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RESEARCH ARTICLE

Study on the phytochemical, antioxidant and *in vitro* anticancer activity on root extract of *Simarouba glauca* DC

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Abstract

Plant made medicine plays main role in drug formulation and synthesis with moderate or no side effects. *Simarouba glauca* is exotic to India and known for its phytomedicine property belonging to the Simaroubaceae family prominent for its leaves arrangement and is called "Lakshmi taru" or "Paradise tree". The goal of this study was to present the preliminary phytochemicals, antioxidants, and cytotoxicity mechanism of *S. glauca* root extracts in terms of apoptosis in cell cycle. Phytochemical, qualitative, and antioxidant assay analyses were performed with the standard protocol using HPTLC and cytotoxicity studies were analysed using a flow-cytometer. The Phytochemical screening revealed the presence of saponins, flavonoids, steroids, and triterpenoids. Furthermore, the extracts demonstrated potent antioxidant activity and could serve as a lead source of natural antioxidants. In a concentration-dependent manner, anticancer study demonstrated cytotoxicity against the A549 cell line by stopping the cells at the S-phase. The main investigation reveals the antiproliferative properties of *S. glauca* methanolic root extract. Identification of cytotoxic compounds and their mode of action require further in-depth research. The outcome of our investigation offers compelling evidence that *S. glauca* methanolic root extract can be used as an effective ethnomedicinal agent with the ability to treat human disorders.

Keywords

Phytoconstituents; antioxidant activity; HPTLC; anti-cancer; flow-cytometry

Introduction

The history has demonstrated that plants serve humankind in a variety of ways, mainly by providing food and medicine (1). Pharmaceutical firms need a supply of specialised metabolites from the plant kingdom such as alkaloids, saponins, flavonoids, steroids, phenolic compounds, poisons, and volatile oil. Since they treat conditions without exhibiting any adverse effects on people, phytochemicals are also alluded to be "Man Friendly Medicines" (2). According to Indian traditional medicine, these illnesses are broadly categorised based on the plant components utilizing Siddha, Rasa shastra, Unani, Sa-Rigpa, Ayurveda, Yoga, and Chinese techniques (3).

Due to inadequate early detection methods and inappropriate medicine, cancer is a fatal disease that is spreading quickly and is one of the world's major health challenges (4). Bio-medical sciences have advanced, still cancer remains cryptic, and one of a major challenge to humankind (5). Additionally, it is one of the common causes of mortality that aids in the

invasion and unchecked proliferation of aberrant cells, which causes tumor formation (6). Organic materials and bioactive substances made from healing plants and herbs have exceptional pharmacological abilities to serve as a various cancer suppressor (7).

S. glauca foreign to India belongs to the family Simaroubaceae is specified by many names i.e.- Lakshmi taru, bitter ash, Paradise tree, Mountain Damson, Dysentery bark, and Aceituno in different countries (8). The National Bureau of Plant Genetic Resources (NBPGR) brought this plant in the Research Station at Amravathi, Maharashtra in 1966 for its oil use and at the University of Agricultural Sciences, Bangalore in 1986 (9). *S. glauca* plant is well known for its anticancer properties. The major bioactive phytochemicals working in leaves, bark and seed are Quassinoids, which include ailanthinone, glaucarubinone, and thin-6-one. Other chemicals include benzoquinone, holacanthone, glaucarubine, glaucarubolone, melianone, tricaproin, simarolide, scopoletin, sitosterol, and tirucalla (10). *S. glauca* mediates cell death either by upregulation of apoptosis or tumor suppressors; arrest of cells in cell cycle stages; inhibition of cyclins and induction of p21 and p27 inhibitors reduce spreading with changes of autophagy and last by downregulation of oncogenes (11–13).

Despite having potent anticancer properties, to date, there is no systematic HPTLC phytochemical, antioxidant and cancer study has been performed on roots of *Simarouba glauca*. HPTLC is chromatographic technique that not only provides the analysis and quality control data of a particular species but also give us additional information needed for the separation, isolation, characterization, and identification of phytochemical compounds (14). This study also emphasizes on *in vitro* effects of *S. glauca* methanolic root extract, by treating the A549 lung cancer cell line with respect to apoptosis induction and cell cycle inhibition by flow cytometry. Hence, the goal of this investigation is to showcase fingerprinting with the validation of phytochemical constituents, antioxidant properties and analyse the anticancer activity of the roots of *Simarouba glauca*.

Materials and Methods

Protocol

The standard protocols outlined in The Ayurvedic Pharmacopoeia of India, with several physicochemical characteristics, including moisture content, pH, ash values, hot extraction and extractive values, were considered and followed (15). All the chemicals used were of the analytical category and came from Himedia and Merk.

