Original Article

Comparative phytochemical analysis of *Senna alata* (L.) Roxb. and *Gliricidia sepium* (Jacq.) Walp. leaf extracts

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Abstract

Phytochemicals remain a cornerstone of drug development due to their natural origin, cost-effectiveness, and lower toxicity. *Senna alata (S. alata)* and *Gliricidia sepium (G. sepium)*, both belonging to the family Fabaceae, are recognized for their diverse bioactivities, including pharmacological and insecticidal properties. However, the limited and seasonal availability of medicinal plants like *S. alata*, a perennial shrub with an uneven distribution in Kerala, poses challenges for large-scale extraction of compounds with therapeutic and insecticidal value. In contrast, *G. sepium* is a fast-growing, widely distributed tree that can be a promising source of bioactive compounds, owing to its abundant biomass and year-round availability. The present study compares the phytochemical profiles of *S. alata* and *G. sepium* to evaluate their potential as sources of valuable bioactive compounds. In this study, sequential solvent extraction using hexane, chloroform, and ethanol was performed on dried leaves, followed by both qualitative and quantitative analyses through biochemical assays and thin-layer chromatography (TLC). The findings of the present study demonstrate substantial overlap in the qualitative and quantitative profiles of major bioactive compounds. This study further signifies the need for exploration of underutilized plant species to overcome supply constraints in phytopharmaceuticals and for facilitating drug discovery and the development of related products. Further research involving advanced analytical techniques and bioactivity assays is recommended to confirm functional equivalence.

Keywords: Bioactivity, biopesticide, *Gliricidia sepium,* medicinal plant, phytochemicals, *Senna alata*, thin layer chromatography.

Introduction

Traditional as well as modern medicine often rely on the phytochemical compounds from the medicinal plant that show various bioactivities, offering a wide range of benefits. Phytochemicals have long been the choice of drug development as they are easily available, natural, and have less nontarget action and toxicity. Low cost and easy availability make these plant-based products attractive. *Senna alata (S. alata)* and *Gliricidia sepium (G. sepium)* are two economically important plants known for their pharmacological properties, like antimicrobial, anti-inflammatory, and antioxidant activities[1,2] and antifeedant properties.[3] The phytochemicals, such as alkaloids, flavonoids, tannins, and phenolic compounds found in these plants have been shown to exhibit significant bioactivity and are often targeted in natural product research.[4] *G. sepium* is a fast-growing, medium-sized tree available throughout the season and has a wide distribution, whereas *S. alata* is a perennial shrub typically found along riversides, near water bodies, and in cultivated areas. Both plants belong to the family Fabaceae. This study aims to evaluate and compare the

phytochemical compositions of *G. sepium* and *S. alata* to assess the degree of similarity that may justify their analogous uses, an approach commonly employed in medicinal plant research. Compounds with potential bioactivity have been serially extracted from the dried leaves of *S. alata* and *G. sepium* using hexane, chloroform, and ethanol as solvents. Qualitative and quantitative analyses were subsequently conducted using biochemical assays, thin layer chromatography (TLC), and spectrophotometric techniques.[5,6] This is a comprehensive and comparative account of the phytochemical profiles of *S. alata* and *G. sepium*.

Materials and Methods

The leaves of *S. alata* (Figure 1) and *G. sepium* (Figure 2), collected from Thiruvananthapuram and surrounding areas, were shade-dried and subjected to serial hot extraction using hexane, chloroform, and ethanol. The extracts were dried in a vacuum concentrator.

Figure 1: *Senna alata*

Figure 2: *Gliricidia sepium*

Qualitative phytochemical analysis

Various standard tests were employed for the qualitative phytochemical analysis of the extract by mixing it with an equal volume of the appropriate reagent and observing the resulting colour change indicative of specific phytochemicals. Alkaloids were detected using Hager's[7] and Wagner's reagents;[8] flavonoids with ferric chloride test[9] and alkaline reagents;[10] and glycosides using Baljit's and foam tests.[11] Steroids were identified by Hesse's response and Liebermann-Burchard's test.[12] Tannins and phenols were detected with Braymer's[9] and Ferric chloride tests[13] respectively; proteins with Biuret and Ninhydrin tests;[8,13] lipids with spot and filter paper tests[13,14] and carbohydrates with Fehling's and Benedict tests.[9,13]

Thin layer chromatography profiling and staining

Thin layer chromatography was used to separate and visualize phytochemicals in each of the fractions, with silica gel as the stationary phase and a solvent system of petroleum ether, cyclohexane, ethyl acetate, acetone, and methanol in the ratio 6: 1.6: 1: 1: 0.4 as the mobile phase. The bands separated were observed under ultraviolet as well as visible light, and Rf values were recorded.

