Ind. J. Pharm. Edu. Res., 2026; 60(4):1656-1669.
https://www.ijper.org

Original Article

Chemical Characterization and Biological Potential of *Simarouba glauca* Leaf Extract: GC-MS, *In vitro*, and Molecular Docking Studies

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ABSTRACT

Background: Medicinal plants remain an important source of therapeutic agents due to their rich phytochemical composition and broad pharmacological activities. *Simarouba glauca* is a traditionally used medicinal plant; however, its leaf extract has not been comprehensively investigated for combined antioxidant, antimicrobial, anticancer, and molecular docking potential against oral cancer targets. **Objectives:** This study aimed to characterize the phytochemical constituents of ethanolic *Simarouba glauca* leaf extract and evaluate its antioxidant, antimicrobial, and anticancer activities, supported by molecular docking analysis. **Materials and Methods:** Dried leaves of *S. glauca* were extracted using ethanol, and the phytochemical constituents were identified by GC-MS analysis. Antioxidant activity was assessed using DPPH, Nitric Oxide (NO), and Ferric Reducing Antioxidant Power (FRAP) assays. Antimicrobial activity was evaluated against selected bacterial and fungal pathogens using the agar well diffusion method. Cytotoxic activity was determined against human oral cancer KB cells using the MTT assay. To explore the possible anticancer mechanism, major phytocompounds identified by GC-MS were subjected to molecular docking against Cyclin-Dependent Kinase 4 (CDK4), a key regulator of cell-cycle progression. **Results:** GC-MS analysis identified 23 bioactive phytocompounds in the ethanolic leaf extract. The extract exhibited concentration-dependent antioxidant activity by significantly scavenging DPPH and NO radicals and enhancing ferric reducing power. It also showed notable antimicrobial activity against the tested pathogens. In the MTT assay, the extract significantly decreased KB cell viability in a dose-dependent manner, indicating potent cytotoxic effects. Molecular docking revealed strong binding interactions of selected phytocompounds with the active site of CDK4, suggesting possible inhibition of cancer cell proliferation. **Conclusion:** Ethanolic *Simarouba glauca* leaf extract possesses significant antioxidant, antimicrobial, and anticancer properties. The integrated *in vitro* and *in silico* findings highlight the therapeutic potential of *S. glauca* leaves as a promising natural source for the development of novel antimicrobial agents and targeted therapies against oral cancer.

Keywords: Antioxidants, CDK4 receptors, *In silico* analysis, Oral cancer, Secondary metabolites, *Simarouba glauca*.

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Received: 27-04-2026;
Revised: 11-05-2026;
Accepted: 06-07-2026.

INTRODUCTION

Medicinal plants have been extensively used in traditional systems of medicine and remain a major source of novel therapeutic agents in modern drug discovery. They contain a wide range of structurally diverse phytochemicals, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolic compounds, which are known to possess antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties.[1] In contrast to many synthetic drugs that generally act through a single target, plant-derived compounds often exert multitarget mechanisms, thereby offering broader therapeutic efficacy and reduced adverse effects. This multifunctional nature has generated renewed scientific interest in medicinal plants as safer and more effective alternatives for the treatment of chronic and infectious diseases.[2] Among these medicinal plants, *Simarouba glauca* DC, commonly known as paradise tree or Lakshmi taru, is an evergreen tropical tree belonging to the family Simaroubaceae. It is native to Central and South America and is

DOI: 10.5530/ijper.20264744

widely cultivated in India because of its medicinal value, drought tolerance, and economic importance. Traditionally, various parts of the plant, including leaves, bark, roots, and seeds, have been used to manage fever, diarrhea, dysentery, malaria, inflammation, diabetes, and skin disorders.[3]

Phytochemical investigations have revealed that *S. glauca* is rich in bioactive constituents such as quassinoids, alkaloids, flavonoids, terpenoids, steroids, saponins, and phenolic compounds. Among these, quassinoids are considered characteristic metabolites of the Simaroubaceae family and have been widely reported for their antimalarial, anticancer, anti-inflammatory, antiviral, and antiparasitic activities. Recent studies employing advanced analytical techniques such as GC-MS, HPLC, and metabolomics have further confirmed the chemical richness of *S. glauca* leaves and identified several compounds with significant pharmacological potential.[4,5] Oxidative stress and microbial infections are major contributors to the progression of chronic diseases, including cancer, while the increasing prevalence of antimicrobial resistance has created an urgent need for novel therapeutic agents. Natural antioxidants can scavenge reactive oxygen species and protect biomolecules from oxidative damage, whereas plant-derived antimicrobials may provide effective alternatives to conventional antibiotics.[6] Previous reports have also demonstrated that *S. glauca* extracts exhibit cytotoxic and apoptosis-inducing effects in various cancer models, highlighting their therapeutic relevance.[7,8] However, comprehensive studies integrating phytochemical profiling, antioxidant, antimicrobial, anticancer, and molecular docking analyses of *S. glauca* leaf extract remain limited. Therefore, the present study was undertaken to characterize the phytochemical constituents of ethanolic *Simarouba glauca* leaf extract using GC-MS analysis and to evaluate its antioxidant, antimicrobial, and cytotoxic effects against human oral cancer KB cells. In addition, molecular docking analysis was performed to investigate the interaction of major phytocompounds with Cyclin-Dependent Kinase 4 (CDK4), a crucial regulator of cell-cycle progression and a promising target for oral cancer therapy.[4,7]

Oral cancer represents a pivotal global health challenge, characterized by high incidence and death rates despite several developments in conventional therapies. In 2020, over 377,000 cases of oral squamous cell carcinoma were reported globally, and projections indicate a nearly 40% increase in incidence by 2040, alongside a corresponding rise in mortality.[9] This escalating trend highlights the unmet need for more effective prevention strategies and novel therapeutic interventions to combat the complex tumorigenesis process, which involves genetic alterations, epigenetic modifications, and a dysregulated tumor microenvironment. Late-stage oral squamous cell carcinoma is particularly devastating, often leading to chronic pain, uncontrolled bleeding, and profound debilitation.[10] While current first-line treatments, such as surgery and chemo-radiotherapy, are available, they frequently demonstrate insufficient anti-tumor activity and are associated with considerable toxicity. Consequently, there is a critical need for alternative therapies that offer improved potential with fewer systemic adverse effects. This imperative has catalyzed extensive research into natural compounds, particularly phytochemicals, which have demonstrated promising anticancer properties with selective cytotoxicity against neoplastic cells, thereby minimizing adverse effects on healthy tissues.[11]

