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PHYTOCHEMICAL PROFILING AND BIOLOGICAL ACTIVITIES OF *SWERTIA CHIRAYITA*: ANTIOXIDANT, ANTIDIABETIC, AND TLC FINGERPRINT ANALYSIS

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Keywords:

Swertia chirayita, Phytochemical analysis, Antioxidant activity, α-amylase inhibition, Thin layer chromatography

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ABSTRACT: Natural products from medicinal plants remain a valuable source of therapeutic drugs. The current study was designed to assess the phytochemical content, *in-vitro* antioxidant properties and antidiabetic activity, and also chromatographic fingerprint of the ethanolic extract of *Swertia chirayita*. Phytochemical screening of the extract confirmed the presence of various secondary metabolites such as flavonoids, phenolic compounds, tannins, saponins, and terpenoids. Quantitative analysis of the phytochemicals showed that the ethanolic extract had higher flavonoid and phenolic compound content than the aqueous extract. Antioxidant activity of the extract was determined by the DPPH radical scavenging and metal chelating methods, which showed strong free radical scavenging activity. Furthermore, the α-amylase inhibition assay showed strong antidiabetic activity, suggesting that the plant extract may be useful in the regulation of carbohydrate metabolism. Thin Layer Chromatography (TLC) analysis of the extract showed the presence of various phytochemicals with different Rf values, suggesting the presence of compounds with varying polarity. The findings of this study demonstrate the pharmacological significance of *Swertia chirayita* and its potential use as a natural source of antioxidants and antidiabetic agents.

INTRODUCTION: Medicinal plants have been utilised to cure specific disorders since antiquity. Since ancient times, numerous parts of medicinal plants have been employed to cure various disorders. Ayurvedic, Unani, and Siddha are three indigenous medical systems that have been practised for many centuries. Seventy percent of the villagers' medical needs are met by the traditional indigenous medical systems. Additionally, the demand for therapeutic herbs from the contemporary pharmaceutical industry has increased numerous times [14].

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Medicinal herbs are commonly utilised to treat many health issues worldwide [14, 19]. In industrialised countries, over 25% of prescribed medicines are derived directly or indirectly from medicinal plants. In developing countries, 80% of populations rely entirely on plants for their primary health care [21, 28]. Plant treatments have been shown to effectively treat infections caused by microorganisms. Approximately 70,000 plant species have been utilised to treat various microbial infections [19, 30].

A multidisciplinary approach combining botanical, ethnobotanical, phytochemical, and biological techniques has led to drug discovery from plants. Plants provide new lead molecules for the development of drugs against various pharmacological targets [21, 28]. Medicines based on plants were earlier dispensed in the form of crude drugs such as tinctures, teas, powders, and other

herbal formulations, which now serve as the basis of novel drug discovery. Discovery of drugs from plants has traditionally been time-consuming, so faster methods for plant collection, bioassay screening, isolation, and development of compounds must be adopted (Karan *et al.*, 1996). The search for novel compounds to be isolated from medicinal plants has emerged as a fascinating field of study in recent years. Due to their therapeutic qualities as well as their effective antibacterial, antidiabetic, and antioxidant properties, plants of ethnopharmaceutical significance are being investigated [26].

Swertia chirayita is a well-known herb that thrives in the Himalaya's temperate climate and belongs to the Gentianaceae family of plants. It is also known as Indian gentian [3, 11]. Gentianaceae is a blooming family with diverse floral patterns and hues. *Swertia chirayita* is an annual or biennial herb with seasonal growth. Stems vary in size from 60 to 150 cm and are upwardly quadrangular with a cylindrical base [15]. When a plant is young, its stems are greenish brown; when it reaches maturity, this colour shifts from light brown to violet. Its 10-cm-long, opposing, pair-pointed leaves lack stems [2, 13].

Swertia chirayita grows well in marshy areas and forest gardens with a sunny border and partial shade. Due to the presence of several chemical constituents such as triterpenoids, mangiferin, swerchirin, flavonoids, terpenoids, saponins, lignans, pentacyclic triterpenoids, etc., the plant is well-known for its medicinal properties (Kaloo & Bhat, 2020; Khalid *et al.*, 2011) [12].

