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Phytochemical Profiling, GC-MS Characterization, Antimicrobial, Antioxidant and Antidiabetic Activities of Stem Bark Extracts of *Glycosmis pentaphylla* (Retz.) DC.

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Abstract

Background: *Glycosmis pentaphylla* (Retz.) DC. is a medicinal plant widely used in traditional medicine for the treatment of various ailments. The present study aimed to investigate the phytochemical composition, GC–MS profile, antimicrobial, antioxidant and antidiabetic activities of the stem bark extracts of *G. pentaphylla*.

Methods: Stem bark extracts were prepared using chloroform, ethyl acetate, methanol and aqueous solvents. Qualitative phytochemical screening was performed using standard procedures. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. GC–MS analysis was conducted to identify major phytoconstituents. Antimicrobial activity was evaluated against *Bacillus cereus*, *Escherichia coli* and *Candida albicans* using the agar well diffusion method. Antioxidant activity was assessed through DPPH and hydrogen peroxide scavenging assays, while antidiabetic potential was evaluated by α-amylase inhibition and glucose uptake assays.

Results: Phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, terpenoids, tannins, glycosides, sterols, saponins and carbohydrates in varying proportions among the solvent extracts. The aqueous extract exhibited the highest total phenolic content (68.20 ± 0.01 mg GAE/g extract) and total flavonoid content (59.01 ± 0.05 mg QE/g extract), followed by the methanolic extract. GC–MS profiling identified β-myrcene, β-pinene and sabinene as major volatile constituents, while vitexin was recognized as an important non-volatile flavonoid phytoconstituent. The stem bark extract demonstrated concentration-dependent antimicrobial activity against *B. cereus*, *E. coli* and *C. albicans*. Furthermore, the extract exhibited appreciable antioxidant activity and significant α-amylase inhibitory and glucose uptake activities, indicating promising antidiabetic potential.

Conclusion: These findings demonstrate that *Glycosmis pentaphylla* stem bark is a rich source of bioactive phytochemicals with antimicrobial, antioxidant and antidiabetic activities. The observed biological effects may be attributed to the synergistic actions of phenolic compounds, flavonoids and terpenoid constituents. These results support the traditional medicinal use of this plant and highlight its potential for the development of novel phytopharmaceutical agents.

Introduction

Plant-derived bioactive compounds are particularly attractive because they possess a wide range of biological properties and are often associated with fewer adverse effects compared with synthetic drugs. Among these compounds, phenolics, flavonoids, alkaloids, terpenoids, tannins, glycosides and other secondary metabolites have been extensively investigated for their antimicrobial, antioxidant, anti-inflammatory and antidiabetic activities. (Feng, 2025)

Glycosmis pentaphylla (Retz.) DC., belonging to the family Rutaceae, is a medicinal shrub widely distributed in tropical and subtropical regions of Asia, including India, Sri Lanka, Bangladesh, Thailand and other neighboring countries.

(Hatti, 2025) The plant is commonly found in forests, hilly regions and rural landscapes, where it has been used for generations in traditional medicine systems. (Dincheva *et al*., 2023) Different parts of the plant, including leaves, roots, fruits and stem bark, have been employed in folk remedies for the treatment of fever, inflammation, cough, liver disorders, skin diseases, microbial infections and various metabolic ailments. (Wani & Upadhyay, 2026) The widespread traditional use of this species suggests the presence of biologically active constituents that may contribute to its therapeutic effects. Previous phytochemical studies on *G. pentaphylla* have revealed the occurrence of several classes of secondary metabolites, including alkaloids, flavonoids, coumarins, terpenoids, essential oils and phenolic compounds. (Babu & Radhamany, 2020) These phytochemicals exhibit diverse pharmacological activities, such as antioxidant, antimicrobial, anticancer, anti-inflammatory, hepatoprotective and antidiabetic effects. Despite the growing body of evidence supporting the medicinal value of the plant, most investigations have focused on the leaves, fruits, or aerial parts, while comparatively limited attention has been given to the stem bark. (Wimmer, 2025) Since different plant parts often vary in their phytochemical composition and biological activities, detailed studies on the stem bark are necessary to fully understand its medicinal potential. (Sarkar *et al*., 2025) Phenolic compounds and flavonoids are among the most important groups of plant metabolites because of their remarkable antioxidant capacity. These compounds can neutralize reactive oxygen species and free radicals, thereby protecting biological systems from oxidative damage. Oxidative stress has been implicated in the development and progression of numerous chronic diseases, including cardiovascular disorders, neurodegenerative diseases, cancer and diabetes mellitus. (Chatterjee & Majumdar, 1954) Therefore, plants rich in phenolic and flavonoid constituents are considered promising candidates for the development of natural antioxidant therapies. In addition to their antioxidant properties, these compounds have also been reported to possess antimicrobial and antidiabetic activities through various mechanisms, including enzyme inhibition, modulation of cellular signaling pathways and enhancement of glucose metabolism. (Hatti, 2025) The identification of phytochemical constituents is essential for understanding the therapeutic potential of medicinal plants. Gas chromatography–mass spectrometry (GC–MS) is a powerful analytical technique widely used for the characterization of volatile and semi-volatile compounds present in plant extracts. GC–MS profiling provides valuable information regarding the chemical composition of medicinal plants and facilitates the identification of compounds that may be responsible for the observed biological activities. The integration of phytochemical screening, quantitative estimation of bioactive compounds and advanced analytical techniques, such as GC–MS, offers a comprehensive approach for evaluating the medicinal significance of plant materials. (Aadesariya *et al*., 2017)

