Journal of Young Pharmacists, 2025; 17(1):89-96.
https://www.jyoungpharm.org

Original Article

Phyto-chemicals and Spectral Characterization of Novel Glaucarubinone Quassinoid from *Simarouba glauca* Leaves: *In vitro* Anti-proliferative Potential against Breast Adenocarcinoma Cell line

Vanitha Subburaj[1,*], Umaa Kuppuswamy[2], Veintraimuthu Sankar[3]

[1]Department of Pharmacognosy, PSG College of Pharmacy (Affiliated with the Tamil Nadu Dr. M G R Medical University), Coimbatore, Tamil Nadu, INDIA.
[2]Department of Pharmaceutical Chemistry, PSG College of Pharmacy (Affiliated with the Tamil Nadu Dr. M G R Medical University), Coimbatore, Tamil Nadu, INDIA.
[3]Department of Pharmaceutics, PSG College of Pharmacy (Affiliated with the Tamil Nadu Dr. M G R Medical University), Coimbatore, Tamil Nadu, INDIA.

ABSTRACT

Background: *Simarouba glauca* belonging to the Simaroubaceae family and rich in quassinoids was traditionally used to treat cancer. Our aim is to quantify quassinoid in its leaf's bioactive extract and perform *in vitro* screening to assess its potential against breast cancer. **Materials and Methods:** The ultrasonic assisted extraction was used to prepare chloroform, methanol and aqueous leaf extracts of *Simarouba glauca,* phytochemical and spectral characterizations were performed. The quassinoid enriched methanol fraction was subjected to an *in vitro* anti-proliferative assay against MDA-MB 231 breast adenocarcinoma cell line. **Results:** The fingerprint analysis indicated that glaucarubinone was isolated from methanol fraction 4 of *Simarouba glauca* leaf with an R_f value of 0.22, closer to the standard R_f (0.24). Liquid chromatography mass spectroscopy analysis indicated that the isolated glaucarubinone peak was observed at m/z 496.33 g/mol $[M^+H]$. *In vitro* cytotoxicity assay results showed that all three extracts were proved cytotoxic in nature, with IC_{50} values of 123.05 µg/mL, 117.81 µg/mL and 155.06 µg/mL respectively on the breast adenocarcinoma cell line. **Conclusion:** This study concluded that glaucarubinone quassinoid is present in the methanol fraction. Further, it possessed anti-cancer potential against MDA-MB 231 breast adenocarcinoma cell line.

Keywords: Novel Quassinoids, Spectral characterization, HPTLC, *in vitro* anti-proliferative assay.

Correspondence:
Mrs. Vanitha Subburaj
Department of Pharmacognosy, PSG College of Pharmacy (Affiliated with the Tamil Nadu Dr. M G R Medical University), Combaitore-641004, Tamil Nadu, INDIA.
Email: vanithas2002@gmail.com

Received: 28-04-2024;
Revised: 02-05-2024;
Accepted: 15-05-2024.

INTRODUCTION

Breast cancer is the second most prevalent type of cancer in women.[1] The compromised immune system brought in by radiation and surgery, together with its high expense, contributes to the growing cancer burden worldwide.[2] One strategy to decrease the incidence of cancer globally is to find a greater availability of innovative anticancer phytomolecules that can be combined with chemotherapy. Because evidence-based, safe and effective are thought to be useful in treating and preventing a wide range of illnesses.[3,4]

The *Simarouba glauca* leaf is a member of the Simaroubaceae family, also referred to as "Laxmitaru," "Paradise tree," or dysentery bark.[5] The leaf decoction of SG has been claimed to anticancer, antimalarial, antiviral, antibacterial and anthelmintic properties in traditional medicine.[6] Quassinoids of SG include dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone,benzoquinone, holacanthone, melianone, canthin, simaroubidin, simarolide, simarubin, simarubolide, sitosterol and tirucalla.[7] Quassinoids are triterpene lactones used in folk medicine for their pharmacological properties and seem to be well documented.[8] Anticancer studies on the crude leaf decoction of SG have been recorded but have not been proven scientifically. Furthermore, in folk practice, the aqueous leaf decoction of SG has to be prepared daily, which leads to non-compliance.

The current research has been focused on the isolation of a novel lead phytochemical, 'Quassinoid' in the extract of *Simarouba*

DOI: 10.5530/jyp.20251210

Copyright Information :
Copyright Author (s) 2025 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

glauca leaf and evaluating the anticancer potential of the isolated extract using an *in vitro* assay against the MDA-MB 231 breast adenocarcinoma cell line.

MATERIALS AND METHODS

All chemicals used for this research were of analytical grade from Pure Chemicals, Loba Chemicals and Merck; Standard Quassinoid-Glaucarubinone was from (NCI) Frederick, 21702, United States. MTT (Himedia), all cell culture reagents and disposable dishes were purchased from Thermo Scientific, Olympus and Tarson's India Limited respectively. MDA MB-231 cell lines were purchased from NCCS in Pune. FTIR, UV-Spectrophotometer (1650 PC Shimadzu, Japan), UPLC (Waters, USA), Acetonitrile and $CDCl_3$ (Sigma Aldrich), NMR spectrometer (BRUKER 400MHz), Superfit Vacuum Rotary Evaporator, HPTLC (Camag, Muttenz, Switzerland), Digital Ultra Bath Sonicator (Labman Scientific).