S. chirayita is used in traditional medicine to treat chronic fever, malaria, anemia, bronchial asthma, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcer, scanty urine, hypertension, melancholia, mental disorders, bile secretion, blood purification, and diabetes [23]. According to Ayurveda, *S. chirayita* has a bitter (tikta) taste and a cooling (shita), easily digested (laghu), and dry (ruksha) thermal action [11]. It is a key component in numerous Ayurvedic health tonics, supplements, liver tonics, skin creams, soaps, anti-diabetic and anti-cancer treatments, and even hair oils [23]. The objective of this study is to identify the major phytochemicals present in the ethanolic extract of *Swertia chirayita*. The study also aims to investigate the *in-vitro* antioxidant and antidiabetic activity of *Swertia chirayita.*

MATERIAL AND METHODS:

Test Sample:

Collection and Identification of Plant: The *Swertia chirayita* whole dried plant was obtained from Anant Ayurveda Bhandara, Goregaon, Mumbai, India, in October 2025. The plant material used in the present study consisted of the whole plant. The plant was identified and authenticated by the botanist Dr. Kamlakar H. Patil, Department of Botany, Shivaji University, Kolhapur. A voucher specimen (SSAK-001) was deposited in the departmental herbarium for future reference.

Preparation of Plant Extract:

Preparation of Ethanolic Extract: The entire dried plant was powdered and stored. 95% ethanol was used to macerate the weighed powder (50 g), and the mixture was agitated by a shaker for 24 hours. Afterwards, the extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at low temperature and pressure to afford crude ethanol extract. The concentrate was further evaporated and dried by a hot water bath at 75°C. The percentage yield of the ethanolic extract was calculated and found to be 16% w/w. Lastly, the extract was ready for the study.

Preparation of Aqueous Extract: The powdered plant material of *Swertia chirayita* (50 g) was extracted with distilled water (500 mL) by maceration. The mixture was heated at 60–70°C for 2 hours with occasional stirring and then allowed to stand at room temperature for 24 hours. The extract was filtered using Whatman No. 1 filter paper to remove plant debris. The filtrate was concentrated using a water bath at 70°C until a semi-solid mass was obtained. The extract was further dried to constant weight, and the percentage yield (13% w/w) was calculated. The dried extract was stored in an airtight container until further use.

All experimental assays were performed using extracts normalised based on dry extract weight (mg/mL), not crude volume, to ensure consistency and reproducibility.

Phytochemical Screening: The ethanolic extract of *Swertia chirayita* was subjected to phytochemical screening to detect major secondary metabolites such as terpenoids, flavonoids, saponins, tannins, and phenols [6]. All tests were performed on prepared extracts (not raw plant powder) using standard protocols with slight modifications. Each test was carried out in triplicate for accuracy.

Test for Terpenoids: Approximately 0.5g of dried ethanolic extract of *Swertia chirayita* was dissolved in 2mL of chloroform. Then, 3mL of concentrated sulfuric acid was carefully added to create a layer.

Observation: Formation of a reddish-brown colouration at the interface indicated the presence of terpenoids [3, 22].

Test for Flavonoids: 5 mL of 10% diluted ammonia and 1 mL of concentrated sulfuric acid were added to 0.5 mL of the extract solution (1mg/mL).

Observation: Development of a yellow colour that disappears upon standing confirmed the presence of flavonoids [3, 12, 22, 25].

Test for Tannins: In a test tube, 0.5g of plant extract was heated in 10mL of distilled water for 5 mins before the solution was filtered. 2-3 drops of 0.1% ferric chloride were added to the 2 mL of filtrate.

Observation: Appearance of a blue-black or greenish colouration indicated the presence of tannins [5, 10, 27].

Test for Saponins: In test tubes, 5 mL of distilled water was added to 0.5 g of extract and shaken vigorously for 2 minutes. Then, 2-3 drops of olive oil were added and shaken again.

Observation: Formation of a stable, persistent froth and emulsion confirmed the presence of saponins [4, 5, 7, 27].

Keller-Killiani Test (Cardiac Glycosides Test): 0.5g of extract was dissolved in 5mL of distilled water. To this, 1-2 drops of 5% ferric chloride solution and 2mL of glacial acetic acid were added. Shake the mixture gently for proper mixing. 1mL of concentrated sulfuric acid was added along the side of the test tube.

