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Isolation and therapeutic profiling of alkaloid-enriched leaf fractions from *Simarouba glauca* DC.

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Abstract
Plant-derived antioxidants are recognized for their ability to scavenge free radicals and mitigate the adverse effects of conventional tumour therapies. Combinatorial approaches integrating phytochemicals offer promising strategies against drug resistance and complex diseases. The present study explores the alkaloid fraction of *Simarouba glauca* DC. Leaf methanol extract, focusing on its antimicrobial and germination inhibition potential. Crude extracts were prepared by Soxhlet extraction and phytochemical screening using Mayer's and Dragendorff's tests confirmed abundant alkaloids. TLC analysis (toluene:ethyl acetate:methanol:water; 30:30:15:1) revealed orange bands at Rf 0.61 and 0.96 with Dragendorff's reagent. GC-MS analysis identified diverse phytochemicals, including the indole alkaloid 2-methoxy-6H-indolo [3,2,1-de] [1,5] naphthyridin-6-one (RT 4.37 min). Antibacterial activity was evaluated by the Disc Diffusion Method against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus subtilis* and *Escherichia coli*. The crude extract exhibited the highest inhibition against *B. subtilis* (24 mm), while the alkaloid fraction inhibited *S. typhimurium* (8 mm) and *P. aeruginosa* (10 mm), with no effect on *E. coli* or *B. subtilis*. Germination assays on mung bean seeds demonstrated dose-dependent inhibition, with complete suppression at 250 mg/mL. Collectively, these findings highlight the potential cytotoxic, antimicrobial and anticancer activities of *S. glauca* alkaloids, supporting further pharmaceutical exploration.

Keywords: *Simarouba glauca*, alkaloids, Mayer's, Dradendorff's, Thin Layer Chromatography (TLC), GC-MS analysis, Germination Inhibition Assay

Introduction
The increasing demand for natural and sustainable healthcare solutions has revitalized interest in phytochemical research, with a focus on exploring their potential as therapeutic agents. Phytochemicals have been a cornerstone of traditional medicine for centuries, offering a rich source of bioactive compounds having potential for therapeutic applications. Phytochemicals provide several advantages over synthetic compounds, including reduced toxicity, enhanced biocompatibility and cost-effectiveness. Furthermore, plant-based medicines promote sustainable healthcare and conservation, aligning with the principles of green pharmacy. The diverse array of phytochemicals, including alkaloids, glycosides, flavonoids, terpenoids and phenolic acids, has been extensively studied for their medicinal properties. These compounds have demonstrated remarkable efficacy in providing antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, anti-malarial, cardioprotective, hepatoprotective, neuroprotective and anticancer properties [1].

Plant-derived pharmaceuticals have the potential to function as agents that prevent DNA damage, as well as serve as antioxidants, mitotic disruptors, histone deacetylase inhibitors and methyltransferase inhibitors. Among the various phytocompounds, alkaloids exhibit significant therapeutic potential. Both plant-derived alkaloids and their synthetic derivatives are employed globally as fundamental medicinal agents due to their antimicrobial, antispasmodic, analgesic and a particular emphasis on their anticancer efficacy [2].

Alkaloids are cyclic compounds that contain nitrogen in a negative oxidation state and are characterized by their restricted occurrence among living organisms [3]. Alkaloids are bitter-tasting and often optically active substances. These substances are generally colourless and can exist in either crystalline or liquid form at room temperature, showcasing their diverse physical properties [4]. Based on their biosynthetic precursors and heterocyclic ring structures, the compounds are classified into several categories, including indole, purine, tropane, piperidine, pyrrolidine, pyrrolizidine, quinolizidine, isoquinoline and imidazole alkaloids. This classification highlights the diversity and complexity of the alkaloid compounds within these frameworks [5]. Alkaloids have been shown to induce autophagy, apoptosis, reduce tumour

volume, inhibit cell proliferation and migration. They can also be utilized as part of a combination therapy. Matrine, Palmatine, Fangchinoline, Piperlongumine, Berbamine, Harmine, Liriodenine, Brucine, Capsaicin, Noscapine, Mahanine, Alpha-Tomatine, Vincristine and Vinblastine are examples of therapeutically proven anticancer alkaloids derived from plants [6,7].

