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Phytochemical Profiling and *In-Vitro* Evaluation of Biological Activities of *Solanum diphyllum* L

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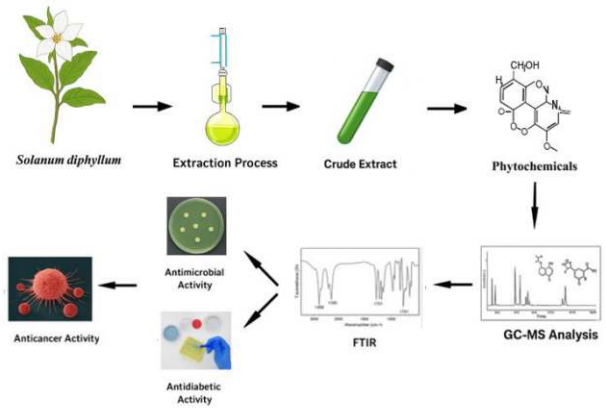
ABSTRACT

This investigation is to study the biological activity and preliminary phytochemical screening of Solanum diphyllum L. By using various solvents. The preliminary phytochemical studies show the occurrence of tannins, glycosides, flavonoids, saponins, alkaloids, and steroids. GC-MS analysis reveals important bioactive substances associated with a number of health benefits. GC-MS analysis shows total 12 compounds with respect to their retention time, the FTIR shows a significant functional group between 4000 cm^{-1} to 600 $cm^{-1}$with compound class. Antimicrobial activity shows significant activity in the aqueous and methanol extracts, the antimicrobial activities (Escherichia coli and Staphylococcus aureus) fungi (Aspergillus nigar and Aspergillus flavus), exhibiting a substantial zone of inhibition. The IC_{50} value for methanol and aqueous extract, which measures the antidiabetic activity by α-glucosidase inhibition, with inhibition value (IC_{50}) of 112.25µg/ml and 71.492µg/ml correspondingly. In relative to methanol, the aqueous extract displays a zone of inhibition propensity. The cytotoxic assay shows a significant effect towards the aqueous extract with an IC_{50} of 454.58 µg/ml against the A549 cell line, and HeLa cells with IC_{50} of 318.07 µg/ml for methanol. These outcomes imply the potential pharmacological uses of S. diphyllum L. leaf extract.

Keywords: *Cytotoxicity (A549, HepG2), Antidiabetic, Antimicrobial, FTIR, GC-MS, Phytochemicals.*



Graphical Abstract

INTRODUCTION

The Solanaceae family, comprising approximately 98 genera and 3000 species, is a particularly fascinating group of angiosperms. Its members show a diverse range of morphological as well as ecological characteristics and have a cosmopolitan distribution, largely due to their utilisation as food, decorative plants, and medicinal resources. Members of the Solanaceae family are beneficial to people's health as well as nutrition. They consist of tobacco, jimson weed seeds, black nightshade, aubergine, tomato, potato and capsicum (commonly known as chilli peppers). Throughout growth and during post-harvest distribution, these plants emit positive compounds along with potentially hazardous compounds. These active compounds consists of glycosides and alkaloids [1]. While some of these substances can be beneficial, such as antioxidants, others may offer health hazards if ingested in excess. Understanding the balance between nutritional advantages and potential toxicity is critical for safe consumption and use in food systems. Antiherpes and cancer drugs are produced from *Solanum nigrum* and *Solanum lyratum*. Glycosides like spirosolane, solanidane, spirostane, and furostane are abundant in 45 Solanum plant species, according to extensive research on these plants. The extract displayed considerable cancer-cell-growth inhibition across different cell lines and showed promising anti-herpes [2]. In conventional Indian medicine, this shrub is extensively to treat skin infections, respiratory issues, and intestinal parasites. The genus *Solanum L.* is the largest in the Solanaceae family. It has almost 1500 species and lives in tropical and subtropical parts of the world. They are found mostly in the south and southeast of Brazil [3]. Originally in Mexico and extending south to Costa Rica in Central America, the medicinal herb species *Solanum diphyllum* L., also referred to as two-leaf nightshade, has evaded cultivation and naturally spread over many tropical and subtropical regions of the world. *S. diphyllum* was originally discovered in India in 1995 in two lactation sites in West Bengal's Howrah District [4]. There are no published scientific data on its micromorphological characteristics. Against breast and colon cancer cell lines, the plant showed encouraging cytotoxicity, and it may be a powerful source of anticancer compounds. The ultrastructural characteristics of the pollen, seed sporoderm, and leaf were studied using scanning electron microscopy.