Observation: Formation of a brown ring at the interface, along with possible green or violet rings, indicates the presence of cardiac glycosides [1].

Test for Phenols (Ferric Chloride Test): 2-3 drops of a 10% ferric chloride solution were added to small amounts of extracts (1 mL of 1 mg/mL solution) that had been diluted in 2ml of distilled water.

Observation: Development of a blue, green, or violet colouration confirmed the presence of phenolic compounds [26].

Antioxidant Activity:

Quantitative Analysis of Total Flavonoids: According to the procedures outlined by Ebrahimzadeh *et al.* (2008) [9], the flavonoid content of plant extracts of *Swertia chirayita* was determined by mixing 0.5 ml of each extract with 1.5 ml of methanol, adding 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate, and finally adding 2.8 ml of distilled water to the mixture. After that, the mixture was allowed to incubate at room temperature for 30 minutes. Total phenolic content was determined using quercetin as a standard. A calibration curve was prepared using quercetin at concentrations ranging from 10–100 µg/mL. The absorbance was measured at 415 nm, and the calibration curve equation obtained was $y = 0.005x + 0.012$ with a correlation coefficient ($R^2 = 0.998$). A reagent blank was used for correction. A spectrophotometer was used to measure the reaction mixture's absorbance at 415 nm. The total flavonoid content was expressed in mg quercetin equivalent (QE)/g of dry extract. Every determination was performed in triplicate (n=3), and results are expressed as mean ± SD.

Quantitative Analysis of Total Phenols: A method outlined by Kim et al. (2003) was used to assess the total phenolic content of the plant extract of *Swertia chirayita*. This involved mixing 4.5 ml of distilled water with 0.5 ml of extract, followed by the addition of 0.5 ml of Folin Ciocalteu reagent. 5ml of 7% sodium carbonate and 2ml of distilled water were added to the mixture after it had been mixed and allowed to stand at room temperature for five minutes. The solution was mixed and then incubated at 23°C for 90 minutes.

At 750 nm, the absorbance was measured using a spectrophotometer. A calibration curve was constructed using gallic acid in the concentration range of 10–100 µg/mL. The absorbance was recorded at 750 nm, and the calibration equation was y = 0.006x + 0.015 with a correlation coefficient (R^2 = 0.997). The absorbance of the blank was subtracted from all readings. The total phenolic content was stated in mg gallic acid equivalent (GAE)/g of dry extract. The amount of total phenolic content was measured in triplicate (n=3), and results are expressed as mean ± SD.

Estimation of Antioxidant Activity through 1,1-Diphenyl-2-Picrilhydrazyl (DPPH) Reduction: The method suggested by Von Gadow et al. (1997) was used to assess the DPPH radical scavenging activity of plant ethanolic and aqueous extract of *Swertia chirayita* at different concentrations. This involved placing each plant extract sample in a separate cuvette of different concentration (10–50 µg/mL), adding 2 ml of a 6×10^{-5} M methanolic solution of DPPH radical immediately, and measuring absorbance at 517 nm. Following a 16-minute incubation period, the decrease in absorbance for all samples was then tested once more. The standard was an ascorbic acid methanolic solution at a concentration of (10–100 µg/mL). Triplicates of each determination were made. The percentage decrease in colour was used to quantify the DPPH radical-scavenging activity. The formula proposed by Yen and Duh (1994) was used to determine the percentage inhibition of the DPPH radical. The control was taken at time zero, meaning before the antioxidant activity of plant extracts commenced. The percentage inhibition was calculated for each concentration, and a dose–response curve was plotted between concentration and percentage inhibition. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined from the graph.

