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Original Article

Exploring the Glycosidic Compounds in *Ipomoea sagittifolia* Stems: A High-Performance Liquid Chromatography Approach

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ABSTRACT

Background: The abstract provides a comprehensive overview of the study on secondary metabolites from Ipomoea sagittifolia, emphasizing the extraction, isolation and characterization processes. Secondary metabolites, including alkaloids, flavonoids, phenols and glycosides, were identified and analyzed using various analytical techniques. **Materials and Methods:** Ethanol extraction followed by Soxhlet apparatus yielded a crude extract, subsequently purified through column chromatography using a solvent mixture of n-butanol, glacial acetic acid and water. Thin Layer Chromatography (TLC) confirmed the presence of compounds across 108 eluted fractions, further validated by UV-vis spectroscopy revealing absorption peaks characteristic of glycosides at 288 nm. **Results:** Fourier Transform Infrared (FTIR) spectroscopy highlighted functional groups such as alcohols, phenols, carboxylic acids and ketones, typical of glycoside structures. High-Performance Liquid Chromatography (HPLC) confirmed the presence of cardiac glycosides through retention time analysis, aligning closely with Scillaren A standards. **Conclusion:** These findings underscore the pharmacological potential of Ipomoea sagittifolia in traditional medicine and pharmaceutical applications, supported by its diverse secondary metabolite profile. Future research could delve deeper into bioactivity assays to elucidate specific therapeutic properties linked to these metabolites, thereby enhancing its relevance in healthcare and biotechnological innovations.

Keywords: Bioactive, Elucidation, High Performance Thin Layer Chromatography, *Ipomea*.

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INTRODUCTION

Secondary metabolites have substantial ramifications for many different scientific areas; study into them as biomarkers has attracted a lot of attention. Alkaloids, flavonoids, terpenoids and phenolics are examples of secondary metabolites, which are organic substances produced by fungi, plants and microbes that are essential for both ecological interactions and organism adaptation.[1] Their bioactive qualities along with their distinctive and varied architectures make them important instruments for environmental monitoring, medication development, illness diagnostics and agricultural advancements. Secondary metabolites provide vital insights that propel innovation in biotechnology, medicine, agriculture and ecological conservation by acting as markers of biological processes, environmental changes and disease states.[2] This rapidly expanding field of study emphasizes how crucial it is to locate and use these substances as biomarkers in order to improve our comprehension of intricate biological systems and to create novel, beneficial uses for society.[3]

A variety of cutting-edge analytical methods are used in the study of secondary metabolites found in plants in an effort to fully understand the diversity and complexity of these bioactive substances.[4] Alkaloids, flavonoids, terpenoids and phenolics are examples of secondary metabolites that are important for a plant's defence, adaptability and interactions with its surroundings.[5] These substances have a lot of potential uses in business, agriculture and medicine, which makes them quite interesting. Accurate and advanced approaches are needed for the identification, quantification and structural elucidation of secondary metabolites in plants.[6]

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High-performance liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy and other spectroscopic techniques are frequently used to offer in-depth understandings of the molecular makeup and biological roles of these metabolites. Numerous substances are found in complex matrices in plants and no one active ingredient is in charge of the system's total effectiveness.[7] This makes standardizing final herbal medication formulation and setting quality control requirements for raw components difficult. Traditionally, the quality and validity of complex herbal remedies were determined by a limited number of indicators of pharmacologically active ingredients.[8] Hence the current work explores the characterization of *Ipomea sagittifolia* Stem extract.

Ipomoea sagittifolia is commonly known as the "Arrowleaf Morning Glory." This flowering plant belongs to the Convolvulaceae family and is more widely known for its importance in medicine and ecology. This species, like its other relatives in the genus *Ipomoea*, is climbing or trailing by very slender, vine-like stems. The leaves are in the shape of an arrow, which is how this species got its name. It grows in the tropics and subtropics with open, moist, sandy, or loamy soil.

MATERIALS AND METHODS

Materials and Reagents

Acetonitrile, Ortho phosphoric acid, Methanol, Silicagel, n-butanol, glacial acetic acid. All the chemicals were analytical grade.

Extraction

Stems of *Ipomoea sagittifolia* was collected in the month of November in the surroundings of Gudivada and the plant material was authenticated by Prof. K. Madhava Shetty, Dept. of Botany, SV University, Tirupati, Andhra Pradesh, India. The stems were washed and shade dried for 7 days. The dried Stems were finely powdered by mechanical grinder. The powdered leaf material (100 g) was extracted with ethanol using hot percolation process by Soxhlet apparatus. Excess solvent was evaporated by rotary evaporator; crude extract was obtained and stored in airtight container.

Isolation and Characterization

Column Chromatography

The ethanolic stem extract, weighing 5 g, was subjected to chromatographic separation using a silica gel medium with a particle size range of 60-120 mesh. The chromatographic column used had a length of 60 cm and a diameter of 3 cm, providing an adequate surface area for the separation process. The elution of the extract was performed using a solvent mixture consisting of n-butanol, glacial acetic acid and water in the ratio of 4:1:5. This specific solvent combination was chosen for its efficacy in separating the compounds present in the leaf extract.

As the elution proceeded, the fractions were systematically collected in 5 mL portions. Each collected fraction was then monitored using Thin Layer Chromatography (TLC) to assess the presence and distribution of various compounds. Fractions displaying similar spots on the TLC plates, indicating the presence of similar or identical compounds, were combined to streamline further analysis and ensure consistency.

In total, 108 fractions were successfully eluted and collected. Each of these fractions was subsequently subjected to TLC analysis. The TLC studies were crucial in identifying and confirming the presence of various phytocompounds within the fractions. The TLC results provided a visual representation of the separated compounds, allowing for the identification and characterization of the phytochemicals present in the ethanolic leaf extract.