Simarouba glauca DC., commonly known as 'Paradise tree or Laxmitaru', belongs to the family Simaroubaceae. It is a tropical plant species native to Central and South America, now widely distributed in tropical regions of Asia, including India. The plant is a deciduous tree, growing up to 10-15 meters in height, with a broad canopy and smooth, grey bark. *S.glauca* has been used in traditional medicine for centuries, particularly in Ayurveda and Unani systems [8]. Various parts of the plant are employed to treat diverse ailments, such as antibacterial, antifungal, antiparasitic, haemostatic, antipyretic, antihelminthic, antidysentric and anticancerous [9]. The ethanopharmacological significance of the plant is attributed to its rich phytochemical composition. Quassinoids are one of the major active phytochemical constituents present in the plant. Glaucarubin, glaucarubol, glaucarubolone and the two esters of glaucarubolone- ailanthinone, glaucarubinone - are reported to treat gastrointestinal disorders and cytotoxicity [10].

The germination response study serves as a preliminary assessment for the cytotoxic effect of a plant extract or sample being analysed. This test is economical and suitable for laboratories with limited resources, making it an effective screening tool when a large number of samples require cytotoxicity assessment. Mung bean seeds are utilized for this analysis as they have widespread availability, ease of handling, high nutritional value and rapid germination compared to other seed varieties.

The present study focuses on the isolation of the alkaloid fraction from the crude extract of *Simarouba glauca* leaves to investigate its potential cytotoxic properties by evaluating its effects on the growth patterns of mung bean seeds. Phytochemical profiling of the isolated alkaloid fraction was carried out using Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses to confirm the presence of alkaloids and to identify their chemical constituents. In addition, the antibacterial activity of the alkaloid fraction was assessed using standard methods against selected bacterial strains to evaluate its potential as an antimicrobial agent. These analyses collectively aim to provide a comprehensive understanding of the bioactive properties of the alkaloid fraction from *S.glauca*, supporting its potential application in pharmaceutical and agricultural sectors.

Materials and Methods

Sample Collection

Fresh leaves of *Simarouba glauca* were collected from the Botanical Garden of Mar Ivanios College (Autonomous), Thiruvananthapuram, Kerala (Figure 1). The voucher specimen deposited at the Department of Botany, Mar Ivanios College, authenticated the study material. They were cleaned thoroughly with running water and later with double-distilled water to remove debris or other organic contents. Water-drained leaves were allowed to shade dry.

Fig 1: Plant and leaf of *Simarouba glauca*

Soxhlet Extraction

50 g of shade-dried leaves of *S.glauca* were powdered and extracted with 500 mL of methanol using Soxhlet apparatus for 24 hours. The solvent from the extract was removed under reduced pressure using a rotary vacuum evaporator (Heidolph, Germany), and the crude sample was stored in a sterile pre-weighed, screw-capped container at 4°C until use [11].

Separation of Alkaloid Fraction

The alkaloid fraction from the methanol extract of *S. glauca* leaves was separated using a modified protocol described by Singh *et al*., (2000) [12]. The crude extract was diluted with distilled water and kept overnight. The supernatant liquid was carefully decanted from the resinous gum. The supernatant was basified with sodium bicarbonate (Na_2CO_3) and extracted with chloroform using a separating funnel. The chloroform extract was treated with anhydrous sodium sulphate to remove moisture and subsequently distilled for further analysis.

Phytochemical Profiling of Alkaloids

The presence of alkaloids in the obtained fraction was assessed by qualitative phytochemical analysis as per the methods of Harborne [13].

Mayer's Test: A few drops of Mayer's reagent (K_2HgI_4) were added to the extract. The formation of a creamy white precipitate suggests the presence of alkaloids in the sample.

Dragendorff's Test: Added 1 mL of Dragendorff's reagent ($KBiI_4$) to 2mL of the extract and the formation of orange-red precipitate indicated the presence of alkaloids.