Collection and Authentication of *Simarouba glauca* leaves

Fresh leaves of *Simarouba glauca* were collected from the herbal garden maintained and voucher specimens were stored by PSG College of Pharmacy, PSGIMSR and Hospital Campus, Coimbatore, India. Plant were identified and authenticated (BSI/SRC/5/23/2021/Tech./3L8) by plant taxonomist Dr. M. U. Sharief, Botanical Survey of India (BSI), TNAU Campus, Coimbatore, Collected SG leaves were washed with tap water to remove the dust and adhering debris before being shade dried and pulverized into coarse powder for the extraction process.

Extract Preparation

Simarouba glauca leaf extracts (chloroform, methanol and aqueous) were produced using a Digital ultra-bath sonicator and temperature controller (Frequency 40 kHz) at 30°C with a power of 60-100W. To maximize the extraction efficiency, the defatted SG leaf marc was extracted using 250 mL of solvents employing fine ultra-wave contact duration of about 40 min. The prepared SG leaf extracts were concentrated using a rotary evaporator and calculate the percentage yield of the SG leaf extracts.[9]

Qualitative Phytochemical analysis

The acquired SG leaf extracts performed phytochemical analysis to identify the following phytocompounds such as proteins, alkaloids, glycosides, sterols, terpenoids, carbohydrates, flavonoids, saponins, tannins, volatile oils and mucilage.[10]

In vitro anti-proliferative study

The MDA MB-231 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium. The culture was carried out in a CO2 incubator at 37°C with a 5% CO_2 atmosphere and 18-20% O2. SG leaf extracts were assessed for their *in vitro* antiproliferative potentials using the MTT assay.[11] 20,000 cells per well were seeded into 96-well plates. Different doses of SG leaf extracts were added (12.5, 25, 50, 100, 200 µg/mL) and 1 µM/mL of Doxorubicin was used as standard. The plate was incubated for 24 hr at 37°C. After the incubation period, the plates were filled with 0.5 mg/mL MTT reagent. To protect the plate from light, they are covered with aluminium foil and incubated for 3 hr. Then, 100 µL DMSO solution was added to enhance solubility and read the absorbance at 570 nm. Table 1 and Figure 1 provide % cell viability data analysis along with IC_{50} value of the respective SG leaf extracts.

$$\% \text{ Cell viability} = [\text{Mean absorbance of treated cells/Mean absorbance of Untreated cells}] \text{ X } 100$$

The IC_{50} value was calculated using a linear regression equation.

$$Y=Mx+C$$

Where,

Y=50, M and C values are taken from the viability.

Chromatographic analysis

Preparative Thin Layer Chromatography

The prepared SG leaf extracts were analyzed using TLC.[12] Preparative TLC analysis was done to isolate the quassinoid. The TLC plates were made manually and activated. Then, the bioactive extract of SG leaf was dissolved in methanol and applied to the activated plates for separation of quassinoid. A chromatogram was developed using n-hexane: ethyl acetate (2:8) as a mobile phase. Then the separated chromatograms were marked with 10 distinct colour bands under daylight, at 254 nm and 366 nm using a UV lamp.[13] It was scraped off dissolved in methanol and filtered. After the eluent was evaporated, the calculated yield was assigned the following names as Fr1 (220 mg), Fr2 (140 mg), Fr3 (90 mg), Fr4 (190 mg), Fr5 (110 mg), Fr6 (14 mg), Fr7 (19 mg), Fr8 (43 mg), Fr9 (20 mg) and Fr10 (32 mg). The fractions were then stored in eppendorf tubes. Using the HPTLC fingerprint technique, the amount of quassinoid present in bioactive fractions of SG leaf was estimated and displayed in Figure 2.

High-Performance Thin Layer Chromatography

SG leaf fractions were subjected to quantification using the HPTLC method. The mobile phase used was n-hexane: ethyl acetate (2:8)[14] and standard quassinoid concentrations of 2, 3, 4, 5 and 6 µL of (Glaucarubinone). Fingerprint profiles were obtained by scanning the tracks at a wavelength of 254 nm. Table 2 and Figure 2 display 3D overlay, standard and fraction 4 chromatograms with R_f values. The amount of quassinoid in the bioactive fraction (Fr4) of SG leaf, or the QEAF (Quassinoid Enriched Active Fraction), was calculated using a calibration curve with standard quassinoid (GB).

Table 1: % Cell Viability of SG leaf extracts treated with MDA MB 231 cells after 24 hr.

Culture condition	% Cell viability Chloroform	% Cell viability Methanol	% Cell viability Aqueous
Untreated	100±0.09	100±0.03	100±0.03
Dox-1 uM	54.19±0.02	44.92±0.02	44.92±0.02
12.5 µg	97.13±0.01	91.09±0.02	93.38±0.02
25 µg	82.03±0.02	81.24±0.01	86.35±0.01
50 µg	66.23±0.03	69.88±0.01	79.89±0.01
100 µg	53.68±0.03	54.35±0.08	67.74±0.02
200 µg	41.58±0.01	23.19±0.03	36.37±0.03
IC_{50}Conc (µg/mL)	123.05	117.81	155.06

The values are mean± SD (*n*=3).

