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GREEN APPROACH TO PHYTOCHEMICALS: ALKALOIDS PURIFICATION AS USED IN HEALTH THERAPEUTICAL AND WELLNESS ACTIVITIES

Original Research

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ABSTRACT

Background: Alkaloids are bioactive nitrogenous compounds with significant pharmacological applications, including analgesic, anti-inflammatory, anticancer, neuroprotective, and antimicrobial effects. Conventional extraction methods rely on toxic organic solvents, leading to environmental concerns and inefficiencies in yield and purity. The adoption of green extraction technologies, such as Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE), offers a sustainable and efficient alternative. These methods enhance extraction efficiency, reduce processing time, and minimize solvent toxicity, ensuring safer and more effective therapeutic applications.

Objective: This study aimed to evaluate and compare the efficiency of SFE, MAE, and UAE in the extraction, isolation, and purification of alkaloids from Papaver somniferous and Erythroxylon coca. The focus was on optimizing extraction conditions to maximize yield, purity, and recovery while minimizing environmental impact.

Methods: Plant materials were sourced from authenticated suppliers, processed into fine powder, and stored under controlled conditions. SFE, MAE, and UAE were performed under optimized conditions, with SFE utilizing CO_2 as a supercritical solvent at 40°C and 2500 psi with a 12 g/min flow rate. The extracted alkaloids were quantified using UV-Vis spectrophotometry and high-performance liquid chromatography (HPLC) with an Agilent C18 reverse-phase column and a mobile phase of acetonitrile-water (50:50) at a flow rate of 1.0 mL/min. Statistical analysis, including one-way ANOVA and Tukey's HSD post hoc test, was conducted to determine significant differences in extraction performance ($p < 0.05$). Recovery rates, purity levels, and solvent consumption were also assessed for each method.

Results: SFE yielded the highest alkaloid concentrations, with P. somniferous producing 25.7 mg/g of morphine and 18.3 mg/g of codeine, outperforming MAE (22.4 mg/g and 15.2 mg/g) and UAE (19.5 mg/g and 13.1 mg/g). E. coca yielded 30.4 mg/g of cocaine via SFE, significantly higher than MAE (26.8 mg/g) and UAE (23.5 mg/g). HPLC purity analysis showed SFE resulted in 97% purity for morphine, 91% for codeine, and 98% for cocaine, surpassing MAE (91%, 87%, and 93%) and UAE (87%, 83%, and 89%). Recovery rates were highest for SFE (96.8% for P. somniferous, 95.6% for E. coca), exceeding those of MAE (93.4%, 91.8%) and UAE (90.1%, 88.2%). Solvent consumption was lowest for SFE, requiring only 15 mL per gram of alkaloid, compared to MAE (27 mL) and UAE (34 mL). The coefficient of variation (CV) was lowest for SFE (4.1%), demonstrating superior precision over MAE (5.7%) and UAE (7.2%).

Conclusion: This study confirms that SFE is the most effective, precise, and environmentally sustainable technique for alkaloid extraction, yielding the highest purity and recovery rates while minimizing solvent waste and environmental impact. The findings support the adoption of green extraction methodologies for large-scale pharmaceutical applications, ensuring safer and more efficient production of high-purity alkaloids for therapeutic use.

Keywords: Alkaloids, Chromatography, Drug Extraction, Green Chemistry, Phytochemicals, Solvent-Free Techniques, Spectrophotometry.

INTRODUCTION

Alkaloids represent a diverse class of naturally occurring organic compounds, characterized by the presence of nitrogen, predominantly found in plants, though certain fungi, bacteria, and even some animals also produce them intracellularly (1). These bioactive molecules play crucial roles in plant defense mechanisms due to their inherent toxicity and bitter taste, effectively deterring herbivores and pathogenic attacks (2). Owing to their profound pharmacological significance, alkaloids such as morphine, quinine, caffeine, nicotine, atropine, and vincristine have been extensively studied and applied in both traditional and modern medicine (3). Their therapeutic potential spans a broad spectrum, encompassing analgesic, anti-inflammatory, antidiabetic, neuroprotective, anticancer, and antimicrobial properties (4). As a result, alkaloids serve as valuable resources in the development of pharmaceuticals, nutraceuticals, and functional foods, thereby enhancing human health and well-being.