A significant challenge with conventional cancer therapies, including chemotherapy, lies in their limited selectivity for tumor cells over normal cells, leading to insufficient drug concentrations at tumor sites, systemic toxicity, and the emergence of drug resistance. This non-specificity often results in dose-limiting side effects and may fail to eradicate all malignant cells, increasing the risk of tumor recurrence. These challenges emphasize the importance of exploring alternative therapeutic avenues that can selectively target cancer cells while decreasing systemic harm, thereby improving patient outcomes and quality of life.[12] Simarouba *glauca* is a well-known edible oil tree, usually referred to as 'Lakshmi Taru' or 'King Oil Seed Tree,' and belongs to the Simaroubaceae family. *S. glauca* has been utilized for various therapeutic purposes in several countries.[13] S. *glauca* is a significant herbal remedy employed in the treatment of numerous ailments. The bark, leaves, and seeds of *S. glauca* are abundant in bioactive chemicals exhibiting diverse pharmacological effects.[14] Several previous studies have already mentioned that *S. glauca* extracts showed anticancer properties against various cancers, including colorectal cancer,[15] leukemia,[16] pancreatic cancer,[17] and lung cancer.[18] Various parts of *S. glauca* have been used to treat a broad spectrum of diseases. Nonetheless, there are very limited studies that have documented the broad-spectrum antimicrobial, antioxidant, and anticancer effects of *S. glauca* leaf extract against oral cancer. Therefore, this study involved phytochemical analysis and examined the antioxidant, antimicrobial, and anticancer effects of ethanolic leaf extract of *S. glauca* on oral cancer cells.

MATERIALS AND METHODS

Plant collection and authentication

The leaf sample of the *S. glauca* plant was collected during June 2025 from the Erode district, Tamil Nadu, India. The collected plant sample was authenticated by the expert botanist.

Extraction of plant material

The leaf samples were taken from the *S. glauca* plant and sectioned into fragments. The chopped pieces were rinsed with running tap water, subsequently cleaned with distilled water, dehydrated at 40°C, and ground into a fine powder using an electric grinder. Approximately 10 g of fine powder was incorporated into 100 mL of ethanol (70%) and macerated for 24 hr at 30°C for extraction. The filtrate was gathered and evaporated at 40°C. The desiccated

extract was preserved in sealed containers at 4°C for subsequent utilization.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of *S. glauca* leaf ethanolic extract was conducted using the GCMS-TQ8040 instrument (Shimadzu, Japan), characterized by high sensitivity and performance, in accordance with the outlined procedure. A DB-5 ms non-polar column with an internal diameter of 30×0.25 mm and a sheet thickness of 0.25 µm was utilized. The column temperature was initially maintained at 50°C for 1 min, and thereafter elevated to 300°C over a duration of 20 min. 0.5 µL of the extract was administered for analysis. Helium was the carrier gas with a flow rate established at 1 mL/min in the splitless mode. The split ratio was 5, with the sample injector and detector temperatures sustained at 250°C and 230°C, respectively. Electrons with an energy of around +0.50 kV were utilized in electron ionization mass spectrometry. Additionally, 40 min were allocated to recording mass spectra within the 50 m/z to 600 m/z range. The identification and quantification of individual phytoconstituents in *S. glauca* leaf extract was conducted by comparing retention indices and mass spectral fragmentation patterns with reference samples from the NIST libraries.

Antimicrobial activity

The well diffusion technique was utilized to investigate the antimicrobial characteristics of the *S. glauca* leaf extract. The diverse pathogens, including *S. pneumoniae, B. subtilis, K. pneumoniae*, and *E. coli*, were cultured individually in the Mueller-Hinton agar plates, and Sabouraud Dextrose agar medium was used for the *C. albicans*. Subsequently, 6 mm wells were created on the agar plate surfaces. The wells were filled with 10, 30, and 60 µg/mL of *S. glauca* ethanolic leaf extracts, respectively, and incubated for 24 hr. The standard antibiotic streptomycin (10 µg/per well) was utilized as a positive control. The inhibitory zones were then evaluated, and the results are documented.

DPPH radical scavenging assay

The *in vitro* antioxidant effect of *S. glauca* extract was investigated using the DPPH radical scavenging assay. Briefly, test tubes containing 1 mL of extract were prepared at dosages between 2.5 and 25 µg/mL. Each test tube was allocated a freshly produced DPPH reagent (2 mL) in methanol. The reaction mixtures, together with the reference standard (Vitamin C), were vortexed for 30 min. The absorbance of the resultant solutions was quantified at 520 nm, utilizing water as a blank reference. The percentage decrease in DPPH scavenging effect was determined using the formula:

DPPH scavenging percentage (%)=(Absorbance of control (Ao) - Absorbance of sample (As) / Ao × 100)

Nitric Oxide (NO) scavenging assay

The NO scavenging effect of the *S. glauca* extract was assessed using the previous method.[19] Sodium nitroprusside (5 mM) in saline solution was combined with several quantities of the extract and incubated for 150 min. The suspension was subsequently combined with Griess reagent. Lastly, the absorbance was taken at 546 nm.

Ferric-Reducing Antioxidant Potential (FRAP) assay

The efficacy of *S. glauca* leaf extract in reducing ferric ion generation was investigated using the previously outlined method by Benzie and Strain (1999).[20] A 50 µL of different dosages of extract was combined with 3 mL of FRAP reagent, consisting of ten parts of acetate buffer (300 mM), one part TPTZ, and one part $FeCl_3 \cdot 6H_2O$ (20 mM) solution. The suspension was then incubated for 30 min, and finally, the absorbance was quantified at 593 nm. The antioxidant effect of the extract was determined by its efficacy to decrease ferric ions.

Collection of cell line

The oral cancer KB cells were acquired from ATCC, USA. Thereafter, cells were grown in DMEM with 10% FBS in a CO_2 incubator at 37°C. Upon achieving 80% confluency, cells were trypsinized and employed for subsequent tests.

MTT assay

The MTT assay was executed to assess the cytotoxicity of *S. glauca* leaf extract on oral cancer KB cells. The cells were loaded into the 96-well plate and incubated for 24 hr. Subsequently, cells were subjected to various concentrations (2.5, 5, 7.5, 10, 12.5, and 25 µg/mL) of the extract for a duration of 24 hr. After that, MTT (20 µL) reagent and 100 µL of DMEM were combined in the well for 4 hr. Subsequently, 100 µL of DMSO was mixed with the wells to liquefy the formazan deposits, and the absorbance was taken at 570 nm.[21]

In silico studies

Ligand molecules

The bioactive phytochemicals detected via GC-MS analysis in the *S. glauca* leaves, including 1,2-Benzenedicarboxylic acid bis (2-methylpropyl) ester, Dibutyl phthalate, 2-Propenoic acid 3-(4-methoxyphenyl)-ethyl ester, N-[3,5-Dinitropyridin-2-yl]valine,[22] and Alpha-Chamigrene were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/) (accessed on 23rd April 2025) and stored in a structural data file format (*.sdf file). The structures were subsequently transformed into PDB file format (*.pdb) utilizing OpenBabel 2.4.1 software and readied for docking studies.[23]