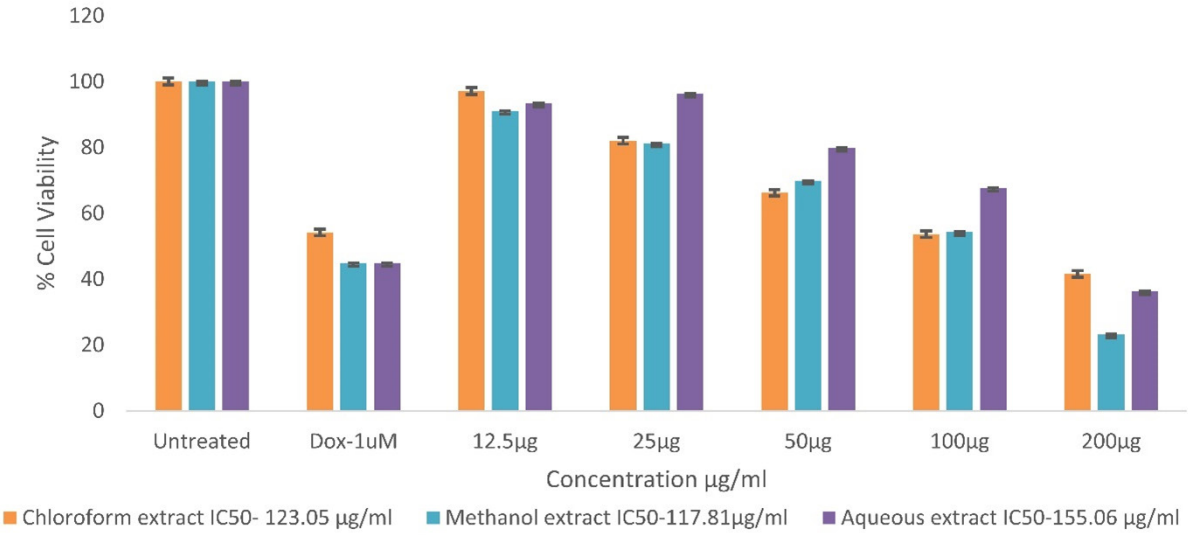

Figure 1: % Cell Viability of SG leaf extracts treated with MDA MB 231 cells after 24 hr.

The values are mean± SD (*n*=3).

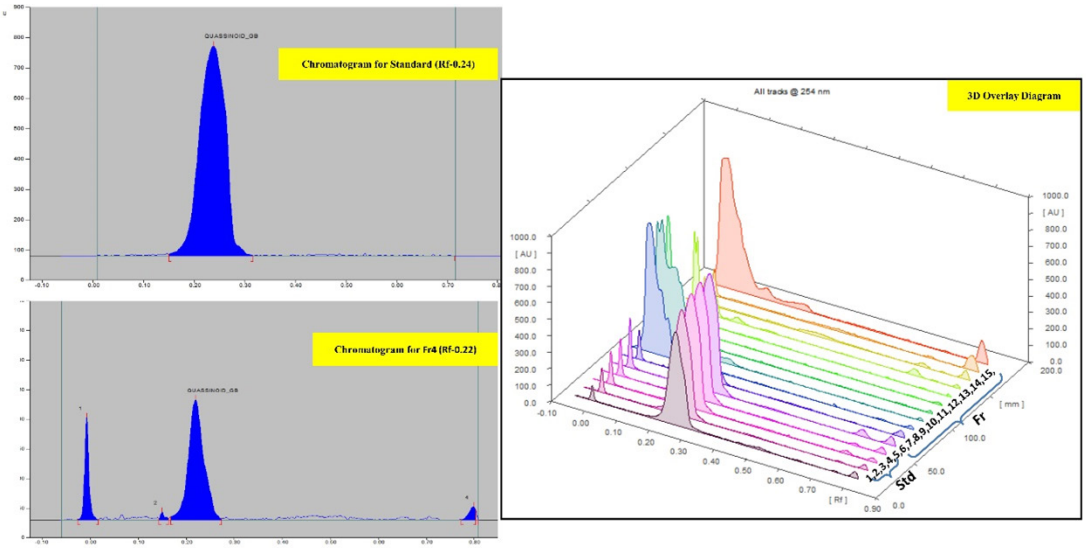

Figure 2: Quantification of Quassinoid from bioactive fractions of SG leaf extract by HPTLC.

Spectral Characterization

The isolated quassinoid in QEAF of SG leaf was measured for UV absorption spectra at 250 nm. FT-IR analysis was performed and significant functional groups were characterized as illustrated in Table 3. LC-MS phytochemical profiling of isolated quassinoids in QEAF of SG leaf was performed. The compound was identified using the BEH C18 column. The gradient elution was done with solvent A: 0.1% formic acid in water and solvent B: acetonitrile. The solvents ratio (A: B) was varied from 0-1 min (A: 98% and B: 2%), 1-6 min (A: 50% and B: 50%), 12-16 min (A: 5% and B: 95%) and 17-20 min (A: 98% and B: 2%). The sample injection volume (QEAF) was set to 5 µL, with a flow rate of 0.2 mL/min. The m/z was measured at 496 g/mol [M+H],[15,16] and shown in Figure 3 and Table 4.[13]C and [1]H NMR analysis of isolated quassinoid in QEAF of SG leaf was predicted by BRUKER 400MHz using $CDCl_3$ as solvent.[17,18] The [13]C and [1]H NMR peaks of the isolated quassinoid in the bioactive fraction of SG leaf are shown in Figure 4.

