BIODIVERSITAS
Volume 25, Number 12, December 2024
Pages: 4971-4977

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251234

Characterizing the chemical composition and variations of several medicinal plant leaves using ^{1}H NMR Spectroscopy

HANAN ALMAHASHEER♥
Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University. Dammam 31441-1982, Saudi Arabia.
Tel.: +966-133337329, ♥email: halmahasheer@iau.edu.sa

Manuscript received: 24 September 2024. Revision accepted: 22 December 2024.

Abstract. *Almahasheer H. 2024. Characterizing the chemical composition and variations of several medicinal plant leaves using ^{1}H NMR Spectroscopy. Biodiversitas 25: 4971-4977.* This study utilizes ^{1}H NMR spectroscopy to characterize the chemical composition of nine medicinal plant leaves, focusing on spectral intensity differences across four distinct regions based on organic functional groups. The analysis revealed that the carbohydrate region (50-110 ppm) had the highest spectral intensity at 0.655374, indicating a predominant presence of cellulose. The aliphatic region (0-50 ppm) displayed an intensity of 0.222092, while the aromatic region (110-150 ppm) was lower at 0.047311. The carboxyl region (150-200 ppm) exhibited an intensity of 0.075224, reflecting lower concentrations of carboxyl compounds. Significant variations in chemical group ratios were observed among the samples. *Circaea lutetiana* exhibited the highest carbohydrate ratio (0.82), while *Peganum harmala* showed the lowest (0.48). Aromatic ratios were notably higher in *P. harmala* and *Crataegus* sp. (0.10) compared to *Cassia senna* and *Ficus carica* (0.01). Additionally, *P. harmala* had the highest aliphatic ratio (0.35), while *C. senna* exhibited the highest carboxyl ratio (0.12). The robust application of NMR spectroscopy enhances our understanding of phytochemical diversity. It highlights the diverse chemical profiles and potential functional adaptations of the studied plants, suggesting significant implications for their medicinal applications. This research aligns with Saudi Vision 2030, a vision that we, as a scientific community, seek to boost the agricultural sector's role in the economy while encouraging sustainable practices and biodiversity conservation. By elucidating the molecular characteristics of these plants, we provide critical insights into their ecological roles and responses to environmental changes. Ultimately, this study advances sustainable resource management and fosters innovative applications in healthcare, nutrition, and the preservation of plant biodiversity in Saudi Arabia.

Keywords: Carbohydrates, *Circaea lutetiana*, NMR spectroscopy, *Peganum harmala*, phytochemical diversity

INTRODUCTION

Traditional plant herbs have been an integral part of human life and culture for thousands of years. Herbal medicine practices have been documented in ancient texts from cultures around the world, including Ayurveda in India, traditional Chinese medicine, and Egyptian, Greek, and Roman medicine (Abourashed and El-Alfy 2016). The World Health Organization estimates that 80% of people globally rely on herbal medicines for their primary healthcare (WHO 2019; Emwas et al. 2021).

Studies have identified numerous bioactive compounds in medicinal plants that exhibit anti-inflammatory, antimicrobial, and antioxidant properties (Rodríguez-Yoldi 2021; AlShwyeh and Almahasheer 2022). For instance, compounds in garlic and ginger have been linked to cardiovascular health benefits and digestive support, respectively (Ajanaku et al. 2022). As well as a number of integrative medicine programs in hospitals and clinics (Mortada 2024), and spiritual contexts (Hausermann 2021). In addition, the sustainability of herbal practices is crucial in an era of climate change, where the loss of biodiversity threatens the availability of traditional medicinal resources (Fayiah et al. 2024). Furthermore, the globalization of herbal medicine has increased interest in ethnobotanical research, leading to the discovery of novel compounds and therapeutic applications (Domingo-Fernández et al. 2023). Beyond medicinal uses, herbs and edible plants provide nutritional and culinary benefits (Almahasheer 2020). This led to unique regional cuisines and food traditions still popular today (Pieroni et al. 2021; Almahasheer 2024). The global trade and cultivation of botanical commodities like tea, coffee, spices, and essential oils have shaped economies and history (Pilcher 2023).

Analysis of the wide range of phytochemicals in herbs continues to reveal their pharmacological activities and mechanisms of action. Advanced analytical techniques are necessary to characterize the complex components of plant extracts fully. Nuclear magnetic resonance (NMR) spectroscopy has become an indispensable tool for structural elucidation of bioactive compounds from herbs and other natural sources (Zhao et al. 2022). NMR provides qualitative and quantitative data that aids identification of novel chemical entities for drug discovery research (Emwas et al. 2021).

Carbon fingerprints of solid materials are effectively analyzed using ^{1}H NMR, which offers several advantages over other spectroscopic methods. Unlike liquid-state ^{1}H NMR, this technique requires less time and provides direct insights into the carbon skeleton without the need for sample dissolution. Additionally, it allows for high-resolution signal detection due to significant chemical shifts associated with solid materials. A notable research gap exists concerning the need to explore the interactive

effects within herbal mixtures and the underutilization of advanced analytical techniques like ^{1}H NMR to elucidate the complex phytochemical profiles of these plants. This study aims to address this gap by systematically analyzing a collection of plant samples using the efficient ^{1}H NMR technique. By doing so, the goal is to unravel the intricate chemical profiles of these botanical specimens, thereby enhancing our understanding of their phytochemical composition.