Quantitative phytochemical analysis

The phenol and tannins were quantitatively measured using Folin-Ciocalteu method at 725nm,700nm, respectively, with gallic acid as standard.[14] Flavonoid content was estimated by the aluminium chloride colorimetric assay at 415nm, using quercetin as the standard.[15] Protein content was determined using Bradford assay at 595nm, with bovine serum albumin as the standard.[16] For all assays, stock solutions of hexane, chloroform and ethanolic leaf extracts were prepared in methanol (1mg/mL stock concentration), serially diluted, and analysed in Varioscan. Appropriate controls and enzyme blanks were used, and absorbance were compared against calibration curves of

respective standards to determine the exact concentrations phytochemicals in the samples.

Yield of crude extracts

For calculating the yield of extracts, hexane, chloroform, and ethanol extracts of *S. alata* and *G. sepium* were transferred into petri dishes and allowed to air dry. Upon complete solvent evaporation, the remaining residues were weighed, and the percentage yield of each extract was determined. For calculating the yield of TLC fractions, the bands from developed TLC plates were scraped off, extracted using ethyl acetate, and separated from silica by centrifugation (at 10000 rpm for 10 mins). The yield of each fraction was calculated by evaporating the solvent in a vacuum concentrator and subsequently weighing the residue.

Results and Discussion

Qualitative analysis

The results of qualitative phytochemical analysis of *S. alata* and *G. sepium* leaf extracts are summarised in Table 1. Analysis of leaf extracts of *S. alata* revealed that the hexane extract was rich in glycosides, tannins, phenols, carbohydrates, and fats. The chloroform extract consisted of higher levels of alkaloids, flavonoids, glycosides, phenols, tannins, carbohydrates, fats, and oils. This observation agrees with the study by Akinmoladun *et al.,* which also reported chloroform as an effective solvent for extracting alkaloids and flavonoids from medicinal plants.[17] In the case of *G. sepium*, the hexane extract contained alkaloids, flavonoids, steroids, glycosides, phenols, carbohydrates, proteins, fats, and oils. The chloroform extract was rich in alkaloids, flavonoids, glycosides, tannins, phenols, carbohydrates, and fats, as reported in an earlier study on *G. sepium* leaf extracts.[18] The ethanolic extracts of *G. sepium* leaf, contained alkaloids, flavonoids, glycosides, steroids, carbohydrates, and proteins, which agreed with Karthika *et al.,* who reported the presence of bioactive constituents with antioxidant and anti-inflammatory properties in ethanol extracts of *G. sepium*.[1] Extraction of phytochemicals from the plant material mainly depends on the type of solvents used.[19] In the present study, the ethanolic extract was found to contain alkaloids, flavonoids, glycosides, tannins, carbohydrates, and proteins, indicating that ethanol is effective in the extraction of both polar and moderately polar compounds, consistent with the findings of Harborne.[5]

Thin layer chromatography profiling and visualization

Thin layer chromatography analysis of *S. alata* and *G. sepium* leaf extracts in different solvents revealed distinct differences in band patterns under UV-visible light. In *S. alata*, the hexane extract showed a maximum of nine bands, with Rf values ranging from 0.98 to 0.31 (Figure 3).

