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Phytochemical Analysis and Profiling of Possible Compounds from *Simarouba glauca* Leaf Extracts

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ABSTRACT

Simarouba glauca is one of the important medicinal plants used in the treatment of many diseases by traditional healers. In particular, it has medicinal properties such as anti-inflammatory, antimicrobial, antioxidant, antimalarial, skin-moisturizing and antidiabetic potential. Despite having potential medicinal value, the detailed analysis of its phytoconstituents and the systematic evaluation of its biological activities is lacking. Thus, the present study is aimed at the estimation and profiling of bioactive compounds from *S. glauca* leaves extracts using High-Performance Liquid Chromatography (HPLC) and High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS). The quantitative analysis demonstrated that ethanol was the most effective solvent, extracting significant concentrations of alkaloids (94.17 mg/g), flavonoids (82.36 mg/g), phenols (49.75 mg/g) and saponins (133.21 mg/g). In contrast, aqueous and ethyl acetate extracts exhibited lower concentrations of these phytochemical compounds. HR-LCMS analysis revealed the presence of several bioactive compounds in the ethanolic extract, including kaempferol, quercetin-3β-D glucoside and gallic acid, all known for their antioxidant and anti-inflammatory properties. The aqueous extract additionally contained catechin and chlorogenic acid, while azelaic acid was identified in the ethyl acetate extract. These findings suggest that ethanol and water extracts of *S. glauca* leaves contain a rich array of bioactive ingredients. Future research should be carried out to isolate these compounds and explore their pharmacokinetics and toxicity profiles to develop effective pharmaceutical products.

Keywords: *Simarouba glauca*, Characterization, Bioactive, Phytochemical.

Introduction

Medicinal plants have played a pivotal role in maintaining human health since ancient times, providing a foundation for many modern pharmaceuticals. The field of ecological ethnobotany delves into the intricate relationship between humans and plants, particularly focusing on the utilization of plants for medicinal purposes. Traditional medicine, deeply ingrained in various cultures, harnesses the power of nature through empirical knowledge and practices passed down through generations.[1,2] These practices often involve the careful selection and preparation of plant-based remedies, reflecting a profound understanding of the natural world and its healing, the concept of a medicinal gradient in plants reveals the diverse therapeutic potentials and varying efficacies among different species. By exploring this gradient, we bridge traditional knowledge with modern scientific research, uncovering how specific phytochemical profiles contribute to their medicinal properties.[3,4] Phytochemicals are bioactive compounds produced within plant cells through complex biosynthetic pathways.

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These compounds such as flavonoids, alkaloids, saponins, and terpenoids, exhibit a wide range of chemical structures and biological activities.[5,6] Their diverse properties contribute significantly to the health-promoting effects of plants by influencing various physiological processes. The structural diversity among phytochemicals facilitates a broad spectrum of biological activities. Such a range of biological activities is a direct consequence of the diverse functional groups and molecular frameworks present within these compounds.[7] Consequently, phytochemicals can interact with various biological targets and pathways, leading to their potential therapeutic properties in combating many diseases such as oxidative stress, inflammation, microbial infections and cancer.[8] The intricate relationship between phytochemical structure and function emphasizes the importance of detailed phytochemical profiling and characterization in the development of novel therapeutic agents.[9,10]Among important therapeutic plants, *Simarouba glauca* (*S. glauca*) which belongs to the family *Simaroubaceae*, is a significant medicinal plant that has a long history of traditional use among indigenous people known for its rich metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids.[11] These compounds exhibit a wide range of medicinal properties, encompassing anti-inflammatory, antimicrobial, antioxidant, antimalarial, skin-moisturizing, antidiabetic, and anticancer effects.[12]Despite the established medicinal uses of plants, the identification and characterization of specific bioactive compounds have often been inadequately evaluated in most of these plants. Traditional applications of these plants typically rely on crude extracts, which may contain a complex array of compounds with varying levels of activity. This imprecision in compound identification can result in inconsistent therapeutic outcomes and limits the potential for developing targeted effective treatments.[13,14] The characterization and profiling of phytochemical compounds are crucial for enhancing our understanding of medicinal plants and optimizing their application in modern medicine. Employing advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass

