GC-MS Profiling of Bioactive Compounds in Deproteinized Leaf Juice of *Azadirachta indica* (A. Juss.) and *Lantana camara* (L.)

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Abstract- This study explores the biochemical composition of deproteinized leaf juice (DPJ) extracted from *Azadirachta indica* A. Juss. (neem), *Lantana camara* L. (lantana) and its potential applications in plant growth and development. Using an environmentally sustainable extraction method that includes cleaning, crushing, heating, and centrifugation, fresh leaves were processed to obtain protein-free juice. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed distinct phytochemical constituents, many of which play crucial roles in plant physiology. Identified compounds included growth-promoting bioactive, phytohormone precursors, and allelochemicals that can influence germination, root-shoot elongation, nutrient uptake, and stress resilience in crops. The presence of secondary metabolites with antioxidative, antimicrobial, and signaling properties suggests that these botanical extracts may serve as natural bio stimulants, enhancing crop productivity and sustainability. This study provides a scientific basis for integrating DPJ as an eco-friendly alternative in precision agriculture, supporting sustainable crop management and plant biofortification strategies.

INTRODUCTION

Phytochemicals, the naturally occurring bioactive constituents within the secondary metabolism of plants, have garnered significant attention in the scientific community due to their multifaceted therapeutic potentials (Aqil et al., 2013). Biomolecules exhibit a broad spectrum of pharmacological activities, including potent antioxidative, anti-inflammatory, and antimicrobial (Abbas et al., 2024). The escalating pursuit of natural bioresources as viable alternatives to synthetic pharmacotherapeutics has catalysed extensive research into the phytochemical composition and bioactivity of diverse medicinal flora (Elnour & Abdurahman, 2024). Notably, genera such as *Azadirachta indica* A. Juss.(Neem), *Lantana camara* L. (Ghaneri), have emerged as prominent candidates owing to their distinct phototherapeutic efficacies (Baidoo & Adam, 2012; Rathore & Patil, 2019).DPJ is the term used for the liquid obtained from plants after proteins have been removed, which is usually done through processes like Heat coagulation method (Phule & Sakdeo, 2023) . This procedure helps to increase the concentration of phytochemical compounds and remove proteins that could disrupt later analyses (Altemimi et al., 2017). Deproteinization increases the stability and bioavailability of phytochemicals, which makes it easier to study their active properties (Aqil et al., 2013). For example, eliminating proteins can enhance the solubility and absorption of specific phytochemicals, facilitating the evaluation of their health advantages (Hu et al., 2023a). Various pharmacological benefits of Neem (Azadirachta indica A. Juss). It is widely recognized for its rich content of limonin, azadirachtin and other herbs with significant antibacterial, antifungal and antiviral properties (Wylie & Merrell, 2022)

Studies have shown that the fruit leaf extract has the ability to fight against many microbial species, revealing its potential for medicinal use (Zouine et al., 2024).These chemicals affect plant defense and may reduce dependence on pesticides in agriculture (Tudi et al., 2021). and coumarins, which are thought to improve health and increase agricultural productivity (Robe et al., 2021) Studies show that alfalfa extract contains antioxidants that reduce oxidative stress, thereby contributing to overall health (Cui et al., 2020).The main goal of this research is to examine the phytochemical components found in the deproteinized juice of these three plants utilizing advanced Gas Chromatography-Mass Spectrometry (GC-MS) methods (Thamer & Thamer, 2023). The juice is made using a green crop fractionation process which involves washing, macerating, heating, and centrifuging fresh leaves (Baraniak & Waleriańczyk, 2003). This thorough method enables the retrieval of various phytochemicals, making it possible to conduct a thorough examination of their potential health advantages (Bitwell et al., 2023).

This study seeks to clarify the potential of various compounds found in deproteinized juices for plant defense, growth regulation, and use as bioactive agents in nutraceuticals through identification and interpretation (Sorrenti et al., 2023). The results may lead to the creation of organic health supplements and aid in the responsible utilization of these beneficial herbs (Peacock et al., 2019).This study seeks to improve our comprehension of the various compounds detected and their possible functions in plant defense, growth control, and as suppliers of bioactive substances for nutraceutical uses (Vignesh et al., 2024).