Collection of *S. glauca* roots

The fresh roots of *S. glauca* plant were acquired from National Bureau of Plant Genetic Resources (NBPGR), Akola rural, Amravati, Maharashtra. The plant material was collected from Mota Laija area of Kutch district of Gujarat (2200' N, 7000' E to 2400'N, 7200' E) India in October, 2013 and authenticated with the help of taxonomist Dr. Devang Chachad, Professor at Department of Botany, Jai Hind College (Autonomous), Church Gate, Mumbai and one from a local wholesale plant nursery, MTech gardens, Kerala was acquired for studying the geometric effects seen in fingerprint.

Preparation and extraction of *S. glauca* roots

Extraction of *S. glauca* roots by conventional extraction process was carried out (thakur *et al.,* unpublished work). The fresh acquired roots were washed immediately in distilled water thrice to remove dust and extraneous matter. Later sun dried for 1 week and powdered using mixer grinder. The powder mass of desired size was obtained by using mesh sieve of various size ranges. 10g of the plant powder was subjected for heat extraction using Soxhlet apparatus for 12 hours cycle with 250 ml of 99% methanol, at 65°C. The apparatus was on standby till it come to room temperature. The thick extract was then obtained by evaporating the excess solvent in a shallow dish with a flat bottom over a water bath heated at 50-100° C as shown in Fig 1. Stored in amber color glass bottle for all the further subsequent analytical tests.

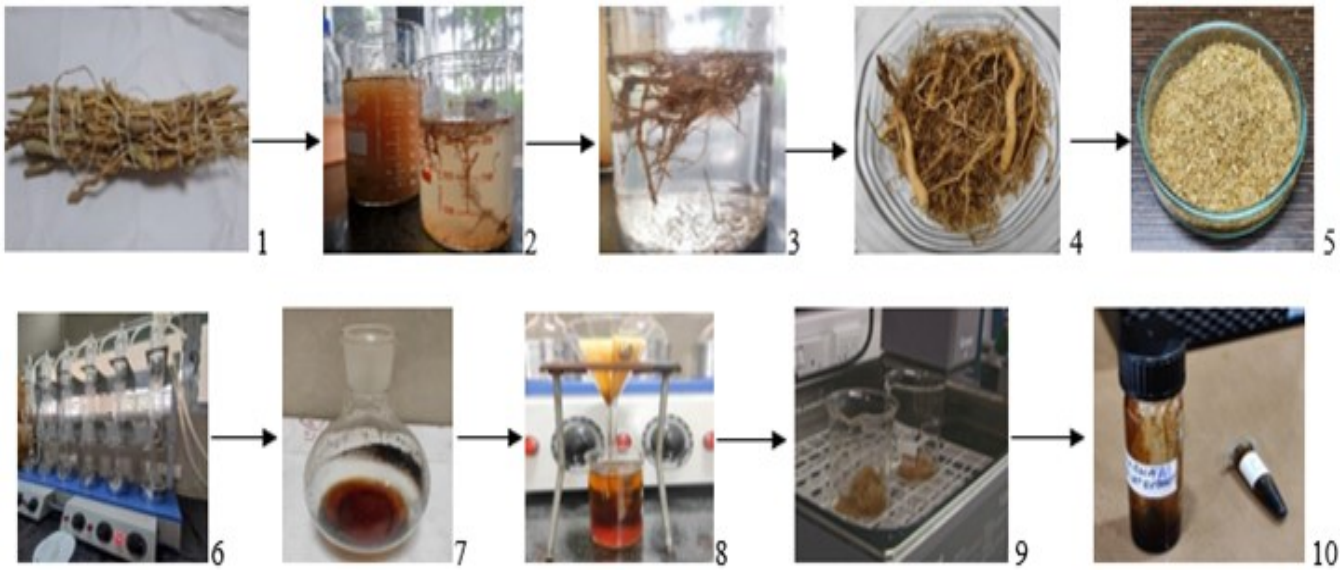

Fig. 1. Steps wise preparation of sample powder and extract

Instrumentation

A full CAMAG HPTLC unit (Muttenz, Switzerland) composed of a CAMAG win CATS software server (version 3.2.22308.1), CAMAG Automatic TLC Sampler 4 (ATS 4), A full CAMAG HPTLC unit (Muttenz, Switzerland) composed of a CAMAG win CATS software server (version 3.2.22308.1), CAMAG Automatic TLC Sampler 4 (ATS 4), Hamilton syringe (100 µL; Bonaduz, Switzerland), developing chamber with twin troughs and a flat floor (10 × 10 cm), CAMAG TLC Scanner IV, CAMAG TLC visualizer, CAMAG Derivatizer, CAMAG TLC Plate Heater 3 and ultrasonic bath (Frontline FS4, Mumbai, India) was used in the study.