In addition to phytochemical characterization, the assessment of biological activities is crucial for validating traditional medicinal claims. (Bashkin *et al*., 2021) Antimicrobial studies help determine the effectiveness of plant extracts

against pathogenic microorganisms, whereas antioxidant assays evaluate their ability to scavenge free radicals and reduce oxidative stress. (Džamić & Matejić, 2022) Similarly, antidiabetic investigations provide insights into the potential of plant-derived compounds to regulate glucose metabolism and inhibit carbohydrate-digesting enzymes. Such studies contribute to the identification of natural therapeutic agents that may be useful in the prevention and management of chronic diseases. (Pant *et al*., 2021) Therefore, the present study was designed to investigate the phytochemical composition and biological activities of different solvent extracts of the stem bark of *G. pentaphylla*. The study involved qualitative phytochemical screening, determination of total phenolic and flavonoid contents and GC–MS profiling to identify the major bioactive constituents.(M *et al*., 2021) Furthermore, the antimicrobial, antioxidant and antidiabetic properties of the stem bark extracts were evaluated to assess their therapeutic potentials. The findings of this study are expected to provide scientific evidence supporting the traditional use of *G. pentaphylla* and contribute to the development of plant-based therapeutic agents for the management of infectious diseases, oxidative stress-related disorders and diabetes mellitus.

Materials and Methods

Chemicals

All chemicals and reagents used in this study were of analytical grade and sourced from Sigma-Aldrich (Merck) and HiMedia, India.

Plant Collection and Identification

Plants were collected from the Chakra, Hosanagara Talu, Shivamogga District. A herbarium specimen was deposited in the Department of Botany at Sahyadri Science College under the reference number SSC/DB/2024/0055.

Solvent extraction

The dry stem bark of *Glycosmis pentaphylla (Retz.) DC* was coarsely pulverized and serial solvent extraction was conducted using a Soxhlet apparatus. Extraction was performed with increasing polarity using chloroform, methanol, ethyl acetate and distilled water. The extracts were then concentrated using a rotary evaporator. The concentrated extracts were stored in airtight containers on ice until further use.

Phytochemical analysis

Crude extracts of *Glycosmis pentaphylla (Retz.) The DC* stem bark was screened for the presence of various phytochemical components, including alkaloids, tannins, phenols, sterols, terpenoids, glycosides, saponins, flavonoids and carbohydrates, using standard procedure. (K *et al*., 2020)

Estimation of total polyphenols content (TPC)

To estimate the total phenol content (TPC), 1.5 mL of Folin–Ciocalteu's (FC) reagent and 7.5% sodium carbonate (Na_2CO_3) solution were used to treat the plant extract, which had a concentration of 1 mg/mL in the respective solvent. The mixture was subjected to oxidation and the absorbance was measured at 750 nm after 1 h of incubation at room temperature. The experiment was conducted in triplicate and the results are expressed as the mean ± standard deviation. The number of phenolic

compounds was determined using a gallic acid calibration curve and the results were expressed as gallic acid equivalents (GAE) in mg/100 mL of the sample. (Shrivastava & Daharia, 2026)

Estimation of total flavonoid content (TFC)