$$\%\text{inhibition} = (A_{C\,(0)} - A_{C\,(t)} / A_{C\,(0)} \times 100$$

Where,

$A_{C\,(0)}$ = Absorbance of control at time, t = 0 min

$A_{C\,(t)}$ = Absorbance of sample at time, t =16 min

Estimation of Antioxidant Activity through Metal Chelating Activity: A method proposed by Dinis *et al.* (1994) [8] was slightly modified to evaluate the metal (ferrous ion) chelating activity using ethanolic and aqueous extracts of *Swertia chirayita* at different concentrations (10–50 µg/mL). This involved adding roughly 100 µl of samples of plant extracts to 50 µl of a 2 mM FeCl2 solution. The reaction was then initiated by adding 200 µl of 5 mM ferrocyanide, shaking briskly, and allowing it to sit at room temperature for 10 minutes. At 562 nm, the absorbance of each sample for reducing the colouring of the solution was determined using a spectrophotometer. A reagent mixture devoid of sample was used as a negative control, and ethylenediaminetetraacetic acid solution (EDTA-0.1 mM) was utilised as a positive control instead of sample extracts. The percentage decrease in colour indicates the metal chelating activity. Each estimation was carried out in triplicate. The IC_{50} value was determined from the dose–response curve. The extracts' metal chelating capabilities were evaluated using the equation below:

$$\text{Metal chelating capacity (\%)} = (A_0 - A_t) / A_0 \times 100$$

Where,

A_0= Absorbance of the negative control

A_t = Absorbance of the sample extracts

Antidiabetic Activity:

***In-vitro* Enzyme Inhibition Assay for Antidiabetic Activity:** The antidiabetic potential of *S. chirayita* was evaluated *in-vitro* using a method proposed by Prabhakar *et al.* (2013) [4]. The inhibitory activity of the enzyme α-amylase (Porcine pancreas), which breaks down starch to create glucose, was used to measure the *in-vitro* anti-diabetic activity. Using this procedure, 1 mL of the ethanolic and aqueous extract of different concentrations (10–50 µg/mL) was introduced to 1 mL of the enzyme α-amylase (1 U/mL) solution prepared in phosphate buffer (0.02 M, pH 6.9, containing 0.006 M NaCl). The mixture was incubated for 10 minutes at 37°C. Followed by the addition of 1 ml of 1% starch solution as a substrate, and further incubated at 37°C for 15 minutes. After that, 2 ml of 3,5-dinitrosalicylic acid (DNSA) reagent was added to stop the reaction. After being incubated in a boiling water bath for five minutes, the reaction mixture was allowed to

cool to room temperature. Next, using a spectrophotometer, the absorbance of the reaction mixture was determined at 546 nm. Acarbose was used as a positive control. A control reaction without extract represented 100% enzyme activity. The IC_{50} value (concentration required to inhibit 50% of enzyme activity) was determined from the dose–response curve using regression analysis. The percentage of induced inhibition of α-amylase enzyme activity was calculated as follows:

% of inhibition of α-amylase = Enzyme activity of control - Enzyme activity of extract / Enzyme activity of the control × 100

Thin Layer Chromatography: Thin-layer chromatography (TLC) was performed using silica gel TLC plates (60 F254) as the stationary phase. The plates were activated in a hot air oven at 105°C for 30 minutes before use. Different solvent systems **Table 4** were prepared as the mobile phase, and the TLC chamber was saturated for 20 minutes before development. Sample extracts (10 µL) were applied onto the plates using a capillary tube, and the plates were developed up to a distance of 8 cm. After development, the plates were air-dried and exposed to iodine vapours in an iodine chamber, followed by heating at 105°C for 10 minutes to enhance spot visibility. The separated components were finally visualised under UV light at 366 nm. The Rf value of the spots was calculated using the given formula [18].

RF =Distance travelled by spot (cm) / Distance travelled by solvent front (cm)

Statistical Analysis: Results were represented as mean ± SD. The data was analysed using Student's t-test for comparison between two groups. $P < 0.05$ was considered significant.

Observation:

Phytochemical Screening: The therapeutic benefits of plant extracts are mostly attributable to physiologically active chemical components found in them. Qualitative Analysis of the phytochemical reveals the presence of Terpenoids, flavonoids, tannins, saponins, glycosides and phenols presented in **Table 1**. The pharmacological activities listed are based on previously reported literature and not confirmed by the present study.