Chromatographic conditions

The samples were applied in the form of bands (width-8mm) and track position 16mm using a 100µL micro syringe (Spray-on needle for dosing syringe (695.0046) on gently brush cleaned silica gel coated aluminium plate 60 F254 (size 10×10 cm, 100µm thickness) (E.Merck, Darmstadt, Germany) with the help of a CAMAG Automatic TLC Sampler 4 (ATS 4) (16). Then, the plates were dried for at least 5mins with a cold air dryer. The sample-loaded plates were developed in a pre-saturated twin trough chamber with suitable mobile phases of minimum 10ml for 20minutes with a developing distance of 70mm and relative humidity of 33% for the analysis. Visualization and photo documentation was performed with CAMAG TLC Visualizer 2 at white light, at UV 254nm and UV 366nm wavelengths for the clean plates and the developed plates. Densitometric scanning was carried at 254 nm (deuterium lamp) filter: K320; 366nm florescence (Mercury lamp), filter: K320; and 540nm (tungsten lamp), all with K320 filter. The data resolution was set to 1nm, the spectrum acquisition speed was 20 mm/s, and the slit interference was maintained at 6×0.45mm, micro. To ensure reproducible separation, the findings were evaluated for optimal band separation and migration. To prepare the sample of 100µg/ml, 100mg of Soxhlet extract was weighed and dissolved in 5ml HPTLC grade methanol, later sonicated for 10minutes, and spined at 25000rpm (10min). The clear upper solution was separated and again diluted in 1:5 parts methanol and used as a test solution for the HPTLC study (Table 1).

HPTLC Fingerprint of *S. glauca* root extract

The 2 different area of *S. glauca* methanolic root samples named as sample A (1:10 part diluted) and sample B (1:5 part diluted) (NBPGR) were applied in form of bands with respective volumes of 2µl, 5µl, and 10µl of concentration 100µg/ml on the TLC plate by ATS 4 with a 25µl syringe as shown in table 2. The plate was pre-marked with developing distance of 70mm, the sample-loaded plate was kept in developing chamber with twin troughs saturated with solvent mixture (Toluene: Methanol; Ethyl acetate 4:1:4 (v/v/v)) followed by derivatizing with anisaldehyde sulphuric acid reagent (ASR). Later, 3min heating at 100^{O}C before visualizing (17, 18)(Table 2).

Qualitative phytochemical evaluation

Using the sample B formulated extract further qualitative HPTLC phytochemical investigations were conducted. These phytochemical screening comprised examinations for alkaloids, saponins, tannins, triterpenoids, flavonoids, phenols, steroids, bitter principles. The main objective was to play with polarity to fine tune and get good results. Except for the mobile phase and the derivatization reagents, all chromatographic settings were the same for all phytochemical detections (18).

Antioxidant DPPH radical scavenging assay

A 25ml syringe with filling speed of 15µl/s was used by ATS 4 to dispense 2, 5, 10 and 15µl of the sample with a concentration of 100 µg/ml onto the TLC AI plate silica gel 60 F254. With an application position of 8mm and solvent front of 70mm marked, the sample-loaded plate was kept in developing chamber with twin troughs and saturated mobile phase of Toluene: Methanol: Ethyl acetate, with concentration of 4:1:4 (v/v/v) till it runs up to solvent front. The plate was dried and derivatized using DPPH (2,2-diphenylpicrylhydrazyl) reagent in the dark after visualization at R white, 254nm (absorbance, deuterium lamp), and 366nm (florescence, mercury lamp). The derivatized plate was then wrapped in aluminium foil to incubate in the dark for five minutes before being checked for a white, fluorescent zone (19).

Table 1. Tracks indicating sample volume and position

Track	Vial ID	Description	Volume	Position	Type
1	SB223257-02	*S. glauca* root extract sample B (1:5)	2.0 µl	C1	Sample
2	SB223257-02	*S. glauca* root extract sample B (1:5)	5.0 µl	C1	Sample
3	SB223257-02	*S. glauca* root extract sample B (1:5)	10.0 µl	C1	Sample
4	SB223257-02	*S. glauca* root extract sample B (1:5)	15.0 µl	C1	Sample

Table 2. Tracks indicating sample volume and position

Track	Vial ID	Description	Volume	Position	Type
1	SB223257-02	*S. glauca* root extract sample A (1:10)	2.0 µl	C1	Sample
2	SB223257-02	*S. glauca* root extract sample A (1:10)	5.0 µl	C1	Sample
3	SB223257-02	*S. glauca* root extract sample A (1:10)	10.0 µl	C1	Sample
4	SB223257-02	*S. glauca* root extract sample B (1:5)	2.0 µl	C2	Sample
5	SB223257-02	*S. glauca* root extract sample B (1:5)	5.0 µl	C2	Sample
6	SB223257-02	*S. glauca* root extract sample B (1:5)	10.0 µl	C2	Sample