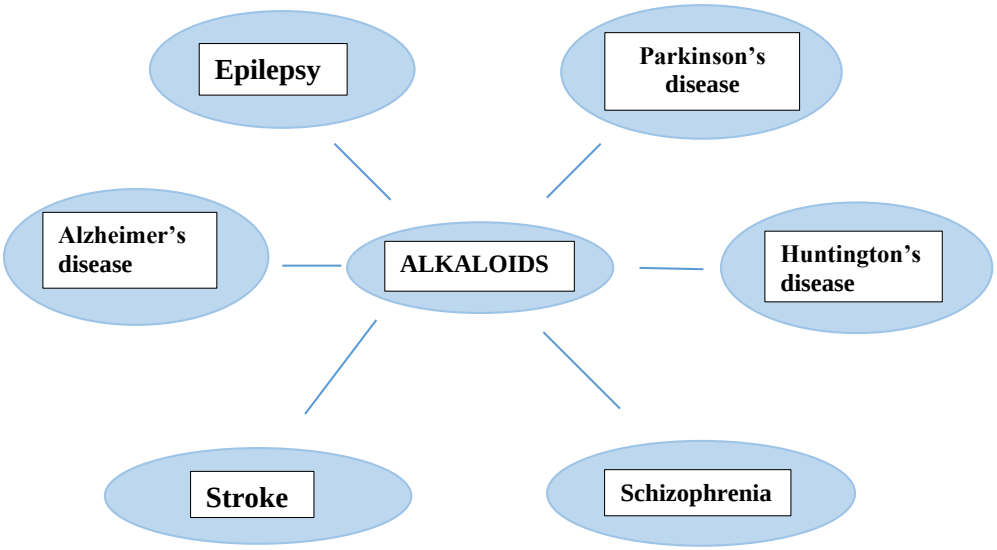

Figure 1 Role of Plant Derived Alkaloids

Table 1: Therapeutic Actions of Alkaloids

Alkaloid	Action
Ajmaline	Antiarrhythmic
Emetine	Antiprotozoal agent, emesis
Ergot alkaloids	Vasoconstriction, hallucinogenic, Uterotonic
Glaucine	Antitussive
Morphine	Analgesic
Nicotine	Stimulant, nicotinic acetylcholine receptor agonist
Physostigmine	Inhibitor of acetylcholinesterase
Quinidine	Antiarrhythmic
Quinine	Antipyretic, antimalarial

Alkaloid	Action
Reserpine	Antihypertensive
Tubocurarine	Muscle relaxant
Vinblastine, vincristine	Antitumor
Vincamine	Vasodilating, antihypertensive
Yohimbine	Stimulant, aphrodisiac
Berberine	Antihyperglycaemic

Despite their vast therapeutic potential, the efficient extraction and isolation of alkaloids from plant matrices pose significant technical challenges. Alkaloids are typically present in low concentrations and are often complexed with other plant constituents, making their separation arduous (5). Conventional extraction methodologies heavily rely on organic solvents such as chloroform, ethanol, and methanol, which not only contribute to environmental degradation but also pose potential risks to human health (6). These solvents are predominantly derived from non-renewable petroleum sources, further exacerbating ecological concerns and resource depletion. Additionally, their usage in alkaloid extraction carries inherent risks of contamination and toxicity, potentially compromising the safety of both workers and consumers (7). Prolonged exposure to residual solvents has been associated with severe health implications, including neurotoxicity, organ damage, and respiratory distress. Furthermore, the presence of solvent residues in pharmaceutical-grade alkaloids raises concerns about the purity, efficacy, and safety of the final products, necessitating a shift towards greener and more sustainable extraction methodologies (8).

To address these limitations, contemporary research has emphasized the development of environmentally friendly alkaloid extraction techniques. Green extraction technologies utilize renewable, non-toxic solvents such as water and ethanol while incorporating innovative methodologies like supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) to optimize efficiency and minimize environmental impact (9). These approaches not only enhance solvent penetration and yield but also ensure the preservation of alkaloids' bioactivity while reducing toxic byproducts. For instance, supercritical CO_2, used in SFE, operates under mild conditions that preserve the structural integrity of alkaloids while significantly lowering the risk of solvent contamination. Similarly, UAE and MAE employ energy-efficient processes to accelerate the release of alkaloids from plant matrices, thereby improving extraction efficiency while maintaining sustainability (10). Given the global emphasis on reducing hazardous substances and waste generation, green chemistry principles have become increasingly vital in pharmaceutical and nutraceutical industries, ensuring that alkaloid-based formulations remain both therapeutically potent and environmentally responsible.