Table 2: R_f value of Standard Quassinoid and the fractions of bioactive extract of SG leaf.

Sl. No.	Particulars	Reports (R_f value)
Solvent system: n-Hexane - Ethyl acetate (2:8)		
1	R_f value of Standard Quassinoid (Glaucarubinone)	0.24
2	Fraction 1 (Fr1)	-
3	Fraction 2 (Fr2)	0.05, 0.12
4	Fraction 3 (Fr3)	0.12, 0.52
5	Fraction 4 (Fr4)	0.15, 0.22, 0.80
6	Fraction 5 (Fr5) and Fraction 6 (Fr6)	-
7	Fraction 7 (Fr7)	0.05, 0.12, 0.67
8	Fraction 8 (Fr8)	0.05, 0.34, 0.36, 0.67
9	Fraction 9 (Fr9)	0.67, 0.78
10	Fraction 10 (Fr10)	0.13, 0.25, 0.79

RESULTS

Authentication and Preparation of leaf extracts of *Simarouba glauca*

Simarouba glauca is an evergreen flowering tree belongs to the Simaroubaceae family. The current investigations used with SG leaves were collected from the PSGCP Herbal Garden, India. It was acknowledged and authenticated (No.BSI/SRC/5/23/2021/

Table 3: IR Interpretation of QEAF of *Simarouba glauca* leaf.

Wave number (cm^{-1})	Functional group	Wave number (cm^{-1})	Functional group
3371.68	N-H Stretching	1284.63	C-O Stretching
2933.83	C-H Stretching	1232.55	C-O Stretching
2730.33	C=C Stretching	1210.37	C-O Stretching
2340.7	O=C=O Stretching	1072.46	C-O Stretching
1702.24	C=O Stretching	1044.49	CO-O-CO Stretching
1612.54	C=C Stretching	818.81	C=C Stretching
1510.31	C=C Stretching	642.32	C-Br Stretching
1448.59	C=C Stretching	608.56	C-Br Stretching
1361.79	C-H Bending	551.66	C-I Stretching

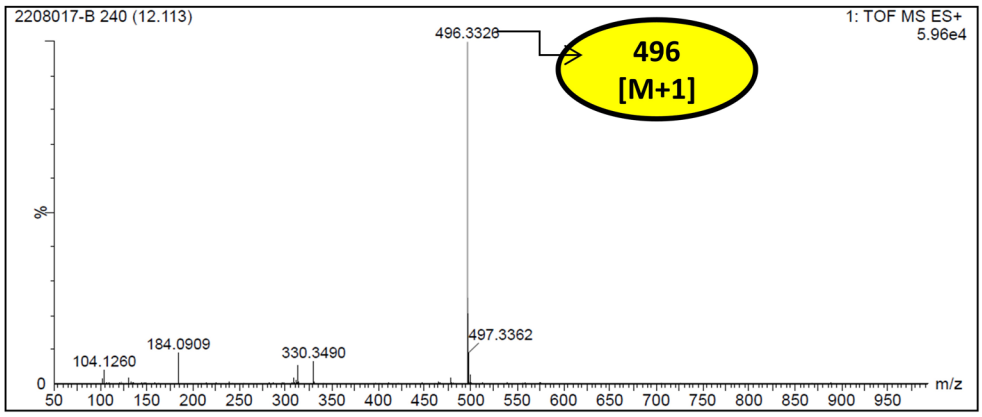

Figure 3: LC-MS analysis of Quassinoid in QEAF of Simarouba glauca leaf.

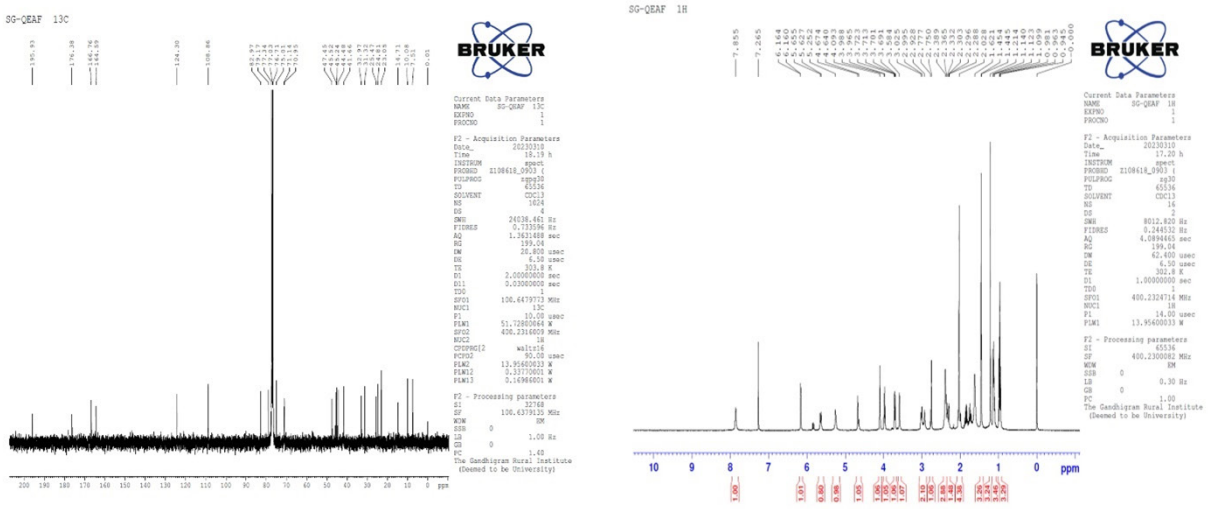

Figure 4: ^{13}C & ^{1}H NMR spectra of Isolated Quassinoid in QEAF-SG leaf (Solvent: $CDCl_3$).

