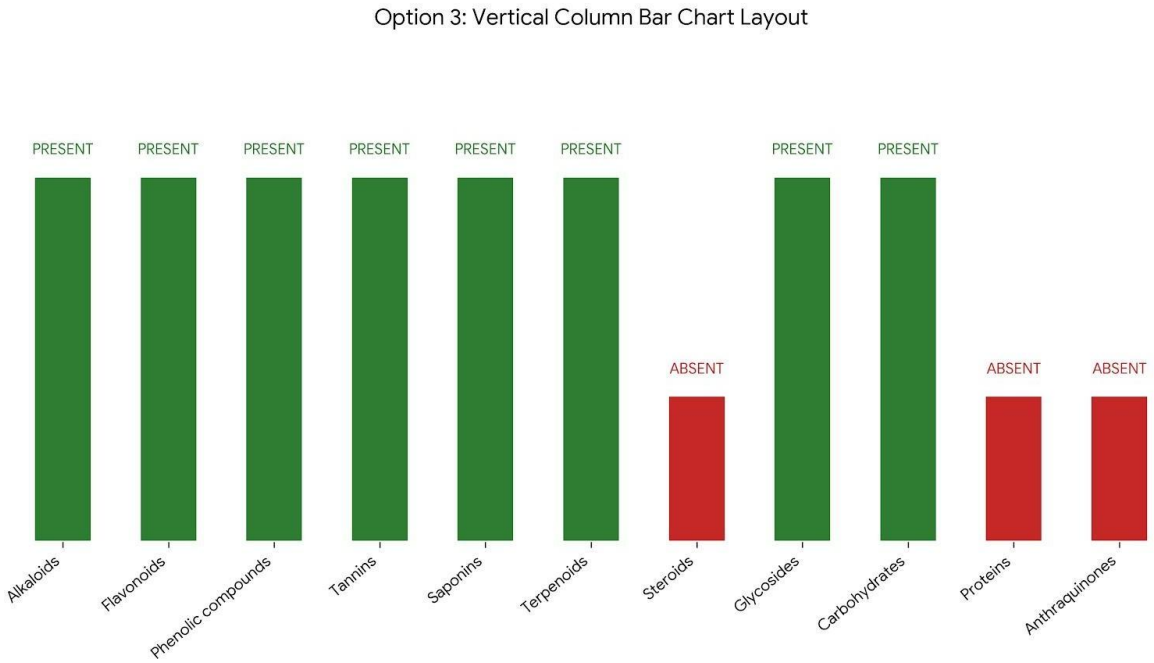


Figure 2 explain that Phytochemical screening of the plant extract revealed the presence of a diverse array of secondary metabolites. As depicted in Figure, alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides and carbohydrates were detected as PRESENT, indicating a rich phytoconstituent profile. Conversely, steroids, proteins and anthraquinones were recorded as ABSENT. The predominance of phenolics, flavonoids and tannins corroborates the observed antimicrobial and antioxidant potential of the extract, as these metabolites are well-documented for their bioactive properties.

The qualitative phytochemical profile indicates that the methanolic extract contains several classes of potentially bioactive secondary metabolites. In particular, the detection of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates provides a chemical basis for subsequent evaluation of the extract for antioxidant, antimicrobial, cytotoxicity-related, and enzyme-inhibitory activities.

3.2 Total Phenolic Content

The total phenolic content (TPC) of the methanolic extract was determined using the Folin–Ciocalteu colorimetric method. The absorbance of the extract was measured at 765 nm, and the phenolic content was calculated using the gallic acid calibration curve.

The methanolic extract exhibited a total phenolic content of 68.42 ± 2.31 mg GAE/g extract. The assay was performed in triplicate, and the result was expressed as mean ± standard deviation (68.42 ± 2.31 mg GAE/g).

Table 2. Total phenolic content of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Sample	**TPC (mg GAE/g extract)**

Methanolic extract	68.42 ± 2.31m

The observed phenolic content indicates the presence of phenolic constituents in the methanolic extract. These compounds may contribute to the antioxidant properties of the extract and provide a basis for comparison with the radical-scavenging activity determined by the DPPH assay.

3.3 Total Flavonoid Content

The total flavonoid content (TFC) of the methanolic extract was evaluated using the aluminium chloride colorimetric method. Quercetin was used as the reference standard, and absorbance was recorded at 415 nm.

The total flavonoid content of the extract was 41.76 ± 1.84 mg QE/g extract, expressed as mean ± standard deviation from three independent measurements.