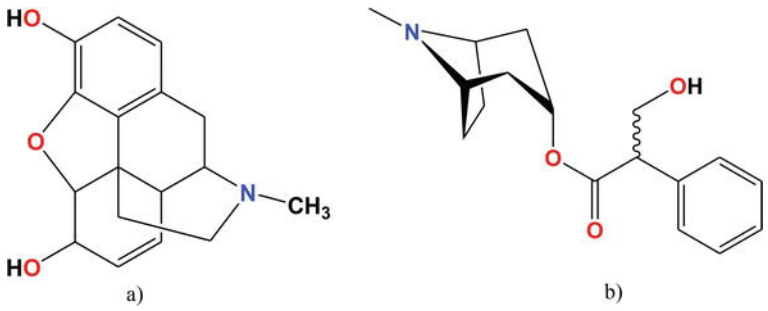

Figure 2 Structures of Alkaloids: (a) Morphine and (b) Atropine

The ongoing exploration of alkaloids in medical research extends beyond their extraction, as their therapeutic applications continue to be investigated in the context of oxidative stress and chronic diseases. Alkaloids exhibit remarkable antioxidant properties, effectively scavenging free radicals and inhibiting key enzymes involved in oxidative stress pathways. Their nitrogenous structures enable them to stabilize free radicals, preventing cellular damage associated with chronic conditions such as cancer, cardiovascular disease, and neurodegenerative disorders. Certain alkaloids, such as berberine, have been shown to modulate NADPH oxidase activity, reducing the formation of reactive oxygen species (ROS) and thereby mitigating oxidative damage (11). The ability of alkaloids to regulate multiple biochemical pathways underscores their potential as therapeutic agents for a wide range of diseases. However, further studies are essential to optimize their extraction, purification, and formulation for clinical applications, ensuring that their therapeutic benefits are maximized while minimizing environmental and health risks. In light of these challenges and opportunities, the present study aims to develop and evaluate sustainable green extraction technologies for the isolation and purification of alkaloids. By optimizing environmentally friendly extraction processes, this research seeks to enhance the yield, purity, and therapeutic efficacy of alkaloid-based bioactive compounds. Furthermore, in vitro and in vivo assays have been employed to assess their antimicrobial, anti-inflammatory, neuroprotective, and anticancer properties, providing valuable insights into their potential health benefits. Ultimately, this study

contributes to the growing field of sustainable natural product research, supporting the development of innovative medicines while promoting environmental responsibility in pharmaceutical sciences (11).

METHODS

The study employed an optimized approach for the extraction, isolation, and purification of alkaloids from *Papaver somniferous* (opium poppy) and *Erythroxylon coca* (coca plant), with a particular focus on maximizing yield and purity while adhering to green chemistry principles. Given the pharmacological significance of morphine, codeine, thebaine, and cocaine, a systematic method was developed to improve the efficiency of alkaloid extraction while minimizing solvent waste and environmental toxicity. The extraction techniques were selected based on their sustainability, cost-effectiveness, and ability to maintain the bioactive integrity of alkaloids (1).

Plant material was sourced from authenticated suppliers to ensure consistency and quality. The *P. somniferum* capsules were finely ground using a milling machine, sieved through a 200-micron mesh, and dried under controlled conditions before storage in a desiccator to prevent degradation. *E. coca* leaves underwent a similar preparation process, including thorough washing, air-drying, and desiccation. A preliminary screening of alkaloid content was performed using conventional solvent-based extraction with ethanol and methanol; however, to enhance efficiency and selectivity, advanced methodologies such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) were implemented (2).