These detailed chromatographic and TLC analyses are essential steps in isolating and identifying the bioactive compounds within the extract, contributing valuable information towards understanding its chemical profile and potential applications.[9,10]

UV Spectroscopy

The eluted fractions were subjected to detailed analysis for their maximum absorption properties using UV spectroscopy. To begin with, the elution's were carefully vacuum filtered to ensure the removal of any particulate matter. Following filtration, the supernatant was collected for further analysis. A precise volume of 0.5 ml from each eluted fraction was then mixed with a solution consisting of acetonitrile and water in a specific ratio of 40:10. This mixture was prepared to facilitate the appropriate environment for UV-vis analysis.

The resulting solution from each elution was then subjected to UV-vis spectrophotometry using a Shimadzu spectrophotometer. The measurements were taken over a broad wavelength range, spanning from 200 to 800 nm. This comprehensive scan allowed for the detection of characteristic absorption peaks across the entire UV and visible spectrum.[11]

In addition to the samples, a blank solution was also prepared and analyzed under the same conditions to serve as a control and ensure the accuracy of the measurements. The blank helped in establishing a baseline for the absorbance readings, thereby allowing for the precise determination of the sample peaks.[12]

The spectrophotometer provided detailed absorbance spectra for each sample, from which the maximum absorption values were identified and recorded. These characteristic peaks were crucial for understanding the molecular interactions and properties of the substances present in the eluted fractions. The recorded UV-vis peak values offer vital information on the chemical composition and potential applications of the samples analyzed.

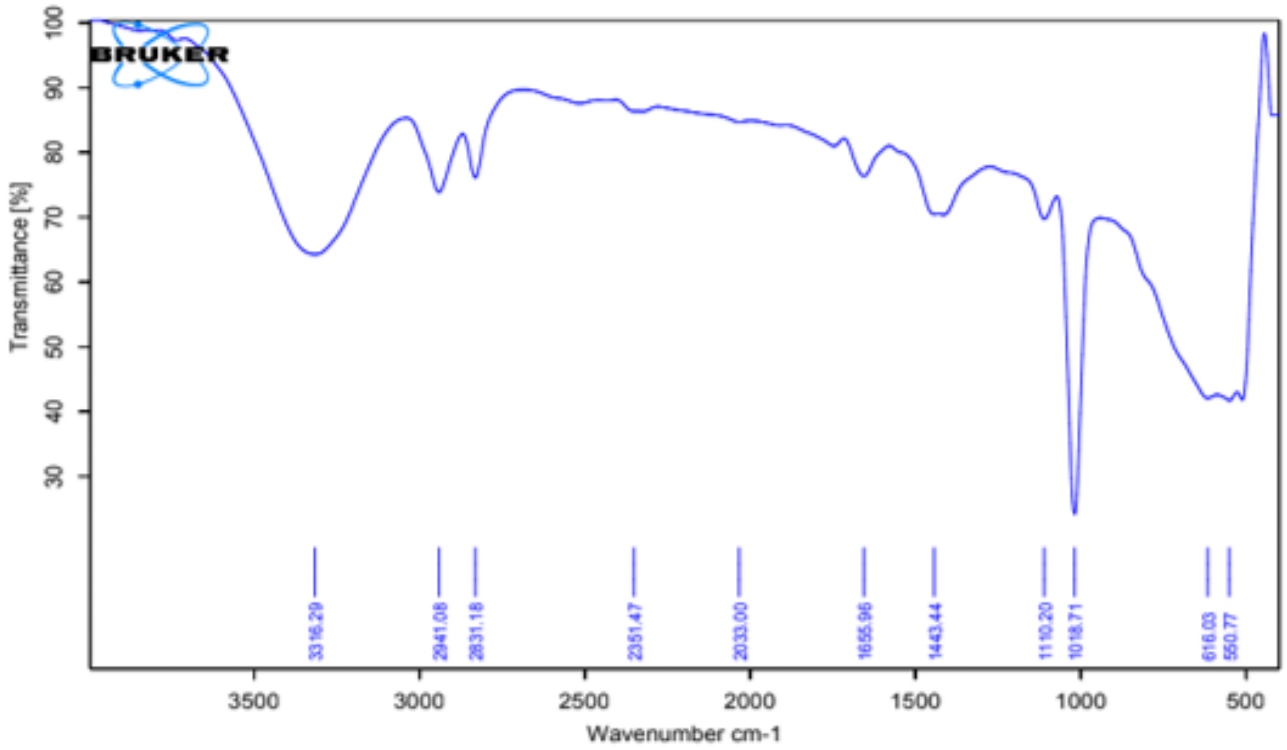

Figure 1: FTIR Spectra for ethanolic leaf extract of *Ipomea sagittifolia*.

FT-IR Analysis

The dried stem extract of *Ipomoea sagittifolia* was subjected to Fourier Transform Infrared (FTIR) analysis to elucidate its chemical composition and identify specific functional groups present.[13-16] The analysis began by encapsulating 1 mg of the dried extract powder into 10 mg of Potassium Bromide (KBr) pellets. This encapsulation process ensured the formation of translucent sample discs suitable for FTIR spectroscopy, facilitating accurate and reproducible measurements.

The prepared sample discs, containing the powdered extract and KBr, were then loaded into an FTIR spectroscope, specifically a Shimadzu instrument. The spectrometer was configured to scan across a wide spectral range from 400 to 4000 cm^{-1} with a spectral resolution set at 4 cm^{-1}. This broad range and high resolution allowed for detailed analysis of the infrared absorption spectra, which are characteristic of different functional groups present in the phytoextract.