spectrometry (MS) allows researchers to systematically identify and quantify the bioactive constituents within plant extracts. This meticulous analysis helps to gain insights into the specific compounds responsible for therapeutic effects, thereby facilitating the development of standardized and potent formulations.[15,16] This study performed a thorough quantification and identification of bioactive phytochemical compounds in *S. glauca* leaf extracts, using ethanol, ethyl acetate and water as solvents. Modern analytical techniques, including high-performance liquid chromatography (HPLC) and high-resolution liquid chromatography-mass spectrometry (HR-LCMS), were employed to elucidate the biological compounds and their therapeutic properties the growing recognition of *S. glauca's* medicinal potential, there is a significant gap in understanding the specific phytochemicals responsible for its therapeutic effects. While traditional uses of *S. glauca* are well documented, scientific evidence supporting its bioactive compounds and their therapeutic properties remains limited. This research aims to address this gap by employing advanced analytical techniques to identify key bioactive compounds. The findings may facilitate the development of targeted therapies optimized for specific health conditions, bridging the divide between traditional herbal remedies and modern medicinal applications.

Materials and Methods

Plant material collection

The leaves of *Simarouba glauca* were collected in March 2020 from the Kuvempu University campus garden located in Jnana Sahyadri, Shimoga (13°43'60.0"N 75°37'45.1"E, India). The botanical identification of the specimen was verified as voucher specimen number 95212 by Dr. N. Sasidharan, Emeritus Scientist at the Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India. Following collection, the leaves were carefully cleaned to remove any impurities and then subjected to shade drying for seven days to preserve their phytochemical components.

Preparation of S. glauca leaf extracts

The extraction and isolation of therapeutically potent compounds from *S. glauca* leaves involve several steps. In the initial stage, Soxhlet extraction was conducted using the Soxhlet apparatus (500 mL borosilicate glass), employing 75 g of dried leaf material. To ensure the efficient extraction of a broad spectrum of phytochemicals, 500 mL of both ethyl acetate and ethanol were selected as solvents, chosen for their polarity and efficacy in isolating diverse bioactive compounds. The continuous Soxhlet extraction method was employed to efficiently isolate a majority of the compounds. The extracts were subsequently concentrated using a rotary evaporator (Rotavapor R-100) at 40 °C, resulting in yields of 6.4 and 5.4% in the ethyl acetate and ethanol extracts, respectively. Additionally, an aqueous extraction was carried out by utilizing 30 g of dried leaves and 300 mL of distilled water in a round-bottom flask. The flask was placed in a pressure cooker at 180 °C until the initial pressure release, after which the temperature was reduced to 60 °C and allowed to cool for 15 min. The cooled sample was then filtered through Whatman filter paper No. 1. The filtrated sample was subsequently centrifuged (REDMI CPR-24i Plus Refrigerated Centrifuge) at 10000 rpm for 10 min and the supernatant was transferred into a clean flask for further concentration using a lyophilizer (Mini-Lyodel - 1.5 kg), resulting in an 11.7% yield of the water extract. The concentrated crude extracts from both methods were then stored for subsequent analysis.[17]

High-performance liquid chromatography analysis

To ensure the accuracy of the results, the HPLC technique was employed for the quantitative analysis of bioactive compounds known for their therapeutic properties. The combination of advanced devices, carefully selected columns and improved operating conditions facilitated precise HPLC analysis, providing valuable insights into phytochemicals present in *S. glauca* leaf extracts across three different solvents such as ethanol, water and ethyl acetate. The analysis was made by using HPLC Waters model no. 486; Waters Corp., Milford, MA, USA) to achieve accurate and reproducible results. For separating plant constituents and ensuring the best accuracy, the C-18 RP-HPLC column (4.6mm × 250mm) was employed with a mobile phase consisting of methanol and water (7:3 ratio), while maintaining the flow rate of 1 mL per min.

For the quantitative analysis, standard solutions of alkaloids, phenols, flavonoids, saponins, and tannins were prepared at a concentration of 0.4 mg/mL of each separately. Simultaneously, *S. glauca* leaf extracts obtained from water, ethanol, and ethyl acetate solvents were prepared at a concentration of 5 mg/mL. Both the standard solution and leaf extract were homogeneously suspended in the mobile phase, and 20 µL of this carefully calibrated mixture was injected into the HPLC column, the elution process was closely monitored at specific wavelengths as shown in Table 1.