In line with Saudi Vision 2030, employing advanced techniques like ^{1}H NMR is essential for expanding our understanding of plant materials. This precise analytical method aids scientists in uncovering the chemical composition of various plants and herbs, revealing new insights into their pharmacological properties and potential applications across multiple industries. Such research aligns with the vision's objectives of fostering innovation, promoting research, and advancing economic diversification and sustainable development within the Kingdom. The application of this advanced spectroscopic method not only deepens the understanding of the phytochemical composition of these plants but also explores their potential medicinal, nutritional, and industrial applications. This research contributes significantly to the scientific discourse on plant chemistry and supports broader goals of advancing knowledge and innovation. Ultimately, the findings may lead to more sustainable and effective uses of plant resources, bolstering the Kingdom's aspirations for a diversified and sustainable economy.

MATERIALS AND METHODS

Plant collection and experimental details

Nine medicinal plants were gathered from a neighborhood market in April 2024, Dammam, Saudi Arabia (Table 1 and Figure 1). The samples were ground using a pre-cooled pestle and mortar under liquid nitrogen, and the dried samples were carefully prepared for NMR analysis following (Kim et al. 2010). Analysis performed on solids state probe. The samples were finely ground, then packed evenly into a 4 mm zirconia rotor, and sealed at the open end with a Vespel cap. The spectra were recorded using a cross-polarization (CP) pulse program from Bruker pulse library with a recycle delay time of 5 s as described in (Alkordi et al. 2015; Chisca et al. 2015). The spectral data were collected and analyzed using Bruker Topspin 4.1.4 software (Bruker BioSpin, Rheinstetten, Germany).

The NMR experiments were conducted using a Bruker Avance III 400 MHz spectrometer equipped with a triple-resonance 4 mm Bruker MAS probe (BrukerBioSpin, Rheinstetten, Germany, Figure 2) at King Abdullah University of Science and Technology (KAUST).

Chemical shift analysis

For data analysis, the ^{1}H NMR spectra of these samples were divided into four regions according to their organic functional groups, with amendments following (Hatcher et al. 1983; Chung et al. 2012; Dupree et al. 2015; Al-Faiyz 2017). These regions were the aliphatic region (0-50 ppm), the carbohydrate (cellulose) region (50-110 ppm), the aromatic region (110-150 ppm), and the carboxyl region (150-200 ppm).

Statistical analysis

The sample differences were tested using general linear models, descriptive statistics (Mean, Std Error, Lower 95%, Upper 95%), and Tukey's HSD (honest significant difference) post-hoc test to evaluate pairwise differences. JMP v13 was used to compute all statistics.

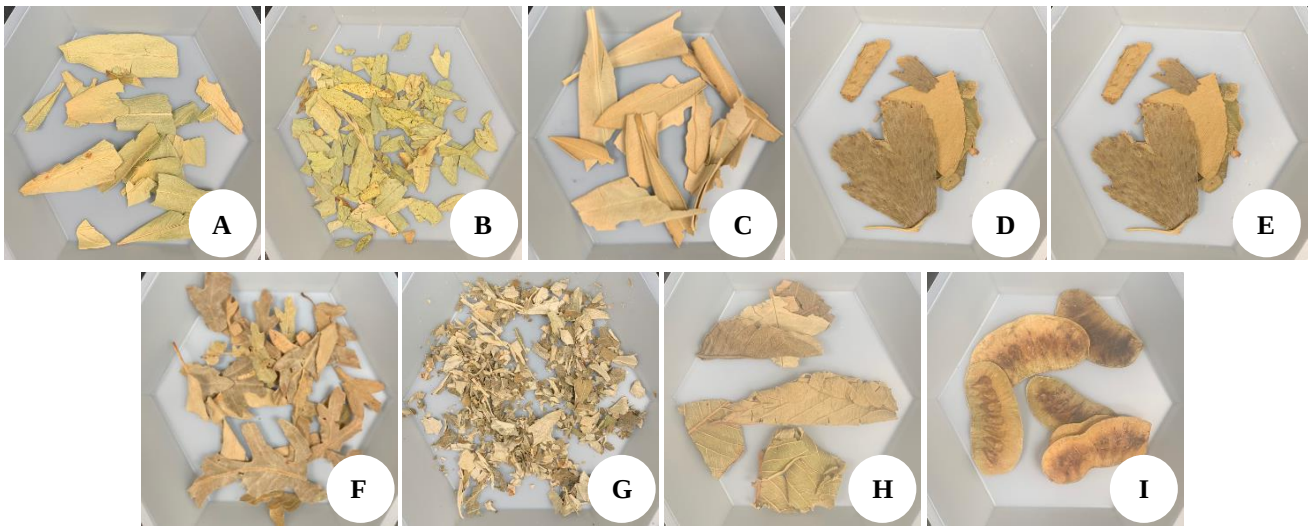

Figure 1. Displays the assortment of various herbal plant samples: A. *Peganum harmala*; B. *Cassia senna*; C. *Salix* sp.; D. *Ginkgo biloba*; E. *Ficus carica*; F. *Crataegus* sp.; G. *Tilia* sp.; H. *Psidium guajava*; I. *Circaea Lutetia*