Thin Layer Chromatography (TLC)

It was performed using the conventional one-dimensional ascending method [14]. TLC Plate Silica Gel 60 F254 (Merck) was used. Extracts were applied using glass capillaries and plates were developed in pre-saturated chambers. Solvent systems such as Toluene: Ethyl Acetate: Methanol: Water (3.9:3.9:1.97:0.13), Hexane: Ethyl Acetate (6:4), and Ethyl Acetate: Methanol: Water (20:27:2) were selected for the profiling. After development, plates were dried and sprayed with Dragendorff's reagent for alkaloid detection and the retention factor (R_f) values were calculated.

$$Rf = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed using a Shimadzu QP2020 with an SH-Rxi-5Sil MS column. Samples (1.0 µL) were injected with a split ratio of 10 at 250°C, using helium (99.999% purity) as the carrier gas at 1 mL/min. The oven was held at 60 °C for 8 min. Ionization was performed at 70 eV, and mass spectra were recorded over the m/z range of 10-200. Compounds were identified by comparing the mass spectra with the NIST 17 Library.

Antibacterial activity

Antibacterial activity was evaluated using the Disc Diffusion method against *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (MTCC 98), *Staphylococcus aureus* (MTCC 96) and *Pseudomonas aeruginosa* (ATCC 27853). Nutrient agar and broth (HIMEDIA) were prepared and sterilized for culturing and inoculum preparation. Fresh bacterial cultures were adjusted to 0.5 McFarland standards and swabbed uniformly onto nutrient agar plates. 100 mg/mL concentration of plant crude extract and alkaloid fraction was impregnated onto sterile paper discs. The plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the zones of inhibition in millimetres.

Germination Inhibition assay

Germination Inhibition Assay is a preliminary evaluation study on the cytotoxic effect of the tested plant extract. It was conducted with minor modifications inspired by the approach outlined by Kumar and Singhal [15]. Green gram/ Mung bean seeds were used for the analysis and they were purchased from a Horticrop (Kerala State Government grocery store), Thiruvananthapuram. Good quality seeds of equal weight were taken in a 24-well plate. The seeds were allowed to soak in distilled water (control) and in different concentrations of the crude alkaloid extract for 24 hours. The plate was closed with the lid and left at room temperature for the imbibition. At the end of the test period (24 hours), the seeds were taken from the wells and gently dried on clean, dry tissue paper. The seeds treated under the same conditions were placed on a glass slide and set on moistened tissue paper inside a Petri dish. The morphological changes in the germination of seeds were observed by maintaining the seeds at room temperature under moist conditions for 96 hours.

Results and Discussion

Yield and phytochemical screening of the extracted alkaloid fraction

The shade-dried leaves of *S.glauca* were powdered and subjected to hot extraction using the Soxhlet apparatus, with methanol as solvent. From 50 g of leaf powder, a yield of 13 g of crude sample was achieved. Using the partial separation technique, 3.76 g of the alkaloid fraction was isolated from 10g of crude methanol extract. Qualitative phytochemical screening with Mayer's and Dragendorff's tests confirmed a high concentration of alkaloids in the extracted fraction. Figure 2 illustrates the separation of the alkaloid fraction from the crude extract. Table 1 and Figure 3 present the results of the phytochemical analysis of the alkaloid fraction.