Table 1: HPLC analysis of *S. glauca* leaf extracts

Metabolite	Standard	Wavelength (nm)	Ethanol Extract (mg/g)	Water Extract (mg/g)	Ethyl Acetate Extract (mg/g)
Alkaloids	Caffeine	230	94.17	57.87	40.66
Flavonoids	Rutin (Rutoside)	272	82.36	50.39	33.26
Phenols	Gallic acid	254	49.75	34.38	20.12
Saponins	Saponin	203	133.21	75.06	43.11
Tannins	Tannic acid	270	18.42	23.94	7.41

High-Resolution Liquid Chromatography and Mass Spectroscopy analysis

The crude leaf extracts of *Simarouba glauca* were characterized using the High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) technique, a contemporary analytical method employed for comprehensive investigation of the chemical composition of extracts. The phytochemical profiling was performed utilizing a Thermo Scientific Xcalibur HR-LCMS system (Version 4.2.28.14, Thermo Fisher Scientific, USA). The analytical instrument consists of two electrodes: an outer electrode and an inner electrode. The electrodes assume the functions of an analyzer and detector, respectively. Both positive and negative ionization modes were used for direct infusion, covering a mass range (*m/z*) of 50 to 8000 amu. The column material employed was Hypersil Gold 3 µm 100 × 2.1 MM (Thermo Scientific), along with the mobile phase consisting of acetonitrile and water (5:95 ratio). The employed methodology, encompassing the separation and identification of chemical constituents, highlights the complexity of the analytical process. This involves meticulous considerations, such as retention time, metabolite categorization in the database, variations within the software library, estimation of *m/z* values, and positive and negative ionization modalities of MS data.[18]

Identification of compounds
Identification of bioactive compounds obtained by HR-LCMS analysis was performed by matching unidentified compounds with the spectrum of known compounds utilized by the SAIF-IIT Bombay database, which contains more than 62000 spectrum patterns. The name, molecular weight, and structure of the components of the trial materials were also determined.[19] The characteristics of identified compounds were determined using websites that are accessible for searching chemical databases, such as Pub-Chem and Chem-spider, etc.

Statistical analysis
Phytochemical compounds were quantified using an HPLC profile, and the concentrations were calculated from peak areas, using standard data and sample parameters. Statistical analysis was performed in Microsoft Excel (Microsoft Office Professional Plus 2019). HR-LCMS data was analysed using the mzCloud software library, comparing spectra to a large database and calculating similarity scores. This enabled precise compound identification, validated by additional databases.

Results and Discussion

Natural products used for medicinal purposes have an essential role in improving health, more particularly when taking into consideration the adverse effects posed by synthetic medications. Thus, exploring nature and its products for safer therapeutic alternatives is becoming an increasingly important research area.[20,21] This study was aimed at contributing to the area of exploring *S. glauca* leaf extracts for medicinal advantages by quantification and characterization of its phytochemical gradient to identify their potential biological compounds. The three commonly used solvents were taken to perform the HPLC quantitative analysis and HR-LCMS characterization of *S. glauca* leaf extracts based on the polarity of the solvents from low to high. The ethyl acetate solvent used is nonpolar, while ethanol and distilled water are polar solvents.[22]