Table 4: LC-MS Analysis of Isolated Quassinoid in QEAF of SG leaf.

Time	Flow	%A	%B	Retention Time	Measured mass (m/z) of isolated quassinoids in QEAF of SG leaf	Exact mass (m/z) of known quassinoid (Glaucarubinone) with Molecular formula	Other Fragmented peaks (m/z)
Initial	0.200	98.0	2.0	12.113 min	496.3326	494.5 g/mol (495) $C_{25}H_{34}O_{10}$	104.1260, 184.0909, 330.3490, 497.3362
1.00	0.200	98.0	2.0				
6.00	0.200	50.0	50.0				
12.00	0.200	5.0	95.0	12.315 min	496.3326	494.5 g/mol (495) $C_{25}H_{34}O_{10}$	313.2817, 421.2737, 465.2990
16.00	0.200	5.0	95.0				
17.00	0.200	98.0	2.0				
20.00	0.200	98.0	2.0				

Tech./328) from plant taxonomist Dr. M. U. SHARIEF, Botanical Survey of India (BSI), TNAU Campus. The percentage yields of *Simarouba glauca* leaf extracts (chloroform, methanol and aqueous) obtained from UAE (Ultrasonic assisted extraction) method. In this study highest percentage (%) w/w yield was obtained from aqueous SG leaf (23.08%) when compared to other two extracts chloroform (9.18%) and methanol (15.84%) respectively.

Qualitative Phytochemical Analysis

The prepared SG leaf extracts were subjected to phytochemical analysis and they showed the presence of flavonoids, alkaloids, glycosides, saponins, terpenoids, tannins, steroids and proteins. However, alkaloids, terpenoids and steroids were absent from the aqueous SG leaf extract. Saponins and carbohydrates were absent from the chloroform and methanol SG leaf extracts.

In vitro anti-proliferative assay

In vitro cytotoxicity assay of *Simarouba glauca* leaf extracts was done using MTT assay and the results are shown in Table 1 and Figure 1. All were effectively cytotoxic with significant IC_{50} values of 123.05 µg/mL, 117.81 µg/mL and 155.06 µg/mL on MDA-MB 231 respectively. The results suggested that methanol SG leaf extract proved to possess effective cytotoxic potentials on MDA MB 231 cells with an IC_{50} value of 117.81 ug/mL. Chloroform and aqueous SG leaf extracts were observed to have moderate cytotoxic potentials. Hence, the valuable bioactive extract was found to be methanol SG leaf extract. Hence, it was purified and isolated through the preparative TLC method.

Chromatography analysis

The quantification of quassinoid content in 10 distinct fractions of the bioactive SG leaf extract was achieved via the HPTLC fingerprint technique. The mobile phase, hexane-ethyl acetate

(2:8), was chosen based on the TLC study. Afterward, the quassinoid chemical compound found in the bioactive SG leaf extract was isolated using a preparative TLC study. The calibration curve was generated in HPTLC using different concentrations of standard quassinoid (GB) and to determine the amount of quassinoid (12.573 µg) present in the SG leaf fraction (Fr4). The results of this technique demonstrate that the separated quassinoids in fraction 4 of the bioactive extract's SG leaf had an R_f value of 0.22, which is comparable to the Rf value of 0.24 found for standard quassinoids. The standard and the bioactive fraction 4 chromatograms with Rf values and 3D overlay are shown in Table 2 and Figure 2.

Spectral Characterization

The UV absorption spectrum (λ_{max}) of isolated Quassinoid in Enriched Active Fraction 4 (QEAF) of SG leaf was seen at 250 nm. FT-IR analysis was employed to analyze the functional groups of the isolated quassinoid in QEAF. Table 3 shown the outcome of the IR functional groups interpretations of bioactive SG leaf fraction 4.

The Mass spectra of the isolated quassinoid peak were observed at m/z 496.33 g/mol [M+H], as well as other fragmented peaks (m/z) were also measured with a retention period of 12.113 min and their m/z values were annotated in positive mode and shown in Figure 3 and Table 4. This study compares the measured mass value m/z, 496 of the isolated quassinoid found in the QEAF of SG leaf to the precise mass value of a known quassinoid (Glaucarubinone) 495 g/mol.

