Importance of collection of drug substance during the peak season and off season: A Review

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ABSTRACT
The seasonal variations in alkaloid distribution patterns have been investigated in this study. Seasonal fluctuations play a crucial role in influencing the active principles of medicinal plants. Hence, therapeutic efficacy influenced. Alkaloids are primary compounds, unique to drugs which enhance the main action of the drug. Seasonal variation has direct influence on chemical constituents of the plants. It is analyzed by using Liquid chromatography mass spectrometry (LCMS), High-Performance Liquid Chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography mass spectrometry (GCMS), Co-chromatography, UV spectra etc. Based on these articles LC-MS is predominantly used for the detection of alkaloids. On comprehensive evaluation of 40 articles around 16 articles reported LC-MS and 4 articles HPLC, 4 articles GC-MS, rest of articles reported of other methods. This study concluded that seasonal variation has direct influence in alkaloids of the plants reviewed by collection of various plant alkaloids in off season and on season is recorded.
Keywords- Alkaloids, on season, off season, LCMS, seasonal variation

INTRODUCTION

Nitrogenous organic compounds are a class of alkaloids with inherent alkaline properties. Initially isolated from botanical sources, since it has been established that alkaloids are diverse array of living beings, such as fungi, insects, animals. As of current knowledge, the compendium of identified natural products classified as alkaloids number approximately 12,000. The primary criterion for the taxonomic categorization of a compound is alkaloid contingent upon the fundamental nitrogen atom present within its molecular structure, distinct from nitrogen atoms participating in amine or peptide bonds. This foundational nitrogen atom underpins the alkaline characteristics of

alkaloids and frequently governs their biological functionalities. Alkaloids are subject to classification based on their molecular structure, facilitating the elucidation of their chemical attributes and functional roles. Furthermore, a customary taxonomical approach involves the stratification of alkaloids predicated on their botanical origins, given that distinct plant species yield an assortment of alkaloids with divergent biological properties and applications. Alkaloids are essential compounds with unique properties with water-soluble in acidic condition and fat-soluble in alkaline condition, which is helps in protonated form for dissolution and deprotonated form for membrane permeation. Alkaloids are renowned to protection. Most of the drugs are made from alkaloids which structurally modified to enhance the primary action and minimize the unwanted side effects and also play a vital role. Approximately 20% of the secondary metabolites are from plant kingdom, which protect plants. Alkaloids are anesthetics, cardio protectors, and anti-inflammatory agents in medical field. They also exhibit various pharmacological effects, including antidepressant, anxiolytic, convulsive, and hallucinogenic activities. Furthermore, certain alkaloids within this class of compounds have been reported to possess anti-tumor, mutagenic, and cytotoxic properties. Some clinically used alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine, with well-defined pharmacological applications. However, it's important to note that many alkaloids are toxic, making their biosynthesis a general defense mechanism in producing organisms. For the production of alkaloids in many plant species use fungal inducers. The tetra-hydro iso quinoline and mono-terpeneindole alkaloids is remarkable which origin from a single central biosynthetic agent. Organisms that continually develop the ability to produce new structures can thrive in their environments. Plants represent to some of the most common alkaloids such as *morphine, Quinine, Strychnine, Cocaine,* and *Carpine.* The medicinal significance of many alkaloids is crucial in biosynthesis research.

Recent reviews indicate a growing emphasis on targeting major metabolic pathways, aiming to generate highly optimized single molecules. However, it's worth noting that many sectors dealing with secondary or natural products remain variety-oriented. In numerous species, natural products function as a kind of immune system.

In recent years, significant progress has been made in the discovery of alkaloids, the elucidation of biosynthetic pathways, and metabolic engineering. Metabolic engineering has proven effective in increasing alkaloid production levels, recreating alkaloid structures by manipulating biosynthetic enzymes. Despite these advancements, alkaloids remain somewhat underrepresented as lead compounds in the discovery, commercialization, and licensing of new drugs. The field of pharmacokinetic studies demands high throughput, sufficient sensitivity, and rapid analysis times, all of which can be met by LC-MS/MS technology. Given the substantial diversity of flavonoids found in individual plant tissues, the low concentrations of certain compounds, and the cost-effectiveness of LC/MS, this method remains the preferred choice for studying this group of secondary metabolites.