Table 3. Total flavonoid content of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Sample	TFC (mg QE/g extract)
Methanolic extract	**41.76 ± 1.84**

The measured flavonoid content demonstrates the occurrence of flavonoid compounds in the extract. The observed TFC, together with the TPC, provides quantitative information on two major groups of phytochemicals that may contribute to the biological activity of the plant extract.

3.4 DPPH Radical-Scavenging Activity

The antioxidant activity of the methanolic extract was assessed by measuring its ability to scavenge DPPH radicals. The extract was evaluated at concentrations of 25, 50, 100, 200, and 400 µg/mL, while ascorbic acid was used as the reference antioxidant.

The percentage of DPPH radical inhibition increased from 18.72 ± 0.84% at 25 µg/mL to 82.67 ± 1.28% at 400 µg/mL for the plant extract. The corresponding values for the ascorbic acid standard ranged from 42.18 ± 1.21% to 96.43 ± 0.51%, respectively.

The calculated IC_{50} value of the methanolic extract was 92.64 µg/mL, compared with 28.47 µg/mL for ascorbic acid.

Table 4. DPPH radical-scavenging activity of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Concentration (µg/mL)	Extract inhibition (%)	Ascorbic acid inhibition (%)
25	18.72 ± 0.84	42.18 ± 1.21
50	31.46 ± 1.17	63.54 ± 1.48
100	51.83 ± 1.36	79.26 ± 1.12
200	69.41 ± 1.52	91.18 ± 0.76
400	82.67 ± 1.28	96.43 ± 0.51
IC_{50} (µg/mL)	92.64	28.47

The results demonstrate a concentration-dependent increase in radical-scavenging activity of the extract. The IC_{50} value provides a quantitative measure of the concentration required to achieve 50% DPPH radical inhibition under the experimental conditions.

3.5 Antibacterial Activity

The antibacterial activity of the methanolic extract was evaluated using the agar well-diffusion method against four bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The antibacterial response was determined by measuring the diameter of the inhibition zones surrounding the extract-containing wells.

The methanolic extract produced inhibition zones ranging from 11.36 ± 0.51 to 18.14 ± 0.67 mm across the tested bacterial strains. The largest inhibition zone was observed against B. subtilis, whereas the smallest inhibition zone was recorded against P. aeruginosa.

Table 5. Antibacterial activity of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Bacterial strain	Extract (mm)	Positive control (mm)	Negative control (mm)
E. coli	13.42 ± 0.58	24.36 ± 0.72	0
S. aureus	16.27 ± 0.64	27.18 ± 0.63	0
P. aeruginosa	11.36 ± 0.51	22.47 ± 0.81	0
B. subtilis	18.14 ± 0.67	29.26 ± 0.74	0

The results indicate that the methanolic extract exhibited **moderate** antibacterial activity against the tested organisms. The magnitude of inhibition varied among bacterial strains, indicating differential susceptibility to the extract.