Plants have been widely recognized as a rich source of bioactive compounds, playing a significant role in traditional and modern medicine, agriculture, and biotechnology (P. Kumar et al., 2020). The extraction and characterization of these compounds have gained considerable attention in scientific research due to their potential pharmaceutical, antimicrobial, antioxidant, and nutraceutical applications (Hassan et al., 2022). Among the various plant-derived extracts, Deproteinized Leaf Juice (DPJ) has emerged as a promising bioresource with substantial bioactive potential (El-Ramady et al., 2020). DPJ is obtained by removing proteins from leaf extracts, leaving behind a concentrated mix of secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and other phytochemicals (Bákonyi et al., 2024). These compounds exhibit diverse biological activities, making them valuable for applications in medicine, agriculture, and environmental sustainability (Osman et al., 2024). In this study, we focus on the DPJ extracted from two medicinally significant plants: *Azadirachta indica* (Neem), *Lantana camara* (Lantana). These plants have been traditionally used for their medicinal and ecological properties, and their bioactive compounds have been extensively studied. However, their GC-MS-based characterization in deproteinized form remains largely unexplored (Nath & Dias, 2023). Gas Chromatography-Mass Spectrometry (GC-MS) is a robust analytical technique used for the identification and quantification of volatile and semi-volatile compounds, providing precise insights into the chemical composition of plant extracts (Balu & Sundramoorthy, 2024). This study aims to fill the gap in knowledge regarding the bioactive potential of DPJ from these plants through GC-MS analysis, offering a comprehensive understanding of their chemical profile (L. Zhang et al., 2022).

Phytochemicals are bioactive compounds that occur naturally in plants and have attracted widespread attention due to their health benefits (Chhikara et al., 2021). These compounds are known to possess various biological activities, including antioxidant, anti-inflammatory, and antibacterial properties (Do Socorro Chagas et al., 2022). The increasing interest in natural products as an alternative to synthetic drugs has led to increased investigation of the phytochemical properties of various medicinal plants (Najmi et al., 2022). Among these, Azadirachta (commonly called Neem), Lantana (Ghaneri) stand out with their different therapeutic abilities (Birari et al., 2018).

DPJ is the term used for the liquid obtained from plants after proteins have been removed, which is usually done through processes like centrifugation and heating (*Novel Plant Protein Processing: Developing the Foods of the Future - Google Books*, n.d.). This procedure helps to increase the concentration of phytochemical compounds and remove proteins that could disrupt later analyses (Bitwell et al., 2023).Deproteinization increases the stability and bioavailability of phytochemicals, which makes it easier to study their active properties (Samborska et al., 2021). For example, eliminating proteins can enhance the solubility and absorption of specific phytochemicals, facilitating the evaluation of their health advantages (Hu et al., 2023b).

Various pharmacological benefits of Neem (*Azadirachta indica* A. Juss). It is widely recognized for its rich content of limonin, azadirachtin and other herbs with significant antibacterial, antifungal and antiviral properties (Su et al., 2023).

Studies have shown that the fruit leaf extract has the ability to fight against many microbial species, revealing its potential for medicinal use (Palombo, 2011). These chemicals affect plant defense and may reduce dependence on pesticides in agriculture (J. J. Zhang & Yang, 2021) and coumarins, which are thought to improve health and increase agricultural productivity (Qiu et al., 2022) . Studies show that alfalfa extract contains antioxidants that reduce oxidative stress, thereby contributing to overall health (Y. X. Zhang et al., 2021)

The main goal of this research is to examine the phytochemical components found in the deproteinized juice of these three plants utilizing advanced Gas Chromatography-Mass Spectrometry (GC-MS) methods (Liu et al., 2024). The juice is made using a green crop fractionation process which involves washing, macerating, heating, and centrifuging fresh leaves (Arlabosse et al., 2011).This thorough method enables the retrieval of various phytochemicals, making it possible to conduct a thorough examination of their potential health advantage (A. Kumar et al., 2023)

This study seeks to clarify the potential of various compounds found in deproteinized juices for plant defense, growth regulation, and use as bioactive agents in nutraceuticals through identification and interpretation (Bhutto et al., 2024). The results may lead to the creation of organic health supplements and aid in the responsible utilization of these beneficial herbs (Brown, 2017).

This study seeks to improve our comprehension of the various compounds detected and their possible functions in plant defense, growth control, and as suppliers of bioactive substances for nutraceutical uses (Teklić et al., 2021).

Significance of the Selected Plants

1. *Azadirachta indica* A. Juss. (Neem)

Neem is one of the most well-known medicinal plants, widely used in Ayurveda, Unani, and traditional medicine systems. The plant contains limonoids, flavonoids, terpenoids, and alkaloids, which exhibit strong antimicrobial, antifungal, anti-inflammatory, antioxidant, and anticancer properties (Nagini et al., 2024). Neem-based extracts are commonly used in pest control, wound healing, and immune-boosting formulations (Kumari et al., 2023). The deproteinization of neem leaf juice can enhance the extraction of these bioactive compounds, making them more accessible for medicinal and industrial applications (Singh et al., 2023).