Apoptosis/necrosis and cell cycle detection by flow cytometry

Utilizing FITC Annexin V Apoptosis Detection Kit of BioLegend, CA, USA, human adenocarcinoma alveolar basal epithelial cell line A549 from the American Type Culture Collection, Pune, was used to analyse necrosis/apoptosis (cell death). The concentration of 2×10^5 cells per well were incubated in 6 well culture dish for 24hr. Later, 1.5mg/ml of *S. glauca* methanolic root extract was employed as a stock solution, and from there, different concentrations (5%, 10%, 15%, and 20%) and controls were introduced. Following 48 hr of incubation, % of apoptotic/necrosis were evaluated using annexin V-FITC/PI staining assay. Later cell phase distribution was performed as outlined in with slight alteration. Using PI/RNase staining solution, the A549 cell lines were examined at various stages of the cell-cycle (BD Biosciences, San Jose, CA). The media from the 24-hour-old culture was removed, and the cells separated were fixed in 70% chilled ethanol for an hour (at or below -20 °C). Cells were then suspended in PI staining buffer (0.5ml, 50g/ml) for 30 minutes. Using a MACS flow cytometer, cell profiling was carried out (MACSQuant® Analyzer 10, Miltenyi biotech, Germany) (20). Unstained samples were used as a control. All data were analysed using FlowJo 8.8.6 and 8.8.10 software (Tree Star).

Statistical analysis

There were three copies of each experiment run. The findings were presented as mean standard deviation. The Kolmogorov-Smirnov test was used to establish normal distribution.P<0.05 indicates that one-way ANOVA analyses the intergroup differences.

Result and Discussion

Physicochemical Evaluation

We measured the root's powder moisture content, swelling index, ash value, and extractive value, among other physicochemical characteristics. As stated in Table 3, the following values for the following variables were obtained: moisture content (0.03±0), swelling index (1±0), total ash (0.0936±0.000548), acid insoluble ash (0.053±0.00095), water soluble ash (0.78 0±00095), alcohol extractive value (29.0667±1.77), water extractive value (0.78±0.00095), and ethanol (4.48%).

Table 3. Results of physicochemical studies

SL. No.	Parameters	% w/w ± SD (at 90% C.I.; n=3)
1	**Moisture content**	0.03±0
2	**Swelling index**	1±0
3	**Ash values**	
	Total ash	0.09367±0.000548
	Acid insoluble ash	0.053±0.00095
	Water soluble ash	0.078±0.00095
4	**Extractive value**	
	Alcohol extractive value	29.0667±1.771
	Water extractive value	19.7333±1.793
5	**Hot extraction**	
	Ethanol	4.48%

HPTLC Fingerprinting

The methanolic extract of *S. glauca* roots was used in the study to create an optimal fingerprint profile that was displayed as a chromatogram and densitogram (Fig 2). There was no overlap with any other component observed in the sample under examination, and it was determined that the mobile phase utilised provided compact bands for extracts at numerous Rf values. The developed plate after derivatization were scanned at white light and 366nm (florescence) producing a chromatogram revealing the occurrence of 6 phytoconstituents seen in track 5 with ascending order Rf values found to be 0.17, 0.35, 0.5, 0.67, 0.76, 0.88 respectively and densitogram peak at 540nm including the retention factor values, peak height, peak area, and percent area of the unidentified compounds (Table 4), which gave significant positive prominent bands and peaks that were later used for analysis. This also indicated that environmental conditions alter and impact the plant's secondary metabolite formation.

Table 4. HPTLC desitogram peak table with corresponding Rf values, height and percentage values of methanolic root extract of *S.glauca*

Peak #	Start		Max			End		Area	
	Rf	H	Rf	H	%	Rf	H	A	%
1	0.008	0.0000	0.045	0.0503	9.08	0.073	0.0000	0.00154	4.98
2	0.085	0.0000	0.129	0.0797	14.41	0.166	0.0000	0.00266	8.59
3	0.279	0.0324	0.311	0.0564	10.19	0.361	0.0191	0.00318	10.38
4	0.410	0.0077	0.463	0.0435	7.87	0.511	0.0128	0.00251	8.14
5	0.511	0.0128	0.544	0.0268	4.85	0.574	0.0144	0.00124	4.02
6	0.574	0.0144	0.627	0.0530	9.57	0.658	0.0318	0.00299	9.67
7	0.658	0.0318	0.721	0.1104	19.95	0.765	0.0421	0.00693	22.42
8	0.765	0.0421	0.848	0.1332	24.08	0.881	0.0900	0.00986	31.90