The objective of our work is to analyze and characterize secondary metabolites in complex extracts obtained from various plants using LC-MS methods.

LC-MS is an analytical tool determining the mass and structure of individual compounds within a sample by combining two techniques. The basic procedure involves separating samples into individual compounds through high-performance liquid chromatography (HPLC) and then detecting these compounds, along with their fragments, using mass spectrometry (MS). LC-MS instrumentation has become more user-friendly, making it accessible to a broader scientific community. This enhanced ease of operation has significantly contributed to the widespread adoption of LC-MS technology, particularly within the sphere of the pharmaceutical industry, where it plays a pivotal role in the detection of drug metabolites. It is a multifaceted analytical technique that harmoniously amalgamates the potent capabilities of separation. In this analytical paradigm, liquid chromatography facilitates the physical separation of compounds, while mass spectrometry meticulously scrutinizes the mass-to-charge ratio of ionized particles. Conventionally, physical separations are conducted through either high-performance liquid chromatography (HPLC) or LCMS, the latter also denoted as HPLC-MS.

LCMS stands out as a dominant analytical modality renowned for its extraordinary precision, sensitivity, and specificity when juxtaposed with HPLC. Consequently, it has

found extensive utility across diverse domains, including scientific research, pharmaceutical analysis, and food quality assessment. It excels in the isolation, detection, characterization, and quantification of biochemical entities within complex chemical matrices, thereby representing a formidable fusion of liquid chromatography and mass spectrometry. One pivotal advantage is the circumvention of chemical derivatization procedures, thereby culminating in accelerated analysis durations and enabling high-throughput operations. Furthermore, the emergence of ultra-performance liquid chromatography-mass spectrometry has witnessed substantial growth within metabolomics research, precipitated by the advent technology and mass spectrometry. Remarkable strides have been taken in advancing the development of reliable and user-friendly LC-MS instrumentation. The analysis through targeted liquid chromatography-mass spectrometry methods of plant alkaloids represents a crucial aspect of botanical research and pharmacological investigations. This analytical approach is instrumental in the precise identification, quantification, and characterization of specific alkaloid compounds within plant samples.

Targeted LC-MS employs selective methodologies to focus on predefined alkaloids of interest, allowing for a comprehensive examination of these bioactive molecules. The method typically involves the extraction of alkaloids from plant materials, followed by chromatographic separation and mass spectrometric detection. This targeted approach provides researchers with a powerful tool to explore the presence, concentration, and variations of specific alkaloids in different plant species or under varying environmental conditions. Such studies hold significant implications for our understanding of the ecological roles, medicinal properties, and biosynthetic pathways of alkaloids in plants. Moreover, targeted LC-MS methods contribute to the development of new pharmaceuticals and therapeutic agents derived from plant alkaloids, thereby bridging the gap between botanical science and medical research.

AIM

A comparative review of a collection of various alkaloids in off-season and on-season

OBJECTIVE

Plant alkaloid analysis by targeted LC-MS method

MATERIALS AND METHODS

The articles about species and their respective total alkaloid content were systematically gathered. A comprehensive search encompassing the years from 1983 to 2023 was conducted on both Google Scholar and PubMed databases. Key phrases including seasonal variations, alkaloid content, and LCMS, among others, were employed for the search. Subsequently, a total of 40 articles were meticulously selected for comprehensive review.