The morphology and anatomy of *Solanum diphyllum* L. were similar to those of its genus' near relatives. The presence of lenticel-like structures may be a response to the plant's defence needs and/or a result of an adaptation to its surroundings; for example, the plant discards excess storage materials to reduce the effect of stored osmolytes, and the presence of epidermal wax and tannins may aid the plant's acclimatisation to the surrounding light intensities. *Solanum diphyllum* L. shows remarkable cytotoxicity against colon (HCT 116) and breast cancer cells (MCF7) indicating a potential source of anticancer agents [5]. Only a few number of phytochemicals from solanaceous plants are studied for biological activity, even though many have been isolated and identified. In their study, they describe the identification and isolation of the steroidal alkaloid that was recently found in Egyptian flora (*Solanum diphyllum*) and its potent cytotoxic action from (rel. int.) 575 [m], 557 [m-h2o], 542 [m-h2o-ch3], and 396 [m-c6h11o6] [6]. *Solanum diphyllum* L. exhibits anti-inflammatory, anticancer, antiulcer, antioxidant, antimicrobial, anti-infection, anti-alpha amylase, and anti-alpha glucosidase activities, as well as immunomodulatory effects, and additionally encourages cytotoxic effects on breast and colon cancer cell lines, indicating its potential as an important reservoir of anticancer compounds [7]. The objective of this investigation is to identify secondary metabolite classes using phytochemical tests and GC-MS Analysis, and characterisation of *Solanum diphyllum* L. species, as well as to evaluate in vitro antibacterial efficacy and cytotoxicity on different tumor cells.

Experimental Study

Procurement and processing of plant samples

A plant, *Solanum diphyllum* L. It was collected from the Uttara Kannada district, Karnataka, India. The plant surface was thoroughly cleaned using running tap water; the plant's surface was thoroughly cleaned to get rid of any impurities and particulates. Ground the samples after shade drying. A Soxhlet extraction was performed on about 25 g of plant powder using Distilled Water and Methanol as solvents at varying temperatures. To obtain an adequate extract for additional research, the extraction procedure was repeated multiple times. The semisolid extract was kept in glass vials for further analysis.

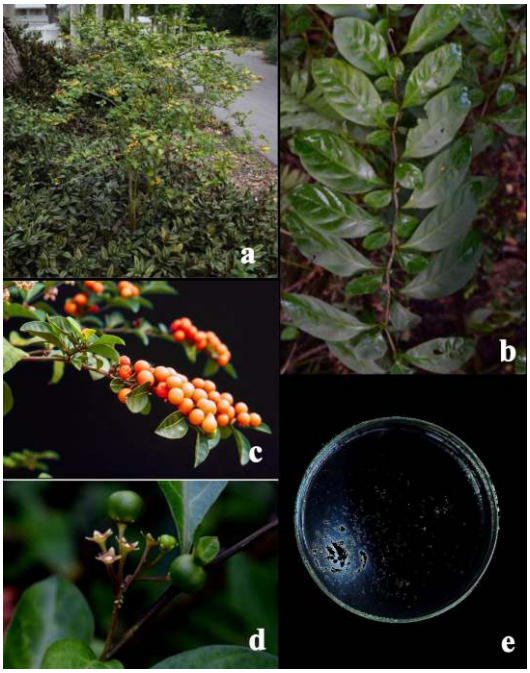


Fig. 1: Showing S. diphyllum A. Habitat, B. Twig, C. Fruit, D. Flower, E. Crude extract

Qualitative phytochemical analysis

The following standard protocols were used to assess the extract for the determination of bioactive compounds from [8] [9].

Protein test:

Millon test

When two mL of Millon reagent were combined with the initial extract, a white sediment was formed that turned red when heated slightly, signifying the presence of protein.

Test for Ninhydrin

The crude extract becomes violet when heated with two mL of a 0.2% Ninhydrin solution, indicating the existence of amino acids and proteins.

Test for Carbohydrates:

Benedict's reagent test

When extract was heated along with 2 ml of Benedict's reagent, carbohydrates are showed up, by forming a reddish-brown precipitate.

Molisch's reagent test

2 ml of extract was thoroughly agitated with Molisch's reagent, then concentrated H_2SO_4 (2 mL) added. At the interphase, a violet ring was formed suggesting the existence of a carbohydrate.

Flavonoids test

Alkaline test

The extract was combined with 2% NaOH (2 mL) solution. A strong yellow tint was produced; the yellow colour becames colorless when few drops of neutralised acid was added, shows the existence of flavonoids.

Saponins test

Foam test

The steady foam was formed by combining the extract with 5 mL deionized water by vigorous shaking indicated the existence of saponins.

Test for steroids

Urine test

After combining crude, extract with two mL of chloroform and concentrated suphuric acid (H_2SO_4 along sides of the test tube). An crimson hue formed at the bottom layer of the chloroform suggesting the steroids are present.

Terpenoids test

Burchard Test

After the extract was dissolved in 2 ml of chloroform, and dried off. To this, add concentrated H_2SO_4 and boil for roughly 2 minutes. Terpenoids were identified by their greyish colour.

Test for alkaloids

Dragendroff's test

After the extract was dissolved in 2 ml of chloroform, and dried off. To this, add concentrated H_2SO_4 and boil for roughly 2 minutes. alkaloids were identified by their greyish colour.

Wagner's reagent test

When the extract was combined with Wagner's reagent, the formation of reddish-brown sediment indicates the existence of alkaloids.