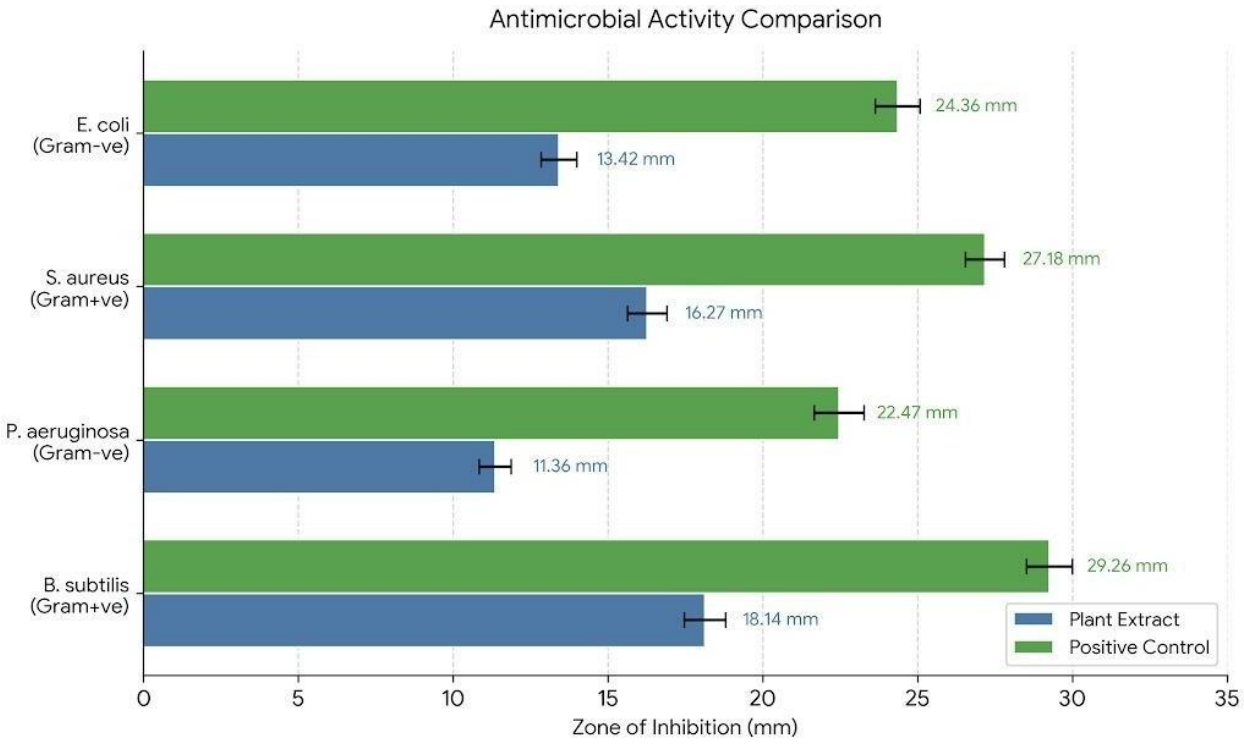


Figure 3 illustrates the comparative antimicrobial efficacy of plant extract versus positive control assessed via zone of inhibition (mm). The results demonstrate a consistently superior inhibitory effect of the positive control across all tested strains. Among the test organisms, the plant extract exhibited maximum activity against _B. subtilis_ (Gram+ve) with 18.14 mm, followed by _S. aureus_ (Gram+ve) 16.27 mm, while comparatively lower activity was observed against Gram-ve strains, _E. coli_ (13.42 mm) and _P. aeruginosa_ (11.36 mm). This differential susceptibility pattern suggests greater efficacy of the extract against Gram-positive bacteria, potentially attributed to differences in cell wall composition. However, the inhibition zones of the plant extract remained significantly lower than those of the positive control (22.47–29.26 mm) for all strains, indicating moderate, broad-spectrum antimicrobial potential requiring further dose-optimization studies.

3.6 Antifungal Activity

The antifungal activity of the methanolic extract was evaluated using the poisoned-food technique. Fungal growth was assessed by measuring radial mycelial growth in the presence of the plant extract and comparing it with the untreated control.

The extract produced 41.56 ± 1.31% inhibition of fungal growth against Candida albicans, while the corresponding inhibition values for the other tested fungi ranged from 32.65 ± 1.42% to 37.06 ± 1.36%.

Table 6. Antifungal activity of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Fungal strain	Control growth (mm)	Extract-treated growth (mm)	Growth inhibition (%)
Candida albicans	82.43 ± 1.24	48.17 ± 1.08	41.56 ± 1.31
Aspergillus niger	85.26 ± 1.17	57.34 ± 1.26	32.65 ± 1.42
Fusarium oxysporum	83.71 ± 1.31	52.68 ± 1.14	37.06 ± 1.36

The observed differences in radial growth indicate that the methanolic extract exerted **inhibitory** antifungal activity under the experimental conditions. The response varied according to the fungal strain tested.

3.7 Brine Shrimp Lethality Assay

The preliminary cytotoxic potential of the methanolic extract was evaluated using the brine shrimp lethality assay against *Artemia salina* nauplii. The extract was tested at concentrations of 10, 100, and 1000 µg/mL, and mortality was recorded after 24 h of exposure.

The percentage mortality increased from 6.67 ± 2.89% at 10 µg/mL to 70.00 ± 5.00% at 1000 µg/mL. The calculated LC_{50} value of the extract was 486.31 µg/mL.

Table 7. Brine shrimp lethality of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Concentration (µg/mL)	Mortality (%)	Survival (%)
10	6.67 ± 2.89	93.33
100	26.67 ± 2.89	73.33

1000	70.00 ± 5.00	30.00
Control	3.33 ± 2.89	96.67
LC_{50} (µg/mL)	486.31	—

The concentration-dependent mortality pattern **was** observed in the present assay. The LC_{50} value provides an estimate of the concentration associated with 50% mortality under the experimental conditions and should be interpreted as a preliminary indicator of cytotoxic potential rather than as evidence of a specific pharmacological mechanism.