Protein retrieval

The 3D structure of the protein receptor molecule was sourced from the PDB public domain. The structural data for the protein targets, Crystal Structure of CDK4 in complex with a D-type cyclin (2W9F), were obtained from the PDB. The receptor molecule was prepared before docking with the ligand by removing water molecules and heteroatoms pertinent to the docking process, deleting missing atoms in amino acid residues, and eliminating the ligand-bound active site from the PDB file using AutoDock Tools.[24]

Molecular Docking

The docking was conducted utilizing the Autodock 4.2 tool. The target protein was produced by extracting water molecules from them. Subsequently, polar hydrogens, Gasteiger charges, and Kollman charges were incorporated, and AD4-type atoms were designated. The torsion tree was established for the ligand. The produced proteins and ligands were stored in PDBQT format (*.pdbqt file). Blind docking was performed, treating the entire protein molecule as the binding site. The outcomes were evaluated according to binding energies.[24,25]

Statistical analysis

The statistical tests were conducted utilizing GraphPad software, with data illustrated as mean±SD of triplicate measurements. One-way ANOVA and Tukey's *post hoc* test were performed to evaluate the data, with significance set at $p<0.05$.

RESULTS

GC-MS analysis of the ethanolic extract of *S. glauca* leaves

The GC-MS chromatogram of the ethanolic leaf extract of *S. glauca* displayed 25 peaks (Figure 1), which correspond to the phytocompounds, detected by comparing their peak retention time, area (%), height (%), and mass spectral patterns with known compounds from the NIST library. The findings indicated that 23 phytochemicals were discovered in the *S. glauca* leaf extract (Table 1).

Antimicrobial effect of ethanolic extract of *S. glauca* leaves

The antimicrobial efficacy of *S. glauca* ethanolic leaf extract against diverse pathogens, including *S. pneumoniae, B. subtilis, K. pneumoniae, E. coli*, and *C. albicans*, was evaluated employing the well diffusion method. The findings illustrated that the administration of *S. glauca* leaf extract at varying doses markedly inhibited the growth of these pathogens in a dose-dependent manner. The highest zone of inhibition was noted at *K. pneumoniae, E. coli*, and *C. albicans* (Figure 2). The increased inhibition zone surrounding the *S. glauca* leaf extract-treated wells indicated a reduction in the growth of these strains.

Effect of ethanolic extract of *S. glauca* leaves on the *in vitro* free radical scavenging activity

Figure 3 demonstrates the antioxidant effects of *S. glauce* leaf extract, which is assessed using various free radical scavenging effects. The findings of the various free radical scavenging assays indicated that the various concentrations of the extract of *S. glauca* leaves effectively reduced the DPPH, NO, and ferric ion radicals

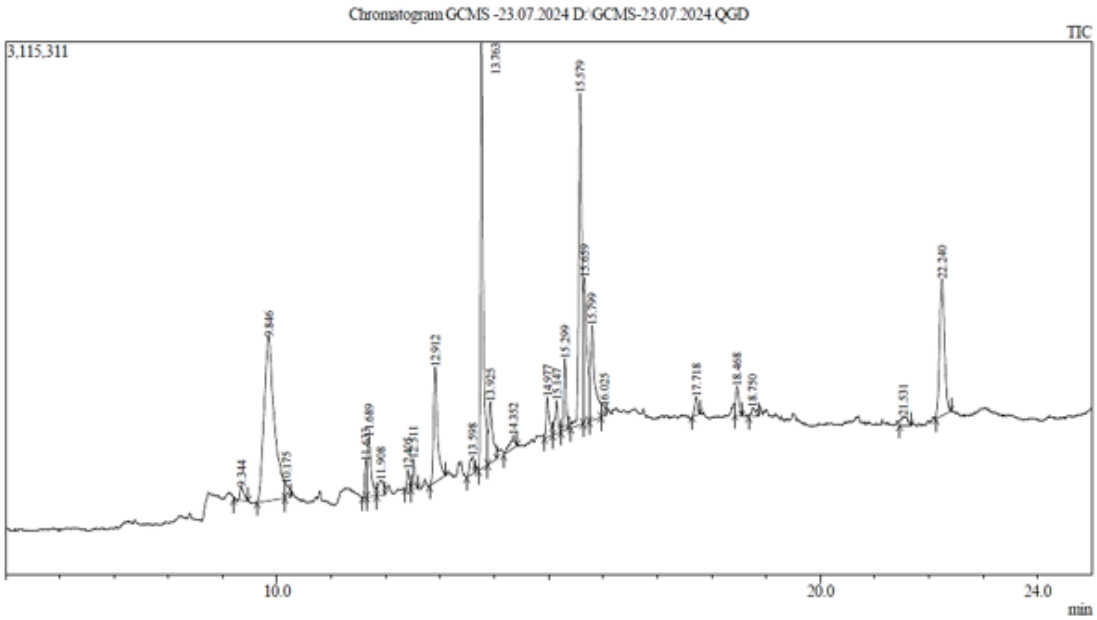
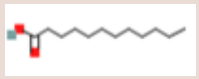
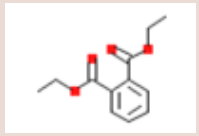
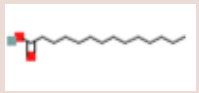
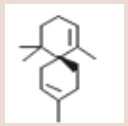
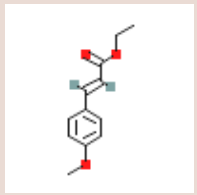
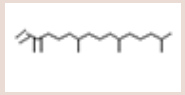
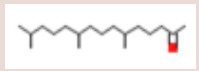
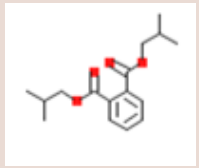
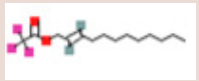
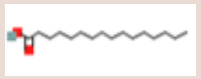
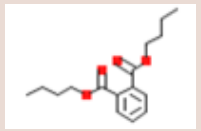
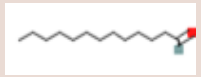
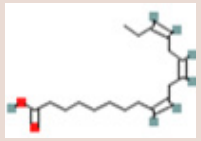
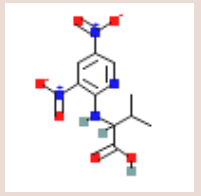
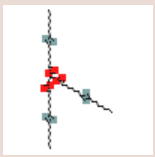
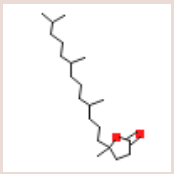
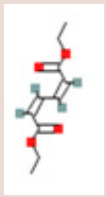
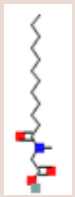


Figure 1: GC-MS analysis of ethanolic extract of *S. glauca* leaves.

Table 1: GC-MS analysis of the ethanolic extract of *S. glauca* leaves.