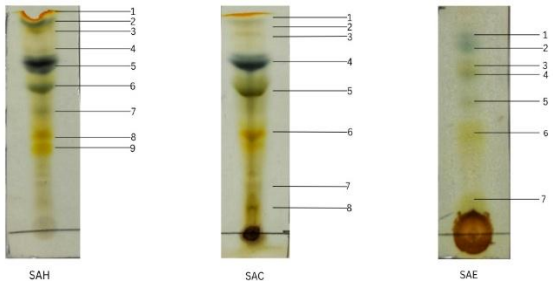

Figure 3: Thin layer chromatography profiles of extracts of *Senna alata* with hexane (SAH), chloroform (SAC) and ethanol (SAE).

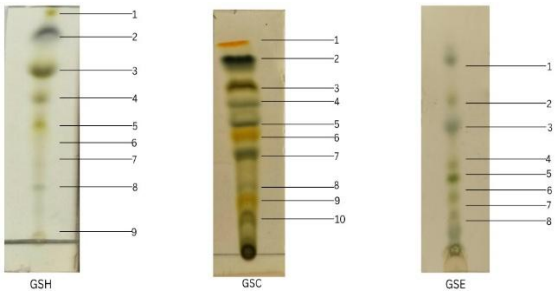

Figure 4: Thin layer chromatography profiles of extracts of *Gliricidia sepium* with hexane (GSH), chloroform (GSC) and ethanol (GSE).

The highest Rf value recorded was 0.98 in the hexane extract, while the lowest was 0.10 in the chloroform and ethanol extracts. In *G. sepium*, the hexane extract revealed nine bands, and chloroform extracts had 10 bands (Figure 4). The highest Rf value of 0.98, and the lowest Rf

Table 1: Qualitative analysis of phytochemicals identified in the thin layer chromatography fractions of *Senna alata* and *Gliricidia sepium* leaves.

Phyto-chemicals	Tests	*Senna alata*			*Gliricidia sepium*		
		Hexane extract	Chloroform extract	Ethanol extract	Hexane extract	Chloroform extract	Ethanol extract
Alkaloids	Hager's Test	+	+	++	+	+	++
	Wagner's Test	+	++	++	++	++	++
Flavonoids	$FeCl_3$ Test	+	++	++	+	++	++
	Alkaline Reagent Test	+	+	++	+	++	+
Glycosides	Baljet's Test	+	+	++	+	+	++
	Foam Test	++	++	++	++	++	+
Steroids	Salkowski Test	+	++	+	++	+	++
	Hesse's Response	+	++	+	++	+	++
Tannins	10% NaOH Test	++	+	+	+	++	+
	Braymer's Test	+	++	++	+	++	++
Phenols	Iodine Test	++	++	++	+	++	+
	$FeCl_3$ Test	+	++	++	++	+	+
Carbo-hydrates	Fehling's Test	++	++	++	++	++	++
	Benedict's Test	+	+	++	++	+	++
Proteins	Biuret's Test	+	+	++	+	+	++
	Ninhydrin Test	+	++	++	++	+	++
Fats and Oil	Spot Test	++	++	+	++	++	+
	Filter paper Test	++	++	+	++	++	+

+ indicates the presence of the compound; ++ indicates a relatively higher level. $FeCl_3$ = Ferric chloride; NaOH=Sodium hydroxide

value of 0.08 were obtained with the ethanol extract.

The TLC profiles of *S. alata* and *G. sepium* observed in this study may be compared to the patterns in previous studies. Alvarez *et al.,* reported three bands with Rf values of 0.123, 0.708, and 0.831 in methanol extract of *G. sepium* leaf extract, suggesting the presence of flavonoid, phenolic, and alkaloid compounds.[20] Likewise, Lahare *et al.,* performed TLC on methanol, chloroform, and aqueous extracts of *S. alata* and observed up to six bands in the methanol extract, comprising flavonoids, tannins, alkaloids, and phenols, the Rf values of which corresponded to those obtained in the current study.[21] These reports support our findings of multiple bands in both plant extracts. It is noteworthy that the chloroform extracts of both plants exhibit identical Rf values viz., 0.98, 0.74, and 0.63 for alkaloids, phenols, and tannins, respectively. This observation is further supported by staining with appropriate reagents. The similarity in Rf values across species suggests the potential presence of identical or structurally analogous bioactive compounds. Such cross-species similarity in phytochemical profiles has also been reported in comparative studies of certain medicinal plants.[22,23] The present study indicates that *S. alata* shares similarities in its phytochemical profile with *G. sepium* leaves, as revealed by TLC analysis. Given that the availability of *S. alata* is limited to certain seasons, while *G. sepium* leaves are available throughout the year, the latter could serve as a viable source for extracting several of the beneficial bioactive components found in *S. alata*. This suggestion agrees with the ethnobotanical practices that promote the use of alternative plant species during seasonal unavailability and to conserve overexploited medicinal plants.[24-27] However, such a