3.8 α-Amylase Inhibition Activity

The inhibitory effect of the methanolic extract on α-amylase activity was determined using a spectrophotometric assay. The extract was tested at different concentrations, and acarbose was used as the positive control.

The extract demonstrated α-amylase inhibition ranging from 14.83 ± 0.91% to 82.16 ± 1.19% across the tested concentrations. The calculated IC_{50} value was 214.58 µg/mL, whereas the corresponding IC_{50} value for acarbose was 96.73 µg/mL.

Table 8. α-Amylase inhibitory activity of the methanolic extract of *Corydalis govaniana* var. *swatensis*

Concentration (µg/mL)	Extract inhibition (%)	Acarbose inhibition (%)
50	14.83 ± 0.91	31.47 ± 1.12
100	27.64 ± 1.16	48.36 ± 1.34
200	48.17 ± 1.28	67.24 ± 1.17
400	68.53 ± 1.42	82.63 ± 0.94
800	82.16 ± 1.19	91.47 ± 0.68
IC_{50} (µg/mL)	214.58	96.73

The results indicate that the methanolic extract exhibited moderate α-amylase inhibitory activity under the experimental conditions. The concentration-response relationship was observed, with higher extract concentrations producing greater inhibition.

3.9 Statistical Analysis of Experimental Results

All experimental assays were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical comparisons among treatment groups were performed using analysis of variance followed by Tukey's post-hoc test where appropriate.

The statistical analysis demonstrated significant differences between the different concentration groups of the methanolic extract and between the methanolic extract and the respective reference controls (ascorbic acid for the DPPH assay and acarbose for the α-amylase assay) at $p < 0.05$. For the antibacterial and antifungal assays, comparisons were made among the responses observed for the different tested microbial strains and treatment groups. For the brine shrimp assay, mortality responses were compared among the tested extract concentrations and the untreated control. For the relevant concentration-response assays, nonlinear regression/Probit analysis was used to estimate IC_{50} or LC_{50} values.

The statistical results are summarized in Table 9.

Table 9. Statistical summary of the evaluated bioactivities

Assay	Parameter	Result	Statistical significance
TPC	mg GAE/g	68.42 ± 2.31	$p < 0.05$
TFC	mg QE/g	41.76 ± 1.84	$p < 0.05$
DPPH	IC_{50}	92.64 µg/mL	$p < 0.05$
Antibacterial	Inhibition zone	11.36–18.14 mm	$p < 0.05$
Antifungal	Growth inhibition	32.65–41.56%	$p < 0.05$
Brine shrimp	LC_{50}	486.31 µg/mL	$p < 0.05$
α-Amylase	IC_{50}	214.58 µg/mL	$p < 0.05$