Quantitative phytochemical constituents
The quantitative analysis of bioactive compounds in *S. glauca* leaf extracts across the three solvents revealed significant variations in the concentration of alkaloids, flavonoids, phenols, saponins, and tannins, reflective to the solvent polarity and their efficiency in extracting specific phytochemicals. Alkaloids were most effectively extracted from *S. glauca* leaf using ethanol, achieving a concentration of 94.17 mg/g. This suggests that ethanol's intermediate polarity is well-suited for solubilizing these polar nitrogenous compounds which are known for their pharmacological activities such as analgesic, antimalarial, and anticancer effects.[23] In contrast, distilled water, with its high polarity, extracted a moderate concentration of alkaloids (57.87 mg/g), while ethyl acetate, being less polar, demonstrated the lowest extraction efficiency (40.66 mg/g). Flavonoids were also predominantly concentrated in the ethanol extract (82.36 mg/g), indicating its effectiveness in solubilizing these polyphenolic compounds. Water extracted a moderate concentration of flavonoids (50.39 mg/g), probably due to its ability to extract polar flavonoid glycosides. Ethyl acetate, however, showed the lowest flavonoid concentration (33.26 mg/g), reflecting its limited solubility for these compounds which are renowned for their antioxidant properties. [24] Similar trends were observed for phenols and saponins. Ethanol extracted the highest concentrations of both phenols (49.75 mg/g) and saponins (133.21 mg/g), demonstrating its versatility in solubilizing both polar and nonpolar compounds known for their antioxidant, antimicrobial and anticancer properties.[25,26] Water was also effective in extracting saponins (75.06 mg/g) but less so for phenols (34.38 mg/g). Ethyl acetate, due to its lower polarity, exhibited the lowest extraction efficiencies for both phenols (20.12 mg/g) and saponins (43.11 mg/g). Tannins, a class of polyphenolic compounds renowned for their antimicrobial properties,[27] were shown to be extracted most efficiently by water (23.94 mg/g), despite its high polarity. The ethanol extraction yielded a moderate tannin content of 18.42 mg/g, while the ethyl acetate extract revealed the lowest concentration of 7.41 mg/g. (Table 1). The quantitative results indicate that ethanol is the most effective solvent for extracting a wide range of phytochemicals from *S. glauca* leaves, particularly alkaloids, flavonoids, phenols, and saponins. This suggests that ethanol extracts may offer the most comprehensive therapeutic potential due to the broad spectrum of bioactive compounds they contain. In previous research contributions, phytochemical screening of this plant leaf extracts using chloroform, ethyl acetate, methanol, ethanol, aqueous, and hydro alcohol solvents indicated that the polar solvents, ethanol and methanol extracts contained the majority of compounds as reported by Puranik *et al*.[28]

Phytochemical compounds identified by HR-LCMS
The HR-LCMS analysis of *S glauca* leaf extracts, as illustrated in Figures 1-3, reveals characteristic peaks that correspond to a wide range of compounds identified across the three types of extracts employed in this study. These figures represent the chromatographic profiles, while Tables 2-4 provide a detailed list of major compounds identified through both positive and negative ionization modes, molecular formula, mass-to-charge ratios (*m/z*), retention times which span from 1 to 30 min and their reported therapeutic activities. Ethanol extract (EE) exhibited a high concentration of flavonoids and phenolic acids, including kaempferol, quercetin-3*β*-D glucoside, juglanin, caffeic acid, quercetin, and gallic acid. These compounds, known for their antioxidant and anti-inflammatory effects, suggest the potential of *S. glauca* for managing oxidative stress-related conditions.

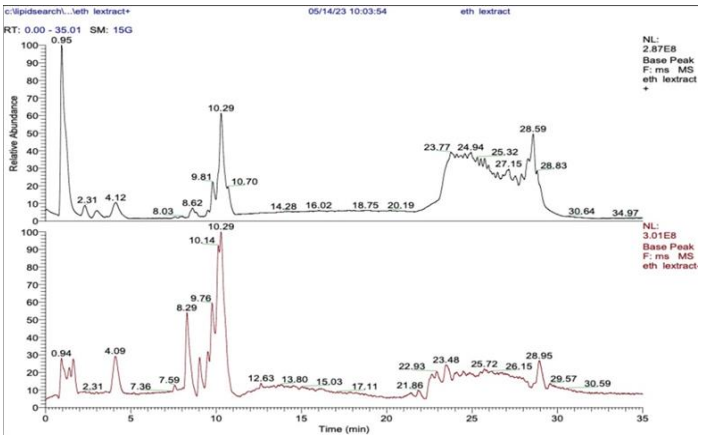

Figure 1: HR-LCMS chromatogram of positive and negative electrospray ionization (ESI) ethanolic leaf extract

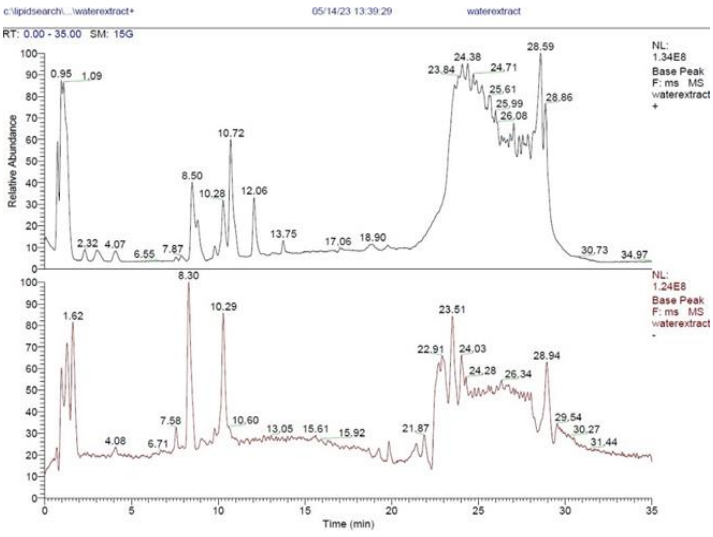

Figure 2: HR-LCMS chromatogram with positive and negative electrospray ionization (ESI) of aqueous leaf extract