Gas chromatography and mass spectrometry (GC-MS)

The chemical composition of *S. diphyllum* L. leaf extract was characterized by GC–MS study were performed using QP2010S, Shimadzu, Japan, mass spectrometry (MS) and gas chromatography (GC) enhanced with an electron ionisation detector. An EC-5 with 30-meter-long column with a 0.25 mm diameter was employed, using a 0.25 µL injection volume and a 2 mm direct injector were included with the chromatograph. A 2 mm direct injector liner was used to inject 2 µL of the sample. The initial temperature was 60°C for two minutes, then increased to 450°C in a level of 20°C per minute [10]. All specimens were injected straight into the capillary column using a 2 mm injector. The identification of the chromatographic peaks was achieved by their relative retention times, and the mass spectrometric analysis was conducted by precisely identifying the compounds through matching the spectral data with the NIST library [11].

Fourier Transform Infrared Spectroscopy (FTIR)

A spectrophotometer, Thermo Fisher Scientifics USA (Waltham, MA, USA), was employed for the analysis of FTIR, to identify the bioactive constituents. The extracts of *S. diphyllum* were paired with potassium bromide (KBr) and formed into pellets. The absorption spectra will be recorded from the 400–4000 cm^{-1} region. The spectral analysis confirms the presence of functional biomolecules in the samples, enabling thorough characterisation of the chemical composition of the nanoparticles [12]

Cytotoxic assay

Cell culture technique

The NCCS (National Centre for Cell Sciences) provided the HeLa and A549 cell lines for this study. The cells are proliferated in DMEM, provided with 2 mM L-glutamine, and 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), along with 1.5 g/L sodium carbonate (Na_2CO_3), has important amino acids of 0.1 mM. For cell proliferation, sodium pyruvate (1mM) and glucose (1.5 g/L) are used in order to avoid contamination from microbes. 10% foetal bovine serum (GIBCO, USA) was added; the antibiotics streptomycin and penicillin were added in 100 IU/100 µg per ml; a moist incubator with 5% carbon dioxide (CO_2) was used to enhance and maintain their physiological conditions for growth and survival; and the cells were kept at 37°C [13].

Evaluation of cytotoxicity

The fifty percent maximum concentration of inhibition measured by the MTT test and the value expressed in IC_{50} to measure the cytotoxicity. A 96-well microplate was loaded with cultured cells (1×10^1), and incubated at 37°C for 48 hours with 5% CO_2 moistened incubator. Plant extracts of *S. diphyllum* with 100 µL of distinct concentrations (0–160 µg/mL) after incubation were added to each well after the monolayer was rinsed with fresh media. The cells were kept under the same settings for additional stimulations. Each well is specified with 100 µL of MTT reagent after discarding of culture media. After that, the incubation was carried out for an extra 4 hours at the same temperature. That ensures precise measurement; to dissolve the formazon crystals 100 µL of (DMSO) was added after removal of supernatant [14].

Glucose Uptake Assay

α-Glucosidase Inhibitory activity

A technique outlined by [15]. It was used to assess the samples' α-glucosidase inhibitory activity. 50 mM of phosphate buffer with a pH of 6.9 contains 50 µL of α-glucosidase 1 U/mL from yeast (SRL, Bangalore, India) are pre-incubated for 10 minutes at 37°C with varying level of samples (0–100 µg/mL). We added 50 µL of a 5 mM p-nitrophenyl-α-D-glucopyranoside solution to the phosphate buffer to start the reaction. For thirty minutes, the catalytic action was conducted at 37°C. Na_2CO_3 (1 M) was added to terminate the reaction, and transmittance was recorded at 405 nm. The formula (OD of blank - OD of test/OD of blank) * 100 was employed to calculate the inhibition percentage, and the findings were shown in Ic_{50}.

Antimicrobial Activity

The samples' antibacterial activity was tested using the agar well diffusion method, according to reports of [16], with small alterations. *Escherichia coli* (MTCC-7410) Gram-negative, and *Staphylococcus aureus (*MTCC-7443), Gram-positive, are used. Two fungus species: *Aspergillus flavus* (MTCC-9606) and *Aspergillus niger*. Using sterile saline solution, the cell suspension was set to approximately 5 × 105 CFU/mL. Samples were suspended in 20 mg/mL DMS stock solution and added at volumes ranging from 100 µg to 400 µg for each well. The Muller Hinton agar is used for bacteria, and for fungal psecies, Czapek s-Dox agar is used. Bacteria were incubated at 37°C, while fungal species are incubated at 28°C for 72 hours, and the zone of inhibition (mm) was evaluated in diameter.