Sl. No.	Compound Name	Pubmed ID	Retention time	Molecular Weight	Molecular formula	Area %	Canonical SMILES	2D structure	Binding Energy (kcal/mol)
1.	Dodecanoic acid (CAS) Lauric acid	3893	9.344	200.32 g/mol	$C_{12}H_{24}O_2$	0.71	CCCCCCCCCCCC(=O)O		-3.9
2.	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	6781	9.846	222.24 g/mol	$C_{12}H_{14}O_4$	22.15	CCOC(=O)C1=CC=CC=C1C(=O)OCC		-5.7
3.	Tetradecanoic acid (CAS) Myristic acid	11005	11.633	228.37 g/mol	$C_{14}H_{28}O_2$	0.96	CCCCCCCCCCCCCC(=O)O		-4.6
4.	Alpha.-Chamigrene	442351	11.689	204.35 g/mol	$C_{15}H_{24}$	2.93	CC1=CC[C@@]2(CC1)C(=CCCC2(C)C)C		-6.2
5.	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	5281783	11.908	206.24 g/mol	$C_{12}H_{14}O_3$	1.04	CCOC(=O)/C=C/C1=CC=C(C=C1)OC		-6.4
6.	Neophytadiene	10446	12.405	278.5 g/mol	$C_{20}H_{38}$	0.52	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C		-4.9
7.	2-Pentadecanone, 6,10,14-trimethyl-	10408	12.511	268.5 g/mol	$C_{18}H_{36}O$	0.82	CC(C)CCCC(C)CCCC(C)CCCC(=O)C		-5.1
8.	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobuty	6782	12.912	278.34 g/mol	$C_{16}H_{22}O_4$	6.00	CC(C)COC(=O)C1=CC=CC=C1C(=O)OCC(C)C		-6.8
9.	trans-2-Dodecen-1-ol, trifluoroacetate	91691549	13.598	280.33 g/mol	$C_{14}H_{23}F_3O_2$	0.92	CCCCCCCCC/C=C/COC(=O)C(F)(F)F		-4.9

Sl. No.	Compound Name	Pubmed ID	Retention time	Molecular Weight	Molecular formula	Area %	Canonical SMILES	2D structure	Binding Energy (kcal/mol)
10.	n-Hexadecanoic acid	985	13.763	256.42 g/mol	$C_{16}H_{32}O_2$	14.65	CCCCCCCCCCCCCCCC(=O)O		-4.4
11.	Dibutyl phthalate	3026	13.925	278.34 g/mol	$C_{16}H_{22}O_4$	3.29	CCCCOC(=O)C1=CC=CC=C1C(=O)OCCCC		-6.5
12.	Tridecanal	25311	14.352	198.34 g/mol	$C_{13}H_{26}O$	0.89	CCCCCCCCCCCCC=O		-4.3
13.	n-Nonadecanol-1	80281	14.977	284.5 g/mol	$C_{19}H_{40}O$	1.59	CCCCCCCCCCCCCCCCCCCO		-4.2
14.	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate	5364509	15.147	296.5 g/mol	$C_{19}H_{36}O_2$	1.34	CCCCCCCC/C=C\CCCCCCCC(=O)OC		-4.7
15.	Phytol Isomer	5280435	15.299	296.5 g/mol	$C_{20}H_{40}O$	2.21	C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C)CCCC(C)C		-4.6
16.	Oleyl Alcohol	5284499	15.579	268.5 g/mol	$C_{18}H_{36}O$	14.23	CCCCCCCC/C=C\CCCCCCCCO		-4.7
17.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	5280934	15.659	278.4 g/mol	$C_{18}H_{30}O_2$	6.77	CC/C=C\C/C=C\C/C=C\CCCCCCCC(=O)O		-5.5
18.	Octadecanoic acid	5281	15.799	284.5 g/mol	$C_{18}H_{36}O_2$	5.49	CCCCCCCCCCCCCCCCCC(=O)O		-4.1
19.	N-[3,5-Dinitropyridin-2-yl]valine	536729	16.025	284.23 g/mol	$C_{10}H_{12}N_4O_6$	0.51	CC(C)C(C(=O)O)NC1=C(C=C(C=N1)[N+](=O)[O-])[N+](=O)[O-]		-6.2

Sl. No.	Compound Name	Pubmed ID	Retention time	Molecular Weight	Molecular formula	Area %	Canonical SMILES	2D structure	Binding Energy (kcal/mol)
20.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)	5364673	17.718	885.4 g/mol	$C_{57}H_{104}O_6$	0.73	CCCCCCCC/C=C/CCCCCCCC(=O)OCC(OC(=O)CCCCCCC/C=C/CCCCCCCC)COC(=O)CCCCCCC/C=C/CCCCCCCC		-4.9
21.	4,8,12,16-Tetramethylheptadecan-4-olide	567149	18.468	324.5 g/mol	$C_{21}H_{40}O_2$	1.25	CC(C)CCCC(C)CCCC(C)CCCC1(CCC(=O)O1)C		-5.6
22.	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl ester) a,a'-dip	6328859	18.750	198.22 g/mol	$C_{10}H_{14}O_4$	0.59	CCOC(=O)/C=C\C=C/C(=O)OCC		-4.7
23.	Glycine, N-methyl-N-(1-oxododecyl)-	7348	22.240	271.4 g/mol	$C_{15}H_{29}NO_3$	8.88	CCCCCCCCCCC(=O)N(C)CC(=O)O		-5.0

in a dose-dependent manner. The increased concentrations of *S. glauca* leaf extract remarkably scavenged the DPPH, NO, and ferric ion radicals. These results suggested the *S. glauca* leaf has considerable *in vitro* antioxidant properties.

Effect of ethanolic extract of *S. glauca* leaves on the viability of oral cancer KB cells

The MTT cytotoxicity experiment was performed to assess the cytotoxic properties of *S. glauca* leaf extract on the KB cell viability. The findings were depicted in Figure 4. The findings indicate that the extract of leaves treatment at diverse dosages (2.5-25 µg/mL) decreased the viability of KB cells. The cell viability showed a marked reduction upon treatment with elevated concentrations of the extract in comparison to untreated cells. The KB cells exhibited greater sensitivity to the extract treatment, with an IC_{50} level determined to be 10 µg/mL.

Molecular docking interactions of phytochemicals in the extract of *S. glauca* leaves against CDK4 in complex with cyclin D1

The findings of molecular docking analyses demonstrated substantial interactions between phytochemicals detected in *S. glauca* and the target protein CDK4 (Table 2). 1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester (Isobutyl) exhibits the strongest binding affinity with a docking energy of -6.8 kcal/mol, highlighting a potentially significant interaction. Other compounds with notable binding energies include Dibutyl phthalate at -6.5 kcal/mol and 2-Propenoic acid, 3-(4-methoxyphenyl)-ethyl ester at -6.4 kcal/mol (Figure 5). In contrast, compounds such as N-[3,5-Dinitropyridin-2-yl]valine and Alpha-Chamigrene displayed lower binding affinities, with docking energies of -6.2 kcal/mol. This indicates that these phytochemicals may considerably interact with CDK4 receptors, facilitating their inhibition. The robust and stable interactions of these phytochemicals with the active sites of CDK4 underscore their potential as natural therapeutic agents.