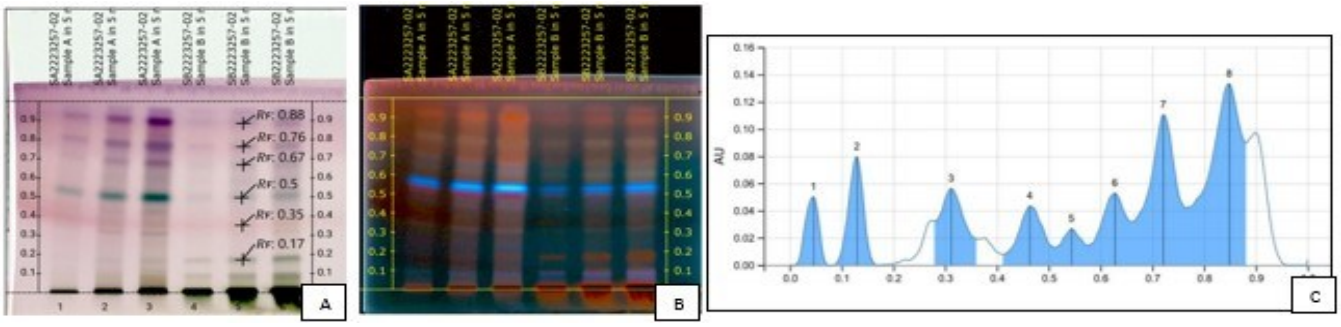

Fig. 2. HPTLC fingerprint with chromatogram a) R white, b) at 366nm and c) HPTLC desitogram at 540 nm showing 8 major peaks.

Qualitative phytochemical investigation

The results of preliminary phytochemical evaluation of *S. glauca* extract are presented in Table 5. The developed and derivatized chromatogram of all 4 phytoconstituent found present including saponins, flavonoids, steroids, triterpenoids with 2, 5, 10, 15µl sample marked with their Rf values in both R white and R366nm after derivatization (Fig 3). HPTLC profiling of saponins under R white with Rf values 0.43, 0.53, 0.65 and 0.6, 0.76 at 366nm; respectively. Profile of flavonoids detected as florescent blue bands at R366nm with Rf 0.2, 0.34, 0.53, 0.66, 0.91. The steroids were detected under R white light at Rf 0.88. The triterpenoids were detected as purple band at R white light with Rf 0.1, 0.49, 0.61, 0.79, 0.87 and Rf 0.21 at R366nm in the methanolic extract of *S. glauca* roots.

Antioxidant DPPH radical scavenging assay

The *S. glauca* methanolic root extracts showed promising high antioxidant activities at all applied sample volumes of 2, 5, 10, 15µg/ml sample with derivations using DPPH reagent. The plate was kept in dark for few minutes before taking the scanned images at R white showing white florescent band zones with Rf at 0.29, 0.47, 0.82 respectively (Fig 4). This indicates that *S. glauca* roots have components that may act as scavengers of free radicals. Scientific investigations have shown that many plant species appear to be trending toward a highly relative connection among total phenols and antioxidants (21). The current investigation, in which *S. glauca* root extracts demonstrated greatest overall antioxidant capacity, has supported the claim.

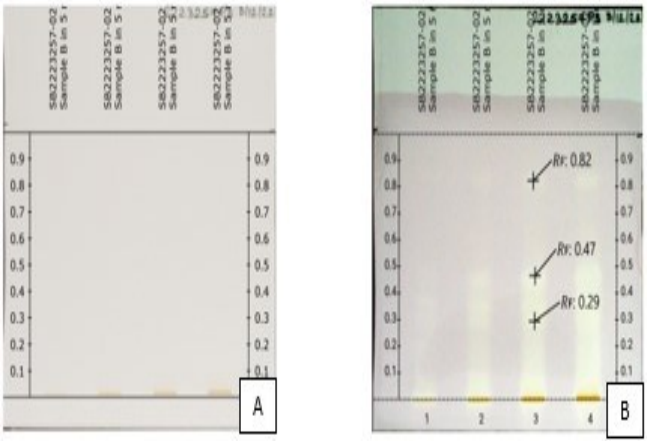

Fig. 4. HPTLC antioxidant DPPH radical scavenging assay at R white A) before derivatization and B) after derivatization

Table 5. HPTLC phytochemical mobile phase, derivatizing agent index of methanolic root extract of *S. glauca*