Adhatoda vasica, along with its alkaloids vasicine and vasicinone, exhibits fluctuations influenced by seasonal variations. These fluctuations are particularly notable from August to September when there is a marked increase, followed by a subsequent decrease after October. These dynamic changes have been observed and analyzed using thin-layer chromatography (TLC) plates, as reported in reference [1]

Researchers have undertaken an examination of the impact of changes on the phenology and liriodenine in *Annona lutescens* according to season. High variability in these aspects was recorded during the months of October to December, utilizing High-Performance Liquid Chromatography (HPLC) as reported in reference [2]

The study of "Goldenseal" (*Hydrastis Canadensis*) biomass and bioactive alkaloid content in various season, harvesting five-year-old plants in late summer or winter resulted highest alkaloid contents. High-Performance Liquid Chromatography measured the alkaloid content [3]

Seasonal variations in tropane alkaloid content in *Duboisia* were investigated, with a focus on comparing individual plants. Alkaloid content in the leaves was analyzed, results a primary peak during May to June and a secondary peak from September to November [4]

This study included the analysis of seasonal changes in yield index and bacoside A content of *Bacopa monnieri (L.)* collected from wild populations was investigated. The examination of bacoside A and biomass in 14 individuals of Bacopa monnieri was

conducted at the end of each season using High-Performance Liquid Chromatography results that the bacoside A content is maximum during the summer and decreased to its minimum during the winter season [5]

Seasonal variations in alkaloid composition from *Crinum moorei* were examined by Gas Chromatography-Mass Spectrometry for the analysis of 11 Amaryllidaceae alkaloids with a major emphasis on Crinamine. The findings revealed that during the summer season, the leaves exhibited the highest quantities of lycorine and crinine among the various plant organs studied [6]

The seasonal variation of bioactive alkaloid contents in *Macleaya microcarpa* was analyzed by High-Performance Liquid Chromatography focusing on isoquinoline alkaloids results in sanguinarine (SA) and chelerythrine (CHE) reached their highest levels in the spring and gradually decreased thereafter. Alkaloid content in the roots peaked in July, followed by a decrease in concentrations of all alkaloids towards the end of summer. These findings were corroborated through isolation and Thin-Layer Chromatography (TLC) experiments. HPLC analyses confirmed the high alkaloid content in the roots [7]

The *Rhizoma coptidis* seasonal variation of alkaloid contents and the anti-inflammatory activity investigated by combination of fingerprint analysis and chemometrics methods. High-Performance Liquid Chromatography determined highest total alkaloid contents in springtime, additionally in July and late October also higher alkaloid contents compared to other months [8]

The *Lippia origanoides Kunth* antimicrobial and seasonal evaluation of carvacrol chemotype oil by GC-MS analysis results higher percentage carvacrol, thymol, p-cymene, and p-methoxy thymol in April. Furthermore, the oil obtained in May 2012 was characterized by a greater number of identified components [9]

The investigation systematically examined seasonal variations in alkaloid composition derived from Crinum macowanii, contextualized within the framework of Phytochemistry. The analysis was conducted organ-to-organ within the Annonaceae family and was determined using capillary gas chromatography. A total of seven

alkaloids were identified in the flowering stalks and leaves. Notably, the bulbs of C. macowanii were found to possess high alkaloid content [10]

The study focused on *Mentha-piperita L*. and aimed to determine the seasonal variation in menthol alkaloid content by GC-MS analysis results Menthone higher concentration during the warmer vegetation season. In peppermint oil Menthol is a major component exhibited an increase from April to July in first year and third year, followed by a decrease in September. Additionally, in 2011, this compound showed an increase in concentration from April to August [11]

In the context of "Lowbush Blueberry" *(Vaccinium angustifolium*), phytochemical variations were investigated seasonally using the HPLC-DAD analysis method. The study aimed to determine the optimal cultivation time for the highest quality. Seasonal adjustments in the leaves of six phenolic compounds and in stems of twelve compounds were examined, results seasonal variation in *V. angustifolium* extracts within the phenolic profile, and the anti-glycation, that late summer is the optimal gathering time [12]

The seasonal variation of *Origanum majorana* essential oil analysed by GC/MS method results that the presence of nutrients has significant impact on both growth and essential oil levels. Notably, these levels were reduced during the summer months compared to the spring season [13]