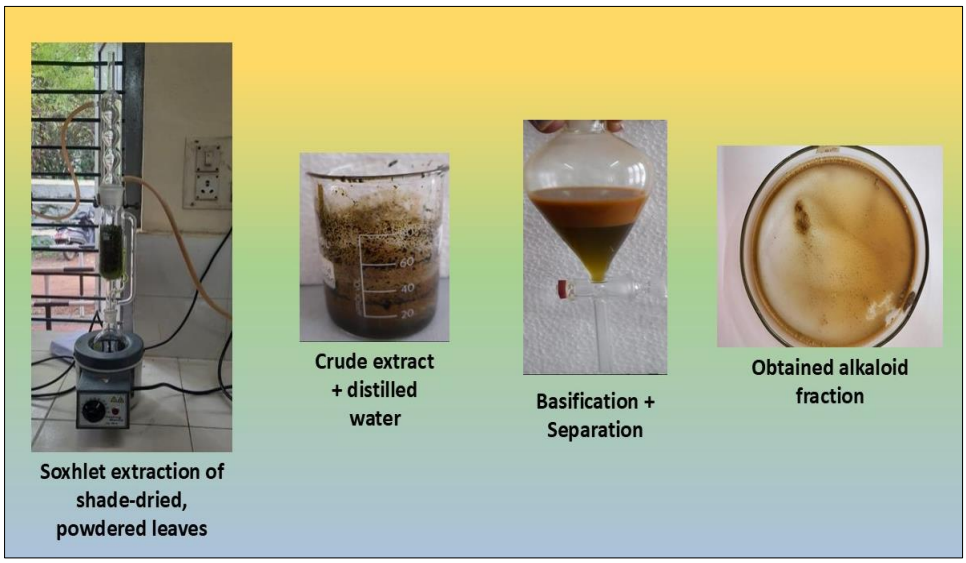

Fig 2: Separation of alkaloid fraction from *S.glauca* leaf methanol extract

Fig 3: Qualitative tests for alkaloids

Table 1: Qualitative phytochemical screening of the obtained alkaloid fraction

Sl.No	Screening test	Observation	Inference
1.	Mayer's test	creamy white precipitate	+++
2	Dragendorff's test	orange-red precipitate	+++

+++ Present in high-concentration

Qualitative phytochemical analysis forms the basis for subsequent quantitative estimations and bioassays. In *Simarouba glauca* leaves, the presence of alkaloids in methanol extracts obtained via Soxhlet extraction has been confirmed through Mayer's test [16,17]. Soxhlet extraction produced a higher crude yield compared to simple maceration [17]. Alkaloids were further validated by positive Hager's and Wagner's assays using Soxhlet-derived extracts [9]. An alkaloid content of 1.2% (w/w) in *S. glauca* leaves has also been reported [18].

Thin-Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was used to separate non-volatile compounds using various solvent systems. Alkaloids were clearly resolved in the Toluene: Ethyl Acetate: Methanol: Water (30:30:15:1) system, showing orange bands at R*f* 0.61 and 0.96 with Dragendorff's reagent, confirming their presence (Fig.4). These findings align with earlier reports indicating the presence of alkaloids, flavonoids, carbohydrates, phenolic compounds, and tannins in the methanolic leaf extract of *S.glauca* [19].

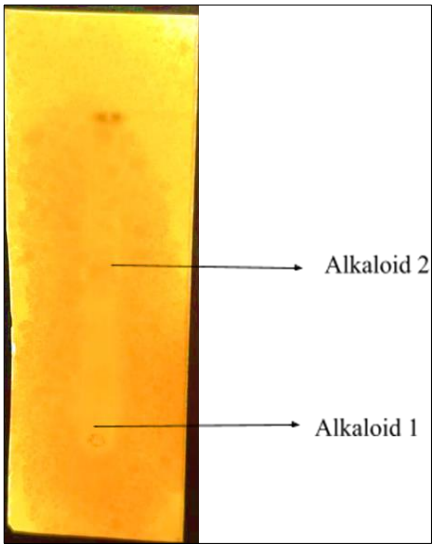

Fig 4: Alkaloid detection by TLC

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Shimadzu QP2020 with an SH-Rxi-5Sil MS column. 1.0 µL of crude methanol extract was injected in split mode (split ratio 10:1) at 250°C, with helium (99.999% purity) as the carrier gas at 1 mL/min. The oven was held at 60°C for 8 minutes and ionization was conducted at 70 eV, scanning a mass range of 10-200 m/z. Compound identification was performed by comparing mass spectra with the NIST17 library. The analysis confirmed the presence of various phytochemicals, including the indole alkaloid 2-methoxy-6H-indolo [3,2,1-de] [1,5] naphthyridin-6-one at RT 4.37 minutes (Figure 5 & Table 2). Canthin-6-one alkaloids, a subclass of β-carboline alkaloids with an additional six-membered ring, were initially isolated from *Pentaceras australis* [20]. GC-MS analyses of *S. glauca* leaf extract have identified alkaloids such as dihydrocodeine and morphinan derivatives [21], which may contribute to the antimicrobial activity observed in the extracts.