Table 1. Herbal herb utilized in this study

Number	Common name	Scientific name	Arabic name	Family name	Part assayed
1	Wild rue	*Peganum harmala* L.	حرمل	Zygophyllaceae	Leaves
2	Alexandrian Senna	*Cassia senna* L.	سنا مكي	Fabaceae	Leaves
3	Willow	*Salix* sp.	صفصاف	Salicaceae	Leaves
4	Ginkgo	*Ginkgo biloba* L.	الجنكة	Ginkgoaceae	Leaves
5	Ficus	*Ficus carica* L.	تين	Moraceae	Leaves
6	Hawthorn	*Crataegus* sp.	زعرور	Rosaceae	Leaves
7	Linden	*Tilia* sp.	زيزفون	Malvaceae	Leaves
8	Guava	*Psidium guajava* L.	جوافه	Myrtaceae	Leaves
9	Enchanter's nightshade	*Circaea lutetiana* L.	عشرق	Onagraceae	Leaves

Figure 2. Nuclear Magnetic Resonance (NMR) Bruker Avance III 400 MHz spectrometer

RESULTS AND DISCUSSION

This study's findings on plant leaves using Nuclear Magnetic Resonance (NMR) spectroscopy focused on analyzing spectral intensity differences in four distinct regions based on their organic functional groups. The NMR spectra obtained from the plant leaves were analyzed by dividing them into these four regions based on their chemical shifts and organic functional groups (Figure 3).

Aliphatic region (0-50 ppm): The spectral intensity in the aliphatic region was measured as 0.222092. This region corresponds to chemical shift values between 0 ppm and 50 ppm and includes resonances associated with aliphatic carbon groups, such as methyl (CH_3) and methylene (CH_2) groups. The measured intensity suggests a relative concentration of aliphatic compounds associated with specific biological processes or pathways.

Aromatic region (110-150 ppm): The spectral intensity in the aromatic region was determined as 0.047311. This region covers chemical shift values between 110 ppm and 150 ppm (Smith and Doe 2020) and exhibits resonances arising from aromatic compounds, including phenylalanine, tyrosine, and tryptophan residues in proteins. The measured intensity reflects the relative abundance of aromatic compounds within the plant leaves.

Carbohydrate (cellulose) region (50-110 ppm): The spectral intensity in the carbohydrate region was 0.655374, the highest among the four regions. This region corresponds to chemical shift values between 50 ppm and 110 ppm and encompasses resonances associated with cellulose, a major component of plant cell walls (Larsen and Engelsen 2015). The measured intensity indicates the highest relative concentration of carbohydrate compounds, specifically cellulose, among the functional groups analyzed.

Carboxyl region (150-200 ppm): The spectral intensity in the carboxyl region was determined as 0.075224. This region covers chemical shift values between 150 ppm and 200 ppm and includes resonances associated with carboxyl groups, such as those found in acetate and protein carbonyls (Zhang et al. 2021b). The measured intensity reflects the relative concentration of carboxyl compounds within the plant leaves (Qian et al. 2015).

The mean value of spectral intensities indicates that the carbohydrate region had the highest intensity (R^2=0.93, F=150.8, P<0.0001), followed by the aliphatic region and then the aromatic and carboxyl regions. This suggests that carbohydrate compounds, specifically cellulose, were the most abundant among the functional groups analyzed in the plant leaves (Table 2; Figures 4.A and 4.B, Tukey HSD post-hoc test, P<0.05).

The analysis of the plant samples revealed significant variations in the ratios of different chemical groups. Sample I: *Circaea lutetiana* exhibited the highest carbohydrate ratio (0.82), indicating a statistically significant increase in the relative abundance of carbohydrates compared to the other samples (p<0.05). In contrast, sample (A: *Peganum harmala*) displayed the lowest carbohydrate ratio (0.48). Regarding the aromatic ratios, samples (A: *P. harmala* and F: *Crataegus* sp.) demonstrated significantly higher values (0.10) when compared to samples (B: *Cassia senna* and E: *Ficus carica*), which had the lowest aromatic ratios (0.01). The aliphatic ratio was significantly higher in sample (A: *P. harmala* 0.35) compared to sample (I: *C. lutetiana* 0.10), indicating a distinct compositional difference in terms of aliphatic compounds. Furthermore, samples (B: *C. senna* exhibited the highest carboxyl ratio (0.12), while samples 6: *Crataegus* sp.) and I: *C. lutetiana* displayed significantly lower values (0.05) for this chemical group. These pronounced differences in the chemical group ratios among plant samples highlight the diverse compositions and potential variations in the chemical properties of the specimens (Figure 5).