To determine the total flavonoid content, a 10% aluminum chloride ($AlCl_3$) solution and 1 M sodium acetate were combined with the plant extract at a concentration of 1 mg/mL in their respective solvents. The mixture was incubated in the dark for 45 min and the absorbance was measured at 415 nm wavelength. The flavonoid content was determined using a quercetin calibration curve and the results were expressed as quercetin equivalents (QE) in mg/100 mL of the sample solution. The experiment was performed in triplicate and the results are reported as mean ± standard deviation. (Tran *et al*., 2020)

GC-MS analysis

GC–MS profiling was performed to identify potential phytochemicals in the methanolic and aqueous extracts of *Glycosmis pentaphylla (Retz.) DC* based on their molecular formulas and retention times [19]. The analysis was conducted using a GC–MS/MS system (Thermo Fisher Scientific, TSQ 9000). The extracts were separated using a capillary column with the following specifications: length, 30 m; ID, 0.25 mm; film thickness, 0.25 µm; and maximum temperature, 330/350°C. A flame ionization detector (FID) was used. The MS transfer line temperature was set to 280°C and the ion source temperature was 250°C. The filter peak width time was 1 s, detector gain multiplier was 1, electron energy was 0 eV and the scan range was from 40 amu to 450 amu with a scan time of 0.2 s. Chemical components were identified by analyzing the mass spectral data of the GC peaks using the National Institute of Standards and Technology (NIST) library. (Dk *et al*., 2025)

Antibacterial and Antifungal Activity Assay

The antibacterial activity of *Glycosmis pentaphylla* extract was evaluated by the agar well diffusion method against Gram-positive *Bacillus cereus* (NCIM 2272) and Gram-negative *Escherichia coli* (NCIM 1168). Nutrient agar medium was prepared and sterilized before being poured into sterile Petri dishes. After solidification, the bacterial cultures were uniformly spread over the agar surface using sterile cotton swabs. Wells of 6 mm in diameter were made in the agar plates using a sterile cork borer. Different concentrations of the test samples (100, 200, 400 and 800 µg/mL) were loaded into the respective wells. Streptomycin was used as the positive control, while the corresponding solvent served as the negative control. The inoculated plates were incubated at 37°C for 24 h, after which the antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding each well. The antifungal activity was assessed against *Candida albicans* (NCIM 1824) using the agar well diffusion method on Sabouraud's dextrose agar (SDA) medium. The fungal inoculum was uniformly spread on the agar surface and wells were prepared using a sterile cork borer. Test samples at concentrations of 100, 200, 400 and 800 µg/mL were introduced into the wells. Fluconazole was used as the standard antifungal drug. The

plates were incubated at 40°C for 24–48 h and the antifungal activity was evaluated by measuring the diameter of the inhibition zones around the wells. All experiments were performed in duplicate and the results were expressed as the mean zone of inhibition (mm). (Babu & Radhamany, 2020)

Antioxidant activity of *methanolic and aqueous extracts of Glycosmis pentaphylla (Retz.) DC stembark*

a) 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

For the DPPH radical scavenging assay, *Glycosmis pentaphylla (Retz.) DC* plant extracts were analyzed using the method described by Rice-Evans *et al*. The test samples, each at a concentration of 100 µg/mL, were mixed with 100 µL of 40 mM DPPH solution in methanol at various concentrations. The mixture was incubated in the dark at room temperature for 30 min to allow the reaction to proceed further. Absorbance was measured at 517 nm using ascorbic acid as the standard. The DPPH scavenging activity of each sample was calculated using the following formula: where As represents the absorbance of the test sample and Ac denotes the absorbance of the control reaction (100 µL of methanol plus 100 µL of DPPH solution). (Md Taib *et al*., 2022)

$$\% \text{ Antioxidant activity} = [(Ac - As)/Ac] \times 100 \text{-------- (1)}$$

b) Hydrogen peroxide (H_2O_2) assay

To evaluate the antioxidant activity of *Glycosmis pentaphylla (Retz.) The ability of DC stem bark extracts* to scavenge hydrogen peroxide was assessed using ascorbic acid. A 40 mM H2O2 solution was prepared using a phosphate buffer (pH 7.4). Extracts at concentrations ranging from 50 to 250 µg/mL in distilled water were mixed with 0.6 mL of a 40 mM H2O2 solution to achieve a final volume of 1.1 mL. After a 10-min incubation, the absorbance of H2O2 was measured at 230 nm using a Labman UV-Vis spectrophotometer, with a blank solution containing only a phosphate buffer as the reference. The extent of H2O2 scavenging was calculated using Equation. (Suwannakul & Chaibenjawong, 2017)

$$\% Scavenged\ [H2O2] = \left[\frac{[(Ac - As)]}{Ac}\right] \times 100 \text{----------(2)}$$

where Ac is the absorbance of the control and Ac is the absorbance in the presence of the extract or standard, respectively.