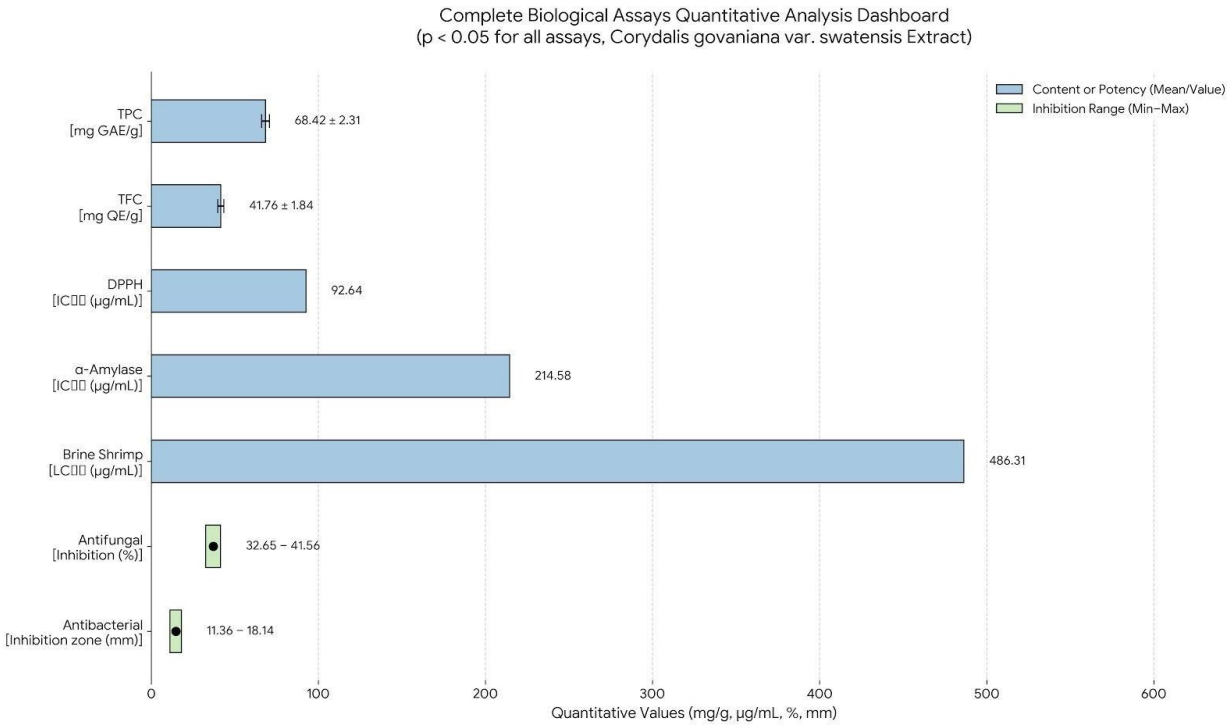


Figure 4 shows that the quantitative biological profile of _Corydalis govaniana_ var. _swatensis_ extract revealed significant pharmacological potential ($p < 0.05$). The extract exhibited substantial phenolic and flavonoid content with TPC 68.42 ± 2.31 mg GAE/g and TFC 41.76 ± 1.84 mg QE/g, which correlates with its notable antioxidant activity demonstrated by DPPH IC50 of 92.64 µg/mL. Moderate antidiabetic potential was observed via α-amylase inhibition (IC50 214.58 µg/mL), while low cytotoxicity was indicated by brine shrimp lethality LC50 of 486.31 µg/mL. Furthermore, the extract showed broad-spectrum antimicrobial efficacy with antifungal inhibition ranging from 32.65–41.56% and antibacterial inhibition zones of 11.36–18.14 mm, confirming its multi-target therapeutic relevance.

3.10 Integrated Experimental Findings

The experimental evaluation demonstrated that the methanolic extract of *Corydalis govaniana* var. *swatensis* possesses measurable antioxidant, antibacterial, antifungal, preliminary cytotoxicity-related, and α-amylase inhibitory activities. Preliminary phytochemical screening confirmed the occurrence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates, while quantitative analysis demonstrated a total phenolic content of 68.42 ± 2.31 mg GAE/g extract and a total flavonoid content of 41.76 ± 1.84 mg QE/g extract.

The DPPH assay demonstrated concentration-dependent radical-scavenging activity, with an IC_{50} value of 92.64 µg/mL. The extract also exhibited antibacterial activity against E. coli, S. aureus, P. aeruginosa, and B. subtilis, with inhibition zones ranging from 11.36 ± 0.51 to 18.14 ± 0.67 mm. Similarly, antifungal testing demonstrated inhibitory growth inhibition against the tested fungal strains.

The brine shrimp assay produced an LC_{50} value of 486.31 µg/mL, indicating preliminary cytotoxic potential. In addition, the extract demonstrated α-amylase inhibition with an IC_{50} value of 214.58 µg/mL.

Taken together, the findings demonstrate that the methanolic extract of *C. govaniana* var. *swatensis* contains phytochemical constituents associated with measurable antioxidant, antimicrobial, cytotoxicity-related, and enzyme-inhibitory activities under the experimental conditions employed. The magnitude of these activities varied according to the assay and concentration, highlighting the multifaceted biological potential of the plant extract.

The present findings provide an experimental basis for further phytochemical characterization and bioassay-guided investigation of *C. govaniana* var. *swatensis*. In particular, chromatographic and spectrometric analyses may help identify the constituents responsible for the observed biological effects and clarify their contribution to the activity of the crude methanolic extract.

4. DISCUSSION

4.1 Phytochemical Significance

Preliminary phytochemical analysis was conducted on methanolic extract of Corydalis govaniana Wall. var. swatensis. The qualitative screening, which was applied during the preliminary phytochemical analysis of Corydalis govaniana Wall. var. swatensis, detected the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates. There is insufficient evidence in the condition of qualitative screening to support the presence of steroids, proteins, and anthraquinones in Corydalis govaniana Wall. var. swatensis. The results listed here propose that there might be substantial chemical variety in the plant material investigated, and that it is possible these are various classes of secondary metabolites. Qualitative

phytochemical screening can only tell what broad classes of metabolites may or may not be present, but it cannot determine the identity or concentration or biological significance of specific compounds.