To distinguish *Psidium guajava* from guava leaf extract, a combination of 1H NMR and LCMS/MS methods was employed. The findings revealed the most significant variation between the months of May and October, with notable distinctions observed in March as well [14]

A comprehensive investigation was undertaken concerning Alstonia scholaris R. The determination of Alstonine was carried out using Co-chromatography, UV spectra, and IR spectra methods. The findings revealed that the extract obtained from the bark collected during the monsoon season exhibited lower potency compared to winter and summer seasons [15]

In *Murraya euchrestifolia*, the analysis of carbazole alkaloids through 1H NMR spectra revealed interesting seasonal variations. The dimercarbazole alkaloids bis-7-hydroxygirinimbine-A and bis-7-hydroxygirinimbine-B were most abundant in winter season. The alkaloids, pentacyclic terminal methylene-substituted carbazoles, including murrayamines D, F(1), G(2), and H(c), murrayazolidine(7) and murrayazolinines(8), were identified in spring.

Furthermore, in the spring season, the C-3 methyl group of many carbazole alkaloids, along with Murray Amines J, M, and N, undergo oxidation to aldehydes. In the fall season, variations in the carbazole alkaloids including the oxidation of the 2,2-dimethyl pyran ring observed as meliamines I and K, which were alcohols. These variations in alkaloid composition were found to be influenced by the changing seasons [16]

The study on tomato fruits aimed to identify Novel Iso-Esculeoside B using the LC−MS/MS method. The research revealed that when comparing the steroidal glycoalkaloids (SGAs) decided in the examined food items, the tomato present with esculeosides exhibited the greatest concentration range. In this research, the standards for esculeosides successfully isolated from tomato products. Based on LC-MS/MS validated for quantifying the nutritionally important SGA compounds in various food items [17]

Pyrrolizidine alkaloid in *Echium plantagineum* was analyzed by Gas chromatography mass spectrometry and Liquid chromatography mass spectrometry/MS method. The GC-MS approach involved several time-consuming derivatization steps and acid reduction procedures, but it did not yield quantitative results. In contrast, LC-MS/MS proved to be the most suitable method for this analysis. With LC-MS/MS, the extract could be directly analyzed, providing valuable structural information that is essential for toxicity assessment. This method revealed the presence of four compounds, with one of them serving as the base peak in the chromatogram [18]

In the study of *Zanthoxylum* for its quaternary alkaloids using LCMS analysis, the highest recovery for quaternary alkaloids (QAs) was observed at approximately pH 7.0. The LCMS phytochemical analysis included the utilization of solid-phase extraction in the experimental process [19]

In a study involving rat plasma, the aim was to determine the concentrations of five active alkaloids found in kushen injection. LC-MS/MS method was performed for the quantitative analysis of these alkaloids, which included matrine, oxymatrine, sophoridine, oxysophocarpine, and N-methylcytosine. This method allowed for the simultaneous measurement of these alkaloids. Importantly, this methodology was successfully applied in a comparative pharmacokinetic study, which involved intravenous administration of compound kushen Injection (CKI) to both normal and non-small cell lung cancer (NSCLC) nude rats. This research marked the first instance of such a comparative pharmacokinetic investigation [20]

In this study LC−MS/MS and LC-HRMS methods used to analyze the glucose-derived β-carboline alkaloids in food. 15 food items have the higher contents β-carboline alkaloids. Interestingly, among these food items, the top five with the highest concentrations of all the β-carboline alkaloids exclusively processed tomato products. The highest levels of Tan E and Tan F, noted 10 out of the 15 food items as well as DH Tan E/F noted 8 out of the 15 food items with the highest concentrations in tomato-based foods [21]

Identification of the medicinal materials from *Uncaria* species with quantitative analysis by LC-MS/MS system by combines DNA bar coding. This approach allows for both accurate species identification and the quantitative analysis of medicinal components in Uncaria species, enhancing the quality control and authentication of these valuable medicinal materials [22]