Antidiabetic activity of *methanolic and aqueous extracts of Glycosmis pentaphylla (Retz.) DC stem bark*

a) Alpha-amylase inhibition assay

In an Eppendorf tube, 1 mL of PBS was combined with 0.5 mL of various sample concentrations (50, 100, 150, 200 and 250 µg/mL) along with a standard solution. Next, 200 µL of 0.5 mg/mL α-amylase solution and 200 µL of 5 mg/mL starch solution were added to the sample. The mixture was incubated at room temperature for 10 min. The control consisted of starch and α-amylase, without any other samples. The reaction was terminated by adding 400 µL of DNS solution and heating the mixture in a boiling water bath for 5 min, followed by cooling. The absorbance was measured at 540 nm using a Labman UV-visible spectrophotometer. The percentage of enzyme inhibition was calculated using the following formula: Enzyme inhibition efficiency was determined by applying the

equation provided below (3). where Ac and As are the absorbance values of the control and sample, respectively. (Freitas *et al.*, 2023)

$$\%\ of\ \propto -amylose\ inhibition = [(Ac - As)/Ac] \times 100 \ldots\ldots\ldots\ldots\ldots (3)$$

b) Glucose take-up measure

Saccharomyces cerevisiae was suspended in double-distilled water and centrifuged (3000 rpm for 5 min) until a clear supernatant was obtained. A 10% (v/v) suspension was prepared using double-distilled water. Plant extracts at various concentrations (50–250 µg/mL) were added to test tubes. Then, 1 mL of a 5 mM glucose solution was added to each tube, which was incubated at 37°C for one hour. Following incubation, the tubes were centrifuged (2500 rpm, 5 min) and the glucose concentration in the supernatant was measured. Absorbance was measured at 540 nm using a Labman spectrophotometer[9]. Metronidazole was used as a reference drug. A standard calibration curve was created by plotting absorbance versus glucose concentration. The percentage increase in glucose absorption by yeast cells was calculated using Equation (3). The experiment was conducted in triplicate to ensure accuracy. where Abs sample is the absorbance of the test sample. Abs control = absorbance of the control reaction. (Sandaruwan *et al.*, 2024)

$$\%\ Glucose\ uptake = Abs\ control - \frac{Abs\ sample}{Abs\ control} \times 100 \ldots\ldots\ldots\ldots\ldots (4)$$

Statistical analysis

The results of the phytochemical analyses and biological activity data were analyzed using SPSS (version 20.0). The significance level was set at $P \geq 0.05$ and the means were compared using Duncan's multiple range test (DMRT) to identify significant differences. Data are presented as the mean ± standard error (SE).

Results

Preliminary phytochemical screening of the chloroform, ethyl acetate, methanolic and aqueous stem bark extracts of *Glycosmis pentaphylla* (Retz.) DC. revealed the presence of a diverse range of secondary metabolites. Alkaloids, phenols, terpenoids and flavonoids were detected in all four solvent extracts, indicating their widespread distribution within the stem bark. Tannins and glycosides were absent in the chloroform extract but were present in the ethyl acetate, methanolic and aqueous extracts. Sterols were detected in the chloroform, ethyl acetate and methanolic extracts, whereas they were absent in the aqueous extract. Saponins were found exclusively in the methanolic and aqueous extracts, suggesting that these polar solvents were more effective in extracting saponin-rich constituents. Similarly, carbohydrates were detected only in the methanolic and aqueous extracts and were absent in the chloroform and ethyl acetate extracts. Among the tested solvents, the methanolic extract exhibited the broadest spectrum of phytochemical constituents, showing the presence of all tested metabolites. The aqueous extract also contained most of the phytochemicals, except for sterols. In contrast, the chloroform extract demonstrated the least phytochemical diversity, lacking tannins, glycosides, saponins and carbohydrates. These findings indicate that polar solvents,

particularly methanol and water, are more efficient in extracting bioactive phytoconstituents from the stem bark of *Glycosmis pentaphylla*, thereby highlighting their potential utility for further pharmacological investigations (*Table 1*).