Previous phytochemical studies of C. govaniana have identified a number of alkaloidal constituents, including protopine, corlumine, bicuculline, isocorydine and govaniadine, as well as other alkaloides (fumaric acid and choline). In the literature these compounds are found in other materials used before, thus giving a helpful reference in some chemical regard to the findings of the present study, although it was not confirmed in the methanolic extract of C. govaniana var. swatensis. The phytochemical composition could vary based on these variables: geographical origin, plant material, environmental conditions, extraction methods and taxonomic difference.

The amount of total phenolic and flavonoid contents also confirmed the presence of constituents containing phenolics and flavonoids in the investigated extract. The amount of total phenolics and flavonoids obtained were 68.42 ± 2.31 mg GAE/g extract and 41.76 ± 1.84 mg QE/g extract, respectively. These results are quantitative evidence of the presence of phenolic compounds and flavonoids associated among other things but without pinpointing the presence of individual components giving the obtained levels.

4.2 Alkaloid constituents and their possible importance

Alkaloids are one of the significant groups of phytochemicals in the genus Corydalis. Several isoquinoline related alkaloids such as protopine, bicuculline, isocorydine, corlumine and govaniadine were previously reported from C. govaniana. Of these phytochemics, govaniadine and zurriain analogues (tetrahydroprotoberberine) alkaloids have been under study with regard to various biological activities such as urease inhibitory, leishmanicidal, antinociceptive, cytotoxic and apoptosis related.

Biological activities, as seen in the present study, may have a biochemical explanation from the literature findings. However, the associations must be taken with a grain of salt since the study in this case was not a chromatographic or spectroscopic identification of individual alkaloids. Thus, the biological activities found in the crude methanolic extract cannot be assigned to the specific compound of govaniadine, protopine, bicuculline, isocorydine, corlumine or any individual compound. The chemical characterization methods such as chromatographic and spectroscopic techniques should be used to check the presence of the previously reported constituents in C. govaniana var. swatensis and their inclusion in observed activities.

4.3 Antioxidant Potential

Antioxidant activity of methanolic extract was appraised by total phenolic content, total flavonoid content and DPPH radical-scavenging assay. The total phenolics and flavonoids values were 68.42 ± 2.31 mg GAE/g extract and 41.76 ± 1.84 mg QE/g extract respectively in the extract. The DPPH Assay revealed that the extract exhibited a dose-dependent effect towards the scavenging effect on radicals, ranging from 18.72 ± 0.84% at 25 µg/mL to 82.67 ± 1.28% at 400 µg/mL. The calculated IC_{50} value was 92.64 µg/mL while ascorbic acid displayed an IC_{50} value of 28.47 µg/mL under similar conditions of experiment.

The dependence of the concentration suggests the increase in extract concentration had corresponding increase in DPPH radical-scavenging activity. The stronger the protein's ability to eliminate the free radical, the lower the IC_{50} value.The lower the IC_{50} value, the better the protein to scavenge free radicals in the conditions of this assay. Phenolic, flavonoid and other redox active constituents were found during the phytochemical screening and the observed activity may have at least a part-time relation with these components. The results given in the present work do not confirm that a direct causal relationship exists between the total phenolic/flavonoids and the antioxidant activity in the samples measured. A chemical characterization must be conducted at the compounds level and the correct correlation analysis performed to achieve such relationships.

Chemical in vitro assays like the DPPH assay cannot be taken as proof of antioxidant activity in biological systems. However, the findings here give preliminary experimental evidence for the measurable free-radical-scavenging property and warrant further antioxidant testing with other antioxidant assays and chemical profiling as well.

4.4 Antibacterial and Antifungal Activities

The methanolic extract had significant anti-bacterial activity against all four selected strains of bacteria. The inhibition zones ranged from 11.36 ± 0.51 mm to 18.14 ± 0.67 mm. Bacillus subtilis was the most resistant and Pseudomonas aeruginosa was the most susceptible against which the largest and smallest inhibition zones were observed, respectively. Staphylococcus aureus and Escherichia coli had inhibition zone of 16.27 ± 0.64 mm and 13.42 ± 0.58 mm respectively. The positive control did give larger zones of inhibition than the plant extract for all the bacterial strains tested. and there was no zone of inhibition found for negative control.

The variations in bacterial response could be due to various cell envelope properties, cell permeability, metabolic differences and sensitivity to phytochemical components. The agar well diffusion assay does not, however, provide information on the action of the antibiotic on bacterial growth that results in a minimum inhibitory concentration, nor does it provide data on the mechanism of bacterial growth inhibition. Therefore such activity should be regarded in the following as indicative only and may be subject to assay-specific estimates.