^{13}C and ^{1}H NMR spectra of isolated quassinoid in QEAF of *Simarouba glauca* leaf was predicated by BRUKER 400MHz using $CDCl_3$ as solvent. The respective peaks associated with quassinoids (Glaucarubinone) present in the QEAF of SG leaf are recorded in Figure 4. Chemical shift (ppm) values of $_{13}C$ and $_{1}H$ NMR are as follows:^{13}C NMR (δ ppm): 7.5 (1C, s), 10.0 (1C, s), 14.7 (1C, s), 23.0 (1C, s), 24.8 (1C, s), 25.46 (1C, s), 31.3 (1C, s), 32.9 (1C, s), 41.6 (1C, s), 44.4 (1C, s), 45.2 (1C, s), 45.5 (1C, s), 47.4 (1C, s), 70.0 (1C, s), 71.1 (1C, s), 75.0 (1C, s), 76.7 (1C, s), 79.1 (1C, s), 82.9 (1C, s), 108.8 (1C, s), 124.3 (1C, s), 164.5 (1C, s), 166.7 (1C, s), 176.3 (1C, s), 195.9 (1C, s).^{1}H NMR (δ ppm): 0.96 (3H, t), 1.11 (3H, d), 1.20 (3H, s), 1.44 (3H,s), 2.39 (3H, s), 2.02 (4H, m), 2.30 (1H, m), 2.75 (1H, s), 2.97 (2H, m), 3.57 (1H, d), 3.70 (1H, q), 3.96 (1H, q), 4.09 (1H, s), 4.64 (1H, t), 5.23-5.63 (1H, d), 6.15 (1H, m), 7.87 (1H, s).

DISCUSSION

Current research has shown that a powerful phytochemical-rich fraction derived from natural sources is effective against cancer.[19] Plant-based anticancer drug discovery stands as a golden mark and gained popularity in the present scenario.[20] In addition to this new finding, other studies have shown that treating many tumours with single therapy leads to the development of drug resistance and the failure of the treatments.[21] Therefore, there is a growing need for phytomedicine made from natural materials because these phytomolecules are thought to be safer, more cost-effective, effective and supported by evidence for the prevention and treatment of a variety of illnesses.[22,23]*Simarouba glauca* is known as the "tree of the solace of cancer," as the traditional medicinal practitioners employed the leaf decoction to treat the first and second stages of cancer, while the quality of life significantly seems to improve in patients with third and fourth stages of many forms of cancer. It also reduces the negative effects of chemotherapy, minimizes appetite loss and guarantees a speedy recovery without causing adverse effects.[24]

Early cancer screening performed by the National Cancer Institute (NCI) in 1976 indicated that an alcohol extract of Simarouba root has toxic effects against leukemic cancer cells at very low concentration (less than 20 µg/mL).[25] The presence of quassinoids in the Simaroubaceae family is a class of bitter compounds with several biological properties including anticancer activity.[26] More research on crude *Simarouba glauca* leaf decoction has been done and the literature review reveals that the crude methanolic SG leaf extract possesses significant anticancer potentials.[27-30] The overall observation of this study, the initial process starts with the collection and authentication of *Simarouba glauca* (SG) leaves. Then, Chloroform, methanol and aqueous extracts of *Simarouba glauca* leaves were prepared by the eco-friendly ultrasonic extraction method. In this study, the highest percentage (%) w/w yield was obtained from aqueous SG leaf (23.08%) when compared to the other two extracts, chloroform (9.18%) and methanol (15.84%) respectively. The prepared SG leaf extracts were subjected to phytochemical analysis and they showed the presence of flavonoids, alkaloids, glycosides, saponins, terpenoids, tannins, steroids and proteins.

In vitro, an anti-proliferative assay of *Simarouba glauca* leaf extracts (chloroform, methanol and aqueous) was done using the MTT method. All three SG leaf extracts were effectively cytotoxic with significant IC_{50} values of 123.05 µg/mL, 117.81 µg/mL and 155.06 µg/mL on Human breast cancer cell lines (MDA-MB 231) respectively. The results suggested that methanol SG leaf extract proved to possess effective anti-breast cancer potentials on MDA MB 231 cells with an IC_{50} value of 117.81 ug/mL compared to other SG leaf extracts (chloroform and aqueous). Furthermore, a qualitative TLC study was carried out using different solvent systems such as n-hexane: ethyl acetate (2:8), chloroform: methanol (3:7), chloroform: methanol (5:5) and chloroform: methanol (7:3). The best solvent was chosen for further chromatography studies both preparative TLC and HPTLC. In these techniques, hexane-ethyl acetate (2:8) was used as a mobile phase to isolate and estimate the quantity of quassinoid from 10 different fractions of the bioactive SG leaf extract. The outcome of this approach proves that the amount of quassinoid in fraction 4 of the *Simarouba glauca* leaf of the bioactive extract was

determined and the R_f value of bioactive Fr4 was found to be 0.22 which is close to the 0.24 R_f value of the standard quassinoid. The UV absorption spectrum (λ_{max}) of isolated Quassinoid Enriched Active Fraction 4 (QEAF) of SG leaf was seen at 250 nm. The FT-IR spectra, ^{13}C and ^{1}H NMR spectral characterization were done for structure elucidation. In LC-MS, the spectrum of the isolated quassinoid peak was observed at m/z 496.33 g/mol [M+H]. This study compares the measured mass value m/z, 496 g/mol of the isolated quassinoid found in the QEAF of SG leaf to the precise mass value of a known quassinoid (Glaucarubinone) which is m/z 495 g/mol.