Table 1. Qualitative phytochemical screening of different solvent extracts of *Glycosmis pentaphylla* (Retz.) DC. stem bark

Phytochemical Constituents	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract	Aqueous Extract
Alkaloids	+	+	+	+
Tannins	-	+	+	+
Phenols	+	+	+	+
Sterols	+	+	+	-
Terpenoids	+	+	+	+
Glycosides	-	+	+	+
Saponins	-	-	+	+
Flavonoids	+	+	+	+
Carbohydrates	-	-	+	+

Note: (+) Present; (−) Absent.

The total phenolic content of the different solvent extracts of *Glycosmis pentaphylla* (Retz.) DC. stem bark was determined using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). Significant variations in phenolic content were observed among the extracts depending on the solvent used for extraction. Among the tested extracts, the aqueous extract exhibited the highest total phenolic content (68.20 ± 0.01 mg GAE/g extract), followed by the methanolic extract (52.00 ± 0.07 mg GAE/g extract). The ethyl acetate extract showed a moderate phenolic content of 25.02 ± 0.02 mg GAE/g extract, whereas the chloroform extract possessed the lowest phenolic content (12.75 ± 0.05 mg GAE/g extract). The results indicate that polar solvents, particularly water and methanol, were more effective in extracting phenolic compounds from the stem bark of *Glycosmis pentaphylla* than less polar solvents such as ethyl acetate and chloroform.

The higher phenolic content observed in the aqueous and methanolic extracts suggests the abundance of polar phenolic constituents, which may contribute significantly to the antioxidant and other biological activities exhibited by these plant extracts (*Table 2*).

Table 2. Total phenolic content (TPC) of different solvent extracts of *Glycosmis pentaphylla* stem bark

Extract	Total Phenolic Content (mg GAE/g Extract)
Chloroform Extract	12.75 ± 0.05
Ethyl Acetate Extract	25.02 ± 0.02
Methanolic Extract	52.00 ± 0.07
Aqueous Extract	68.20 ± 0.01

Values are expressed as mean ± SD (n = 3).

The total flavonoid content of the different solvent extracts of *Glycosmis pentaphylla* (Retz.) DC. stem bark was estimated using the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). The flavonoid content varied considerably among the extracts, reflecting the influence of solvent polarity on flavonoid extraction efficiency. The aqueous extract exhibited the highest total flavonoid content (59.01 ± 0.05 mg QE/g extract), followed by the methanolic extract (32.02 ± 0.30 mg QE/g extract). The ethyl acetate extract showed a moderate flavonoid content of 19.02 ± 0.08 mg QE/g extract, whereas the chloroform extract recorded the lowest value (14.02 ± 0.02 mg QE/g extract). These findings indicate that aqueous and methanolic solvents were more effective in extracting flavonoid compounds from the stem bark of *Glycosmis pentaphylla* compared to the less polar solvents. The elevated flavonoid content observed in the aqueous and methanolic extracts may contribute to their enhanced antioxidant potential and other pharmacological activities, as flavonoids are known to possess strong free radical scavenging, anti-inflammatory, antimicrobial and therapeutic properties (*Table 3*).

Table 3. Total flavonoid content (TFC) of different solvent extracts of *Glycosmis pentaphylla* stem bark

Extract	Total Flavonoid Content (mg QE/g Extract)
Chloroform Extract	14.02 ± 0.02
Ethyl Acetate Extract	19.02 ± 0.08
Methanolic Extract	32.02 ± 0.30
Aqueous Extract	59.01 ±0.05

Values are expressed as mean ± SD (n = 3).

GC–MS Profiling of *Glycosmis pentaphylla* Stem Bark Extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the stem bark extract of *Glycosmis pentaphylla* revealed the presence of several volatile phytoconstituents, predominantly belonging to the monoterpene hydrocarbon class. The mass spectral data were compared with the NIST library database for compound identification. β-Myrcene was identified as the major constituent with the highest matching probability, followed by β-pinene and sabinene. These compounds exhibited characteristic fragmentation patterns with prominent ions

at m/z 41, 69, 91 and 93, which are typical of monoterpene hydrocarbons. The identified compounds have been widely reported to possess antioxidant, antimicrobial, anti-inflammatory and pharmacological activities. In addition to the volatile constituents detected by GC–MS, vitexin, a bioactive flavone C-glycoside, was identified through complementary phytochemical characterization and was considered one of the major non-volatile phytoconstituents of the extract. The combined occurrence of terpenoids and flavonoids may account for the significant biological activities observed in the present study (*Table 4,5*).