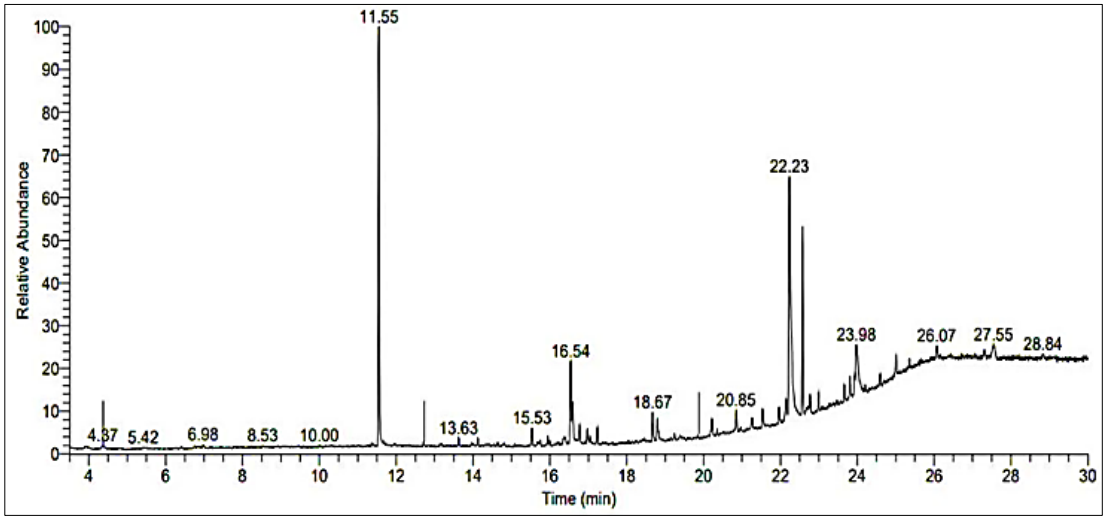

Fig 5: Gas chromatogram of *S.glauca* leaf methanolic extract

Table 2: GC-MS spectral analysis of *S. glauca* leaf methanol extract

Sl. No.	Retention Time	Compound Name
1	4.37	2-Methoxy-6H-indolo[3,2,1-de][1,5]naphthyridin-6-one
2	11.55	2,4-Di-tert-butylphenol
3	13.63	Cyclooctasiloxane, Hexadecamethyl-Octasiloxane
4	15.53	Octadecamethyl-Heptasiloxane
5	15.94	Phthalic acid, butyl tetradecyl ester
6	16.54	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene -2,8-dione
7	16.57	Hexadecanoic acid, methyl ester
8	16.59	à-N-Normethadol
9	16.77	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
10	16.98	Butyl undecyl ester

Antibacterial Activity

The antibacterial activity of the crude and alkaloid extracts (100 mg/mL) was evaluated against *P.aeruginosa*, *S.typhimurium*, *B.subtilis* and *E.coli* using the Disc Diffusion method. The extracts exhibited a zone of inhibition against the tested pathogens (Figure 6 & Table 3). The crude extract showed the highest activity against the Gram-positive *B. subtilis* (24 mm). The alkaloid fraction exhibited inhibition zones of 8 mm and 10 mm against *S.typhimurium* and *P.aeruginosa*, respectively, while no activity was observed against *E.coli* and *B.subtilis*. The results suggest that the antibacterial efficacy of the extracts may improve with higher concentrations. Of note, the findings align with previous studies in which *B.subtilis* showed a maximum inhibition zone of 14 mm when tested with methanolic dried leaf extracts [22].