Class of compound	Mobile phase (10ml)	Derivatizing agent	Observation at R white and R366nm	
Alkaloids	Toluene: Ethyl acetate: Methanol: Ammonia 25% (6:6:3:0.20) v/v/v/v.	Dragendorff Reagent	-	-
Saponins	Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8) v/v/v/v	Anisaldehyde Sulphuric Acid	Yellowish brown bands at R white light	Fluorescent blue violet bands at R366nm
Tannins	Toluene: Ethyl acetate: Formic acid (6:4: 0.3) v/v/v	Alc. FeCl3	-	-
Flavonoids	Ethyl acetate: Water: Formic acid: Acetic Acid (610:2.6:1.1:1.1) v/v/v/v	10% Methanolic sulphuric acid		Blue bands at R366
Steroids	N-butanol: Methanol: Water (6:2:2) v/v/v	Anisaldehyde Sulphuric Acid	Purple band at R white	-
Triterpenoids	N-hexane: Ethyl acetate: (5:5)	Anisaldehyde Sulphuric Acid	Purple band at R white	Blue band at R366
Phenols	Ethyl Acetate: cyclohexane: Formic Acid (6:4:1) v/v/v	Alc. FeCl3	-	-
Bitter principle	Ethyl acetate: Methanol: Water (7.7:1.5:0.8) v/v/v	Vanillin sulphuric acid	-	-

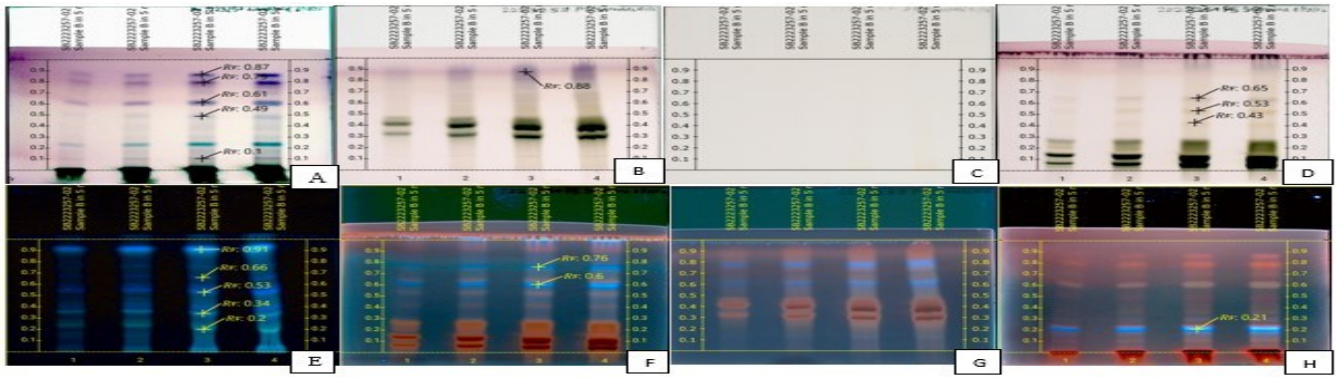

Fig. 3. Representing chromatogram of R white: A) saponins, B) flavonoids, C) steroids, D) triterpenoids and at R366nm E) saponins F) flavonoids, G) steroids, H) triterpenoids.

Flow cytometric analysis with cell cycle analysis

The methanol extract of *S. glauca* root was found to be inhibitory to the A549 cell lines. All the varying concentrations of methanolic *root* extract significantly inhibited the cells at the G_2/M cell-cycle phase ($p = 0.0001$) as the concentration increased compared with corresponding proportions in control cells depicted in fig. 5, and it was also observed that when the concentration of the root extract was increased, the cell inhibition percentage also increased correspondingly. Thus, the concentration of the extract and the cell inhibition is directly proportional, and the cytotoxic activity was found to be concentration-dependent.

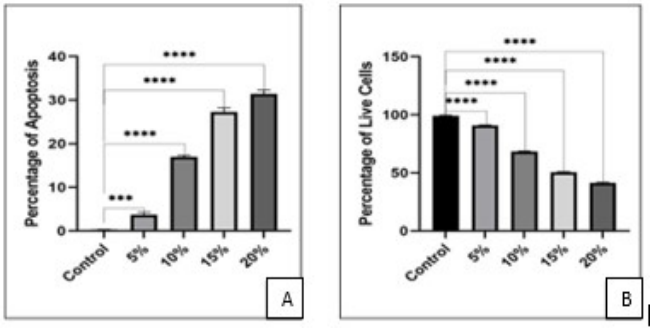

Fig.7. A) Percentages of apoptotic cells and a significant increase in apoptosis (3.42 ± 0.30% vs. control: 16.70 ± 0.20%, 26.97 ± 0.54%, 31.09 ± 0.52% p < 0.0001) a following treatment were vs. vehicle sample seen. B) Percentages of live cells and a significant decrease with 5 to 20% (-8.14 ± 0.41% vs. control: -30.64± 0.46%, -48.46. ± 0.45%, -57.75 ± 0.54% p < 0.0001) treatment were vs. vehicle sample both seen after 48 hr (n=6). *Represents significant result (p < 0.05) compared with untreated cells.