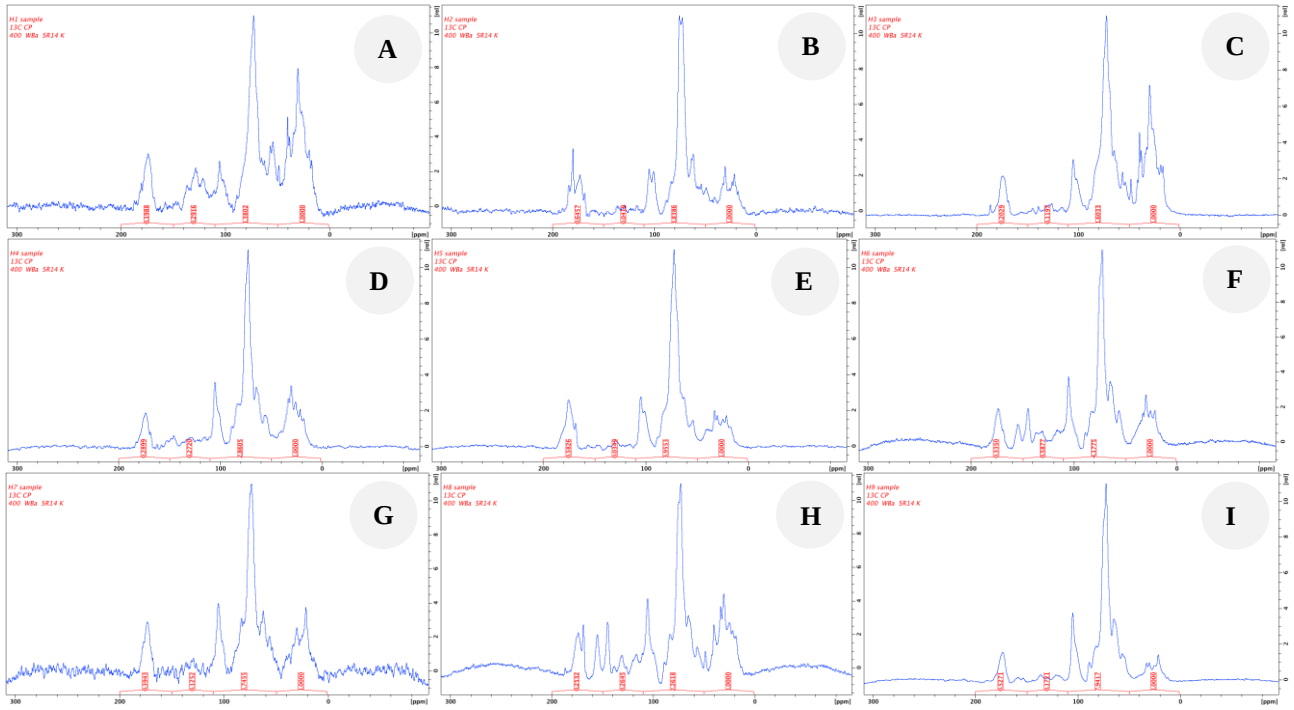

Figure 3. Differences in spectral intensity in the entire chemical shift regions. A. *Peganum harmala*; B. *Cassia senna*; C. *Salix* sp.; D. *Ginkgo biloba*; E. *Ficus carica*; F. *Crataegus* sp.; G. *Tilia* sp.; H. *Psidium guajava*; I. *Circaea lutetia*

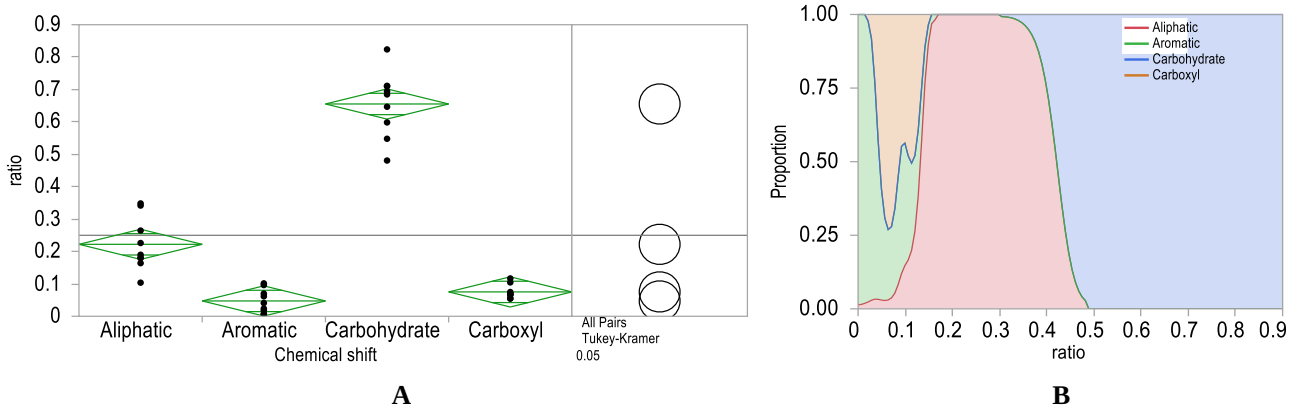

Figure 4. A. Oneway analysis of ratio by chemical shift; B. Proportion of densities. The figure was drawn using JMP v13

Table 2. Mean and ±SE for One-way ANOVA for chemical shift ratio of the nine collected samples. And Tukey HSD post hoc tests for differences between groups, Levels not connected by the same letter are significantly different

Level	Number	Mean	Std error	Lower 95%	Upper 95%	Tukey-Kramer HSD
Aliphatic	9	0.222092	0.02287	0.17550	0.26868	b
Aromatic	9	0.047311	0.02287	0.00072	0.09390	c
Carbohydrate	9	0.655374	0.02287	0.60879	0.70196	a
Carboxyl	9	0.075224	0.02287	0.02864	0.12181	c