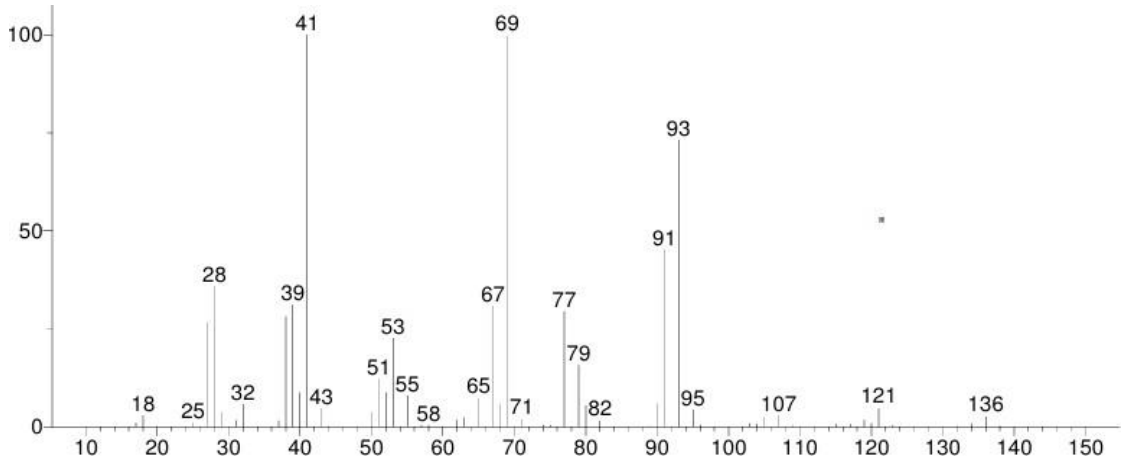

Figure 1. GC–MS mass spectrum of β-myrcene identified from the stem bark extract of *Glycosmis pentaphylla* (Retz.) DC. The compound exhibited characteristic fragmentation ions at m/z 41, 69, 91, 93, 107, 121 and 136, which are consistent with the reference NIST library spectrum, confirming the presence of β-myrcene as a major monoterpene constituent of the extract.

Table 4. Major phytoconstituents identified from *Glycosmis pentaphylla* stem bark extract

Sl. No.	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Chemical Class	Biological Significance
1	β-Myrcene	$C_{10}H_{16}$	136.23	Monoterpene hydrocarbon	Antioxidant, antimicrobial, anti-inflammatory
2	β-Pinene	$C_{10}H_{16}$	136.23	Monoterpene hydrocarbon	Antimicrobial, antioxidant, bronchodilatory
3	Sabinene	$C_{10}H_{16}$	136.23	Monoterpene hydrocarbon	Antioxidant, anti-inflammatory
4	Vitexin*	$C_{21}H_{20}O_{10}$	432.38	Flavone C-glycoside	Antioxidant, antidiabetic, anticancer, anti-inflammatory

Table 5. NIST Library Matching Details of GC–MS Identified Compounds

Compound	CAS Number	Library ID	Match Factor (MF)	Reverse Match Factor (RMF)	Probability (%)
β-Myrcene	123-35-3	14068	774	812	50.7
β-Myrcene	123-35-3	3793	746	748	50.7
β-Myrcene	123-35-3	1330	739	741	50.7
β-Myrcene	123-35-3	13996	731	732	50.7
β-Pinene	127-91-3	13993	734	736	11.8
β-Pinene	127-91-3	65779	732	746	11.8
β-Pinene	127-91-3	13994	723	735	11.8
β-Pinene	127-91-3	13995	717	721	11.8
Sabinene	3387-41-5	13997	724	750	8.29

Antibacterial and Antifungal Activity of *Glycosmis pentaphylla* Stem Bark Extract