RESULTS AND DISCUSSIONS

Preliminary Qualitative Analysis of *S. diphyllum*

In a phytochemical study. How the solvent selection effects the extraction of compounds in leaf extracts of *S. diphyllum*, the methanolic extract reveals the existence of polysaccharides, terpenoids, flavonoids, proteins, alkaloids, steroids, along with saponins, indicating that it has therapeutic potential because of its cytotoxic, antibacterial, and antioxidant qualities. The lack of steroids in the Aqueous extract and methanol extract suggests that they rely on organic solvents for extraction (Table 1). Similarly, it was reported that *S. diphyllum*, the stem and root extract reveals that occurrence of phenols, glycosides, flavonoids, alkaloids, saponins and carbohydrates, while histochemical studies showed the existence of calcium, tannins, starch and mucilage cells [17]. The investigation by [18] the species *Solanum Dasyphyllum* fruit extract reported to possesses alkaloids, tannins, steroids and cardiac glycosides.

Table 1: Qualitative phytochemical analysis of S. diphyllum

Sl.NO	Phytochemical tests	SDLM	SDLA
1	Flavonoids	-	+
2	Alkaloids	+	+
3	Protiens	+	+
4	Terpenoids	-	+
5	Saponins	-	+
6	Steroids	-	-
7	Carbohydrates	+	+

Note: + = Present, - = Absent, DW = Aqueous, M = Methanol SDL= S. diphyllum

Gas chromatography and mass spectrometry (GC-MS)

GCMS profiling of *S. diphyllum* identified the range of compounds with variable area of percentage and Retention time, as demonstrated in (Table 2). And (Fig 2 & 3). The methanol extract shown the total 9 compounds, 2,2-Dimethoxybutane RT 3.192, Neophytadiene RT 18.409,

Squalene RT 32.886, Stigmasterol RT 38.565, Yohimbine RT 38.898, Diosgenin RT 39.233, 5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a- RT 40.010, Lup-20(29)-en-3-ol, acetate, (3.beta.)- RT 40.745 and Methyl (19alpha, 20alpha)-11-methoxy-19-methyl-16, 17-dide RT 42.016. Moreover, the aqueous extract has compounds like 2-Pentanone, 4-hydroxy-4-methyl-RT- RT 4.384, Diphenyl sulfone RT 18.681, Triphenylphosphine oxide RT 28.714 (Table 3). The *solanum villosum* the ethanol leaf extract shown the existence of 12 bioactive compounds the principal chemical compounds are phytol, N-Hexadecanoic acid, 2H-1-Bezopyran-6-ol, 11,14.17-Eicosatrienoate, 4-(3,5-Di-Tert-Butyl-4-Hydoxy Phenyl) Butyle Acrylate, 4,3-Dihydro-2,5,7,8-Tetra methyl-2(4,8,12-Trimethyl Treidecyl)-Acetate and Ethyl ester these compounds are antiflammatory, anticoronary, antieczemic, hepatoprotective, antimicrobial, lypocholestrolemic activity were reported[19]. *Solanum kakasianum*, the leaf and root extract contains 16 and 32 compounds, such as heptadecane-9-hexyle and stigmasterol, compound have antibacterial activity [20].

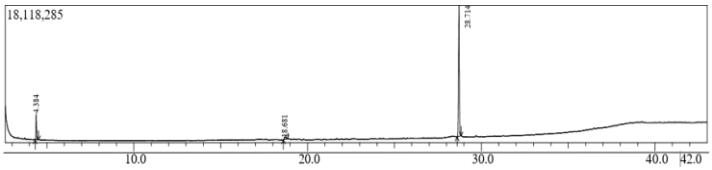


Fig 2: Chromatogram of S. diphyllum methanol leaf extract

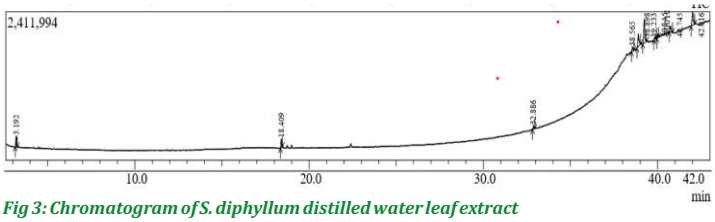


Fig 3: Chromatogram of S. diphyllum distilled water leaf extract

Fig 2: Chromatogram of S. diphyllum methanol leaf extract

Peak#	Retention Time	Area	Area%	Similarity	Base m/z	Compound Name
1	3.192	513978	8.84	90	87.1	2,2-Dimethoxybutane
2	18.409	370014	6.37	95	95.1	Neophytadiene
3	32.886	173403	2.98	81	69.05	Squalene
4	38.565	221383	3.81	65	81.1	Stigmasterol
5	38.898	973003	16.74	84	353.25	Yohimbine
6	39.233	1582781	27.23	90	139.15	Diosgenin
7	40.01	217087	3.73	57	203.15	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-
8	40.745	449654	7.73	77	107.1	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
9	42.016	1124807	19.35	81	382.3	Methyl (19alpha,20alpha)-11-methoxy-19-methyl-16,17-dide
		5813258	100			

Table 3: GC-MS evaluation of Chemically Active Compounds of Aqueous leaf extract

Peak#	Retention Time	Area	Area%	Similarity	Base m/z	Compound Name
1	4.384	9219184	12.6	96	43.05	2-Pentanone, 4-hydroxy-4-methyl-
2	18.681	2273635	3.11	92	125.05	Diphenyl sulfone
3	28.714	61655193	84.29	93	277.1	Triphenylphosphine oxide
		73148012	100			