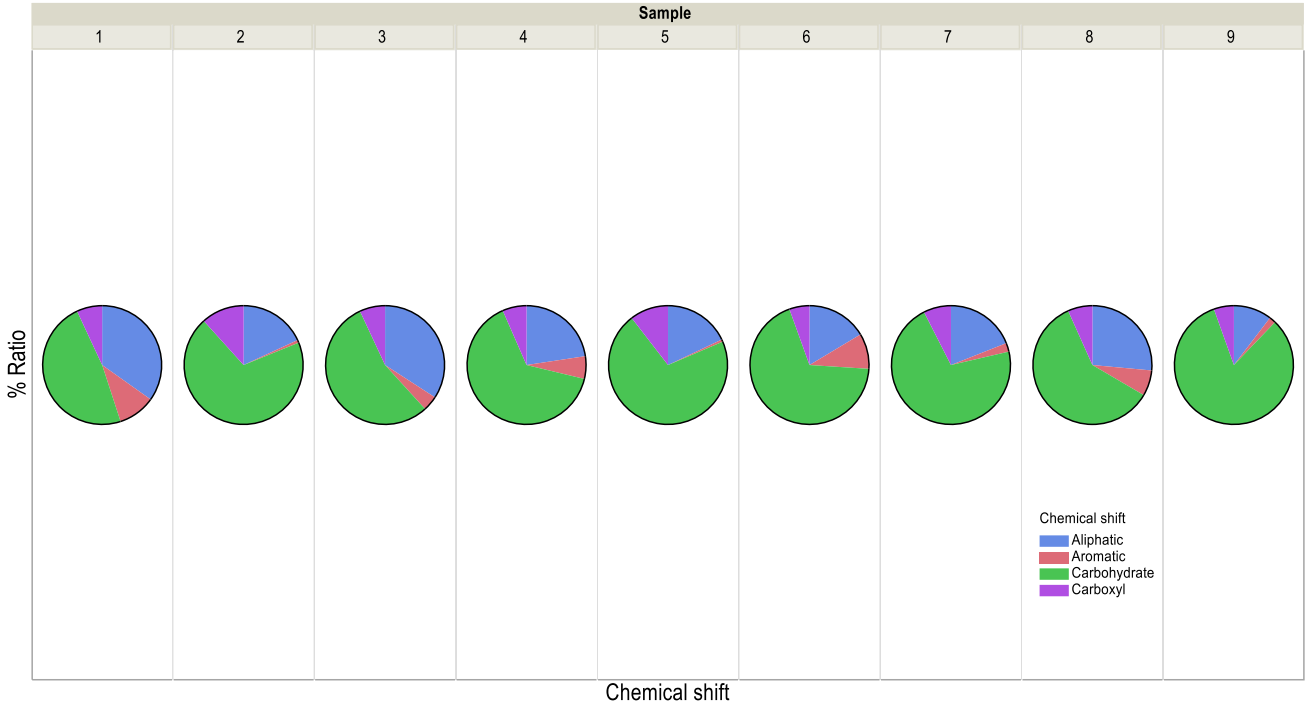

Figure 5. Percent ratio of chemical shifts in the herbal plants examined

Discussion

The analysis of spectral intensities in plant samples highlights the complex biochemical composition of various species, particularly focusing on carbohydrates, aromatic compounds, aliphatic compounds, and carboxyl groups.

The mean value of spectral intensities revealed that the carbohydrate region had the highest intensity, specifically cellulose. Cellulose is a major component of plant cell walls (Zhang et al. 2021a), and is known for its structural role in providing strength and rigidity to plant tissues. This polysaccharide is crucial for the formation of anisotropic cell walls, allowing plants to manipulate the size and shape of their cells as they grow (Cosgrove 2024). The orientation of cellulose microfibrils enables plants to modify the mechanical properties of their tissues, facilitating growth and organ movement. Furthermore, the physical and chemical properties of cellulose can influence the plant's interactions with various biotic and abiotic factors, such as herbivory and water availability. The high abundance of cellulose in the carbohydrate region indicates the importance of this compound in the overall composition of plant leaves.

Variations in chemical ratios

The analysis of plant samples further revealed significant variations in the ratios of different chemical groups among the samples. Sample I: *C. lutetiana* exhibited the highest carbohydrate ratio (0.82), this suggests that *C. lutetiana* leaves may have a higher concentration of carbohydrates, potentially reflecting their role in energy storage or other metabolic processes. On the other hand, sample A: *P. harmala* displayed the lowest carbohydrate ratio (0.48), indicating a relatively lower abundance of carbohydrates in these leaves. The variation in carbohydrate ratios among the samples may be attributed to differences in plant species (Almahasheer et al. 2016a,b; Chen and Chen 2021), developmental stages or environmental factors (Zhang et al. 2024). The broadest variation in phenological patterns is linked to the storage of starch or sucrose as a main reserve carbohydrate (Almahasheer et al. 2016a,b; Roxas et al. 2021). This complex interplay may explain the observed differences in carbohydrate composition and their functional implications in plant physiology.