DISCUSSION

Phytochemicals, recognized as secondary metabolites, are natural compounds in plants that provide substantial therapeutic benefits to humans. This diverse group of bioactive compounds exhibits a wide array of biological effects, including potent anticancer, antimicrobial, and antioxidant effects.[25] This has led to extensive research into their potential applications in mitigating various human ailments, including cancer and microbial infections, leveraging their rich chemical diversity. These compounds interact with nuclear and membrane receptors, modulate metabolic pathways, and induce epigenetic modifications, showcasing their broad biological activities relevant to health promotion and disease prevention.[26] The systematic study of phytochemicals involves a multi-step process, beginning with extraction, followed by isolation, rigorous laboratory testing,

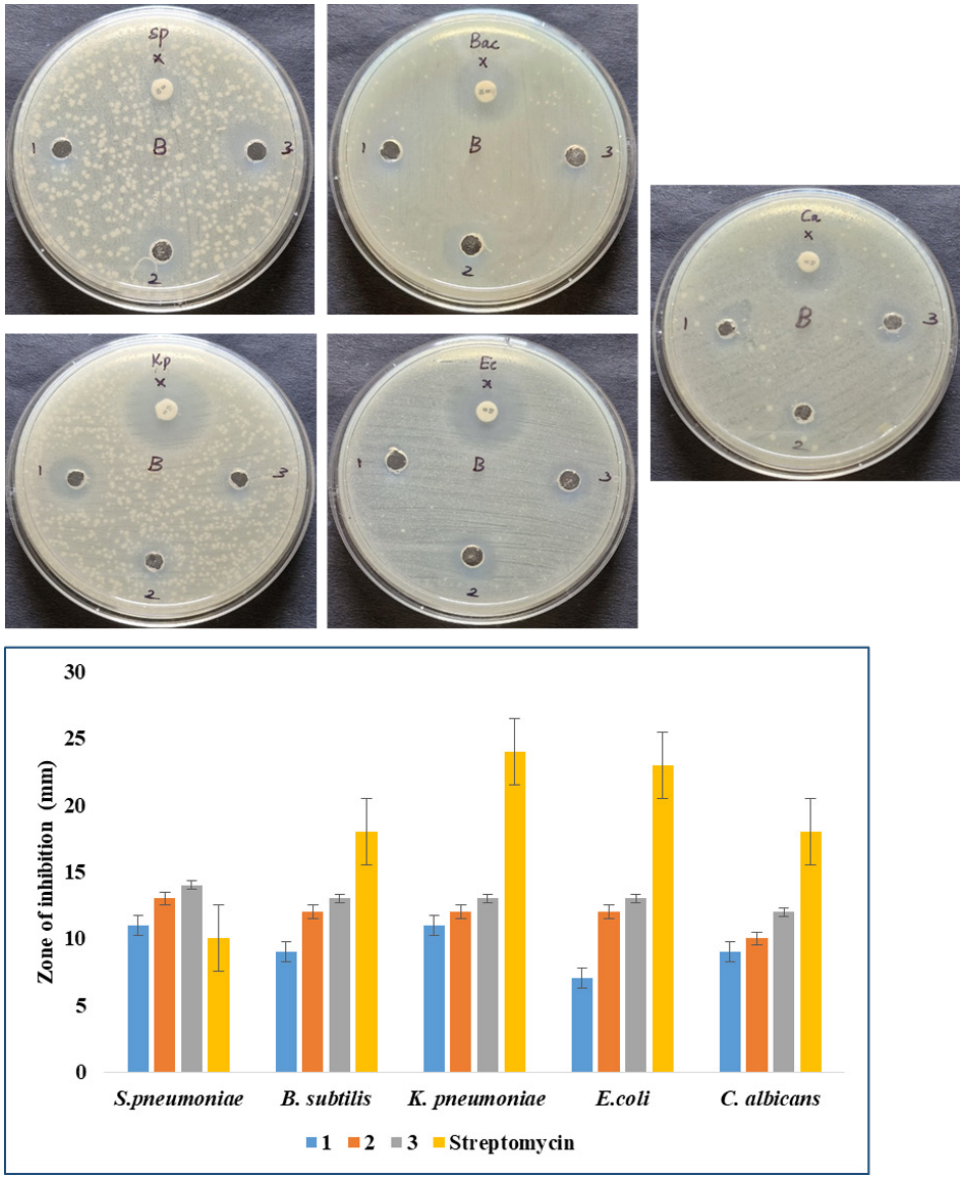


Figure 2: Antimicrobial activity of ethanolic extract of *S. glauca* leaves. The results indicated that the administration of the extract of leaves at varying doses considerably inhibited the growth of all the tested pathogens in a dose-dependent manner. The highest zone of inhibition was noted at *K. pneumoniae, E. coli*, and *C. albicans*.

and ultimately, their application in medicinal formulations. A key challenge in this process lies in the intricate isolation and structural elucidation of these complex compounds, essential steps for understanding their precise mechanisms of action and optimizing their therapeutic utility.[27] Further study is thus crucial to characterize the chemical diversity of phytochemicals and their specific biological targets, paving the way for targeted drug development. This ongoing research aims to overcome challenges such as bioavailability, toxicity, and standardization, which currently limit their broader application in pharmaceutical and functional food development. These investigations are critical for translating the immense potential of phytochemicals into clinically viable therapies that address global health challenges.[28]

Cancer remains a major cause of global mortality, with projections indicating a substantial increase in its burden by 2030, underscoring the critical need for advanced therapeutic strategies. Specifically, oral cancer, ranking as the eleventh most common cancer globally, contributes to over 145,000 deaths annually, disproportionately affecting men and often diagnosed at advanced stages.[29] Despite several developments in surgical, chemotherapeutic, and radiotherapeutic interventions, the overall survival rate for patients with oral cancer has seen only marginal improvement. This stagnation in survival rates is largely attributed to the limited efficacy, significant adverse effects, and substantial cost associated with current treatment regimens, thereby necessitating the exploration of novel and more accessible therapeutic options.[30] This highlights the need

for potential therapies that can mitigate the disease's progression and improve patient outcomes. The limitations of existing treatments, particularly their systemic toxicity and inadequate patient outcomes, necessitate the investigation of alternative, less invasive, and more targeted therapies. This underscores a critical gap in current oncology, necessitating the exploration of novel agents that can enhance therapeutic specificity while minimizing systemic toxicity.[31]

One of the most promising avenues for phytochemical application is in oncology, where their antioxidant, anti-proliferative, and anti-inflammatory properties present viable alternatives or complements to traditional cancer therapies. Conventional chemotherapeutic agents often target DNA synthesis and mitosis, leading to non-selective harm to healthy tissues and significant side effects, whereas phytochemicals offer a promising alternative by exhibiting anticancer effects with potentially fewer adverse reactions.[32] The cytotoxic analysis of phytochemicals is crucial, given their selective action against tumor cells, often without adversely affecting healthy cells, a significant advantage over conventional chemotherapy. Indeed, the rigorous assessments of the cytotoxic effects of these phytochemicals are crucial in oncology.[33,34] Here, the cytotoxicity of the *S. glauca* leaf extract against oral cancer cells was investigated using the MTT assay. The present findings proved that the leaf extract considerably decreased the KB cell viability. These findings highlight that *S. glauca* leaves may have useful effects in mitigating oral cancer progression via inducing cytotoxicity.