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra. specifies the functional groups of chemically active compounds. The significant peaks were found between 4000 cm^{-1} to 600cm^{-1}. The methanolic extract revealed that O-H strong, sharp, O-H very strong, very broad, C-O strong, C-H medium, sharp, S=O strong, sharp, C=C medium, and C-H medium to strong. Corresponding to the Alkane, alkene, alkyl halide, alcohol, secondary alcohol, aromatic substitution, phenol, carboxylic acid, and sulfoxide. The Aqueous revealed that O-H strong, broad, O-H very strong, very broad, C=C medium, CO-O-CO medium, C-Cl strong, sharp, and C-CL strong. Corresponding to Alcohol, alkane, alkyl halide, carboxylic acid, cyclic alkene, anhydride, and halo compound illustrated in (Fig. 4), (Table 4, and 5). It was previously reported that the leaf, seed, and pollen of *S. diphyllum* exhibited the functional peaks, and have the functional groups like aliphatic amine corresponding to peak 1041, 2361 is nitrile, 3118 to aromatic alkene, 3700 with N-H stretch amide group, and this species has nitro compounds and aromatic amino acids in the range of 1550 to 1600 [21]. The studies of *S. lycopersicum* displayed the presence of nitro-compound, carboxylic acid. Imine/oxime, aldehydes and sulfonyl chloride, ketones and alkyl aryl, sulfoxide, correspond to their peaks at 828.888, 821.527(550-850), 700.998 (610-700), and 590.111 (515-690) [22].

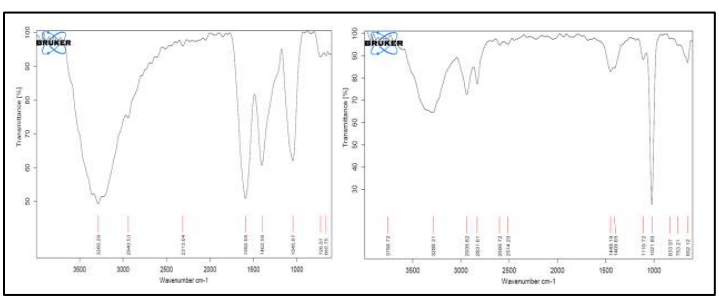


Fig 4: FTIR chromatogram illustrating peak positions of the bioactive functional groups present in S. diphyllum A. Aqueous, B. Methanol

Table 4: Interpretation of FTIR Peak Values for the Bioactive Functional Groups of S. diphyllum methanol leaf extract

Absorption(cm-1)	Peak details	Functional Group	Compound Class
3758.72	Strong, sharp	O-H	Alcohol, phenol
3288.31	Strong, broad	O-H	Alcohol
2939.82	Very strong, very broad	O-H	Carboxylic acid
2831.61	Very strong, very broad	O-H	Carboxylic acid
2599.72	Very strong, very broad	O-H	Carboxylic acid
2514.20	Very strong, very broad	O-H	Carboxylic acid
1449.19	Medium	C-H	Alkane
1409.65	Medium	C-H	Alkane
1110.72	Strong, sharp	C-O	Secondary alcohol
1021.85	Strong, sharp	S=O	Sulfoxide
833.21	Medium	C=C	Alkene
753.21	Strong, sharp	C-Cl	Alkyl halide
652.12	Medium to strong	C-H	Aromatic substitution

Table 5: Interpretation of FTIR Peak Values for the Bioactive Functional Groups of S. diphyllum Aqueous leaf extract

Absorption(cm-1)	Peak details	Functional Group	Compound Class
3286.29	Strong, broad	O-H	Alcohol
2940.53	Very strong, very broad	O-H	Carboxylic acid
2313.04	Very strong, very broad	O-H	Carboxylic acid
1592.05	Medium	C=C	Cyclic alkene
1402.56	Medium	C-H	Alkane
1045.97	Strong, broad	CO-O-CO	Anhydride
726.57	Strong, sharp	C-Cl	Alkyl halide
665.75	Strong	C-Cl	Halo compound