CONCLUSION

At the outset, this particular research was undertaken based on the earlier reports and the inspiration to overcome hurdles in folk practice and scientific evidence to prove the standardized systematic study has not been done till date. Hence, the extraction process needed a change over from conventional to an eco-friendly greener manner using the modern ultrasonic assisted approach of extract preparation by avoiding excess usage of solvents in a much lesser time and for increased percentage yield was followed as the prime process. The current research has proved scientifically that the novel quassinoid, glaucarubinone from *Simarouba glauca* leaf has been identified through the HPTLC fingerprint technique also to evaluate the anti-proliferative potentials of novel glaucarubinone quassinoid; present in the active fraction of *Simarouba glauca* leaf is very much complementing. Futuristic studies will be concentrated on proving the underlying mechanism so that the process may be fruitful for the management and treatment of breast cancer.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Management of PSG College of Pharmacy, Coimbatore, Tamil Nadu for providing the facilities and other research assistance towards the completion of this research work. The authors are thankful to Dr. John A. Beutler, National Cancer Institute (NCI), Chemotherapeutics Repository, Molecular Targets Laboratory, Frederick, USA, for providing Glaucarubinone. The authors are also thankful to The Tamil Nadu Dr. MGR Medical University for the support given during the PhD research process.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SG: *Simarouba glauca*; **MTT:** 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; **MDA-MB 231:** M.D. Anderson and Metastasis Breast cancer; **TLC:** Thin Layer Chromatography; **Prep TLC:** Preparative Thin Layer Chromatography; **IC_{50}:** Inhibitory concentration 50; **HPTLC:** High Performance Thin layer chromatography; **FT-IR:** Fourier transmission infrared spectra; **UV:** Ultra Violet; **LC-MS:** Liquid chromatography-mass spectrometry; **^{13}C and ^{1}H NMR:** Carbon 13 and proton Nuclear magnetic resonance; **DMEM:** Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl sulfoxide; **UPLC:** Ultra-performance liquid chromatography; **$CDCl_3$:** Deuterated chloroform; **PSGCP:** PSG College of Pharmacy; **BSI:** Botanical Survey of India; **TNAU:** Tamil Nadu Agricultural University; **R_f:** Retention factor; **NCCS:** National Centre for Cell Science; **CO_2:** Carbon dioxide 2; **Fr:** Fraction, **GB:** Glaucarubinone; **TOF MS ES+:** Positive electrospray ionization time-of-flight mass spectrometry; **QEAF:** Quassinoids Enriched Active Fraction; **UAE:** Ultrasonic Assisted Extraction; **H:** Hydrogen; **ppm:** Parts per Million; **NCI:** National Cancer Institute; **TCN:** Tricaproin.

REFERENCES

1. Sobhani N, D'Angelo A, Pittacolo M, Roviello G, Miccoli A, Corona SP, *et al.* Updates on the CDK4/6 inhibitory strategy and combinations in breast cancer. Cells. 2019; 2019:20. doi: 10.3390/cells8040321, PMID 30959874.
2. Yue GG, Wong LS, Leung HW, Gao S, Tsang JY, Lin ZX, *et al.* Evaluation of the safety profiles of estrogenic Chinese herbal medicines in breast cancer. Phytomedicine. 2019; 2019:20. doi: 10.1016/j.phymed.2018.10.003, PMID 30668331.
3. Jose A, Kannan E, Vijaya Kumar PR, Madhunapantula SV. Therapeutic potential of phytochemicals isolated from *Simarouba glauca* DC for inhibiting cancers: a review. Syst Rev Pharm. 2018; 2018:20. doi: 10.5530/srp.2019.1.12.
4. Gunasekaran T, Haile T, Nigusse T, Dhanaraju MD. Nanotechnology: an effective tool for enhancing bioavailability and bioactivity of phytomedicine. Asian Pac J Trop Biomed. 20142014 20; 4(Suppl 1). doi: 10.12980/APJTB.4.2014C980, PMID 25183064.
5. Armour RP. Investigations on *Simarouba glauca* DC. in El Salvador. Econ Bot. 1959; 1959:19. doi: 10.1007/BF02860124.
6. Dony EC, Mamatha GS, Srivastava M. Therapeutic potential of *Simarouba glauca* in treatment of Oral diseases. Res J Pharm Technol. 2023; 2023:20. doi: 10.52711/0974-360X.2023.00465.
7. Sameer S, Sudhakar M. Molecular docking and ADME analysis for Anti - HIV1 potential of quassinoids from *Simarouba glauca*. Pharm Clin Res. 2020; 2020:20. doi: 10.19080/JPCR.2020.08.555742 0048.
8. Fiaschetti G, Grotzer MA, Shalaby T, Castelletti D, Arcaro A. Quassinoids: from traditional drugs to new cancer therapeutics. Curr Med Chem. 2011; 2011:20. doi: 10.2174/092986711794839205, PMID 21143123.
9. Tatke P, Rajan M. Comparison of conventional and novel extraction techniques for the extraction of scopoletin from *Convolvulus pluricaulis*. Indian J Pharm Educ Res. 2014; 2014:20. doi: 10.5530/ijper.48.1.5.
10. Kokate CK. Practical pharmacognosy. 4th ed, Vallabh Prakashan. New Delhi; 2005. p. 10-7.
11. Karuppaiah A, Siram K, Selvaraj D, Ramasamy M, Babu D, Sankar V. Synergistic and enhanced anticancer effect of a facile surface modified non-cytotoxic silver nanoparticle conjugated with gemcitabine in metastatic breast cancer cells. Mater Today Commun. 2020; 2020:20. doi: 10.1016/j.mtcomm.2019.100884.
12. Harborne JB. Phytochemical methods. London: Capman and Hall Ltd; 1973. p. 148.
13. Sherma J, Fried B (Chapter 2). Preparative thin layer chromatography. In: Journal of chromatography library. 1987; 1987:19.
14. Kholiya F, Bhatt N, Rathod MR, Meena R, Prasad K. Fundamental studies on the feasibility of deep eutectic solvents for the selective partition of glaucarubinone present in the roots of *Simarouba glauca*. J Sep Sci. 2015; 2015:20. doi: 10.1002/jssc.201500470 [ePub]. PMID 26178388.
15. Gogoi J, Nakhuru KS, Policegoudra RS, Chattopadhyay P, Rai AK, Veer V. Isolation and characterization of bioactive components from *Mirabilis jalapa* L. radix. J Trad Complement Med. 2016; 2016:20. doi: 10.1016/j.jtcme.2014.11.028, PMID 26870679.
16. Khalaf HA, Abbas IS, Tawfeeq AA, Mahdi MF. Determination, isolation and identification of aucubin and verbascoside in the leaves of Iraqi *plantago Lancoleta* L. using different detecting methods. Int J Pharm Pharm Sci. 2018:74-80. doi: 10.22159/ijpps.2019v11i2.30566.
17. Polonsky J, Varon Z, Jacquemin H, Pettit GR. The isolation and structure of 13, 18 dehydroglaucarubinone, a new antineoplastic quassinoids from *Simarouba amara*. Experientia. 1978; 1978:19. doi: 10.1007/BF01922904, PMID 720499.
18. Waterman PG, Ampofo SA. Cytotoxic quassinoids from *Odyendyea gabonensis* stem bark: isolation and high-field NMR. Planta Med. 1984; 1984:19. doi: 10.1055/s-2007-969694, PMID 17340307.