Aromatic compounds and ecological roles

In terms of the aromatic ratios, samples A: *P. harmala* and F: *Crataegus* sp. demonstrated significantly higher values (0.10) compared to samples B: *C. senna* and E: *F. carica*, which had the lowest aromatic ratios (0.01). Aromatic compounds, including phenylalanine, tyrosine, and tryptophan residues, are known for their involvement in various physiological processes, such as defense mechanisms and signaling pathways (Parthasarathy et al. 2018; Chowdhary and Tank 2023). The higher aromatic ratios in *P. harmala* and *Crataegus* sp. leaves suggest a potentially greater presence of aromatic compounds, which may be associated with specific biological functions or adaptations of these plants. This phenomenon could indicate an evolutionary advantage in their respective environments, allowing them to fend off herbivores or pathogens better. Additionally, the presence of aromatic compounds can influence plant interactions with pollinators and microorganisms, further enhancing their ecological roles (Vannette 2020). Phenolic compounds in plants exhibit strong antioxidant properties, helping to neutralize free radicals and protect against oxidative stress, as well as possessing anti-inflammatory effects, contributing to the

reduction of inflammation and related diseases (Yu et al. 2021).

Aliphatic compounds and metabolic processes

The aliphatic ratio was significantly higher in sample A: *P. harmala* (0.35) compared to Sample I: *C. lutetiana* (0.10), indicating a distinct compositional difference in terms of aliphatic compounds. Aliphatic compound groups are involved in diverse metabolic processes, such as lipid metabolism and membrane function (Zill and Cheniae 1962; Schmid 2021). The higher aliphatic ratio in *P. harmala* leaves suggests a relatively higher abundance of aliphatic compounds, potentially reflecting specific metabolic pathways or adaptations in this plant species. Understanding these metabolic pathways can provide insights into the ecological roles and adaptive strategies of these plants. For instance, aliphatic compounds may play crucial roles in plant defense against herbivory or environmental stressors (Mithöfer and Boland 2012), further highlighting their significance in plant resilience.

Carboxyl groups and physiological functions

Furthermore, Sample B: *C. senna* exhibited the highest carboxyl ratio (0.12), while samples F: *Crataegus* sp. and I: *C. lutetiana* displayed significantly lower values (0.05) for this chemical group. Carboxyl groups, such as those found in acetate and protein carbonyls, are involved in various biochemical processes, including energy production and protein modification (Akharume et al. 2021). The higher carboxyl ratio in *C. senna* leaves suggests a relatively higher concentration of carboxyl compounds, potentially indicating specific metabolic activities or physiological functions in this plant. This may also reflect adaptations to environmental stressors, as carboxyl-containing compounds are often implicated in stress responses. For example, increased carboxyl group concentrations can facilitate the regulation of metabolic pathways under adverse conditions (Nabavi et al. 2020).

Implications for conservation and future research

These pronounced differences in the chemical group ratios among the plant samples highlight the diverse compositions and potential variations in the chemical properties of the analyzed specimens. The variations in chemical group ratios may be attributed to genetic factors, environmental conditions, or other factors influencing plant metabolism and physiology (Yadav et al. 2020; Mehalaine and Chenchouni 2021). Such diversity underscores the need for further investigation into how these variations can affect ecological interactions and plant health. Understanding these relationships is vital for conservation efforts and the sustainable use of plant resources.

Finally, further investigations integrating metabolomics and transcriptomics approaches are warranted to elucidate the underlying mechanisms and biological significance of these compositional differences. Employing advanced analytical techniques can enhance our understanding of the metabolic networks within plants, revealing how they adapt to their environments while potentially leading to novel applications in agriculture and medicine. Comparative studies across different ecosystems could provide valuable insights into how environmental factors shape plant chemical compositions and their functional roles within ecosystems. Such research is essential for developing strategies that promote biodiversity conservation while leveraging plant resources sustainably for human benefit. By advancing our understanding of the complex interplay between biochemical composition, environmental variables, and ecological roles, we can cultivate a more nuanced appreciation of the intricate web of life that sustains both plant species and the ecosystems they inhabit. Such knowledge not only enriches our scientific understanding but also informs conservation efforts and sustainable resource management practices, ultimately contributing to the resilience of ecosystems in the face of anthropogenic pressures. The interplay of these factors underscores the necessity for interdisciplinary approaches in ecological research, as they are vital for addressing global challenges related to biodiversity loss and environmental sustainability.

In conclusion, these findings demonstrate the utility of NMR spectroscopy in characterizing the chemical composition and metabolic variations in plant leaves, focusing on the spectral intensities and ratios of different chemical groups. The results revealed that carbohydrate compounds, specifically cellulose, were the most abundant among the functional groups analyzed in the plant leaves. Moreover, these insights align with the goals of Vision 2030, which emphasizes the sustainable use of natural resources and the promotion of biodiversity. Furthermore, significant variations in the ratios of aliphatic, aromatic, carbohydrate, and carboxyl compounds were observed among the plant samples. By understanding these variations, we can better inform conservation strategies and sustainable agricultural practices that are essential for achieving the ambitious objectives outlined in Vision 2030. These findings provide valuable an important insight into the diverse compositions and potential functional differences of plant leaves, highlighting the importance of understanding their chemical properties for various biological and ecological applications.

ACKNOWLEDGEMENTS

I would like to thank Abdelhamid Amwas for his help with the analysis.

REFERENCES

Abourashed EA, El-Alfy AT. 2016. Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans* Houtt.). Phytochem Rev 15: 1035-1056. DOI: 10.1007/s11101-016-9469-x.