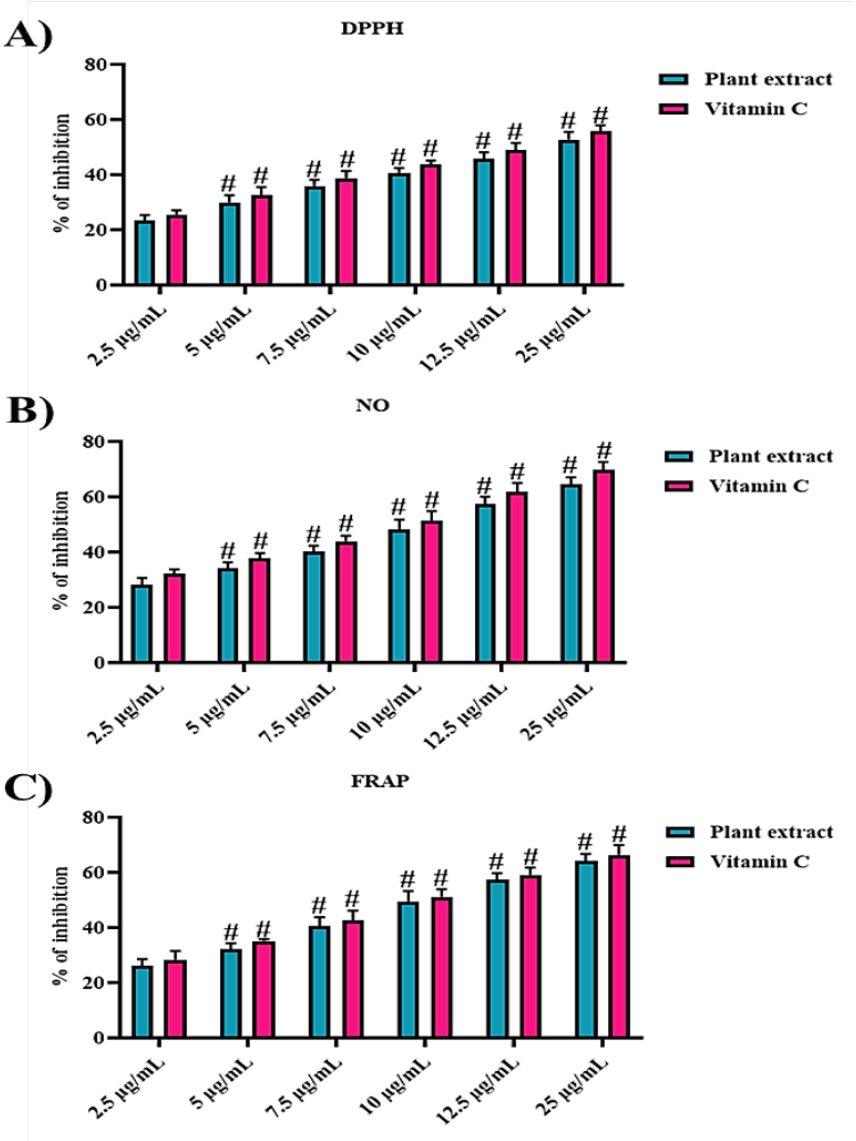


Figure 3: Effect of extract of *S. glauca* leaves on the free radical scavenging activity. The treatment with different doses (2.5-25 µg/mL) of the extract of leaves considerably scavenged the DPPH, NO, and ferric ion radicals in a dose-dependent manner. The results are expressed as mean±SD of triplicate assays, with each value subjected to one-way ANOVA and Tukey's *post hoc* analysis. '#' signifies that the data in treatment groups exhibit a statistically significant difference from the control group at $p<0.05$. (A): DPPH radical scavenging assay; (B): NO radical scavenging assay; (C): FRAP assay.

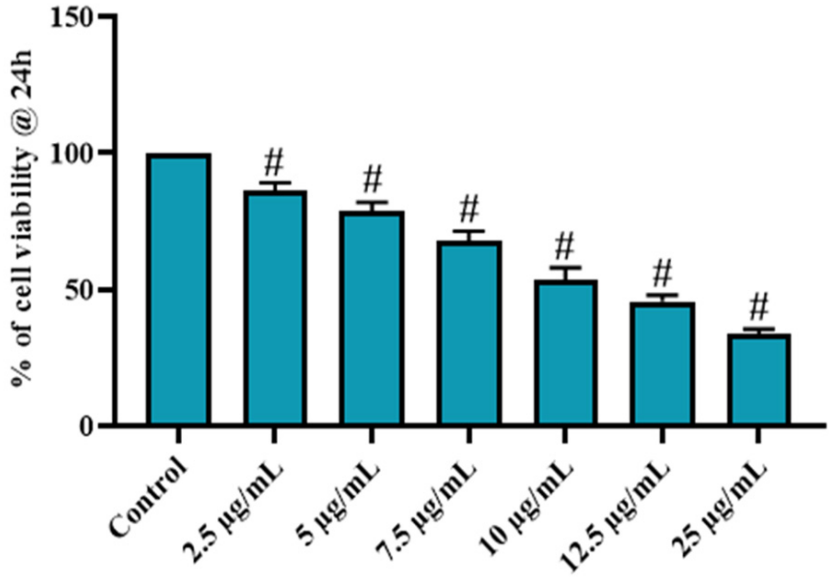


Figure 4: Effect of extract of *S. glauca* leaves on the viability of oral cancer cells. The administration with varying concentrations (2.5-25 µg/mL) of extract of leaves effectively suppressed KB cell proliferation in a dose-dependent manner. The IC_{50} value of the extract of leaves for KB cells was established at 10 µg/mL. The results are expressed as mean±SD of triplicate assays, with each value subjected to one-way ANOVA and Tukey's *post hoc* analysis. '#' signifies that the data in treatment groups exhibit a statistically significant difference from the control group at $p<0.05$.