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF *SWERTIA CHIRAYITA* SHOWING PRESENCE (+) AND ABSENCE (–) OF MAJOR SECONDARY METABOLITES AND THEIR REPORTED PHARMACOLOGICAL SIGNIFICANCE (LITERATURE- BASED)

Phytochemical	Test Observation	Presence (+) / Absence (–)	Reported Pharmacological Properties	References
Terpenoids	reddish-brown colouration at the interface	+	Antioxidant, anti-inflammatory, hepatoprotective	3, 22
Flavonoids	yellow hue that appeared, and as it stands, that colour goes away	+	Antioxidant, antidiabetic, cardioprotective	12
Tannins	brownish green or blue-black hue develops	+	Antioxidant, antimicrobial, anticancer	26 27
Saponins	formation of emulsion	+	Anti-inflammatory, anticancer, immunomodulatory	7
Cardiac Glycosides	No Brown ring at the interface	–	Treatment of cardiac failure	20
Phenols	blue hue developed	+	Strong antioxidant, free radical scavenging	26

Legend: (+) Present; (–) Absent.

Antioxidant Activity:

Quantitative Analysis of Total Flavonoids: The analysis shows the ethanolic extract of *Swertia chirayita* has higher flavonoid content than the aqueous extract, as shown in **Table 2.**

Quantitative Analysis of Total Phenols: The analysis shows the ethanolic extract of *Swertia chirayita* has higher phenolic content than the aqueous extract, as shown in **Table 2.**

1, 1 - Diphenyl – 2 - Picrilhydrazyl (DPPH) Reduction and Metal Chelating Activity: The evaluation indicates that the ethanolic extract of *Swertia chirayita* exhibits superior antioxidant activity compared to the aqueous extract. This is due to a higher concentration of flavonoids and phenolic compounds. Solvent polarity has a substantial impact on the extraction efficiency of bioactive substances.

The ethanol extract shows higher antioxidant capacity, as evidenced by a lower IC_{50} value for DPPH and Metal Chelating activity compared to the aqueous extract. This shows that ethanol is more effective at extracting bioactive antioxidant compounds from *Swertia chirayita.*

TABLE 2: ANTIOXIDANT ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SWERTIA CHIRAYITA*

Extract Type	Total Flavonoids (mg QE/g)	Total Phenolics (mg GAE/g)	DPPH (µg/mL) IC_{50} (mean values)	Metal Chelating (µg/mL) IC_{50} (mean values)
Ethanol	144.60 ± 5.20	90.40 ± 4.35	24.80 ± 1.75	48.35 ± 2.60
Aqueous	70.50 ± 3.80	72.10 ± 3.95	39.60 ± 2.40	64.20 ± 3.55

Values represent Mean±SD of triplicate, *Significant at P<0.05.

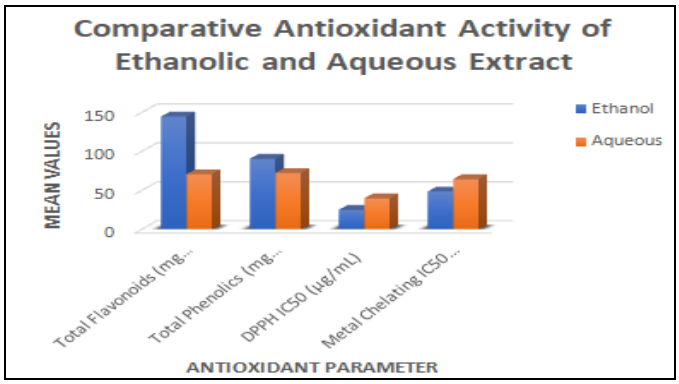

FIG. 1: BAR GRAPH OF COMPARATIVE ANTIOXIDANT ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *SWERTIA CHIRAYITA* SHOWING TOTAL FLAVONOID CONTENT (MG QE/G EXTRACT), TOTAL PHENOLIC CONTENT (MG GAE/G EXTRACT), DPPH RADICAL SCAVENGING ACTIVITY (IC_{50}, µG/ML), AND METAL CHELATING ACTIVITY (IC_{50}, µG/ML). Values are expressed as Mean ± Sd (n = 3).