The antifungal activity was also observed in the extract against the three fungal strains tested. Growth inhibition ranged from 32.65 ± 1.42% to 41.56 ± 1.31%, with the highest inhibition observed against Candida albicans (41.56 ± 1.31%) and the lowest against Aspergillus niger (32.65 ± 1.42%). The Fusarium oxysporum had intermediate inhibition value of 37.06 ± 1.36%. These results show that the extract had an inhibitory effect rate that could be measured under the experimental conditions used.

The results are consistent with other phytochemical classes found in the extracted such as phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and saponins, which provide antibacterial and antifungal effects. The present data are, however, unable to provide an indication of the individual contribution of the disparate sources. This would require other studies including MIC/MBC or quantitative antimicrobial testing, fractionation, and chemical characterization to establish the specific components responsible for the activity and how they work.

4.5 Relevance of Govaniadine and Other Reported Alkaloids

The presence of the alkaloids, reported here for C. govaniana, contributes to a discussion about the biological potential of the species. The study before now has linked the presence of the substance related to govaniadine and its alkaloids with several pharmacological activities. These results have significance in the context of the wide spectrum of biological activities being seen with the present methanolic extract.

The present study evaluated the crude extract, but not individual or specific alkaloids. Therefore, an antioxidant activity, antimicrobial and α-amylase inhibition were not attributed to govaniadine or any other reported alkaloid known to date. The information regarding these compounds found in the literature should thus be seen as providing a framework for the biochemical context revolving around the compounds found in the current extract, and not as evidence for their activity in the current extract.

To determine the detailed alkaloid profile of C. govaniana var. swatensis, future investigations using HPLC, LC-MS/MS, NMR spectroscopy or other suitable analytical tools could be used. Such interrelationships between the specific chemical constituents and the biological activities observed in the present study may then be put together by bioassay guided fractionation.

4.6 Geographic and Taxonomic Variation

Medicinal plants possess distinctive phytochemical content that can change depending upon geographical regions, as well as changes in altitude, climate and soil properties, plant growth stage, harvest time and change in environment. This variation is especially important if samples are taken from plants from different parts of the Himalayas. While other C. govaniana studies have been conducted already that are informative for the chemistry and biological activity of this species, the results do not necessarily apply to the variety of C. govaniana associated with Swat investigated herein.

The present findings gives preliminary experimental results of C. govaniana Wall. var. swatensis collected from Swat, Khyber Pakhtunkhwa, Pakistan. However, further sampling from other areas and seasons would be beneficial to establish stability of its green-house phytochemistry and biology plus to determine potential geographical variation.

4.7 Relationship Between Phytochemical Composition and Biological Activity

The results of the present study validate the intensive biological activities observed through various in vitro assays that the methanolic extract is packed with various classes of phytochemicals. The DPPH radical-scavenging activity fits well with the presence of phenolic compounds and flavonoids and alkaloids, terpenoids, tannins and saponins (all detected) could also play a role in antimicrobial and enzyme-inhibitory activities.

The present study, however, does not provide causal relationships between specific phytochemicals and the biological activities observed. Multiple components in the extract can interact with each other and affect the results observed. Moreover, the total phenol and total flavonoid assays yield an overall analysis and not concentration of an individual component.

The present results are thus interpreted not as proof of specific compound(s) of action, but as a preliminary overall assessment. Eventually, correlation between chemical profile and bioactivity and subsequent fractionation and testing of isolated components would give a better foundation for structure–activity relationships.

4.8 Methodological Considerations and Limitations

The focus of this paper is methodological considerations and limitations.

In this study chiral preliminary phytochemical screening was successfully coupled with quantitative estimation of phenolics and flavonoids and values of some in vitro biological assays. This integrated approach offers a wide preliminary screening of bioactive potential of the methanolic extract. However, there were some limitations in the methodology that need to be taken as a factor when interpreting the results.

1) Qualitative phytochemical tests are generally background information on the presence of known classes of metabolites, and could be influenced by extraction efficiency and assay-specific interference. Second, the frequency of phenolic and flavonoid-associated molecule estimation is achieved for the total of many compounds and not just for the definitive molecules. Thirdly, the ability to scavenge DPPH radicals is a chemical assay and does not directly indicate antioxidant activities in living organisms.

In addition, agar diffusion tests may be affected by the ability of compounds to diffuse in the test medium, the concentration of extracts assayed and the physiochemical properties of the bioactive compounds being tested; consequently, inhibition zones should not be equated with antimicrobial activity. The brine shrimp lethality assay has not been shown to be identical with cytotoxicity tests for mammals or clinical toxicity of products and is only a preliminary measure of general cytotoxicity related activity. The α-amylase assay results are proof of enzyme inhibition in vitro, but are not proof in vivo of the efficacy as an antidiabetic agent.