19. Jose A, Kannan E, Madhunapantula SV. Anti-proliferative potential of phytochemical fractions isolated from *Simarouba glauca* DC Leaf. Heliyon. 2020;6(4): e03836. doi: 10.1016/j.heliyon.2020.e03836, PMID 32373740.
20. Das AP, Agarwal SM. Recent advances in the area of plant-based anti-cancer drug discovery using computational approaches. Mol Divers. 2024; 2024:20. doi: 10.1007/s11030-022-10590-7, PMID 36670282.
21. Khan SU, Fatima K, Aisha S, Malik F. Unveiling the mechanisms and challenges of cancer drug resistance. Cell Commun Signal. 2024; 2024:20. doi: 10.1186/s12964-023-01302-1, PMID 38347575.
22. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta). 2022; 2022:20. doi: 10.1007/s13237-022-00405-3, PMID 36276225.
23. Pan L, de Blanco EJ, Kinghorn AD. Plant-derived natural products as leads for Drug Discovery. In: Osbourn AE, Lanzotti V, editors. Plant-derived natural products. New York: Springer US; 2009. p. 547-67. doi: 10.1007/978-0-387-85498-4_24.
24. Shyamsundar J and J. *Simarouba glauca* DC (paradise tree) [brochure] [Internet]. Bangalore: University of Agricultural Sciences, Department of Agricultural Economics; 1970. [cited Mar 18 2024] Available from: https://ideas.repec.org/p/ags/uasbcp/43624.html.
25. Mahesh CD, Padyanna S, Manjunath JS. *In vivo* AntiCancer studies of *Simarouba glauca* aqueous extract leaves on Ehrlich ascites Carcinoma model in mice. J Ayurveda Integr Med Sci. 2021; 2021:20.
26. Vikas B, Akhil BS, Suja SR, Sujathan K. An Exploration of Phytochemicals from Simaroubaceae. Asian Pac J Cancer Prev. 2017; 2017:20. doi: 10.22034/APJCP.2017.18.7.1765, PMID 28749102.
27. Puranik SI, Ghagane SC, Nerli RB, Jalalpure SS, Hiremath MB. Evaluation of *in vitro* antioxidant and anticancer activity of *Simarouba glauca* leaf extracts on T-24 Bladder Cancer Cell Line. Pharmacogn J. 2017; 2017:20. doi: 10.5530/pj.2017.6.142.
28. Jose A, Chaitanya MV, Kannan E, Madhunapantula SV. Tricaproin isolated from *Simarouba glauca* inhibits the growth of human colorectal carcinoma cell lines by targeting class-1 histone deacetylases. Front Pharmacol. 2018; 2018:20. doi: 10.3389/fphar.2018.00127, PMID 29593526.
29. Prajapati C, Reddy N, Bhatt M. Evaluation of anticancer activity using leaf extract of *Simarouba glauca* on leukemic cancer cell lines. Int J Bot Stud. 2018; 2018:20.
30. Mathew SE, Ramavarma SK, Babu TD, Kuzhivelil BT, Raghavamenon AC. Preliminary assessment on phytochemical composition, cytotoxic and antitumor efficacy of *Simarouba glauca* DC. leaf methanolic extract. Annals Phytomed Int J. 2019;8(2). doi: 10.21276/ap.2019.8.2.15.

Cite this article: Subburaj V, Umaa K, Sankar V. Phyto-chemicals and Spectral Characterization of Novel Glaucarubinone Quassinoid from *Simarouba glauca* Leaves: *In vitro* Anti-proliferative Potential against Breast Adenocarcinoma Cell line. J Young Pharm. 2025;17(1):89-96.