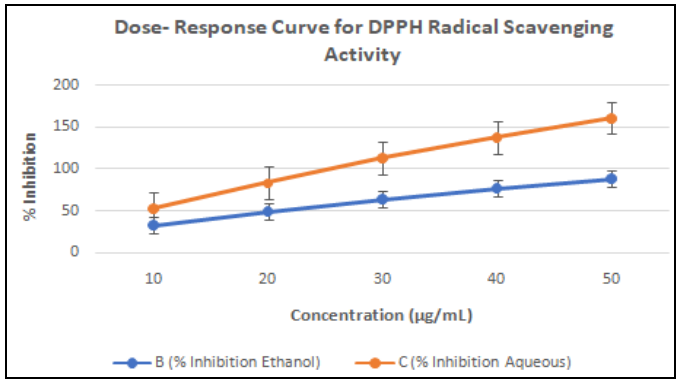

FIG. 2: DOSE–RESPONSE CURVE SHOWING DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SWERTIA CHIRAYITA*. A CONCENTRATION-DEPENDENT (10–50 µG/ML) INCREASE IN PERCENTAGE INHIBITION WAS OBSERVED, AND IC_{50} VALUES WERE DETERMINED FROM THE REGRESSION ANALYSIS. Values are expressed as mean ± SD (n = 3).

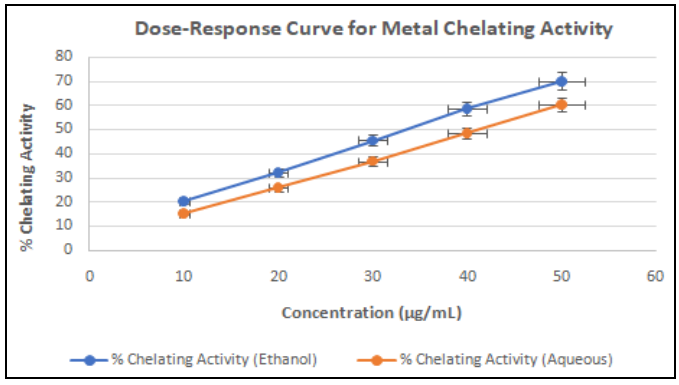

FIG. 3: DOSE–RESPONSE CURVE SHOWING METAL CHELATING ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SWERTIA CHIRAYITA*. A CONCENTRATION-DEPENDENT (10–50 µG/ML) INCREASE IN PERCENTAGE CHELATING ACTIVITY WAS OBSERVED, AND IC_{50} VALUES WERE DETERMINED FROM THE REGRESSION ANALYSIS. Values are expressed as mean ± SD (n = 3).

Antidiabetic Activity:

***In-vitro* Enzyme Inhibition Assay for Antidiabetic Activity:** The result shows that the plant has significant antidiabetic potential, as shown in **Table 2**. The lower IC_{50} value of the ethanolic extract indicates its stronger α-amylase inhibitory potential compared to the aqueous extract. This is due to the significant concentration of bioactive compounds responsible for anti-diabetic activity.

TABLE 3: *IN-VITRO* ENZYME INHIBITION ASSAY FOR ANTIDIABETIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *SWERTIA CHIRAYITA*

Extract type	Mean % α-Amylase Inhibition
Ethanol	52.25 ± 0.61
Aqueous	47.49 ± 0.63

Values represent Mean±SD of triplicate, *Significant at P<0.05.

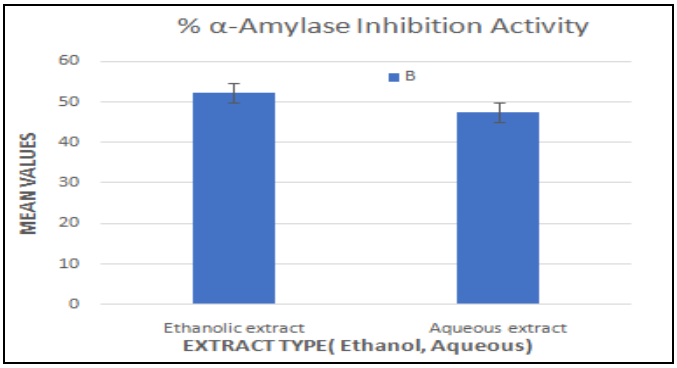

FIG. 4: BAR GRAPH OF COMPARATIVE A-AMYLASE INHIBITORY ACTIVITY OF *SWERTIA CHIRAYITA SHOWING* STRONGER A-AMYLASE INHIBITORY POTENTIAL OF ETHANOLIC EXTRACT WHEN COMPARED TO THE AQUEOUS EXTRACT. Values are expressed as mean ± SD (n = 3).