The increasing prevalence of antimicrobial resistance necessitates the exploration of novel therapeutic avenues, with plant-derived phytochemicals offering a hopeful alternative to current antibiotics. These natural compounds, encompassing a diverse array of secondary metabolites, exhibit a wide spectrum of biological effects, including direct antimicrobial, antifungal, and antiviral effects, alongside immunomodulatory and antioxidant properties.[35] Numerous investigations have demonstrated the antibacterial and antifungal potential of various plant extracts. Specifically, the presence of these useful phytochemicals in plant extracts has been shown to demonstrate strong effects against several pathogenic microorganisms. Moreover, research indicates that plant compounds can either function as direct antimicrobial agents or enhance a pathogen's susceptibility to existing antibiotics, thereby overcoming drug resistance.[36] This multifaceted action is particularly valuable given that the complex phytochemical profiles of plant extracts make it more challenging for microbes to develop resistance compared to standard drugs. This inherent complexity mitigates the evolutionary pressure for resistance development, offering a sustainable approach to antimicrobial discovery. This growing interest in plant-derived antimicrobials stems from their potential to overcome the socioeconomic and long-term negative impacts associated with multidrug-resistant bacteria and fungi. Consequently, scientists are actively seeking novel natural antimicrobial agents that demonstrate efficacy against drug-resistant pathogens and spoilage fungi to mitigate microbial threats and associated challenges.[37] Here, the antimicrobial property of *S. glauca* leaf extract was tested against various pathogens. The present findings revealed that the extract at various concentrations markedly inhibited these tested pathogens. The maximum sensitivity against the extract was observed in the *K. pneumoniae, E. coli*, and *C. albicans*. Therefore, it was evidenced that the leaf extract of *S. glauca* may facilitate the development of novel antimicrobial agents.

Understanding the mechanisms by which plant-derived compounds mitigate oxidative damage is crucial for developing novel therapeutic agents. The study of antioxidants and their implications is of great interest to the scientific community due to the strong link between the presence of free radicals in the body and various human diseases. Consequently, the *in vitro* antioxidant assays provide a robust initial screening platform for identifying promising natural compounds.[38] The DPPH, NO, and FRAP assays are fundamental for evaluating the capacity of plant extracts to neutralize free radicals, which are implicated in various oxidative stress-related pathologies. Among these, the DPPH assay is a broadly adopted technique for evaluating the effect of antioxidants to scavenge stable free radicals through color change measurements. This spectrophotometric approach allows for the quantitative determination of radical scavenging efficiency, reflecting the hydrogen-donating efficacy of the test compound. The change from deep purple to yellow indicates the extent of antioxidant activity.[39] Similarly, the FRAP assay assesses the reducing power of an antioxidant by determining its capacity to reduce ferric tripyridyltriazine to ferrous tripyridyltriazine. The NO radical scavenging assay, conversely, measures the inhibition of nitrite generation, reflecting the ability of plant extracts to neutralize these specific reactive nitrogen species.[40] The complex nature of phytochemicals necessitates the use of diverse assays, like DPPH, FRAP, and NO scavenging, to comprehensively characterize the multifaceted antioxidant properties of plant extracts. This multi-assay approach is essential because no single

assay can fully capture the complex antioxidant mechanisms at play within a plant extract. Thus, researchers frequently employ a combination of these assays to understand the antioxidant activity of plant matrices and biological systems.[41] Here, the antioxidant properties of the *S. glauca* leaf extract were assessed using DPPH, FRAP, and NO scavenging assays. The findings of these free radical scavenging assays suggested that the various concentrations of the extract of leaves effectively reduced the DPPH, NO, and ferric ion radicals. These findings highlight that the *S. glauca* leaf has considerable *in vitro* antioxidant properties.

Oral cancer, a prevalent and aggressive malignancy, necessitates the exploration of novel therapeutic strategies, with molecular docking offering a sophisticated computational approach to identify potential drug candidates from natural sources. This approach is particularly valuable given the persistent challenges of conventional cancer treatments, such as systemic toxicity and the emergence of drug resistance.[42] The urgent need for developing new treatments against cancer is underscored by its status as a leading cause of mortality, with millions of deaths and new cases annually. This pressing demand has propelled the integration of computational tools, like molecular docking, into drug discovery pipelines, especially for identifying lead compounds from natural products known for their diverse chemical structures and pharmacological activities.[43] Molecular docking simulations allow for the prediction of binding affinities and interaction modes between plant-derived compounds and target proteins, such as CDK4/cyclin D1, a crucial regulator of cell cycle progression involved in oral cancer. This *in silico* methodology facilitates the rapid screening of numerous phytochemicals, considerably decreasing the time and cost connected with conventional drug discovery by prioritizing compounds with high therapeutic potential. This computational technique aids in characterizing target mechanisms and optimizing the structure-activity relationship of therapeutic agents, which is crucial for the development of natural product drugs.[44] There is a critical need for innovative strategies that can expedite the identification of effective and less toxic anticancer agents. Molecular docking, therefore, serves as a pivotal tool in addressing this gap by streamlining the discovery of novel small-molecule inhibitors derived from plant extracts. The *in silico* techniques enable researchers to explore vast chemical landscapes and predict the metabolic and toxicity profiles of potential anticancer drugs, thereby mitigating risks in subsequent clinical phases.[45] In the present work, the findings of the molecular docking study suggested the substantial interactions between phytochemicals detected in *S. glauca* leaves and the target protein CDK4/cyclin D. The robust and stable interactions of these phytochemicals with