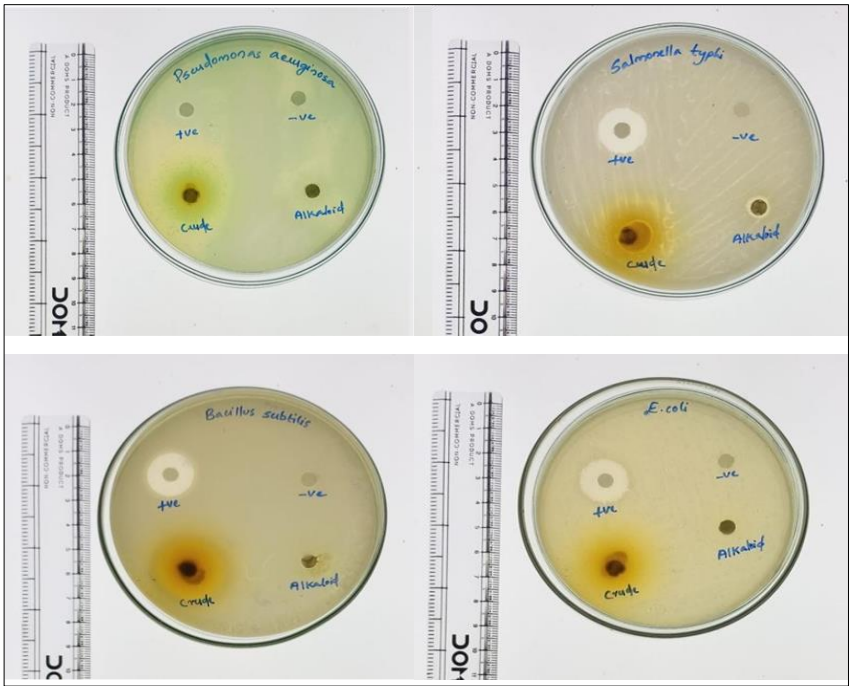

Fig 6: Antibacterial activity of extract against *Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis* and *Escherichia coli*

Table 3: Zone of inhibition of the extracts against the tested bacteria

Bacterial Strain	Zone of Inhibition		
	Crude extract	Crude alkaloid	+ve Control (Streptomycin)
Bacillus subtilis	24mm	Resistant	16mm
Salmonella typhimurium	12mm	8mm	16mm
Pseudomonas aeruginosa	16mm	10mm	14mm
Escherichia coli	21mm	Resistant	16mm

Germination Inhibition Assay

There is an escalating necessity to establish efficient *in vitro* methods for the preliminary screening of potential anticancer agents. These initial assessments can subsequently be validated through studies in animal models [23]. Germination inhibition assay is a simple, rapid and inexpensive model that serves as an excellent choice for *in vitro* preliminary screening. Its accessibility and ease of use make it suitable for a wide range of applications in research and development.

The germination of mung bean seeds was assessed using control (water), alkaloid concentrations of 15.7 mg/mL, 31.3 mg/mL, 62.5 mg/mL, 125 mg/mL and 250 mg/mL. The morphological characteristics of the seeds indicated the inhibitory action of the tested concentrations. The control group demonstrated normal growth of both the radicle and plumule, accompanied by the development of leaflets. In contrast, exposure to increasing concentrations of alkaloids resulted in a dose-dependent inhibition of germination. At the highest concentration, the seeds appeared to be dry, with shrunken radicles and a complete absence of plumule growth. Figures 7-9 display the inhibitory effects of the alkaloid fraction. Further analysis in normal and cancer cell lines extends the properties of the alkaloid fraction in anticancer studies.

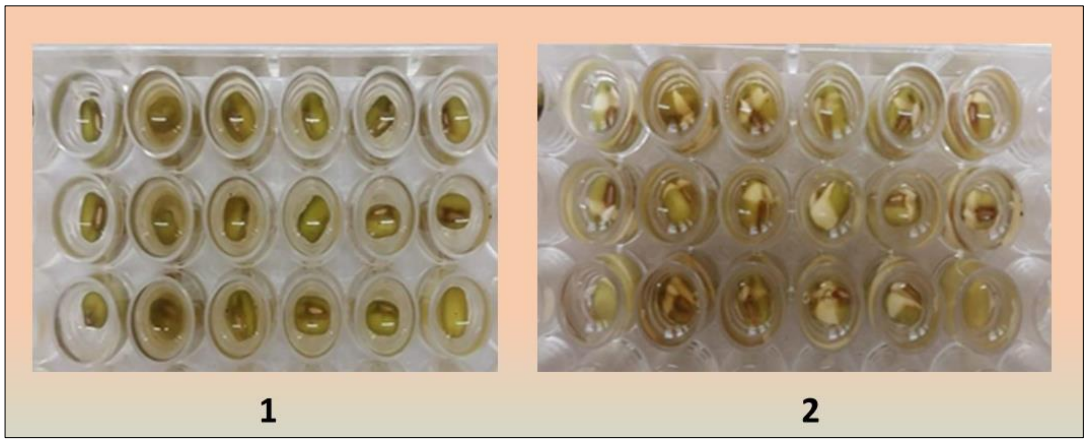

Fig 7: Imbibition of mung bean seeds in the (1) control (water) and (2) varying concentrations of alkaloid fraction