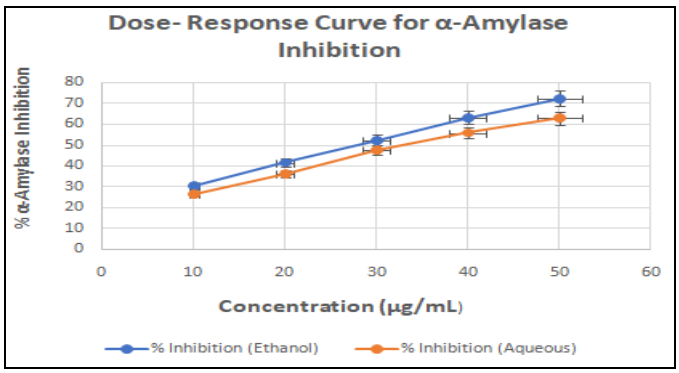

FIG. 5: DOSE–RESPONSE CURVE SHOWING A-AMYLASE INHIBITION ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *SWERTIA CHIRAYITA*. A CONCENTRATION-DEPENDENT (10–50 µG/ML) INCREASE IN PERCENTAGE A-AMYLASE INHIBITION ACTIVITY WAS OBSERVED, AND IC_{50} VALUES WERE DETERMINED FROM THE REGRESSION ANALYSIS. Values are expressed as mean ± SD (n = 3).

Thin Layer Chromatography: Thin-Layer Chromatography (TLC) analysis of *Swertia chirayita* was performed using silica gel 60 F245 plates and multiple solvent systems to achieve optimal separation of phytoconstituents. The solvent systems used benzene:chloroform (3:1), chloroform:methanol (93:7), and toluene:ethyl acetate:formic acid (5:4:1) are consistent with those presented in **Table 4** and were selected to cover a wide range of polarity for effective resolution of compounds. Thin-Layer Chromatography (TLC) analysis of *Swertia chirayita* was performed using silica gel 60 F245 plates and multiple solvent systems to achieve optimal separation of phytoconstituents. The solvent systems used benzene:chloroform (3:1), chloroform:methanol (93:7), and toluene:ethyl acetate:formic acid (5:4:1) are consistent with those presented in **Table 4** and were selected to cover a wide range of polarity for effective resolution of compounds. Although the primary focus of the study was on ethanolic and aqueous extracts, petroleum ether and chloroform fractions were also included in the TLC profiling to obtain a broader phytochemical fingerprint.

These fractions aided in the separation of non-polar and moderately polar constituents, thereby enhancing the overall characterisation of the plant extract.

TABLE 4: THIN LAYER CHROMATOGRAPHIC (TLC) PROFILE OF *SWERTIA CHIRAYITA* EXTRACTS USING DIFFERENT SOLVENT SYSTEMS, SHOWING THE NUMBER OF SPOTS AND CORRESPONDING RF VALUES OBTAINED UNDER UV (366 NM) VISUALISATION, INDICATING THE SEPARATION OF PHYTOCONSTITUENTS

Extract	Solvent System	Visualizing Condition	No. of Spots	Correct Rf Values
Petroleum ether	Benzene: Chloroform (3:1)	UV Long	6	0.11, 0.16, 0.24, 0.42, 0.51, 0.85
Petroleum ether	Chloroform: Methanol (93:7)	UV Long	2	0.18, 0.63
Petroleum ether	Toluene: Ethyl acetate: Formic acid (5:4:1)	UV Long	1	0.20
Ethanol	Benzene: Chloroform (3:1)	UV Long	4	0.24, 0.36, 0.48, 0.57
Ethanol	Chloroform: Methanol (93:7)	UV Long	1	0.13
Ethanol	Toluene: Ethyl acetate: Formic acid (5:4:1)	UV Long	5	0.08, 0.40, 0.50, 0.53, 0.91
Chloroform	Benzene: Chloroform (3:1)	UV Long	2	0.22, 0.42
Chloroform	Chloroform: Methanol (93:7)	UV Long	4	0.12, 0.38, 0.84, 0.92
Chloroform	Toluene: Ethyl acetate: Formic acid (5:4:1)	UV Long	5	0.02, 0.11, 0.14, 0.78, 0.85