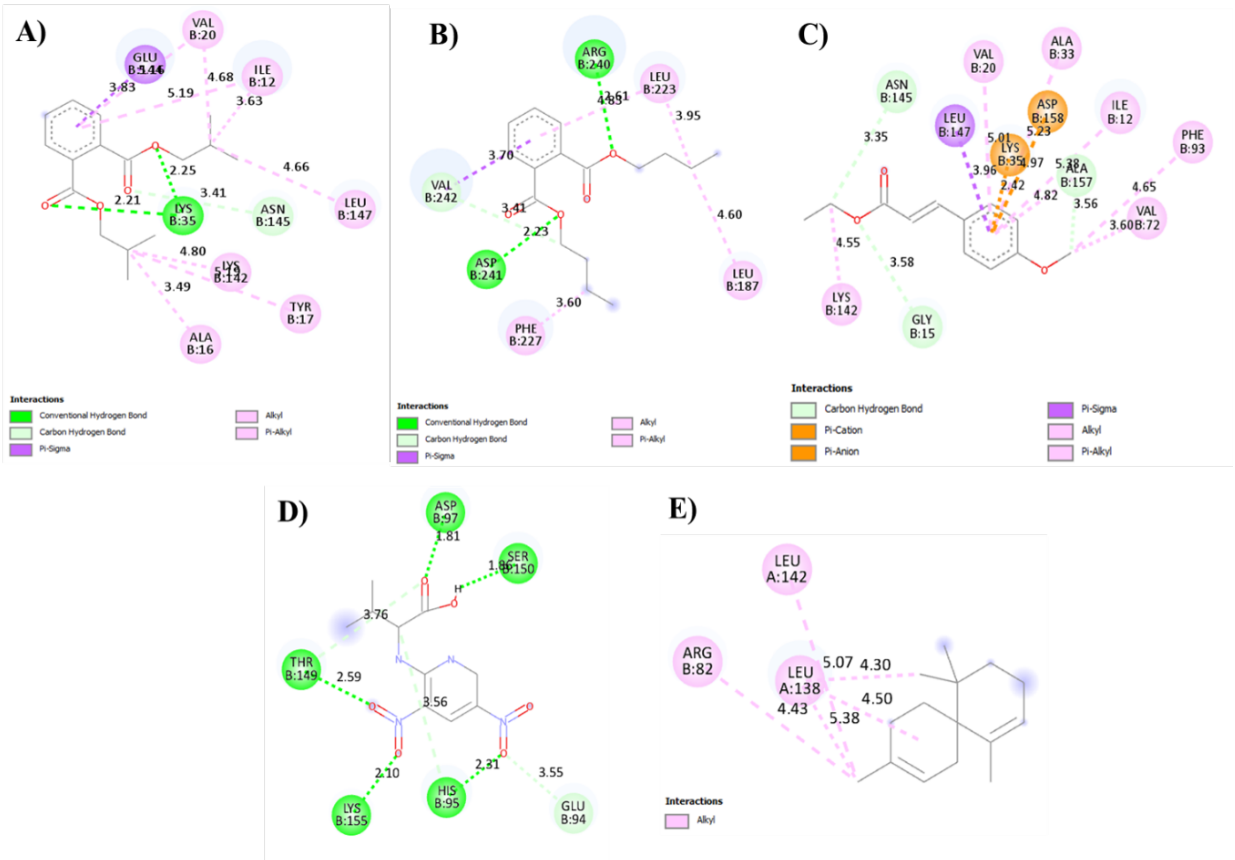


Figure 5: Molecular docking interactions of phytochemicals in the extract of *S. glauca* leaves against CDK4 in complex with cyclin D1. A) 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester; B) Dibutyl phthalate; C) 2-Propenoic acid, 3-(4-methoxyphenyl)-ethyl ester; D) N-[3,5-Dinitropyridin-2-yl]valine; E) Alpha-Chamigrene.

Table 2: Molecular docking scores and residual amino acid interactions of phytochemicals in the ethanolic extract of *S. glauca* leaves against CDK4 in complex with cyclin D1.

Protein	Ligand	Binding energy	Interactions	
			Hydrogen Bond	Residual Hydrophobic/ Pi-Cation/Pi-Anion/Pi-Alkyl Interactions
2W9F	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobuty	-6.8	LYS B:35, ASN B:145	VAL B:20, ILE B:12, GLU B:146, LYS B:35, LEU B:147, LYS B:142, ALA B:16, TYR B:17
2W9F	Dibutyl phthalate	-6.5	ARG B:240, ASP B:241, VAL B:242	LEU B:223, LEU B:187, PHE B:227
2W9F	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	-6.4	ASN B:145, GLY B:15	LEU B:147, VAL B:20, LYS B:35, ASP B:158, ALA B:157, ILE B:12, PHE B:93, VAL B:72, LYS B:142
2W9F	N-[3,5-Dinitropyridin-2-yl]valine	-6.2	ASP B:97, SER B:150, THR B:149, LYS B:155, HIS B:95, GLU B:94	NIL
2W9F	Alpha-Chamigrene	-6.2	NIL	LEU A:142, ARG B:82, LEU A:138

the active sites of CDK4 underscore their potential as natural therapeutic agents for oral cancer.

CONCLUSION

The current research suggests that the *S. glauca* leaf extract showed antimicrobial, antioxidant, and cytotoxic properties against oral cancer KB cells. The results of GC-MS analysis revealed the presence of several secondary metabolites in the extract of *S. glauca* leaves. The extract considerably scavenged the various free radicals, inhibited the growth of several pathogens, and showed cytotoxic effects against KB cells. Consequently, these results indicate that *S. glauca* leaves may facilitate the development of novel therapeutic candidates with diverse pharmacological properties. Furthermore, the results of the *in silico* study indicated that the phytochemicals present in *S. glauca* leaves interacted with CDK4 receptors, which potentially facilitates their inhibition. Hence, *S. glauca* leaves may also facilitate the development of novel anticancer agents against oral cancer.

ACKNOWLEDGEMENT

The authors are grateful to the management and faculty members of Bharathidasan College of Arts and Science, Karpagam Academy of Higher Education, Majmaah University, and Sri Ramachandra Institute of Higher Education and Research for providing the necessary facilities, infrastructure, and support to carry out this research work successfully. The authors also extend their sincere appreciation to the respective departments for their continuous encouragement and academic assistance throughout the study.

ABBREVIATIONS

CDK4: Cyclin-Dependent Kinase 4; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **GC-MS:** Gas Chromatography-Mass Spectrometry; **KB cells:** Human Oral Epidermoid Carcinoma Cells; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **NO:** Nitric Oxide; **S. glauca:** *Simarouba glauca*; **in silico:** Computer-based computational analysis; ***in vitro*:** Experimental study performed outside a living organism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

P. S contributed to investigation, methodology, data curation, formal analysis, and manuscript drafting. S. S contributed to conceptualization, supervision, validation, project administration, and manuscript review and editing. M. A contributed to methodology, resources, data interpretation, and manuscript review. W. A. A contributed to formal analysis, visualization, and manuscript editing. S. M contributed to molecular docking analysis, software validation, and manuscript review. K.S contributed to experimental support, data collection,

and literature review. All authors read and approved the final version of the manuscript.

SUMMARY

The present study demonstrated that the ethanolic leaf extract of *Simarouba glauca* possesses significant antioxidant, antimicrobial, and anticancer activities. GC-MS analysis confirmed the presence of several bioactive phytochemicals in the extract. The extract effectively scavenged free radicals, inhibited the growth of pathogenic microorganisms, and exhibited dose-dependent cytotoxic effects against KB oral cancer cells. In addition, molecular docking studies revealed strong interactions between the identified phytocompounds and CDK4 receptors, suggesting a possible mechanism for anticancer activity through inhibition of cancer cell proliferation. Overall, the findings indicate that *S. glauca* leaves represent a promising natural source for the development of novel therapeutic and anticancer agents, particularly against oral cancer.

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Cite this article: Sundaram P, Sellappan S, Al Aboody MS, Alhoqail WA, Mickymaray S, Suresh K. Chemical Characterization and Biological Potential of Simarouba glauca Leaf Extract: GC-MS, In vitro, and Molecular Docking Studies. Indian J of Pharmaceutical Education and Research. 2026;60(4):1656-69.