proposition necessitates further studies elucidating the structural similarities, biological activities, and pharmacological properties of the compounds in question.[28,29] Careful evaluation and optimization of extraction techniques are also critical in this context.

Quantitative analysis

Quantitative analysis revealed that phenols were the most abundant compounds present in both plants. The phenol content in *S. alata* ethanol extract was estimated to be 0.35 ± 0.010 mg/mL and in *G. sepium* chloroform extract this was 0.18 ± 0.003 mg/mL (Figure 5). Flavonoid content was relatively lower, revealing a maximum at 0.04 ± 0.004 mg/mL in *S. alata* ethanol extract (Figure 6). It was same for the chloroform extract of *G. sepium.* Protein levels were apparent, particularly in *S. alata*, which measured up to 0.30 ± 0.003 mg/mL in ethanol extract, while *G. sepium* chloroform

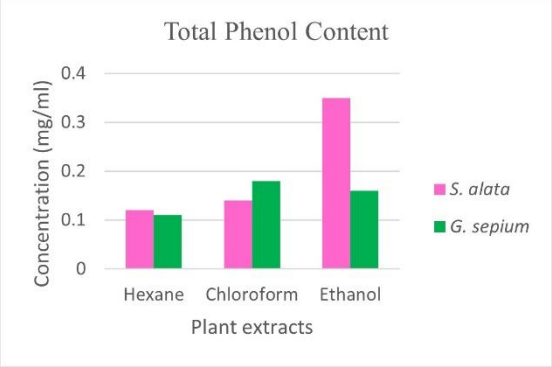

Figure 5: Total phenol in *Senna alata* and *Gliricidia sepium* leaf extracts

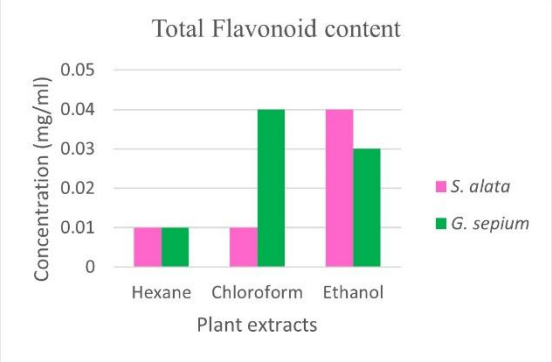

Figure 6: Total flavonoid in *Senna alata* and *Gliricidia sepium* leaf extracts

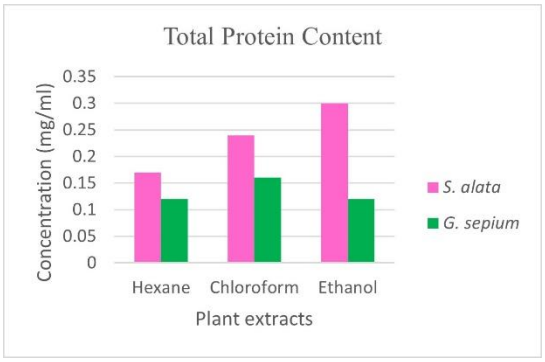

Figure 7: Total protein in *Senna alata* and *Gliricidia sepium* leaf

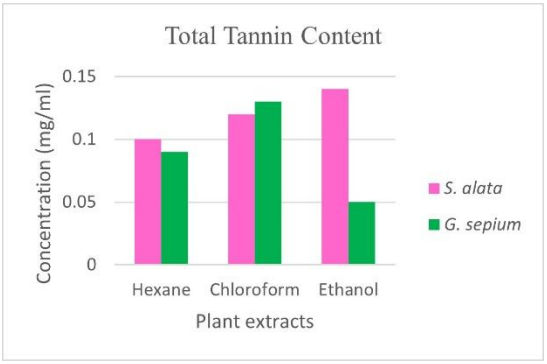

Figure 8: Total tannins in *Senna alata* and *Gliricidia sepium* leaf extracts

extract contained 0.16 ± 0.003 mg/mL (Figure 7). Tannin levels were the lowest among the compounds tested, with 0.14 ± 0.005 mg/mL in *S. alata* ethanol extract and 0.13 ± 0.005 mg/mL in *G. sepium* chloroform extract (Figure 8).

The phenolic content of *S. alata* ethanol extract (0.35 ± 0.010 mg/mL) observed in the present study is consistent with the findings of Rajendran *et al.,* reporting a high phenolic composition of 69.72% in hydroalcoholic leaf extracts.[30] Similarly, the recorded low flavonoid content (0.04 ± 0.004 mg/mL) aligns with their findings (6.96%). Tannin levels in both plants were relatively low, in agreement with previously reported values.[31,32]

Yield of different extracts of *S. alata* and *G. sepium* leaves

Following the extraction, the yield was calculated and compared. In *S. alata*, ethanol produced the highest yield at 0.092 g, followed by hexane at 0.056 g and chloroform at 0.028 g. Similarly, in *G. sepium*, the highest yield was

Table 2: Percentage of yield in *Senna alata* and *Gliricidia sepium* crude leaf extracts.

Plant	Solvent	Colour of extract	Yield of extract* (g)
Senna alata	Hexane	Brown	0.056
	Chloroform	Green	0.028
	Ethanol	Brown	0.092
Gliricidia sepium	Hexane	Brown	0.048
	Chloroform	Green	0.088
	Ethanol	Brown	0.098

* Represents the dry weight of the residue in the extract obtained from 100g of dry leaf powder