Ajanaku CO, Ademosun OT, Atohengbe PO, Ajayi SO, Obafemi YD, Owolabi OA, Akinduti PA, Ajanaku KO. 2022. Functional bioactive compounds in ginger, turmeric, and garlic. Front Nutr 9: 1012023. DOI: 10.3389/fnut.2022.1012023.

Akharume FU, Aluko RE, Adedeji AA. 2021. Modification of plant proteins for improved functionality: A review. Compr Rev Food Sci Food Saf 20 (1): 198-224. DOI: 10.1111/1541-4337.12688.

Al-Faiyz YS. 2017. CPMAS ^{13}C NMR characterization of humic acids from composted agricultural Saudi waste. Arab J Chem 10: S839-S853. DOI: 10.1016/j.arabjc.2012.12.018.

Alkordi MH, Haikal RR, Hassan YS, Emwas AH, Belmabkhout Y. 2015. Poly-functional porous-organic polymers to access functionality-CO_2 sorption energetic relationships. J Mater Chem A 3 (45): 22584-22590. DOI: 10.1039/C5TA05297A.

Almahasheer H, Duarte CM, Irigoien X. 2016a. Nutrient limitation in Central Red Sea mangroves. Front Mar Sci 3: 271. DOI: 10.3389/fmars.2016.00271.

Almahasheer H, Duarte CM, Irigoien X. 2016b. Phenology and growth dynamics of *Avicennia marina* in the Central Red Sea. Sci Rep 6: 37785. DOI: 10.1038/srep37785.

Almahasheer H. 2020. Nutrition in herbal plants used in Saudi Arabia. Scientifica 2020: 6825074. DOI: 10.1155/2020/6825074.

Almahasheer H. 2024. Unveiling the total catechin profile in tea leaves: A novel one-step extraction method empowered by UPLC-IDX-Orbitrap mass spectrometry. Braz J Biol 84: e283740. DOI: 10.1590/1519-6984.283740.

AlShwyeh H, Almahasheer H. 2022. Glucose content of 35 Saudi Arabian date fruits (*Phoenix dactylifera* L.). J Saud Soc Agric Sci 21 (6): 420-424. DOI: 10.1016/j.jssas.2021.11.004.

Chen X, Chen HY. 2021. Plant mixture balances terrestrial ecosystem C:N:P stoichiometry. Nat Commun 12 (1): 4562. DOI: 10.1038/s41467-021-24889-w.

Chisca S, Duong P, Emwas AH, Sougrat R, Nunes SP. 2015. Crosslinked copolyazoles with a zwitterionic structure for organic solvent resistant membranes. Polym Chem 6: 543-554. DOI: 10.1039/C4PY01293C.

Chowdhary VA, Tank JG. 2023. Biomolecules regulating defense mechanism in plants. Proc Natl Acad Sci India Sect B: Biol Sci 93 (1): 17-25. DOI: 10.1007/s40011-022-01387-7.

Chung TL, Chen JS, Chiu CY, Tian G. 2012. ^{13}C-NMR spectroscopy studies of humic substances in subtropical perhumid montane forest soil. J For Res 17 (6): 458-467. DOI: 10.1007/s10310-011-0319-9.

Cosgrove DJ. 2024. Structure and growth of plant cell walls. Nat Rev Mol Cell Biol 25 (5): 340-358. DOI: 10.1038/s41580-023-00691-y.

Domingo-Fernández D, Gadiya Y, Mubeen S Bollerman TJ, Healy MD, Chanana S, Sadovsky RG, Healey D, Colluru V. 2023. Modern drug discovery using ethnobotany: A large-scale cross-cultural analysis of traditional medicine reveals common therapeutic uses. iScience 26 (9): 107729. DOI: 10.1016/j.isci.2023.107729.

Dupree R, Simmons TJ, Mortimer JC, Patel D, Iuga D, Brown SP, Dupree P. 2015. Probing the molecular architecture of *Arabidopsis thaliana* secondary cell walls using two-and three-dimensional ^{13}C solid state nuclear magnetic resonance spectroscopy. Biochemistry 54 (14): 2335-2345. DOI: 10.1021/bi501552k.

Emwas AHM, Al-Rifai N, Szczepski K, Alsuhaymi S, Rayyan S, Almahasheer H, Jaremko M, Brennan L, Lachowicz JI. 2021. You are what you eat: Application of metabolomics approaches to advance nutrition research. Foods 10 (6): 1249. DOI: 10.3390/foods10061249.

Fayiah M, Fayiah MS, Saccoh S, Kallon MK. 2024. Value of herbal medicine to sustainable development. In: Izah SC, Ogwu MC, Akram M (eds). Herbal Medicine Phytochemistry: Applications and Trends. Springer, Cham. DOI: 10.1007/978-3-031-43199-9_32.

Hatcher PG, Breger IA, Dennis LW, Maciel GE. 1983. Solid-state ^{13}C NMR of sedimentary humic substances; new revelations on their chemical composition. In: Christman RF, Gjessing ET (eds). Aquatic and Terrestrial Humic Materials. Ann Arbor Science, Ann Arbor, MI.