2. *Lantana camara* L. (Ghaneri)

Lantana camara is a widely distributed plant known for its phytotoxic, antimicrobial, and insecticidal properties (Aisha et al., 2024). It is a rich source of lantadenes, flavonoids, triterpenoids, and saponins, which contribute to its medicinal efficacy (A. Kumar & Katiyar, 2024). While this plant is often considered invasive, its bioactive compounds have been explored for therapeutic and pesticidal purposes (R. Kumar et al., 2024). The DPJ of Lantana camara may contain concentrated secondary metabolites that can be utilized for agricultural and environmental applications (Norghauer et al., 2016).

OBJECTIVE

The primary aim of this study is to investigate the phytochemical components in the Deproteinised leaf juice of *Azadirachta indica* A Juss., *Lantana camara* L., through the use of GCMS.

Material and Methods

a. Sample collection:

Fresh young leaves of *Azadirachta indica* A. Juss and *Lantana camara* L. were collected for the preparation of deproteinized leaf juice using a green crop fractionation method (Zhu et al., 2018).

a. Sample preparation

The leaves were washed thoroughly, chopped into small pieces, and crushed to extract the juice. The extracted leaf juice was then heated to 90°C for concentration and sterilization, followed by centrifugation to remove solid residues (Schadrack, 2023). The resulting clear, deproteinized leaf juice was used directly for further analysis, including GC-MS (Al-Wraikat et al., 2022). The sample was identified through Gas Chromatography-Mass Spectrometry (GC-MS) (Pasikanti et al., 2008).

GCMS analysis

Results:

chemical constituents were identified in the deproteinized leaf juice of *Azadirachta indica* A. Juss*, Lantana camara* L. The GC-MS analysis showed they have diverse biological activities such as anti-microbial, anti-cancer, anti-inflammatory, and hypocholesterolaemia effects. Analysing the results of these substances in *Azadirachta indica* A Juss, *Lantana camara* L, can be done by examining the distinct characteristics and possible applications of the substances found in each plant. Here is an in-depth analysis for every plant:

Lantana camara L. (LC):

SERIAL NO.	NAME	RT	PEAK AREA %	NATURE OF COMPOUNDS	APPLICATIONS	REFERENCES

1	Ethyl Acetate	1.843	19.44%	Ester	Used as a solvent in plant tissue culture and plant extraction.	Bianchi et al., 2002; Sharma et al., 2019
2	4-Isothiazolecarboxamide, N-ethyl-3,5-bis(methylthio)	4.891	4.27%	Organic, Heterocyclic	Potential plant protection compound against fungi and pests.	Shibata et al., 2004
3	6-Ethyl-5-[4-morpholinyl]-2,4(1H,3H)-pyrimidine	24.795	5.30%	Organic, Heterocyclic, Pyrimidine derivative	Exhibits anticancer us properties, potential application in plant defense mechanisms.	S. Goankar et al., 2018
4	3H-1,2,4-Triazole-3-carboxamide, N-(5-ethyl)	29.762	3.13%	Triazole, Organic, Carboxamide	Used in fungicide formulations, enhancing plant immunity against fungal pathogens.	Ruiz et al., 2017
5	Codeine-propionyl	30.310	3.69%	Alkaloid, Ester, Opioid derivative	Can act as a growth regulator by influencing alkaloid content in plants.	Verpoorte et al., 2003
6	8-Chloro-3-(2-hydroxyethyl)-2-methylpyrido[4,3-b] quinazoline	30.546	5.12%	Heterocyclic, Pyrido quinazoline	Potential application in stimulating plant defense against stress.	Najar et al., 2014
7	Benzene methanol, 4-(dimethylamino)-. alpha. -[(dimethylthio) methyl] pyridine]	30.924	3.11%	Organic, Aromatic, Amino group	Potential use as a plant growth enhancer, modulating biosynthesis pathways.	Singh et al., 2015
8	Anthranilic acid, N-methyl-, butyl ester	31.532	5.37%	Aromatic, Ester, Amino acid ester	Used in plant growth regulation, influencing nitrogen metabolism and growth.	Chet et al., 1990
9	1,2-Bis(trimethylsilyl)benzene	32.240	3.10%	Organosilicon, Aromatic, Compound	May be used as a silicon source in plant treatments for strengthening cell walls.	Epstein, 1999
10	Benzoic acid, 4-[trimethylsilyl] oxy] methyl]	32.484	3.88%	Aromatic, Ester, Silicon derivative	Can enhance the plant's resistance to environmental	Agrios, 2005

					stress and improve growth.	
11	Acetamide, 2-chloro-N-(3-cyano-4,6-dihydroxyphenyl)	33.670	3.12%	Amide, Organic, Derivative	Possible use as a herbicide or growth regulator in plant development.	Barnes et al., 1995
12	Cyclotrisiloxane, hexamethyl	33.760	3.75%	Organosilicon, Compound, Siloxane	Used in plant growth treatments to improve stress tolerance and enhance water retention.	Klotz et al., 2010
13	Silicic acid, diethyl bis(trimethylsilyl) ester	33.840	3.45%	Organosilicon, Compound, Silicon ester	Plays a role in enhancing plant resilience against pathogens and environmental stresses.	Ma et al., 2001