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Table 2: Major identified bioactive compounds from ethanolic extract (EE) using the HR-LCMS technique

Compound	**Formula**	**Mass**	**Best March**	**Class**	**ESI**	**RT/min**	**Medicinal activity**	**References**
Kaempferol	$C_{15}H_{10}O_6$	286.0477	100	Flavonoid	+	10.51	Antioxidant, anti-inflammatory and antimicrobial	41
Quercetin-3β-D glucoside	$C_{21}H_{20}O_{12}$	464.0955	99.9	Flavonoid	+	9.79	Antioxidant, anti-inflammatory and anti-diabetic	30
Juglanin	$C_{20}H_{18}O_{10}$	418.0900	99.9	Flavonoid	+	10.29	Antioxidant, anti-inflammatory and antibacterial	31
Caffeic acid	$C_9H_8O_4$	180.0423	98.8	Hydroxycinnamic acids	+	4.13	Antioxidant, anti-inflammatory and anticancer	40
Quercetin	$C_{15}H_{10}O_7$	302.0427	98.7	Flavonoid	+	9.79	Antioxidant, anti-inflammatory and anticancer	42
Caffeic acid	$C_9H_8O_4$	180.0423	98.7	Hydroxycinnamic acids	-	7.47	Antioxidant and anti-inflammatory	40
Gallic acid	$C_7H_6O_5$	170.0215	98.7	Polyphenols	-	2.14	Antioxidant, anti-inflammatory, and anticancer	33
Trifolin	$C_{21}H_{20}O_{11}$	448.1006	98.4	Flavonoid	+	12.35	Antioxidant and anti-inflammatory	49
Quercetin-3β-D glucoside	$C_{21}H_{20}O_{12}$	464.0955	98.2	Flavonoid	-	10.47	Antioxidant, anti-inflammatory and anti-diabetic	30
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	98.2	Phenolic acids	+	5.64	Antioxidant, anti-inflammatory and antibacterial	34
Phloroglucinol	$C_6H_6O_3$	126.0317	98.0	Polyphenols	+	2.06	Antispasmodic, anti-inflammatory and antioxidants	43
Luteolin	$C_{15}H_{10}O_6$	286.0477	97.4	Flavonoid	-	10.40	Antioxidant, anti-inflammatory and analgesic	44
Trigonelline	$C_7H_7NO_2$	137.0477	95.9	Alkaloid	+	1.07	Antioxidant, anticancer neuroprotective, antibacterial, and antiviral	39
Scopoletin	$C_{10}H_8O_4$	192.0423	95.6	Coumarins	+	9.38	Antimicrobial, antioxidant and anti-inflammatory	45
Taxifolin	$C_{15}H_{12}O_7$	304.0583	94.8	Flavonoid	+	10.34	Antioxidant and anti-inflammatory	46
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	94.5	Phenolic acids	-	7.33	Antioxidant, anti-inflammatory and antibacterial	34
Trifolin	$C_{21}H_{20}O_{11}$	448.006	89.4	Flavonoid	-	10.62	Antioxidant and anti-inflammatory	49
Syringic acid	$C_9H_{10}O_5$	198.0528	83.2	Phenolic acids	-	8.67	Antioxidant and anti-inflammatory	47
Cynaroside	$C_{21}H_{20}O_{11}$	448.1006	76.5	Flavonoid glycoside	-	10.13	Antioxidant, anti-inflammatory and anticancer	48
Phloroglucinol	$C_6H_6O_3$	126.0317	76.2	Polyphenols	-	1.63	Anti-spasmolytic agent and muscles relax	43

"+" and "-" indicate positive and negative Electrospray Ionization (ESI) modes, respectively. RT = Retention Time.