Evaluation of Cytotoxicity Effect from *S. diphyllum*

HeLa and A549 cancer cell lines in a dose-dependent manner. The percentage of Aqueous extract for A549 is 48%, 59%, 65%, 73%, and 84%. At 100, 200, 300, 400, and 500 µg/ml levels, the IC_{50} value is 454.58 µg/ml, relative to reference cisplatin, while HeLa cells have IC_{50} value of 191.58 µg/ml at the same volumes, with percentages of inhibition of 38%, 46%, 65%, 55%, 64%, and 71%. The methanolic extract had no activity against the A549 cancer cell line at the tested doses (Fig. 5). The percentages of inhibition are 41%, 53%, 66%, 77%, and 86% at the volumes 100, 200, 300, 400, and 500 (µg/ml). Sequentially, the inhibition concentration (IC50 value) is 318.07 µg/ml for HeLa cell line, compared to the standard camptothecin. The plant *S. diphyllum* with steroidal alkaloid 3-O-(beta-D-glucopyranosyl) etioline [(25S)-22, 26-epimino-3beta-(beta-D-glucopyranosyloxy) cholesta-5, 22(N)-dien-16alpha-ol], which shows strong Anti-proliferative effects against the HeLa cell line reported by [23]. From previous reported that *S. melangona L* fruit peel methanol extract, in-vitro studies shows the successful inhibition against tested five different cancer cell lines; (HCT116), (HEP2), (MCF7), (HeLa) and (HEPG2) cell lines and isolated compounds are subjected for in-vivo studies which shows significant cell death against (HEP2), (MEP) with IC_{50} value 2.14±0.35 at volume levels of 100 and 200 mg/ml [24]. In contrast, *S. jasminoides,* the alkaloid fractions show dose-dependent strong cytotoxic activity against the tested animals [25].

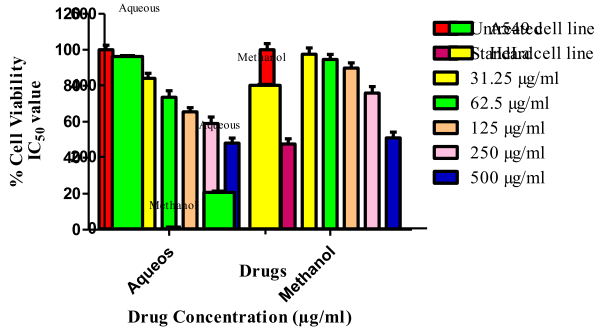


Fig 5: Cytotoxic assay showing different concentrations of S. diphyllum leaf extracts against A549 cell line

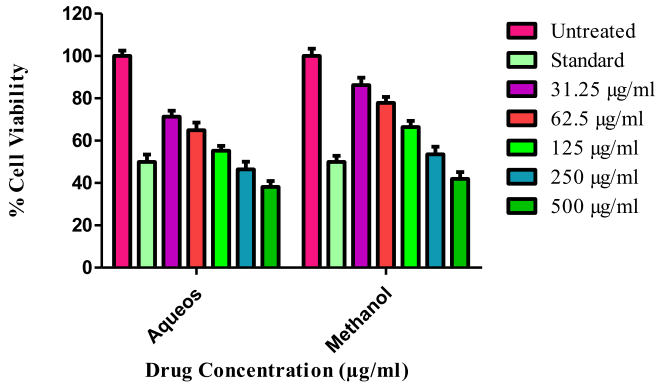


Fig 6: Cytotoxic assay showing different concentrations of S. diphyllum leaf extracts against the HeLa cell line

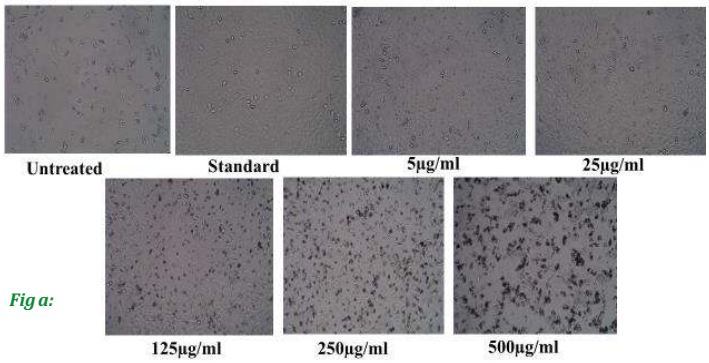


Fig a:

Fig 7: Showing Cytotoxic assay of IC_{50} value of aqueous and methanolic leaf extracts of S. diphyllum against HeLa and A549cell lines

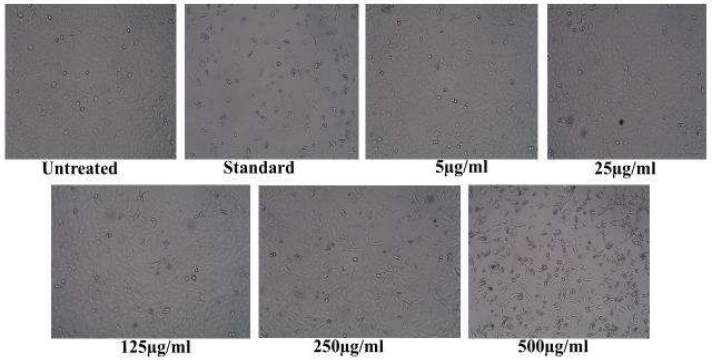


Fig b:

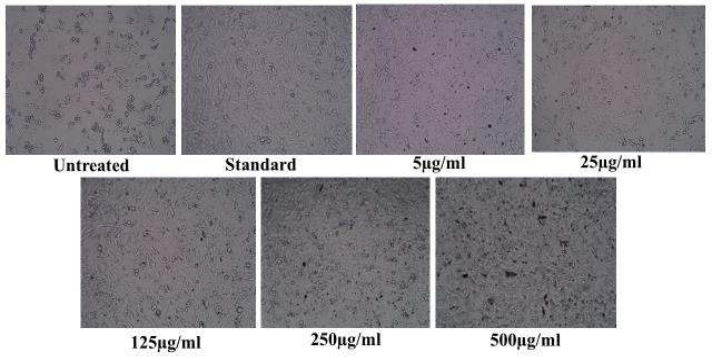


Fig c:

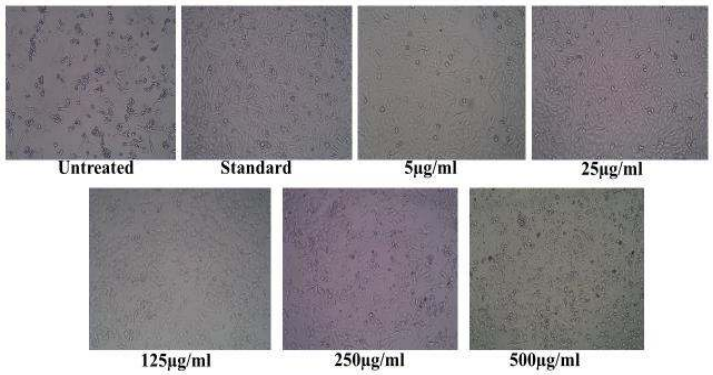


Fig d:

Fig 8: *Microscopic images showing the cytotoxic effects (% cell viability) of methanolic and Aqueous leaf extracts of S. diphyllum in Fig a: Aqueous extract against A549 cancer cell line. Fig b: Methanol extract against A549 cancer cell line, Fig c: dist. water extract against HeLa cancer cell line, Fig d: Methanol extract against the HeLa tumor cells*

Glucose Uptake Assay

Methanolic extracts of *S. diphyllum* showed concentration-dependent inhibition at concentrations of 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL, with percentages of inhibition of 8%, 25%, 41%, 55%, and 73%. The inhibition concentration was (IC_{50} value) was determined to be 71.492 µg/mL (Graph 1), which is displayed in Table 4. The IC_{50} value for Aqueous is 112.25 µg/mL. With inhibition percentages of 3%, 16%, 29%, 43%, and 57%, respectively, at quantities of 25 µL, 50 µL, 75 µL, 100 µL, and 125 µL (Fig. 3). In comparison to standard acarbose, percentage inhibition ranged from 13% to 85% at extract volumes, with 29.51 µg/mL IC_{50} value, These findings imply that plants may have antidiabetic qualities based on earlier research. The α-Amylase activity of *S. incanum L*. extract of root showed the percentage of inhibition at 75.95% at volume 700 µg/ml with IC_{50} 495 µg/ml [26].

Table 6: α-Glucosidase Inhibitory activity, expressed results in IC_{50} µg/ml

Samples	α-Glucosidase
	IC_{50} (µg/mL)
Standard	30.124 µg/mL
Methanol	112.25 µg/mL
Aqueous	71.492 µg/mL

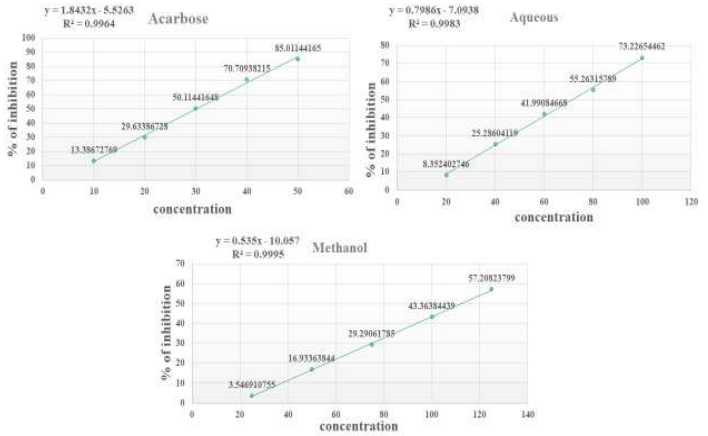


Fig 9: showing Glucose uptake assay of S. diphyllum leaf extract by α-Glucosidase inhibition assay

Antimicrobial activity of *S. diphyllum*

Staphylococcus aureus and *Escherichia coli* pathogens against *S. diphyllum* extract showed increased noticeable antibacterial activity. (Figure 5). The least zone of inhibition was shown by the Aqueous, measuring 8 mm and 12 mm at concentrations of 20 µg and 30 µg for both bacterial strains, while methanolic extract with inhibition of 9 mm, 11 mm, and 14 mm at concentrations of 10 µg, 20 µg, and 30 µg for S. aureus and 8 mm, 11 mm, and 14 mm at the same concentrations demonstrated the greatest inhibition. *S. diphyllum* leaf extract displayed good results for *S. aureus* when compared to *E. coli*. From the studies of [27], *Solanum nigram,* the ethanol extract successfully inhibited the against the pathogens and *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*. Previous studies on *S. nigram* reported against these pathogens: *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Streptococcus agalacticae*, *Streptococcus canis*, *Bacillus cereus* and *Bacillus subtilis*. Gram-positive bacteria, and *Pseudomonas aeruginosa, Escherichia coli*, *Serratia marcescens*, *Pectobacterium atrosepticum*, *Erwinia chrysantemi*, *Candida albicans*, and *Candida parapsilosis* among these bacteria, the highest inhibition was observed in *Pectobacterium* with a 7-11.0 mm zone of inhibition than antibiotics [28]. The strains of *Aspergillus flavus* and *Aspergillus niger* in antifungal activity *S. diphyllum* (Fig.5).