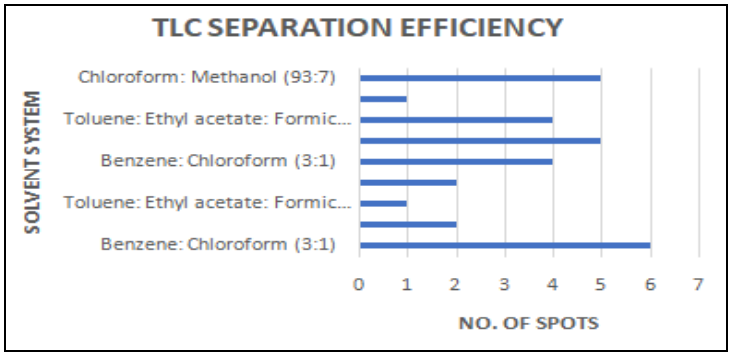

FIG. 6: BAR GRAPH OF TLC SEPARATION PROFILE OF *SWERTIA CHIRAYITA* SHOWING VARIATION IN COMPOUND SEPARATION USING DIFFERENT SOLVENT SYSTEMS

FIG. 7: TLC PROFILE OF *SWERTIA CHIRAYITA* PLANT EXTRACT UNDER UV LIGHT (366 NM) SHOWING SEPARATION OF PHYTOCONSTITUENTS USING DIFFERENT SOLVENT SYSTEMS. DISTINCT BANDS WITH VARYING RF VALUES INDICATE THE PRESENCE OF COMPOUNDS WITH DIFFERENT POLARITIES

DISCUSSION: The present study evaluated the phytochemical composition and *in-vitro* biological activities of *Swertia chirayita*. Qualitative screening confirmed the presence of major secondary metabolites such as flavonoids, phenolics, tannins, saponins, and terpenoids, while cardiac glycosides were not detected. Quantitative analysis demonstrated that the ethanolic extract contained higher levels of phenolics and flavonoids compared to the aqueous extract, which may explain its relatively stronger antioxidant activity observed in DPPH and metal chelating assays. Similarly, the ethanolic extract exhibited higher α-amylase inhibitory activity, suggesting its potential role in modulating carbohydrate metabolism under in vitro conditions. TLC profiling revealed multiple phytoconstituents with a wide range of Rf values, supporting the chemical diversity of the extract; however, these results were limited to fingerprint characterisation due to the absence of reference

standards. Overall, the findings indicate that *Swertia chirayita*, particularly its ethanolic extract, is a promising source of bioactive compounds with antioxidant and enzyme inhibitory potential. However, these observations are based solely on preliminary *in-vitro* assays and should not be interpreted as confirmation of therapeutic efficacy or clinical applicability. The present study is limited by the use of crude extracts without marker-based standardisation, which may result in variability in phytochemical composition. Individual bioactive compounds were neither isolated nor structurally identified, limiting precise attribution of biological activity. The antidiabetic assessment was restricted to a single enzyme (α-amylase) inhibition assay, which does not fully represent the complexity of glucose metabolism. Furthermore, no *in-vivo* studies or toxicity/safety evaluations were conducted to validate the biological effects observed *in-vitro*. Therefore, further investigations involving compound isolation, multi-target studies, *in-vivo* validation, and toxicity assessment are required to substantiate these findings.

CONCLUSION: The current study has reaffirmed that *Swertia chirayita* is a treasure trove of phytochemicals with promising antioxidant and antidiabetic properties. The ethanolic extract showed potent free radical scavenging, metal chelating ability, and significant α-amylase inhibition. Moreover, the Thin Layer Chromatography results showed the varied phytochemical composition of the plant extract. The results of the current study have provided scientific validation for the use of the plant and indicate its potential use in the preparation of natural medicines. Future studies should be aimed at the isolation and characterisation of the phytochemicals, as well as *in-vivo* studies, to tap the plant full potential.

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CONFLICTS OF INTEREST: Nil

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