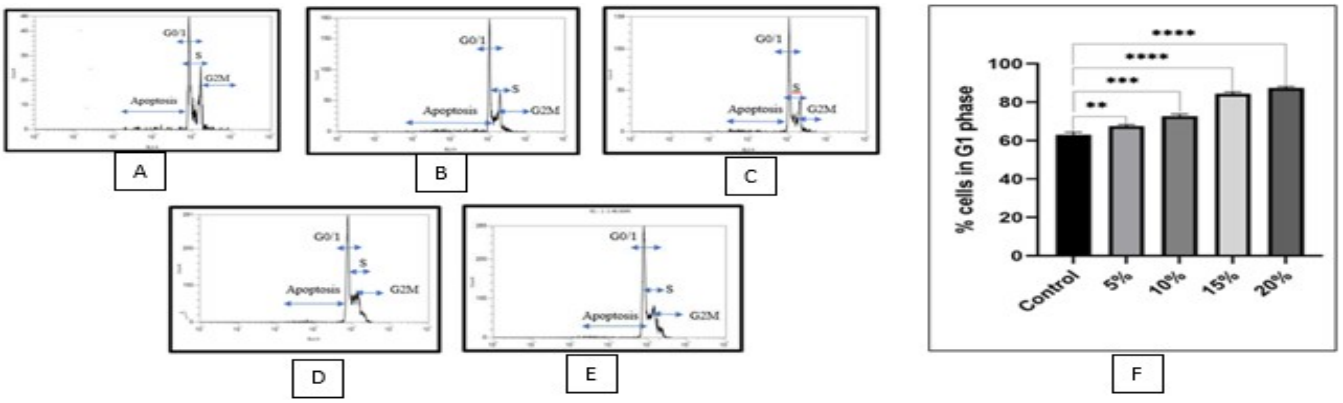

Fig.5. Representation of DNA content distribution of cell cycle phases of A549cell treated with SG root extract for 48 hr (A) represents untreated cells served as a control (B) 5% (C) 10% and (D) represent 15% (E) 20% treated, and (F) cell cycle phases of A549cell treated with *S. glauca* methanolic root extract for 48hr. *Represents significant result (p < 0.05) compared with untreated cells. Percentage of cells in G1 phase significantly increased from 4.72 ± 0.88% (5%), 9.62 ± 0.02 % (10%), 21.37 ± 0.90% (15%) & 24.40± 0.86% (20%) vs. control in a concentration-dependent manner with significant p value <0.05 at all the concentration respectively.

Apoptosis induction

The A549 cells were treated with various concentrations of *S. glauca* methanolic extract(5%, 10%, 15%, and 20%) for 48hr and stained with Annexin V and (PI) were analyzed with flow-cytometer. The results of the evaluation are displayed in fig 6, 7. The methanolic root extract effectively lowered the quantity of alive cells and increased the quantity of dead cells in comparison to control with increase concentration manner ($p < 0.05$). The findings demonstrated that the pathways related to apoptosis are responsible for the development of cytotoxicity.

Discussion

Since the beginning of recorded history, plants have been used as a source of curative medicine for humans. With the present advancements in technology, the significance of plants as sources of therapy-related elements is becoming more and more understood. The evaluation of crude extract is an integral part of correct identity. HPTLC is useful as a phytochemical marker (22,23) and more effective in the field of plant taxonomy and also for the identification of plants through secondary metabolites (24). HPTLC fingerprinting is proved to be a linear, precise, and accurate method for herbal identification (25). Such finger printing is useful in quality control of herbal products and checking for the adulterants (26). Therefore, it can be useful for the evaluation of different marketed pharmaceutical preparations (27–29).

Many marketed medications used in clinical settings come from organic plants (25). In addition to this present development, research investigations show that using monotherapies to treat carcinomas of the colon, rectum, and a variety of cancers results in the rise of reduced drug effectiveness and the unsuccessful