During the scan, the FTIR spectroscope recorded the absorbance of infrared radiation by the sample. The resulting IR spectrum provided a fingerprint of the extract's molecular composition, highlighting absorption peaks corresponding to specific vibrational modes of functional groups such as Hydroxyl groups (-OH), Carbonyl groups (C=O), aromatic rings, aliphatic chains and others. Each peak's position and intensity were analyzed to infer the types and abundance of these functional groups within the extract.

Interpretation of the IR spectrum involved comparing observed absorption bands with established literature data and reference spectra to identify and confirm the presence of characteristic functional groups in the phytoextract. This analytical approach not only characterized the chemical nature of the *Ipomoea sagittifolia* leaf extract but also provided insights into its potential bioactive components and applications in various fields such as pharmaceuticals, nutraceuticals and cosmetics. The FTIR analysis thus served as a powerful tool for comprehensive molecular profiling and functional group identification of the dried leaf extract, contributing to its scientific understanding and potential utilization.[17]

HPLC Analysis

The chromatographic analysis of the High Fat Extract (HFE) was conducted using an Agilent HPLC system equipped with a binary pump, Photo Diode Array (PDA) detector and an Agilent C18 column (25 cm×4.6 mm; 5 µm particle size), maintained at ambient temperature. This setup was chosen for its capability to separate and detect compounds with high sensitivity and resolution. The entire process, including data acquisition and peak integration, was managed using Agilent's Open Lab software, ensuring precise and reliable results.

For the chromatographic separation, an isocratic mobile phase system composed of acetonitrile and water in a ratio of 70:30 was employed. This specific mobile phase composition was optimized to achieve efficient separation of components present in the HFE sample. The pH of the mobile phase was adjusted

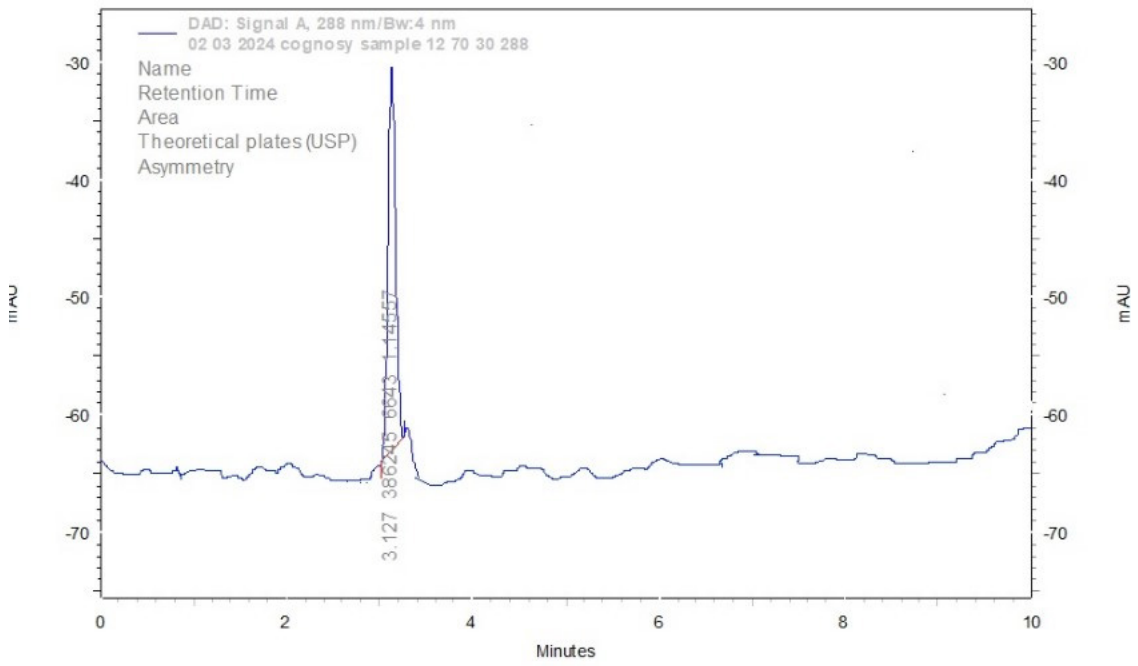

Figure 2: HPLC analysis for Fraction I.

to 3 using orthophosphoric acid, which helped in enhancing chromatographic resolution and stability of the analytes.

During the analysis, the flow rate of the mobile phase was set at 0.8 mL/min to ensure adequate elution of compounds through the C18 column. An injection volume of 20 µL of the HFE sample was introduced into the chromatographic system, allowing for sufficient analyte detection and quantification.

Detection of the separated compounds was carried out using UV detection at a wavelength of 288 nm, which is optimal for detecting UV-absorbing compounds commonly found in natural extracts. The PDA detector enabled the collection of complete UV-vis spectra of eluted peaks, offering additional information about the chemical composition of the separated components.

By comparing the retention times and UV spectra of peaks with those of standard compounds, the identities of specific compounds within the HFE sample were determined. This analytical approach provided valuable insights into the composition and purity of the extract, facilitating its characterization and potential application in various fields such as pharmaceuticals, food supplements, or cosmetics.