For instance, kaempferol's well-documented antioxidant and anti-inflammatory properties align with the plant's potential therapeutic benefits. [29,30] Aqueous extracts (AQE) also contained significant phytochemicals such as kaempferol, quercetin, and juglanin, demonstrating consistent bioactive molecule presence across solvents. In addition, AQE was shown to contain catechin, a flavonoid with significant antioxidant and anticancer capabilities, [31,32] as well as gallic and chlorogenic acids, which are renowned for their antioxidant, anti-inflammatory, and antibacterial properties.[33,34] The ethyl acetate extract (EAE) shared several compounds with EE and AQE, such as kaempferol, juglanin, and quercetin-3β-D glucoside, emphasizing the stability of these phytochemicals across different extraction methods. Additionally, azelaic acid, a dicarboxylic acid known for its antibacterial, anti-inflammatory, and skin-care benefits, was predominantly found in EAE. This compound's presence suggests potential dermatological applications of the extract, particularly in treating conditions like acne and rosacea.[35]

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Table 3: Major identified bioactive compounds from aqueous (AQE) leaf using the HR-LCMS technique

Compound	Formula	Mass	Best Match	Class	ESI	RT/ min	Medicinal activity	References
Kaempferol	$C_{15}H_{10}O_6$	286.0477	100	Flavonoid	+	10.50	Antioxidant and anti-inflammatory	41
Quercetin	$C_{15}H_{10}O_7$	302.0427	99.9	Flavonoid	+	9.79	Antioxidant, anti-inflammatory, and anticancer	42
Juglanin	$C_{20}H_{18}O_{10}$	418.0900	99.9	Flavonoid	+	10.48	Antioxidant, anti-inflammatory and antibacterial	31
Quercetin-3β-D glucoside	$C_{21}H_{20}O_{12}$	464.0955	99.8	Flavonoid	+	9.80	Antioxidant, anti-inflammatory and anti-diabetic	30
Trifolin	$C_{21}H_{20}O_{11}$	448.1006	99.8	Flavonoid	+	10.98	Antioxidant, anti-inflammatory and antibacterial	49
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	99.6	Phenolic acids	-	7.56	Antioxidant, anti-inflammatory and antibacterial	34
Gallic acid	$C_7H_6O_5$	170.0215	98.6	Phenolic acids	-	1.61	Antioxidant, anti-inflammatory, and anticancer	33
Quercetin-3β-D glucoside	$C_{21}H_{20}O_{12}$	464.0955	98.3	Flavonoid	-	9.80	Antioxidant, anti-inflammatory and anti-diabetic	30
Azelaic acid	$C_9H_{16}O_4$	188.1049	98.1	Dicarboxylic acids	-	10.32	Antibacterial, anti-inflammatory and skin care	35
Catechin	$C_{15}H_{14}O_6$	290.0790	97.9	Flavonoid	-	8.35	Antioxidant and anti-cancer	32
Robinetin	$C_{15}H_{10}O_7$	302.0427	97.8	Flavonoid	+	9.79	Antioxidant and anti-inflammatory	50
Caffeic acid	$C_9H_8O_4$	180.0423	97.2	Hydroxycinnamic acids	-	7.54	Antioxidant, anti-inflammatory and anticancer	40
Caffeic acid	$C_9H_8O_4$	180.0423	96.2	Hydroxycinnamic acids	+	4.07	Antioxidant and anti-inflammatory	40
Trigonelline	$C_7H_7NO_2$	137.0477	95.8	Alkaloid	+	1.08	Anti-inflammatory, antioxidative, antibacterial and antifungal	39
Psoralidin	$C_{20}H_{16}O_5$	336.0998	91.2	Flavonoid	+	0.90	Antioxidant, anti-inflammatory and Anticancer	51

"+" and "-" indicate positive and negative Electrospray Ionization (ESI) modes, respectively. RT = Retention Time.

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Table 4: Major identified bioactive compounds from ethyl acetate (EAE) using the HR-LCMS technique