The antimicrobial activity of *Glycosmis pentaphylla* stem bark extract was evaluated against Gram-positive *Bacillus cereus*, Gram-negative *Escherichia coli* and the fungal pathogen *Candida albicans* using the agar well diffusion method. The extract exhibited concentration-dependent inhibitory activity against all tested microorganisms. Against *Bacillus cereus*, the extract showed inhibition zones ranging from 0.2 ± 0.10 cm at 100 µg/mL to 1.4 ± 0.18 cm at 800 µg/mL, indicating strong antibacterial activity at higher concentrations. Similarly, the extract inhibited the growth of *Escherichia coli*, with inhibition zones increasing from 0.2 ± 0.12 cm at 100 µg/mL to 1.0 ± 0.06 cm at 800 µg/mL. The antibacterial activity was more pronounced against Gram-positive than against Gram-negative bacteria. The antifungal activity against *Candida albicans* also increased with concentration, exhibiting inhibition zones of 0.1 ± 0.08 cm, 0.2 ± 0.12 cm, 0.4 ± 0.12 cm and 1.1 ± 0.10 cm at concentrations of 100, 200, 400 and 800 µg/mL, respectively. The results demonstrate that the stem bark extract possesses broad-spectrum antimicrobial activity, which may be attributed to the presence of bioactive phytoconstituents such as phenolics, flavonoids, alkaloids and terpenoids (*Table 6*).

Table 6. Antibacterial and antifungal activity of *Glycosmis pentaphylla* stem bark extract

Concentration (µg/mL)	*Bacillus cereus* (cm)	*Escherichia coli* (cm)	*Candida albicans* (cm)
100	0.2 ± 0.10	0.2 ± 0.12	0.1 ± 0.08
200	0.5 ± 0.13	0.3 ± 0.12	0.2 ± 0.12
400	0.8 ± 0.20	0.7 ± 0.12	0.4 ± 0.12
800	1.4 ± 0.18	1.0 ± 0.06	1.1 ± 0.10

The antioxidant activity increased with increasing concentration of the test sample. The standard exhibited strong DPPH radical-scavenging activity, reaching 89.47% inhibition at 100 µg/mL. The test sample also demonstrated concentration-dependent antioxidant activity in both DPPH and hydrogen peroxide scavenging assays, indicating its potential as a natural source of antioxidant compounds (*Table 7*).

Table 7. Antioxidant activity of *Glycosmis pentaphylla* stem bark extract evaluated by DPPH and hydrogen peroxide scavenging assays

Concentration (µg/mL)	Ascorbic acid Standard (% Inhibition)	DPPH Assay Value	H_2O_2 Assay Value
20	46.86	4.91	0.1514
40	76.33	9.16	0.1575
60	85.40	9.98	0.1604
80	88.60	10.98	0.1622
100	89.47	12.62	0.1671

Values represent the mean observations obtained from the antioxidant assays.

3.6 Antidiabetic Activity

The antidiabetic potential of *Glycosmis pentaphylla* stem bark extract was evaluated using α-amylase inhibition and glucose uptake assays at different concentrations (20–100 µg/mL). The extract exhibited a concentration-dependent increase in both α-amylase inhibitory activity and glucose uptake. In the α-amylase inhibition assay, the extract showed inhibitory activity ranging from 23.04% at 20 µg/mL to 57.49% at 100 µg/mL concentration. The standard drug exhibited higher inhibition, ranging from 92.45% to 96.97% across all tested concentrations. The gradual increase in α-amylase inhibition with increasing concentration indicates the ability of the extract to inhibit carbohydrate hydrolysis and reduce glucose release. Similarly, in the glucose uptake assay, the extract enhanced glucose uptake in a dose-dependent manner, with values increasing from 5.67% at 20 µg/mL to 39.43% at 100 µg/mL concentration. Although lower than that of the standard, the extract demonstrated appreciable glucose uptake activity, suggesting its potential role in improving cellular glucose utilization. The observed antidiabetic activity may be attributed to the presence of phenolic compounds, flavonoids, alkaloids and other bioactive phytochemicals in the stem bark extract (*Table 8*).

Table 8. Antidiabetic activity of *Glycosmis pentaphylla* stem bark extract

Concentration (µg/mL)	Standard (% Inhibition)	α-Amylase Inhibition (%)	Glucose Uptake (%)
20	92.45	23.04	5.67
40	92.90	36.52	14.55
60	93.20	45.51	24.32
80	94.15	53.79	36.29
100	96.97	57.49	39.43

Discussion

The present investigation demonstrated that the stem bark of *Glycosmis pentaphylla* is a rich source of bioactive phytochemicals. (Chen *et al*., 2016) Qualitative phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, terpenoids, glycosides, tannins, sterols, saponins and carbohydrates in different solvent extracts. The variation in phytochemical distribution among solvents highlights the importance of solvent polarity in the extraction of secondary metabolites. Methanolic and aqueous extracts exhibited the broadest spectrum of phytoconstituents, indicating their superior extraction efficiency for polar bioactive compounds. (Ahmed *et al*., 2015) The quantitative estimation of total phenolic and flavonoid contents further supported these observations. The aqueous extract exhibited the highest total phenolic content (68.20 ± 0.01 mg GAE/g extract) and total flavonoid content (59.01 ± 0.05 mg QE/g extract), followed by the methanolic extract. Phenolic compounds and flavonoids are recognized as major contributors to antioxidant activity because of their ability to donate hydrogen atoms and neutralize reactive oxygen species. (Dumrongphuttidecha *et al*., 2021) Therefore, the elevated phenolic and flavonoid contents observed in the polar extracts may account for their enhanced biological activities.