RESULTS AND DISCUSSION

All the fractions were subjected for UV Spectrophotometer analysis to find out maximum absorption. Fraction I resulted in 12 peaks showing the absorbance 0.005, 0.025, 0.009, 0.013, 0.413, 0.451, 0.002, 0.008, 0.006, 0.012, 0.405 and 0.410 at different wavelength 742 nm, 660 nm, 606 nm, 502 nm, 288 nm, 266 nm, 726 nm, 622 nm, 576 nm, 486 nm, 486 nm, 278 nm and 254 nm. Fraction II resulted 12 peaks showing the absorbance 0.005, 0.047, 0.017, 0.021, 0.894, 1.004, 0.003, 0.015, 0.011, 0.021, 0.869 and 0.907 at different wavelengths 738 nm, 656 nm, 604 nm, 498 nm, 288 nm, 266 nm, 728 nm, 620 nm, 576 nm, 490 nm, 278 nm and 252 nm. Fraction III resulted in 12 peaks showing absorbance of 0.005, 0.047, 0.017, 0.025, 0.906, 1.012, 0.004, 0.015, 0.012, 0.024, 0.884, 0.914 at wavelengths 738 nm, 660 nm, 606 nm, 500 nm, 288 nm, 266 nm, 728 nm, 622 nm, 578 nm, 486 nm, 278 nm and 252 nm. Fraction IV shown 12 peaks at absorbance 0.005, 0.039, 0.013, 0.021, 0.818, 0.917, 0.003, 0.011, 0.009, 0.020, 0.799, 0.827 at wavelength 742 nm, 662 nm, 604 nm, 502 nm, 288 nm, 266 nm, 726 nm, 622 nm, 578 nm, 486 nm, 278 nm and 252 nm. Fraction V shown 12 peaks at absorbance 0.006, 0.056, 0.020, 0.034, 1.489, 1.697, 0.004, 0.017, 0.014, 0.033, 1.459, 1.539 at different wavelengths 738 nm, 662 nm, 606 nm, 498 nm, 286 nm, 266 nm, 728 nm, 622 nm, 578 nm, 488 nm, 278 nm, 252 nm. As the every elution is showing the absorbance at 278nm, this wavelength is selected for further identification of glycosides by HPLC analysis.

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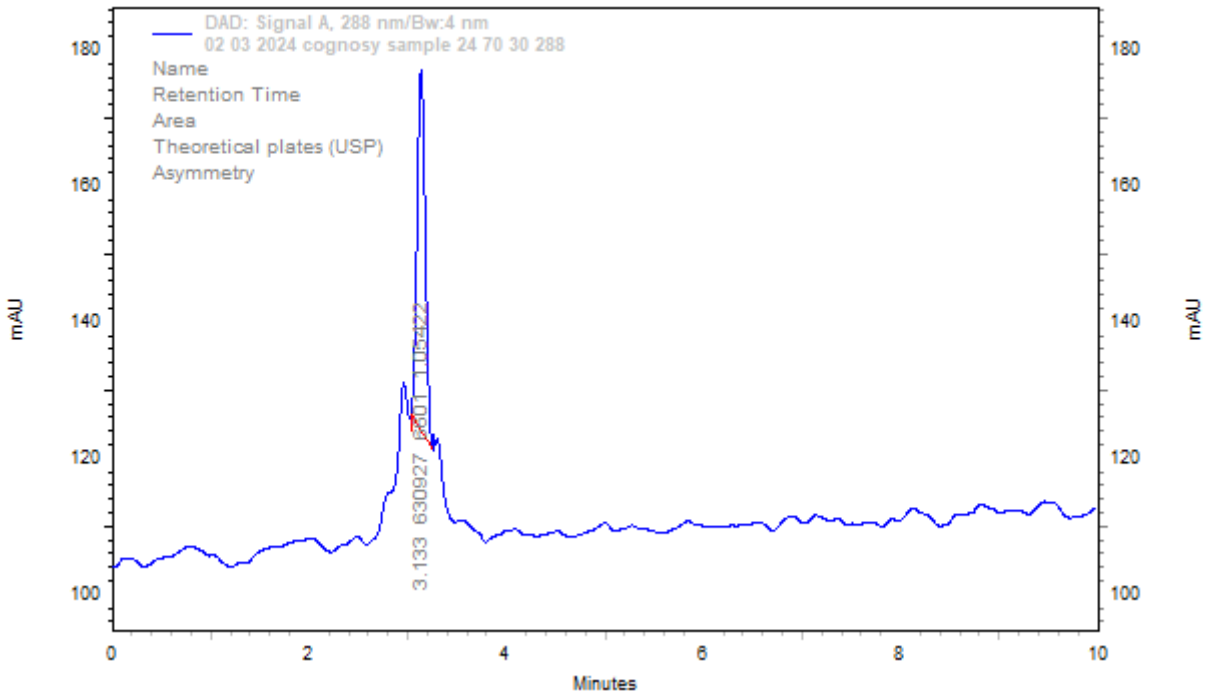

Figure 3: HPLC analysis for Fraction II.

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FT-IR Analysis

Dried Leaf extract of *Ipomeasagittifolia* was used for FTIR analysis. The Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} was selected. IR Spectrum resulted in peaks shown in Figure 1. The interpretiveresults were deputed in Table 1 to identify functional groups in the phytoextract.

The functional group of active components present in the ethanolic extract shown in Table 2, had shown band values identified in the FT-IR spectrum. All potential bands were labeled and possible functional group band values are identified and interpretated. The extract had shown 11 significant bands (between 400 and 4000 cm^{-1}) and most of these bands share their corresponding spectral range.

IR Spectra reveals the presence of functional group alcohol and aromatic hydroxyl group (phenol) as it shown peak at 3316.29 cm^{-1}, carboxylic acid as the peak is at 2941.08 and 2831.18, keto group representing peak at 1655, Alkanes as the peak at 1443.44 and presence of major function groups at the spectra revealed the peaks at 1018.71 and 1110.20. These functional groups represent the presence of glycosides contains both sugar and non-sugar moieties.

HPLC Analysis

The chromatographic equipment of Agilent 1260 HPLC system comprising binary pump, PDA array detector and Agilent C18 column (25 cm×4.6 mm; 5 µm) controlled at ambient temperature was employed for the chromatographic analysis of fractions. The spectras were shown in Figures 2-6 and Table 2.

All the fractions were analyzed at 288nm, the retention time and peak areas were tabulated in Table 2.