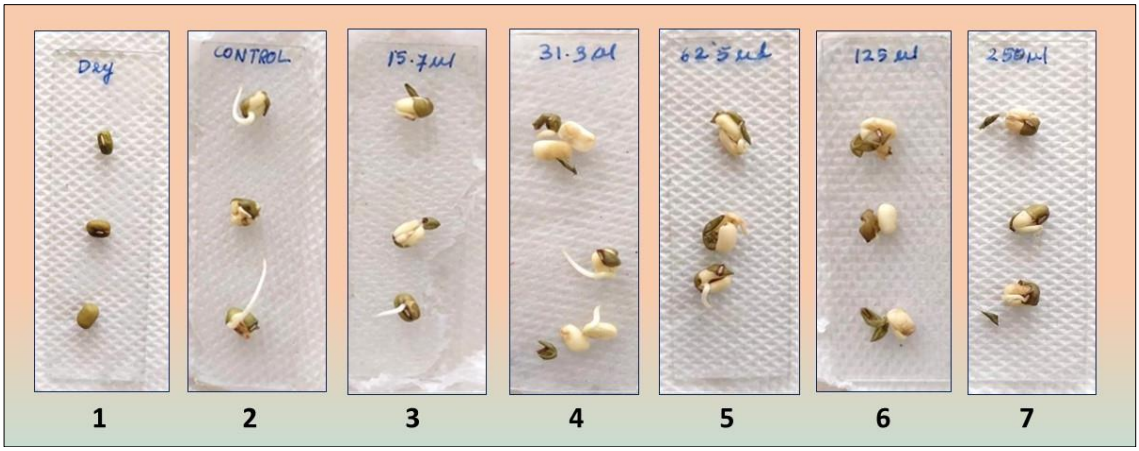

Fig 8: Inhibitory effect of the alkaloid fraction on seed germination after 96 hours -
(a) dry (b) control - water (c) 15.7 µg/mL (d) 31.3 µg/mL (e) 62.5 µg/mL (f)125 µg/mL (g) 250 µg/mL

Fig 9: Morphological changes in mung bean seeds after treatment

Four canthin-6-one alkaloid derivatives have been isolated from twig extracts of *S.glauca*, exhibiting cytotoxicity against human colon, oral epidermoid, prostate and lung cancer cell lines [24]. The methanol leaf extract demonstrated cytotoxic effects on leukemic cell lines K-562, MOLT-3 and KG-1, with IC_{50} values of 74.21, 69.65 and 131.1 µg/mL, respectively, as assessed by the MTT assay [25]. Conversely, another study reported limited cytotoxicity in both short- and long-term assays, despite dose-dependent cell death [26]. This variation was attributed to crude extract composition, where active biomolecules may be masked by other phytoconstituents.

Conclusion

The relevance of the present study is underscored by the isolation of active alkaloid fractions from the crude extract of *Simarouba glauca* and the subsequent evaluation of their cytotoxicity through the mung bean germination assay. This approach is effective in assessing the biological activity of compounds and is economical, making it accessible for future research. Preliminary phytochemical screening using Thin Layer Chromatography (TLC) confirmed the presence of alkaloids in the isolated fractions. Furthermore, GC-MS analysis identified bioactive alkaloid compounds, including dihydrocodeine and morphinan derivatives, in the crude extract of *S.glauca*. The alkaloid fractions also exhibited significant antibacterial activity against selected bacterial strains, indicating their antimicrobial potential. These findings pave the way for further studies using advanced chromatographic techniques to isolate specific compounds, followed by in-depth evaluation on various cell lines to assess their therapeutic potential. This comprehensive research will not only provide scientific validation for the traditional use of *S.glauca* in treating ailments but may also contribute to the development of novel pharmaceuticals. Through continued research, *Simarouba glauca* holds significant potential to be recognized as a "tree of solace" in the realm of cancer treatment.

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Author contributions

All authors contributed significantly to the development and writing of the article.

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No funding was received for this research.

Conflict of Interests

The authors declare no competing interests.

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