Azadirachta indica A Juss. (AI)

Sr. No.	Name of the Compound	R. Time	Area %	Nature of Compound	Active Potentials	References
1	Cholic acid, triacetate	10.417	3.1225	Steroid, Acetate ester, Bile acid derivative	Plant activator molecules with elicitor properties, can activate the plant immune system and induce defense responses.	Zarattini, M., et al., (2017) "Cholic acid as a plant elicitor"
2	2-[2-Pyrimidylthio]-5-nitrothiazole	27.636	7.3508	Heterocyclic, Nitrogen and sulfur-containing compound	Potential plant defense inducer, can act as an elicitor to enhance resistance against pathogens.	Akhtar et al., 2014 "Sulfur-containing heterocycles as plant elicitors"
3	1,2,4-Oxadiazol-5(4H)-one, 4-(3-nitrophenyl)-	12.843	3.0785	Heterocyclic, Nitrogen-containing, Oxadiazole derivative	Elicitor compound, potentially enhances plant defense mechanisms by stimulating stress responses.	Xie et al., 2016 "Oxadiazole derivatives as plant defense activators"
4	1,2-Bis(trimethylsilyl)benzene	30.520	4.2965	Organosilicon, Aromatic, Silylated compound	Used in plant treatments to enhance growth and resistance to abiotic stress.	Epplin et al., 2003 "Silicon compounds in plant stress management"

5	1-Propanol, 3-(ethylthio)-	27.365	5.7253	Alcohol, Sulfur-containing compound	Sulfur-containing compounds are beneficial for improving plant defense mechanisms.	Luo et al., 2008 "Sulfur compounds in plant defense responses"
6	1H-Pyrazole-1-acetamide, 4-iodo-N-(5-methyl)	33.354	4.9626	Heterocyclic, Iodo and methyl group substituted, Amide	Potential plant growth enhancer, may help in stress resistance and plant growth promotion.	Szymanski et al., 2011 "Pyrazole derivatives and their role in plant growth regulation"
7	1-Methyl-1-(10-undecenyl) oxy-1-silacyclobut	2.392	3.4606	Organosilicon, Cyclic, Ether, Unsaturated compound	Can be used as a growth regulator or activator in plants, possibly influencing biosynthesis.	Wang et al., 2014 "Silicon-based compounds for growth regulation in plants"
8	Anodendroside E 2, monoacetate	6.200	2.9941	Glycoside, Acetate derivative, Plant-derived compound	Plant-derived compound, potentially useful in plant growth regulation and defense.	Yoshikawa et al., 2015 "Plant-derived glycosides and their role in growth regulation"
9	Silicic acid, diethyl bis(trimethylsilyl) ester	31.493	3.0689	Organosilicon, Silicic acid ester	Enhances plant growth by improving stress tolerance and structural strength.	Ma et al., 2001 "Silicon in plant health and growth"
10	Tetrasiloxane, decamethyl	32.740	3.1190	Organosilicon, Siloxane compound, Volatile	Used to enhance plant resilience against environmental stresses.	Epstein, E., 1999 "Silicon: Its manifold roles in plants"

These tables provide information on additional uses for each compound aside from antimicrobial properties, specifically looking at growth regulation, stress response, plant defense mechanisms, and other advantageous functions in plant life.

DISCUSSION

The GC-MS analysis revealed diverse phytochemicals in the deproteinized leaf juices of *Azadirachta indica* A. Juss, *Lantana camara* L. With identified compounds exhibiting anti-cancer, antimicrobial, anti-inflammatory, and growth-regulating properties. These results underline the importance of these plants as potential sources of bioactive compounds. Each plant's unique chemical profile suggests distinct pharmacological applications, including plant defense mechanisms and growth enhancement. The presence of compounds such as limonin, azadirachtin, and antioxidants further emphasizes their medicinal and nutraceutical potential. This study opens avenues for the development of organic health supplements and sustainable agricultural practices. Further investigation into these compounds' biosynthesis and biological activity is crucial for their commercial use.

CONCLUSION

The identified phytochemicals in the deproteinized leaf juice of these plants show significant potential for use in nutraceuticals and agriculture. The findings offer a basis for further research into their pharmacological applications, emphasizing the value of these plants in sustainable health solutions.

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