The retention time of all the elusions especially Fraction I (Shown sharp peak) are observed a they were very close to the standard (Scillaren A) retention time represents the presence of cardiac glycosides at ambient temperature.

Ethanolic leaf extract of *Ipomoea sagittifolia* has gained attention in the field of pharmacology and herbal medicine due to its potential medicinal properties.

Table 1: Compounds Identified in the Ethanolic Extract of *Ipomoea sagittifolia* using FT-IR Spectroscopy.

Wave Number			
Band Range	**Standard Value**	**Functional group/Compound Name**	**Observed Peak at**
400 and 4000 cm^{-1}	3500-3200	Alcohols (R-OH)Phenols (Ar-OH)	3316.29
	3300-2500	Carboxylic acid (-COOH)	2941.082831.18
	1650-1770	Keto group	1655
	1470-1450	Alkanes	1443.44
	1000-1320	Alcohols, Carboxylic acid, Esters, Ether	1018.711110.20

Phytochemical screening of the ethanolic leaf extract of *Ipomoea sagittifolia* revealed the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, glycosides and terpenoids. These compounds are known to possess diverse biological activities including antioxidant, anti-inflammatory, antimicrobial and anticancer properties.

To isolate the phytocompounds, Column chromatography is used. A total no. of 108 elutions was eluted out by using mobile phase n-butanol, glacial acetic acid and water (40:10:50). Each fraction subjected for TLC analysis using mobile phasen-butanol, glacial acetic acid and water (40:10:50). Further the elusion is analyzed for maximum absorbance by using UV spectroscopical analysis. The most of the fraction were shown 12 peaks and the absorbance at 288nm represents the presence of glycosides. Hence the absorbance 288nm is considered for further identification of glycosides by HPLC.

Infrared (IR) spectroscopy analysis was conducted to determine the functional groups present in the ethanolic leaf extract of *Ipomoea sagittifolia*. IR spectroscopy helps in identifying bonds and functional groups based on the absorption of infrared radiation by the sample. The ethanolic leaf extract of *Ipomea sagittifolia* revealed the presence of alcohol, phenolic hydroxyl group, ester, keto group and carboxylic acid represents the major functional groups in glycosides. This analysis provides insights into the chemical composition (glycosides) and potential medicinal properties of the extract.

Shui *et al.* conducted a study using HPLC coupled with a PDA detector to analyze flavonoids in various parts of *Abelmoschus manihot*, commonly known as edible hibiscus. Flavonoids are a diverse group of phytochemicals known for their antioxidant and other beneficial biological activities. The researchers aimed to identify and quantify specific flavonoids present in different plant parts, such as leaves, flowers and seeds. HPLC-PDA analysis allowed for the separation of complex mixtures of flavonoids based on their chemical properties and interactions with the C18 column stationary phase.[18-20] The PDA detector provided UV-vis spectra of eluted compounds, enabling the researchers to distinguish between different flavonoids based on their characteristic absorption spectra and retention times. By comparing these data with authentic standards, they were able to identify specific flavonoids such as quercetin, kaempferol and their glycosides in *Abelmoschus manihot* extracts. This study underscored the utility of HPLC-PDA in phytochemical analysis, particularly for profiling flavonoids in plant extracts with high sensitivity and selectivity. The results contributed valuable information to the understanding of the chemical composition and potential health benefits of *Abelmoschus manihot*.

Pothitirat, *et al.* utilized HPLC coupled with Mass Spectrometry (HPLC-MS) to investigate alkaloids in various parts of Mitragyna speciosa, commonly known as kratom. Alkaloids are nitrogen-containing compounds that often exhibit potent pharmacological activities, including analgesic and stimulant effects. HPLC-MS combines the separation power of HPLC with the sensitive detection and structural elucidation capabilities of mass spectrometry.[21-23] This hybrid technique allowed the researchers to separate and detect a wide range of alkaloids present in Mitragyna speciosa extracts with high sensitivity and accuracy. By analyzing the mass spectra of eluted peaks and comparing them with reference standards or databases, they identified alkaloids such as mitragynine and 7-hydroxymitragynine, which are known for their bioactive properties. The study demonstrated the effectiveness of HPLC-MS in profiling complex alkaloid mixtures in botanical extracts, providing comprehensive information on alkaloid composition and contributing to the understanding of kratom's pharmacological effects and potential therapeutic applications.[24]

Wang *et al.* conducted research using HPLC coupled with a diode array detector (HPLC-DAD) to quantify ginsenosides in Panax ginseng products. Ginsenosides are the major bioactive compounds in ginseng known for their adaptogenic and health-promoting properties. HPLC-DAD is widely used for the quantitative analysis of phytoconstituents due to its ability to provide chromatographic separation and simultaneous UV-vis detection of compounds. In this study, the researchers developed a standardized method to determine the content of specific ginsenosides, such as Rg1, Re, Rb1 and Rd, in different ginseng preparations.[25-27] By establishing calibration curves using authentic standards, Wang *et al.* quantified the ginsenoside