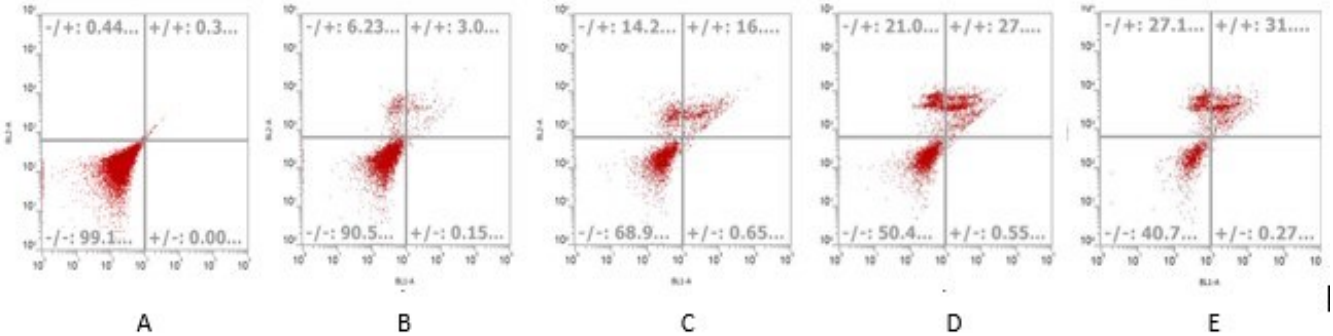

Fig.6. A) A549 cells were without treatment for 48 h and examined using annexin V/PI staining on a flow cytometer vehicle B) treated with 5% *S. glauca* methanolic extract C) treated with 10% D) treated with 15% E) treated with 20%

treatments (26). Therefore, current research is focusing more on generating phytochemical-rich fractions that present better safety and effectiveness for treating cancers. These *S. glauca* leaf and bark crude phytochemical extracts are a possible future source of drugs used in conventional medicine to treat illnesses like diabetes, malaria, worms, gastric, cancer, and conditions related to oxidative damage (27).The existence of quassinoids, a group of intense chemicals with a range of biotic effects, involving anticancer action, is well documented in the Simaroubaceae family (28,29). Our team conducted a study to examine the preliminary phytochemicals, possible antioxidant, and anticancer capabilities of *S. glauca* root extracts.

Our study revealed that there were 4 phytoconstituents present including saponins, flavonoids, steroids, and triterpenoids in the roots of *S. glauca,* which is known to be rich in several phytochemicals. Due to their redox characteristics and chemical makeup, herbal extracts high in secondary metabolites, such as phenolics, flavonoids, and tannins, have antioxidant action (30). The oxidative harm that reactive oxygen species cause to biological systems is a significant factor (31). Due to the simplicity of the reaction, the DPPH radical scavenging method is frequently used to measure free radical scavenging activity. The Rf value revealed significant antioxidant activity in the root extract of *Simarouba glauca*.

The cytotoxicity, anti-malarial, insecticidal, anti-ulcer, hypoglycemic, and anti-tumor property gives potential to the species of this Simaroubaceae family, the existence of quassinoid, alkaloids, phenolic compounds, steroids, flavonoids, and anthraquinone is primarily responsible for the family's rich chemical diversity (32,33). Two of the distinguishing characteristics of cancer cells are reduced apoptosis and deterioration of cell cycle regulation. We used flow cytometry techniques to examine the cell cycle and apoptosis as well as the mechanism underlying the extract's cytotoxic effect on A549 cell lines. According to our findings, *S. glauca* root extract inhibited cell proliferation in A549 cell lines by bringing the cell cycle to an end in the S phase and triggering apoptosis in a concentration-dependent manner. Induction of cancer cell death by the isolated phytochemicals is mediated by the upregulation of apoptosis & arrest of cells in cell cycle stages. Additionally, a recent analysis noted the intricacy of phytochemicals and *S. glauca's* potential anti-cancer properties (22). Another study from Shimoga Cancer Treatment proposed utilizing a decoction made from *S. glauca* leaves to cure cancer. Additionally, it was asserted that the leaf's ethanolic extract included secondary metabolites including phenolic chemicals and had strong anticancer properties against bladder cancer (34). A key paradigm in the development of chemotherapeutic drugs is the ability of the extract to distinguish between healthy and cancerous cells.

Conclusion

Research organizations and pharmaceutical corporations are both commercially interested in phytochemical study of a medicinal plant for the creation of novel medications. The qualitative analysis of *S. glauca* root extracts reveal the presence of medicinally valued bioactive compounds with maximum content of saponins, flavonoids, and triterpenoids and minimum content of steroids. The antioxidant activity was found to be giving good potential for acting as a free radical scavenger. The methanolic root extract inhibits the A549 lung cancer cell line, demonstrating remarkable anticancer potential. Finding novel lead compounds from biological compounds that are less toxic and more effective would be an intriguing alternative for the creation of anticancer medications for the treatment of breast cancer. Therefore, more investigation is needed to determine the method of action and probable lead proportion of the active plant. This will inspire us to develop herbal-based medicines that will treat human ailments.

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Authors' contributions

Conceptualization, M.T., N.V. H.G. and K.S.T; methodology, N.V, K.S.T, B.H, and S.S; software, B.H, and S.S; formal analysis, M.T, N.V. K.S.T; data curation, N.V, B.H, and S.S; writing-original draft preparation, M.T, B.H, N.V; writing review and editing, M.T, K.S.T, B.H & N.V; supervision, M.T and N.V. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

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