also obtained with ethanol at 0.098 g, followed by chloroform at 0.088 g, while hexane yielded the least at 0.048 g (Table 2).

These results from *S. alata* agree with earlier findings that polar solvents like ethanol and methanol are more efficient in extracting phytochemicals due to their ability to solubilize a broader range of polar compounds.[33,34] Similarly, it is reported that Soxhlet extraction using methanol has produced a yield of up to 25.14%, which is significantly higher than that of hexane (11.24%).[21] The present study also showed that the yield was lowest when hexane was used for extraction. When TLC fractions were analysed for yield it was found that the amount of residue obtained was highest in fraction 2 of ethanol extracts in both plants (0.140 mg/mL in *S. alata* and 0.134 mg/mL in *G. sepium*), further supporting ethanol's effectiveness in concentrating bioactive compounds. This agrees with previous studies reporting high antioxidant and phytochemical content in ethanol and hydroethanolic extracts of *S. alata*.[30,34]

Conclusion

The present study demonstrates that *S. alata* and *G. sepium* leaves are rich sources of bioactive compounds, with ethanol and chloroform extracts showing particularly high concentrations of phytochemicals. These findings are consistent with those of a recent study by Veena *et al.,* which has demonstrated the antifeedant properties of *G. sepium* leaves against the larvae of the coconut pest *Oryctes rhinoceros*, thereby underscoring their potential in sustainable pest management.[35] Further research is warranted to isolate and characterize the active compounds, evaluate their biological activities through targeted bioassays, and explore their pharmacological potential for use in both pest control and drug development.

To advance the present findings, future research should focus on the isolation, purification, characterization, and identification of key active constituents using advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance spectroscopy (NMR). Bioactivity validation is recommended through targeted bioassays to establish insecticidal, antimicrobial, or pharmacological properties, thereby confirming efficacy. Another requirement is to elucidate the mechanisms of action of these bioactive compounds to justify their use for a specific application. Studies should also explore the development of formulations for agrochemical or therapeutic applications, with particular attention to safety and scalability. By bridging phytochemical analysis with functional validation, these plants, especially the more abundant *Gliricidia sepium*, could provide sustainable solutions to agricultural and medical challenges, including the issue of herb scarcity.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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