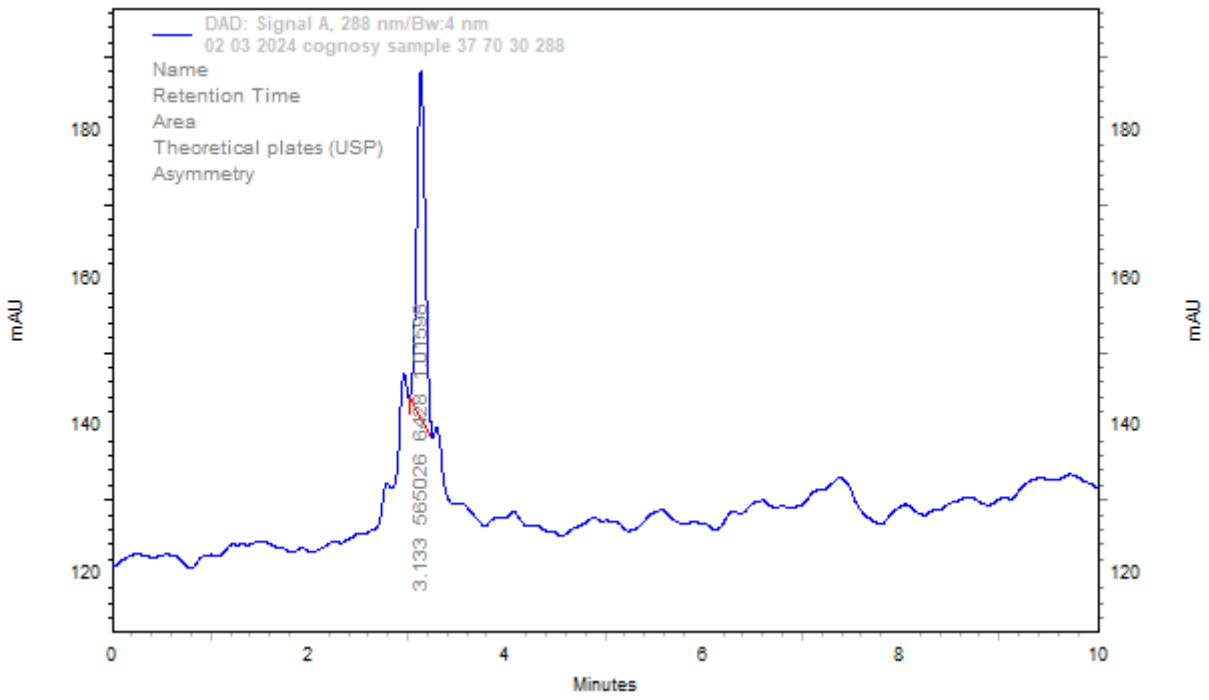

Figure 4: HPLC analysis for Fraction III.

content in Panax ginseng samples, ensuring consistency and quality control in herbal medicine production. This methodological approach provided crucial data for assessing the bioactivity and potency of ginseng products, supporting their use in traditional medicine and nutraceutical applications.

These studies exemplify the diverse applications of HPLC in phytochemical analysis, ranging from the identification and quantification of specific phytoconstituents to quality control and standardization of herbal medicines. HPLC techniques continue to evolve, incorporating advanced detectors and methodologies to further enhance the accuracy, sensitivity and scope of phytochemical analysis in botanical research and product development.

Citrus fruits are renowned for their high content of flavonoids, which contribute significantly to their antioxidant properties and potential health benefits. González-Molina *et al.* (2009) utilized HPLC coupled with a Diode Array Detector (DAD) to investigate flavonoids in different citrus varieties. Specifically, they analyzed compounds like hesperidin and naringin, known for their antioxidant and anti-inflammatory effects. The chromatographic separation provided by HPLC-DAD enabled precise quantification of these flavonoids, allowing for comparisons across different citrus species and varieties. Their findings not only highlighted the variation in flavonoid profiles among citrus fruits but also elucidated how extraction conditions and fruit maturity affect flavonoid content, thereby contributing valuable insights into optimizing citrus fruit processing for maximizing their nutritional and health benefits.[28]

Phenolic acids are important secondary metabolites in plants, renowned for their antioxidant, anti-inflammatory and potential anticancer properties. Lee *et al.* (2010) utilized HPLC to analyze phenolic acids in extracts from Artemisia princeps, a traditional medicinal plant widely used in East Asia. Their study employed HPLC coupled with tandem Mass Spectrometry (LC-MS/MS) to achieve sensitive detection and identification of specific phenolic acids. By quantifying these compounds, such as chlorogenic acid and caffeic acid derivatives, the researchers provided critical data on the composition and concentration of phenolic acids in *Artemisia princeps*. This information is crucial for evaluating the medicinal potential and quality of herbal extracts used in traditional medicine and dietary supplements, emphasizing the role of HPLC in ensuring accurate and reproducible analysis of phenolic acids in medicinal plants (Lee *et al.*, 2010).[29]

Essential oils are concentrated extracts from aromatic plants, rich in terpenoids that exhibit diverse biological activities, including antimicrobial, anti-inflammatory and neuroprotective effects. Baser and Buchbauer (2015) utilized HPLC to analyze the composition of terpenoids in essential oils from lavender and rosemary. Their study focused on identifying individual terpenoid compounds, such as linalool, camphor and α-pinene, which contribute to the characteristic aroma and therapeutic properties of these oils. HPLC's ability to separate and quantify terpenoids with high precision and sensitivity enabled the researchers to assess the chemical diversity and quality of essential oils. This information is invaluable for industries involved in aromatherapy, cosmetics and pharmaceuticals, highlighting HPLC as a key