The identification of secondary metabolites in *Stachybotrys spp*. has been carried out using LC-MS/MS. Spontaneous dialdehyde/lactone isomerization was observed for certain isolated secondary metabolites during the analysis. Additionally, this research marked the first quantitative investigation of novel stachybotrychromenes, providing valuable insights into the chemical composition of these compounds in *Stachybotrys spp* [23]

In the identification of phenolic content in *Mexican lupine* species, the LCMS method was employed. The study aimed to establish relationships between various *Lupine* species. These relationships were calculated based on the flavonoids,

and glycol conjugate content found in both the roots and leaves of the studied plants. This approach allowed for a comprehensive assessment of the phenolic composition across different lupine species [24]

When using targeted LCMS analysis to study plant secondary metabolites, it was observed that a higher number of flavonoid aglycones could be identified and quantified. This suggests that LCMS is a valuable analytical method for detecting and characterizing flavonoid aglycones in plant samples [25]

The study focused on identifying seasonal variations in secondary metabolites of medicinal plants using the DPPH and FRAD methods. Specifically, the research investigated the impact of seasonal variation on the antioxidant and gastroprotective activities of licorice extracts (LE). The findings revealed that samples collected in May and November exhibited the most favorable effects. Additionally, major constituents present in the extracts varied by season. In February and May, liquiritin and glycyrrhizin were the dominant compounds, whereas in November, glabridine and glabrene had the highest relative proportion. These results indicate that the composition and efficacy of licorice extracts can vary significantly based on the time of harvest, highlighting the importance of considering seasonal factors in medicinal plant research [26]

In the identification of mycotoxins and other secondary metabolites in food crops using LC-ESI-MS/MS, the study yielded results obtained by calculating relative standard deviation for repeatability and relative standard deviation for within-laboratory reproducibility (RSDWLR) in seven separate samples and seven technical replicates of the same sample. The findings of the study suggest that when transferring the method to a new matrix, the RSDr (Relative Standard Deviation for repeatability) should take precedence. This indicates that repeatability within the same laboratory is more crucial for maintaining method consistency when applying it to a different matrix. Furthermore, the research also considered the estimation of the contribution of matrix effects on method uncertainty in a new matrix, emphasizing the importance of understanding and accounting for the impact of matrix effects when working with LC-ESI-MS/MS for mycotoxin and secondary metabolite analysis in food crops [27]

The study focused on “Lotus Flower” plant alkaloids and their melanogenesis inhibitory activity, which were determined using LCMS. The research involved measuring the two different regions alkaloids (1–10) specifically NN-1 in Thailand and NN-5 in Taiwan. The study demonstrated that the assay used in the research was highly reproducible, precise, and readily applicable for assessing the quality of “lotus flower” extracts. Furthermore, the results indicated that among the alkaloids (1–10), N-methylcoclaurine was the richest constituent in the extracts. This finding contributes to better understanding of the composition and potential biological activities of “lotus flower” alkaloids [28]

The study aimed at identifying Veratrum alkaloids in *Veratrum Aqua* poisonings using the LC–MS/MS method. The research involved validating the method for the quantification of jervine and proA and the qualitative detection of proB. This approach allows for the precise identification and quantification of specific alkaloids in *Veratrum Aqua* poisonings, which is crucial for toxicological analysis and management in poisoning cases [29]

The study on *Desmodium gangeticum* investigated seasonal variations in cellular characteristics and chemical contents using HPTLC. The moisture content in the plants was found highest in the rainy season, gradually decreasing as progressed. This variation in moisture content coincided with the growth stages of the plant, with the lowest percentage observed in the young stage and the highest percentage in the fully flowered stage. Notably, the plants collected from wild, hilly areas demonstrated superior characteristics compared to those from other regions in the study [30]