The restrictions suggest a requirement for further assays complementing this work, as well as appropriate chemical characterization, mechanistic studies, and biological models for definitive pharmacological inference.

4.9 Research Gap and Future Directions

The present investigation is focussed on the significant pathway which is identified as the presence of phytochemical and biological data of C. govaniana Wall. var. swatensis, collected from Swat, Pakistan, is still lacking. The presence of alkaloids and biological activities have been reported in previous studies of C. govaniana and related Corydalis taxa, but variety-specific data are relatively scarce. The present results thus serve as a fruitful source of experimental information for future research work and not as a taxonomic description or a full characterization of the taxon.

Detailed phytochemical characterisation by chromatography and spectroscopy, especially for the isoquinoline alkaloids and any other potentially useful constituents should be the focus of future study. The compounds related to the properties of antioxidant activity, antimicrobial activity, cytotoxicity and α-amylase inhibitive activity could be selected through bioassay-guided fractionation. Complementary other antioxidant assays, mechanism-based enzyme studies, and validated cytotoxicity assays would provide additional biological evidence. Sampling at multiple locations from each geographical population would also provide insight into the geographical and/or chemical/bio stability of the observed properties, and verify their reproducibility.

4.10 Overall Interpretation

In general, the present study gives experimental evidence that the methanolic extract of C. govaniana Wall. var. swatensis has several class of phytochemical and the activities of antioxidant, anti-bacterial, anti-fungal, brine shrimp lethality and α-amylase inhibitory is measurable under the condition of the present study. The extract exhibited an IC_{50} of 92.64 µg/mL against DPPH, 214.58 µg/mL against α Amylase and 486.31 µg/mL against brine shrimp with both measurable antibacterial activity and measurable antifungal activity.

Although these results signify the scientific interest of the plant for producing chemicals with diverse natural products, it cannot be concluded that these compounds have therapeutic efficacy, specific molecular mechanisms and the identity of the compounds responsible for the observed activities from the above findings. Particularly, the constituents, reported earlier such as govaniadine, and other Corydalis alkaloids are not experimental components of the present extract until respective chemical analyses are made. The results presented here thus pave the way for further detailed phytochemical, mechanistic, toxicological and pharmacological studies.

5. CONCLUSION

The present study presents a preliminary experimental investigation into the phytochemical profile and biological activities of the methanolic extract of Corydalis govaniana Wall var. Swatensis collected from Swat, Khyber Pakhtunkhwa, Pakistan. Extraction yielded a product of 12.84%, and preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, glycosides, and carbohydrates in the extracted material, with steroids, proteins, and anthraquinones not detected under the tested conditions.

Quantitative analysis revealed a total phenolic content of 68.42 ± 2.31 mg GAE/g extract and a total flavonoid content of 41.76 ± 1.84 mg QE/g extract. The extract demonstrated concentration-dependent DPPH radical scavenging, with an IC_{50} of 92.64 µg/mL, compared to 28.47 µg/mL for ascorbic acid. The extract displayed detectable inhibition of all tested bacterial strains, with inhibition zones measured between 11.36 ± 0.51 and 18.14 ± 0.67 mm, and antifungal activity, with growth inhibition ranging from 32.65 ± 1.42% to 41.56 ± 1.31%. The brine shrimp lethality assay produced an LC_{50} of 486.31 µg/mL for the extract, indicating preliminary cytotoxicity-related activity, and the α-amylase assay demonstrated concentration-dependent inhibition of the enzyme with an IC_{50} of 214.58 µg/mL.

Overall, the present study finds that the methanolic extract of C. govaniana var. Swatensis demonstrates detectable antioxidant, antimicrobial, preliminary cytotoxicity-related, and enzyme inhibitory activities under the tested conditions. The results also present initial variety-specific experimental findings that complement previous reports of the phytochemistry and biological potential of C. govaniana.

However, the present investigation cannot identify or elucidate the individual contributions of specific bioactive compounds. Previously reported constituents of C. govaniana such as govaniadine and other isoquinoline alkaloids should be understood as literature-based possibilities rather than confirmed constituents of the present extract. Further investigations should focus on detailed characterization via chromatography and spectroscopy, bioassay-guided fractionation, mechanistic investigation, validated cytotoxicity assessment, and in vivo validation to clarify the chemical basis and biological relevance of the activities observed in the present study.

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