GC–MS profiling revealed the presence of important monoterpene hydrocarbons, including β-myrcene, β-pinene and sabinene, while vitexin was identified as a major non-volatile flavonoid constituent through complementary phytochemical characterization. β-Myrcene and β-pinene have been extensively reported to have antimicrobial, antioxidant and anti-inflammatory properties. Similarly, vitexin is known for its potent antioxidant, antidiabetic and cytoprotective activities. (Babu & Radhamany, 2020)The presence of these compounds provides a phytochemical basis for the pharmacological activities observed in the present study. The stem bark extract exhibited concentration-dependent antimicrobial activity against Bacillus cereus, Escherichia coli and Candida albicans. Greater susceptibility was observed in the Gram-positive bacterium compared with the Gram-negative bacterium, which may be attributed to differences in cell wall structure. The antimicrobial effects are likely associated with the presence of phenolics, flavonoids, terpenoids and alkaloids that can disrupt microbial cell membranes, inhibit enzyme activity and interfere with cellular metabolism. The antioxidant evaluation demonstrated a progressive increase in radical-scavenging activity with increasing concentrations of the extract. The antioxidant potential may be attributed to the abundance of phenolic and flavonoid compounds capable of scavenging free radicals and reducing oxidative stress. Since oxidative stress is implicated in the pathogenesis of numerous chronic diseases, the antioxidant activity observed in this study further supports the therapeutic relevance of the plant.

The antidiabetic investigation revealed significant α-amylase inhibitory activity and enhanced glucose uptake in a

concentration-dependent manner. α-Amylase inhibition delays carbohydrate digestion and glucose absorption, thereby reducing postprandial hyperglycemia. Enhanced glucose uptake suggests improved cellular utilization. These findings indicate that the stem bark extract possesses promising antidiabetic potential, which may be mediated through synergistic interactions among phenolic compounds, flavonoids, terpenoids and vitexin. Overall, the biological activities observed in the present study are strongly correlated with the phytochemical richness of *Glycosmis pentaphylla* stem bark and highlight its potential as a natural source of therapeutic agents for the management of microbial infections, oxidative stress and diabetes.

Conclusion

The present study demonstrated that the stem bark of *Glycosmis pentaphylla* (Retz.) DC. DC possesses a rich phytochemical profile and significant biological activities. Qualitative screening confirmed the presence of diverse secondary metabolites, whereas quantitative analyses revealed high levels of phenolic and flavonoid compounds, particularly in the aqueous and methanolic extracts. GC–MS profiling identified biologically important constituents such as β-myrcene, β-pinene and sabinene, whereas vitexin was recognized as a major non-volatile bioactive flavonoid. The stem bark extract exhibited notable antimicrobial activity against Bacillus cereus, Escherichia coli and Candida albicans, along with appreciable antioxidant and antidiabetic properties. The observed pharmacological effects are likely associated with the synergistic action of the identified phytochemicals. These findings provide scientific evidence supporting the traditional medicinal use of *Glycosmis pentaphylla* and suggest its potential application as a natural source of antioxidant, antimicrobial and antidiabetic agents.

Further studies involving compound isolation, mechanistic investigations, molecular docking, in vivo pharmacological evaluation and toxicity assessment are warranted to fully explore the therapeutic potential of this medicinal plant and facilitate its development into an effective phytopharmaceutical formulation.

Author Contributions: RCK and AS designed the study and; AS Performed experiments. RCK GTM and AS analyzed the data. Surveying and altering MGT and RM. RCK and AS data interpretation and manuscript writing. All the authors have read and agreed to the published version of the manuscript.

Declarations

Ethics approval and consent to participate: Not required.

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Plant Guidelines: The authors confirm that the use of plants in the present study complies with international, national and/or institutional guidelines.

Competing interests: The authors declare no competing interests.

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