This study investigated seasonal variations in the medicinal herb *Stylophorum lasiocarpum*, using liquid chromatography to analyze nineteen alkaloids in the aerial parts and roots of one- and two-year-old plants. The roots had significantly higher alkaloid levels, with peak concentrations during the middle of the growth season. Quaternary benzylisoquinoline alkaloids (QBAs) were particularly high in second-year roots in July. Seasonal variations were observed in the aerial parts, with sanguinarine (SA) and chelerythrine (CR) dominating in the second year, while protopine (PRO) exhibited the opposite trend. The study provides valuable insights into alkaloid

variations in S. lasiocarpum during its growth stages, which are important for understanding its medicinal properties and drug substance collection [31]

High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC) quantified alkaloid content is expressed as aconitine equivalent. In *Aconitum nagarum* root, alkaloid content was assessed during the months of July, August, October, and November. highest levels observed in November. In the case of *Aconitum elwesii* root, The highest levels were significantly observed in November. The analysis of *Aconitum elwesii* leaf crude alkaloid content maximum content was recorded during the pre-flowering stage in August. It is noteworthy that the alkaloid content in plants undergoes dynamic changes throughout their growth period. Both Aconitum species, alkaloid content was higher in the roots than the leaves. It is essential to acknowledge that plant alkaloid contents can vary based on factors such as the species, geographical origin, timing of harvest, and, critically, the method and adequacy of processing [32]

The study revealed significant variation in alkaloid content, particularly *mitragynine* (MG), among the sampled “kratom specimens”. In the first collection phase, MG content ranged from a maximum in June (late summer) to a minimum in October (rainy season). In the second collection phase, MG content exhibited variability across different periods. The data strongly suggested a connection between the season and MG content, with MG content generally higher during late summer and lower during the rainy season. This indicates a clear seasonal fluctuation in alkaloid production. Furthermore, it was observed that geographical locations influenced the variation in MG content in “kratom” leaves. However, this geographical variation did not have an impact on the color of the leaf veins [33]

High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) was used to quantify alkaloid content collected from six locations in Turkey at different growth stages. Interestingly, phenolic content was not affected by the growth season, but higher bulb temperatures and lower leaf temperatures were associated with increased galanthamine and lycorine production. The study aims to determine optimal conditions for cultivating summer snowflakes, including genotype selection and

harvesting timing to maximize galantamine and lycorine content. Environmental factors and plant characteristics play crucial roles in secondary metabolite production [34]

This study investigated the influence of plant genotype, seasonal variations, temperature, and soil type on alkaloid levels (galanthamine and lycorine) and phenolic compounds in summer snowflake plants. The study found that bisindole alkaloids were highest in summer, while vindoline peaked during winter and spring. It also showed that monomeric TIAs were mostly in top-level leaf pairs, while bisindole alkaloids were mainly in the middle-level pairs. Using qRT-PCR, the study examined 10 genes, revealing higher expression in winter, except for sgd. As leaves progressed from the top to the lower level, gene expression decreased. This study offers valuable insights for harvesting *C. roseus* leaves to optimize terpenoid indole alkaloid recovery [35]

This study explored how the changing seasons in the "Brazilian savanna" (*Cerrado*) influence the secondary metabolites in *Duguetia furfuracea*, a plant used in traditional medicine. Over a year, researchers collected leaves monthly and analyzed them for secondary metabolites using advanced techniques. The study found that alkaloids were consistently present throughout the year, with variations in their composition. In contrast, flavonoids accumulate during the rainy season. The dew point temperature was identified as a key factor affecting metabolite changes. This research sheds light on the environmental impact of the chemical composition of *Duguetia furfuracea* and its potential applications in traditional medicine due to its accumulation of valuable secondary metabolite [36]

The study was a detailed analysis of 30 *Buxus sempervirens* extracts using advanced chromatography and mass spectrometry techniques to investigate the temporal variations in alkaloid profiles of two *Buxus* varieties and their leaves and twigs. They employed Principal Component Analysis (PCA), multivariate data analysis methods to reveal seasonal differences in phytochemical composition and distinctions between the two varieties and plant organs. Our models identified eighteen specific compounds indicative of seasons, varieties, or organs, and we proposed fragmentation pathways for known and new alkaloids. This study provides valuable insights for identifying characteristic compounds and optimal harvest times, benefiting future research [37]