Compound	Formula	Mass	Best Match	Class of	ESI	RT min	Medicinal activity	References
Kaempferol	$C_{15}H_{10}O_6$	286.0477	100	Flavonoid	+	10.48	Antioxidant and anti-inflammatory	41
Juglanin	$C_{20}H_{18}O_{10}$	418.0900	99.9	Flavonoid	+	10.49	Antioxidant, anti-inflammatory and antibacterial	31
Azelaic acid	$C_9H_{16}O_4$	188.1049	98.9	Dicarboxylic acids	-	10.32	Antibacterial, anti-inflammatory and skin care	35
Quercetin-3β-D glucoside	$C_{21}H_{20}O_{12}$	464.0955	98.3	Flavonoid	-	9.80	Antioxidant, anti-inflammatory and anti-diabetic	30
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	98.3	Hydroxycinnamic acids	-	4.08	Antioxidant, anti-inflammatory and antibacterial	39
Caffeic acid	$C_9H_8O_4$	180.0423	98.1	Hydroxycinnamic acids	-	10.43	Antioxidant and anti-inflammatory	40
Robinetin	$C_{15}H_{10}O_7$	302.0427	97.8	Flavonoid	+	10.10	Antioxidant and anti-inflammatory	50
Reynoutrin	$C_{20}H_{18}O_{11}$	434.0849	97.8	Flavonoid	+	10.11	Antioxidant and anti-inflammatory	37
Morin	$C_{15}H_{10}O_7$	302.0427	97.5	Flavonoid	+	9.80	Antioxidant and anti-inflammatory	36
Trigonelline	$C_7H_7NO_2$	137.0477	96.7	Alkaloid	+	1.08	Anti-inflammatory, antioxidative, antibacterial and antifungal	42

"+" and "-" indicate positive and negative Electrospray Ionization (ESI) modes, respectively. RT = Retention Time

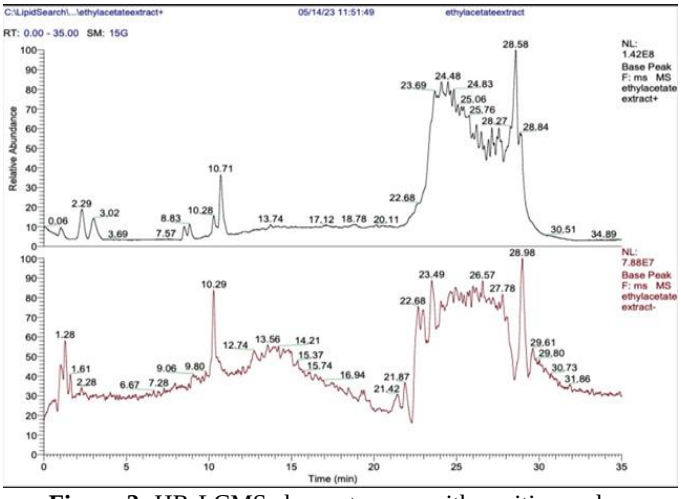

Figure 3: HR-LCMS chromatogram with positive and negative electrospray ionization (ESI) of ethyl acetate leaf extract

The identification of morin and reynoutrin, both flavonoids with documented antioxidant and anti-inflammatory activities.[36,37] The consistent presence of these bioactive compounds across various extracts indicates that *S. glauca* leaves are rich in phytochemicals with a wide range of medicinal activities. The predominance of flavonoid phytochemicals, such as kaempferol, quercetin, and juglanin, across all three extracts emphasizes their significant antioxidant and anti-inflammatory potential. This suggests that these extracts may be valuable in mitigating oxidative stress and inflammatory responses, aligning with the well-documented bioactivities of flavonoids in various therapeutic contexts.[38] Moreover, the presence of multiple bioactive compounds in both positive and negative ionization modes suggests the broad-spectrum therapeutic potential of *S. glauca* leaf extracts. The diversity of identified compounds including phenolic acids (caffeic acid, chlorogenic acid), polyphenols (gallic acid, phloroglucinol), and alkaloids (trigonelline), further emphasizes the extract's multi-targeted approach in exerting medicinal effects. For example, trigonelline, identified in all extracts, is known for its neuroprotective and antimicrobial activities.[39] Caffeic acid, a phenolic compound abundantly found in coffee as well as various fruits and vegetables is renowned for its antioxidant and anti-inflammatory antiproliferative properties.[40]

Conclusion

The data provide strong evidence that *Simarouba glauca* leaf extracts are a rich source of bioactive compounds with significant therapeutic potential. HPLC and HR-LCMS analyses identified a variety of phytochemicals, with ethanol proving particularly effective in extracting alkaloids, flavonoids, phenols, saponins, and tannins. The variation in phytochemical content across solvents highlights the importance of solvent polarity in optimizing extraction. Compounds with known antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activities were confirmed, validating the traditional medicinal uses of *S. glauca*. Future studies should focus on isolating and evaluating these compounds for therapeutic efficacy.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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