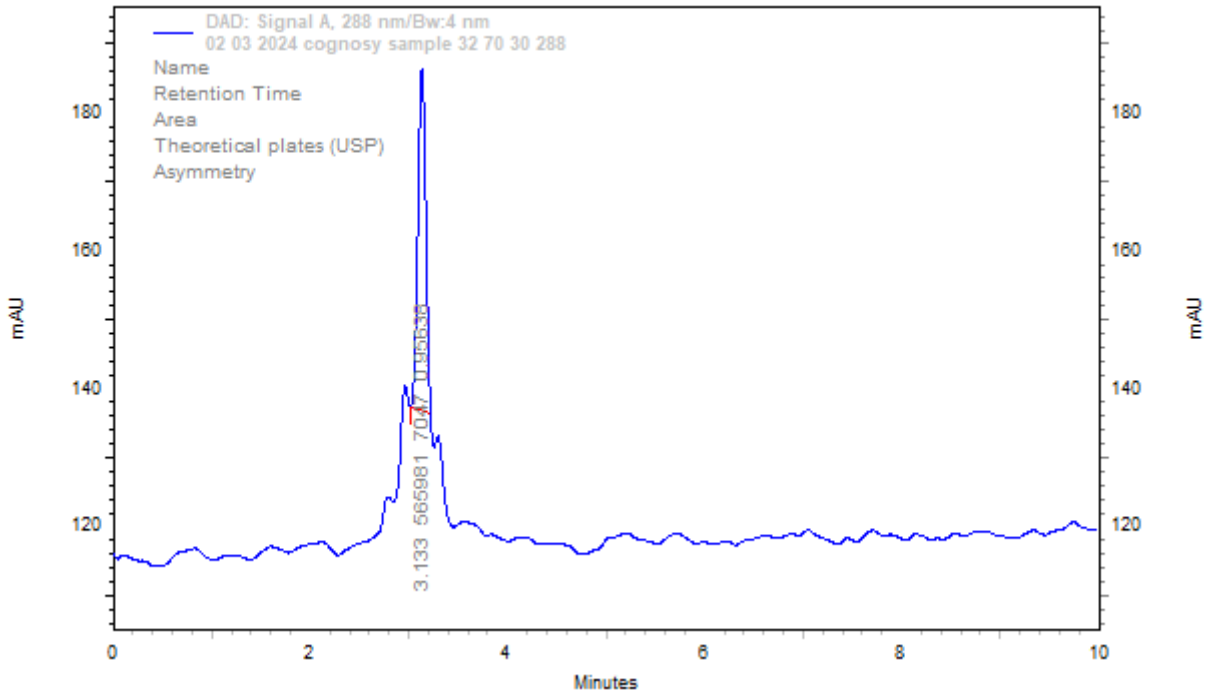

Figure 5: HPLC analysis for Fraction IV.

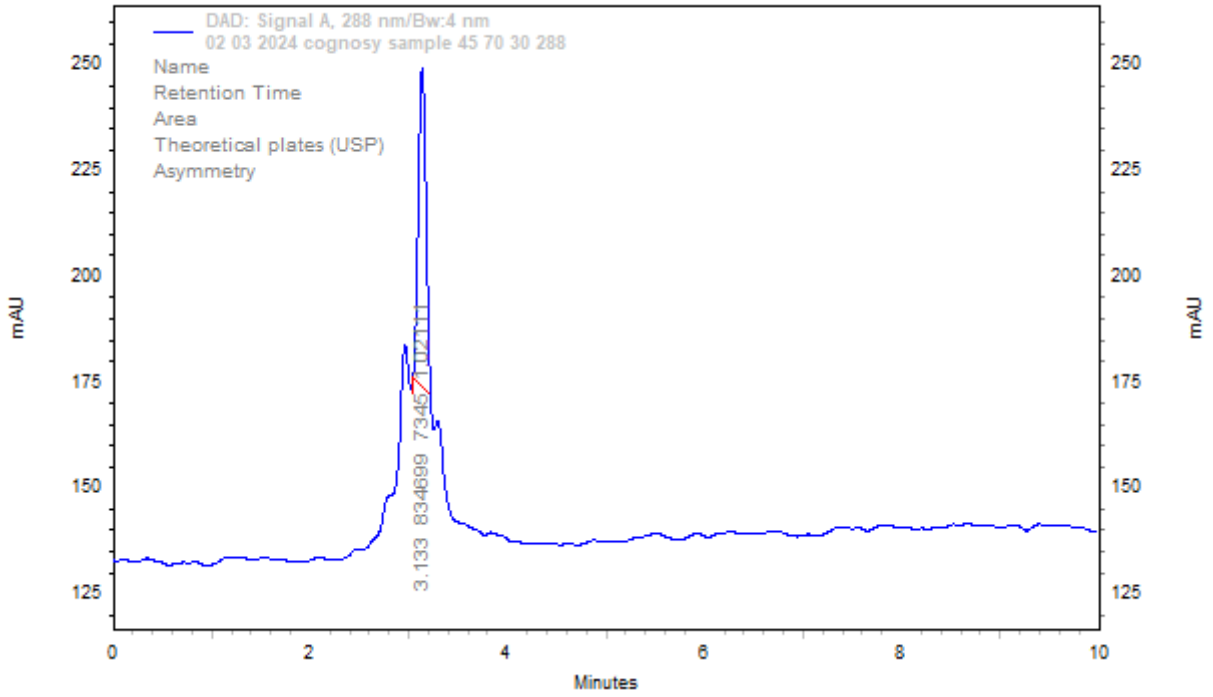

Figure 6: HPLC analysis for Fraction V.

analytical tool for quality control and product development in the essential oil industry.[30]

Alkaloids are nitrogen-containing compounds with diverse pharmacological activities found in various medicinal plants and herbs. Liang *et al.* (2015) employed HPLC coupled with Mass Spectrometry (HPLC-MS) to analyze alkaloids in Uncaria species, including *Uncaria rhynchophylla*, a traditional Chinese herb known for its neuroprotective and anti-inflammatory properties. The study focused on identifying and quantifying alkaloids, such as rhynchophylline and isorhynchophylline, using HPLC-MS. This sophisticated analytical approach provided insights into the alkaloid composition of Uncaria species,

Table 2: Eluted compounds representing Retention Time in HPLC.