This study investigated the Flindersia genus, which consists of 17 tree species mainly found in Australia. It found that Flindersia species adapted to drier, arid environments produced more alkaloids compared to those in rainforests. The research revealed a strong connection between environmental aridity and alkaloid diversity. Rainforest species showed more chemical similarities among themselves than with the four species adapted to semi-arid and arid regions. These findings suggest that the transition of *Flindersia* species from rainforests to drier environments has led to the development of unique alkaloid diversity. This makes plants in arid and semi-arid Australian regions a potentially valuable source of unexplored specialized metabolites. The study also found a rich diversity of alkaloids across the *Flindersia* species, with several new alkaloids identified, including furoquinoline, indole, b-carboline, pyranoquinoline, and alkyl quinolone classes. These results contribute to our understanding of how environmental adaptation can influence the chemical profiles of plant species [38]

This study aimed to analyze seasonal variations in pilocarpine (PIL) and epiisopiloturine (EPI) content in three *P. microphyllus* populations over a year, including dry and rainy seasons. PIL was the predominant alkaloid, with varying levels across populations, except in September. S01 had significantly higher PIL content than S02 and S03. PIL content decreased during the rainy season. EPI content was notably lower in S01 throughout the year, making it a less suitable source for EPI extraction. Molecular and morphological analyses using ISSR markers confirmed these findings, showing the potential of a multidisciplinary approach in both industry and conservation [39]

The study conducted to visualize the eight alkaloids in freeze-fixed stems of *P. amurense* distribution of during both seasons fall and summer, using cryo-TOF-SIMS/SEM. It also quantified alkaloid content with HPLC, revealing seasonal variations. The results showed different alkaloid levels in specific stem positions, suggesting roles in plant physiology and responses to environmental changes. This research contributes to understanding alkaloid biosynthesis and in plant functions, offering valuable insights [40]

RESULT & DISCUSSION

Seasonal and alkaloid variations in the study were interconnected with the specific growth stage of the plant differed with the season. This difference growth stage had a direct impact on the quality of the production of essential oil. Changing seasons influenced the plant's growth and development, which in turn affected the composition and quantity of alkaloids present in the plant. These variations in alkaloid content were then reflected in the essential oil produced, highlighting the intricate relationship between seasonal changes and the quality of essential oils.

It's interesting to note that the study found 16 identified alkaloids out of the 40 species studied by analyzing LCMS and 4 using HPLC, 4 using GCMS and rest of them are various analytical techniques. The research also concluded that seasonal variation is closely linked to the alkaloid composition of the plants, which sheds light on the dynamic nature of plant alkaloids and their sensitivity to seasonal changes. It is important to know how these findings could affect industries that rely on plant alkaloids.

In plants, the concentration of active principles tends to be highest during specific seasons, making it the optimal period for drug collection, as the yield is significantly higher.

LCMS (Liquid Chromatography-Mass Spectrometry) is a prominent analytical technique known for its exceptional precision, sensitivity, and specificity, surpassing HPLC (High-Performance Liquid Chromatography) in these aspects. Consequently, LCMS finds extensive applications in various fields, including research, pharmaceutical analysis, food analysis, and more, owing to its ability to provide accurate and detailed information about complex compounds and substances.

CONCLUSION

The present study undertakes a rigorous comparative analysis to investigate the variances in the collection of a diverse range of plant alkaloids, specifically examining the disparities between off-season and on-season harvests. This research aims to shed light on the temporal dynamics and variations in alkaloid composition within plants, contributing to a deeper understanding of their ecological and pharmacological

significance. The outcomes of this investigation will enhance our knowledge of alkaloid biosynthesis and may have implications for the development of plant-derived pharmaceuticals and therapeutics.

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