The zone of inhibition expressed in concentrations (µg) of 100, 200, and 300 µg for *A. niger* and *A. flavus* is 10 mm and 9 mm at a dose of 300 µg, respectively. 10 mm and 9 mm at a dose of 300 µg exhibitted the inhibition zone by the methanol extract for *A. niger* and *A. flavus*. No activity is seen in distilled water extract at dose of 100 and 200 µg; the aqueous extract shows inhibition for *A. flavus* at a concentration of 300 µg. The inhibition zone ranges from 7 mm to 8 mm. *S. aculeastrum* showed the zone of inhibition at 5µg/ml. Acetone and methanol extracts both inhibited the fungal pathogen *Aspergillus flavus* and *Penicillium notatum* with 56 to 100µg of inhibition percentage [29]. It was reported that the species *Solanum tomentosum* methanol extract shows dose-dependent inhibition for *Aspergillus niger* and *Fusarium oxysporum* with 47.22 to 50.56% percentage of inhibition [30].

Table 7: Antimicrobial activity of S. diphyllum

Sl.	Sample	Conc. (µg)	*S. aureus*	*E. coli*	*A. Flavus*	*A. Niger*
1	Methanol	Blank	---	---	---	---
		1	9.33±0.57	8.66±0.57	---	---
		2	11.33±0.57	11.66±1.15	---	---
		3	14.33±0.57	14.66±0.57	10.66± 0.57	9.66 ± 0.57
2	Aqueous	Blank	---	---	---	---
		1	---	---	---	---
		2	8.66±1.15	8.66±0.57	7.66 ± 0.57	---
		3	12.66±0.57	11.66±0.57	9.66 ± 0.57	8.66 ± 0.57
3	Std	---	16.33± 0.57	15.33±0.57	7.66 ± 0.57	7.66 ± 0.57

Note: A '---' symbol signifies that the tested concentration did not exhibit any activity

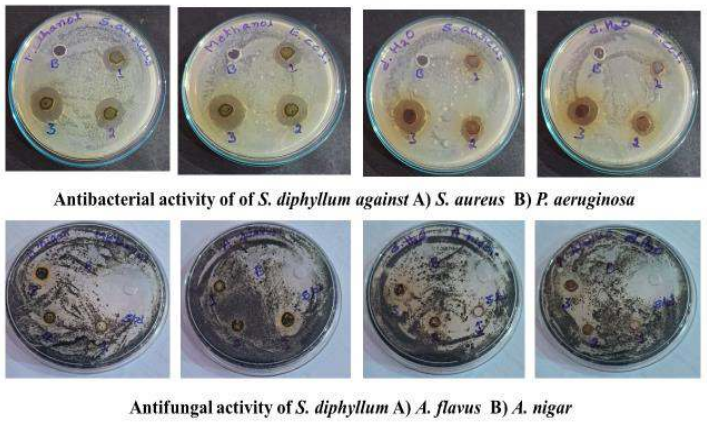


Fig 10: Antimicrobial activity of S. diphyllum methanol and aqueous leaf extracts

CONCLUSION

From the investigations of the above study, *Solanum diphyllum* consists of diverse bioactive compounds and phytochemicals with a variety of physicochemical and pharmacological effects. Their existence supports *Solanum diphyllum* L. Traditional therapeutic value in treating a range of illnesses. These phytoactive compounds are related to numerous pharmacological and biological effects. The many substances found in GC-MS could be a useful source of new medications. The cytotoxic assay of *S. diphyllum* against A549 and HeLa cancer cell lines exhibits the effective results. With IC_{50} of 71.492 µg/ml and 112.25 µg/ml according to their standards, and the existence of the steroidal alkaloid 3-O-(beta-D-glucopyranosyl) etioline [(25S)-22,26-epimino-3beta-(beta-D-glucopyranosyloxy) cholesta-5,22(N)-dien-16alpha-ol] from earlier research, the different compounds are isolated and identified they are potential drug candidate the GC-MS study shows anticancer potential A549 and HeLa cancer cell lines *S. diphyllum* has potential antidiabetic efficacy. Further additional studies are required to isolate, characterise, and investigate the pharmacological effects of reported compounds.

Further investigation is needed on clinical trials, evaluations, and the creation of customised delivery systems should be the top priorities for future research.

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6. Conflict of interest form

The authors declare that they have **no conflict of interest** regarding the publication of this manuscript.

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