Hausermann H. 2021. Spirit hospitals and "concern with herbs": A political ecology of healing and being-in-common in Ghana. Environ Plan E: Nat Space 4: 1313-1329. DOI: 10.1177/2514848619893915.

Kim HK, Choi YH, Verpoorte R. 2010. NMR-based metabolomic analysis of plants. Nat Protoc 5 (3): 536-549. DOI: 10.1038/nprot.2009.237.

Larsen FH, Engelsen SB. 2015. Insight into the functionality of microbial exopolysaccharides by NMR spectroscopy and molecular modeling. Front Microbiol 6: 1374. DOI: 10.3389/fmicb.2015.01374.

Mehalaine S, Chenchouni H. 2021. Quantifying how climatic factors influence essential oil yield in wild-growing plants. Arab J Geosci 14 (13): 1257. DOI: 10.1007/s12517-021-07582-6.

Mithöfer A, Boland W. 2012. Plant defense against herbivores: Chemical aspects. Annu Rev Plant Biol 63: 431-450. DOI: 10.1146/annurev-arplant

Mortada EM. 2024. Evidence-based complementary and alternative medicine in current medical practice. Cureus 16 (1): e52041. DOI: 10.7759/cureus.52041.

Nabavi SM, Šamec D, Tomczyk M, Milella L, Russo D, Habtemariam S, Suntar I, Rastrelli L, Daglia M, Xiao J, Giampieri F, Battino M, Sobarzo-Sanchez E, Nabavi SF, Yousefi B, Jeandet P, Xu S, Shirooie S. 2020. Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering. Biotechnol Adv 38: 107316. DOI: 10.1016/j.biotechadv.2018.11.005.

Parthasarathy A, Cross PJ, Dobson RC, Adams LE, Savka MA, Hudson AO. 2018. A three-ring circus: metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. Front Mol Biosci 5: 29. DOI: 10.3389/fmolb.2018.00029.

Pieroni A, Hovsepyan R, Manduzai AK, Sõukand R. 2021. Wild food plants traditionally gathered in central Armenia: Archaic ingredients or future sustainable foods?. Environ Dev Sustain 23 (2): 2358-2381. DOI: 10.1007/s10668-020-00678-1.

Pilcher JM. 2023. Food in World History. Routledge, New York. DOI: 10.4324/9781003332039.

Qian S, Ding W, Li Y, Liu G, Sun J, Ding Q. 2015. Characterization of humic acids derived from Leonardite using a solid-state NMR spectroscopy and effects of humic acids on growth and nutrient uptake of snap bean. Chem Speciat Bioavailab 27 (4): 156-161. DOI: 10.1080/09542299.2015.1118361.

Rodríguez-Yoldi MJ. 2021. Anti-inflammatory and antioxidant properties of plant extracts. Antioxidants 10: 921. DOI: 10.3390/antiox10060921.

Roxas A, Orozco J, Guzmán-Delgado P, Zwieniecki MA. 2021. Spring phenology is affected by fall non-structural carbohydrate concentration and winter sugar redistribution in three Mediterranean nut tree species. Tree Physiol 41 (8): 1425-1438. DOI: 10.1093/treephys/tpab014.

Schmid KM. 2021. Lipid metabolism in plants. In: Ridgway ND, McLeod RS (eds). Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-824048-9.00011-0.

Vannette RL. 2020. The floral microbiome: Plant, pollinator, and microbial perspectives. Ann Rev Ecol Evol Syst 51 (1): 363-386. DOI: 10.1146/annurev-ecolsys-011720-013401.

WHO [World Health Organization]. 2019. WHO global report on traditional and complementary medicine 2019. World Health Organization.

Yadav S, Modi P, Dave A, Vijapura A, Patel D, Patel M. 2020. Effect of abiotic stress on crops. In: Hasanuzzaman M, Filho MCMT, Fujita M, Nogueira TAR (eds). Sustainable Crop Production. InTechOpen, London. DOI: 10.5772/intechopen.88434.

Yu M, Gouvinhas I, Rocha J, Barros AI. 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci Rep 11 (1): 10041. DOI: 10.1038/s41598-021-89437-4.

Zhang R, Yang D, Li S, Chen J, Hu D, Guo H, Wang C, Wang Y, Cong X. 2024. Coupling of environmental factors and growth stages in simulation of maize biomass allocation. Plant Soil 499 (1): 329-347. DOI: 10.1007/s11104-022-05794-7.

Zhang B, Gao Y, Zhang L, Zhou Y. 2021a. The plant cell wall: Biosynthesis, construction, and functions. J Integr Plant Biol 63 (1): 251-272. DOI: 10.1111/jipb.13055.

Zhang S, Yuan L, Li Y, Zhao B. 2021b. Characterization of ultrafiltration-fractionated humic acid derived from Chinese weathered coal by spectroscopic techniques. Agronomy 11 (10): 2030. DOI: 10.3390/agronomy11102030.

Zhao J, Wang M, Saroja SG, Khan IA. 2022. NMR technique and methodology in botanical health product analysis and quality control. J Pharm Biomed Anal 207: 114376. DOI: 10.1016/j.jpba.2021.114376.

Zill LP, Cheniae GM. 1962. Lipid metabolism. Ann Rev Plant Physiol 13 (1): 225-264. DOI: 10.1146/annurev.pp.13.060162.001301.