Sl. No.	Fraction No.	Retention Time
1	I	3.127
2	II	3.133
3	III	3.133
4	IV	3.133
5	V	2.507
6	Standard	3.00

enhancing understanding of their therapeutic potential and supporting their use in traditional medicine. HPLC-MS's ability to detect and characterize complex alkaloid mixtures underscores its importance in pharmacological and phytochemical research, facilitating the development of herbal remedies with standardized alkaloid content.[31,32]

HPLC plays a crucial role in the quality control and standardization of herbal supplements and botanical preparations. Li *et al.* (2016) conducted research using HPLC-DAD to quantify bioactive compounds, such as ginsenosides, in Panax ginseng products. Ginsenosides are the major bioactive components responsible for the adaptogenic and health-promoting effects of ginseng. By establishing HPLC methods to quantify specific ginsenosides, including Rg1, Re, Rb1 and Rd, the researchers ensured consistency and efficacy in ginseng-based supplements. This analytical approach provided reliable data on ginsenoside content, supporting quality assurance and regulatory compliance in the herbal supplement industry. HPLC's ability to provide accurate and reproducible quantification of bioactive compounds is essential for assessing product potency and ensuring consumer safety, highlighting its indispensable role in herbal supplement manufacturing and quality control (Li *et al*., 2016).

In conclusion, HPLC remains a versatile and indispensable analytical tool for the identification, quantification and quality assessment of phytoconstituents in various botanical extracts and herbal products. The examples discussed demonstrate HPLC's capability to provide precise and reliable data, supporting research and development efforts in pharmaceuticals, nutraceuticalsand natural products. Advances in chromatographic techniques and detector technologies continue to enhance HPLC's analytical capabilities, further advancing our understanding and utilization of phytochemicals for health and wellness applications.

The glycosides from the species *Ipomoea sagittifolia*, in general, display separate structural and functional profiles yet resemble glycosides contained within other *Ipomoea* genus species, besides showing similarity in characteristics between these species in Convolvulaceae and with some other relative families. Such a comparison will show a clue on how it might be potentially pharamacologically potent-usage in traditional drugs besides producing novel drugsSome Glycosides like caffeoylquinic acids contained within *Ipomoea* batatas contain strong antioxidative and anti-inflammatory property and while I. The other type of glycosides that *Sagittifolia* contains is antioxidant.[33-35] The types and the concentration are different, causing the potency and bioactivity to differ. According to research, *I. sagittifolia* has types of glycosides other than I. batatas, which is primarily comprised of phenolic glycosides. *I. carnea* contains indole alkaloids and glycosides and has cytotoxic and neuroactive activity, which does not apply to other types of *Ipomoea. Sagittifolia* appears to have no neuroactive glycosides, but does have glycosides that exhibit some anti-inflammatory and diuretic activity.[36,37] This might indicate that the *I. sagittifolia* glycosides have a safer profile and hence might be used more medicinally.

High-Performance Liquid Chromatography (HPLC) analysis of the ethanolic leaf extract was carried out to separate, identify and quantify the individual components present in the extract. HPLC is a powerful analytical technique widely used in the pharmaceutical and herbal medicine industries for quality control and standardization of herbal extracts. The HPLC analysis revealed the retention time of all eluted fractions is very closer to the standard Scillaren A Retention Time.

Overall, the comprehensive analysis of the ethanolic leaf extract of *Ipomoea sagittifolia* provides valuable insights into its phytochemical composition, potential medicinal properties and therapeutic potential. Further research and clinical studies are warranted to explore its efficacy and safety for various medicinal applications.

CONCLUSION

In conclusion, the studies on *Ipomea sagittifolia* have shed light on its botanical characteristics, traditional uses, pharmacological properties and chemical composition. In conclusion, the ethanolic stem extract of *Ipomoea sagittifolia* shows diverse secondary metabolites. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides and terpenoids, which are known for their antioxidant, anti-inflammatory, antimicrobial and anticancer properties.

The isolation and characterization of glycosides from the extract were carried out using various analytical techniques. Column chromatography facilitated the separation of 108 elutions, which were further analyzed by TLC and UV spectroscopy. UV analysis at 288 nm indicated the presence of glycosides in each fraction, directing subsequent identification using HPLC at the same wavelength. Notably, Fraction I displayed a sharp peak with a retention time close to the standard (Scillaren A), suggesting significant content of cardiac glycosides. FT-IR spectroscopy confirmed the presence of functional groups typical of glycosides, including alcohols, phenolic hydroxyl groups, esters, keto groups and carboxylic acids. These findings corroborate the potential medicinal value of *Ipomoea sagittifolia*, particularly in the context of its glycoside content.

Overall, this study underscores the therapeutic potential of *Ipomoea sagittifolia* as a source of bioactive compounds with diverse pharmacological activities. Further research into the specific mechanisms of action and clinical applications of these compounds could pave the way for their development into novel therapeutic agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENTS

This research did not require ethical statements as no animal studies were conducted.

ABBREVIATIONS

TLC: Thin Layer Chromatography; **HPLC:** High Performance Liquid Chromatography; **UV-Vis:** Ultraviolet-Visible spectroscopy; **PDA:** Photo Diode Array; **HFE:** High fat extract; **KBr:** Potassium Bromide.

SUMMARY

Using a variety of analytical methods, the study identified chemicals such alkaloids, flavonoids, phenols and glycosides by extracting, isolating and characterising secondary metabolites from *Ipomoea sagittifolia*.

Extraction Methodology: A crude extract was obtained by ethanol extraction using a Soxhlet device. This extract was then purified using column chromatography employing a solvent combination consisting of water, glacial acetic acid and n-butanol.

TLC, FTIR and HPLC which identified cardiac glycosides matching Scillaren A standards, were used to confirm the presence of glycosides and other compounds.

The results highlight *Ipomoea sagittifolia*'s pharmacological potential in conventional medicine and point to the need for more bioactivity assay research to examine the particular therapeutic qualities of the identified metabolites, increasing the plant's importance in biotechnology and healthcare.

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