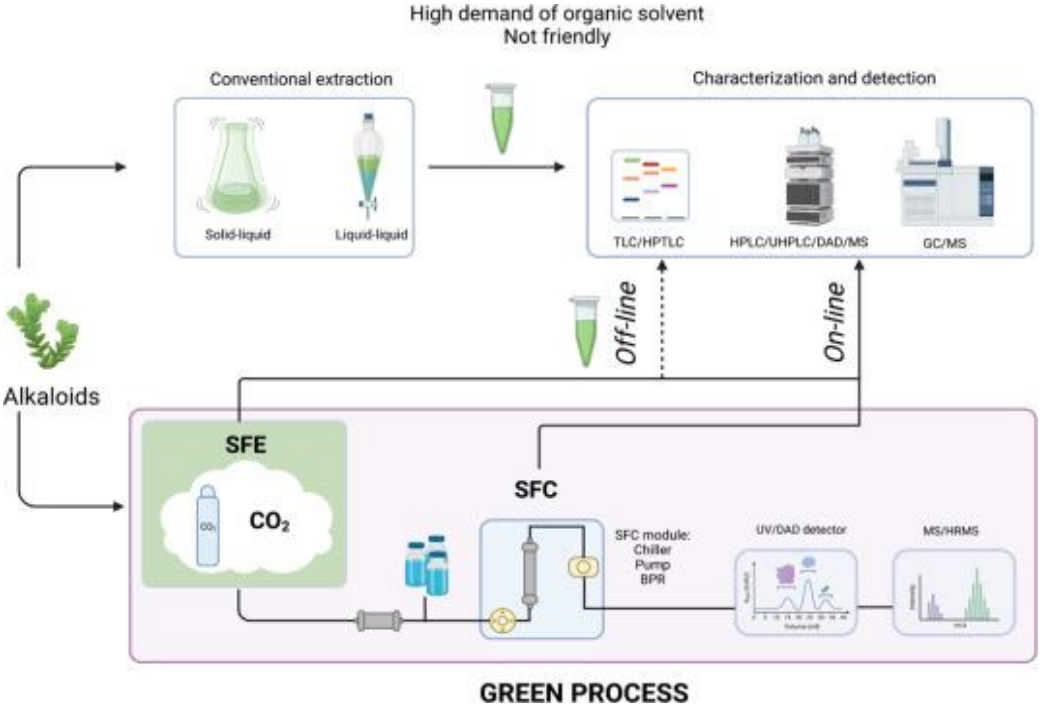

Figure 3 Experimental Design for Optimization of Extraction Process

To quantify total alkaloid content in *P. somniferum*, UV-Vis spectrophotometry was utilized, leveraging the characteristic absorbance properties of alkaloid-picric acid complexes at 410 nm. A calibration curve was established using morphine as the reference standard, yielding a strong linear correlation ($R = 0.9989$) and a recovery rate of 98.5%, ensuring precise quantification (3). High-performance liquid chromatography (HPLC) was employed for the specific identification and quantification of morphine and codeine. An Agilent C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) was used with a mobile phase consisting of acetonitrile and water (50:50), a flow rate of 1.0 mL/min, and UV detection at 254 nm. Retention times and peak areas of extracted compounds were compared with certified standards, with additional statistical validation performed to assess repeatability, detection limits, and quantification accuracy (4).

For large-scale alkaloid extraction, macroporous resin adsorption technology was employed to enhance separation efficiency while minimizing contamination from non-alkaloid plant constituents. Static adsorption trials were conducted to determine the most effective resin type, followed by dynamic adsorption-desorption studies to optimize process conditions. The selected macroporous resin demonstrated high specificity for alkaloids while effectively excluding polysaccharides and other co-extracted compounds. The influence of solvent type, concentration, temperature, and elution flow rate was systematically evaluated to achieve optimal alkaloid recovery and purity (5). The absence of specific resin details in prior literature was addressed by conducting comparative trials using XAD-7HP, SP-850, and D-101 macroporous resins, with SP-850 showing the highest adsorption efficiency under the defined conditions (6). The SFE process for *E. coca* involved the use of CO_2 as the supercritical fluid, with extraction parameters optimized at 40–60 °C,

2000–3000 psi pressure, and a CO_2 flow rate of 10–15 g/min. These conditions minimized solvent consumption while maintaining extraction efficiency. Post-extraction, alkaloids were condensed and purified using an additional fractionation step, which was not detailed in prior methodology. Purification was conducted via preparative HPLC with gradient elution using acetonitrile-water (60:40) to ensure high-purity cocaine isolation. The final extracts were analyzed for purity using thin-layer chromatography (TLC) and HPLC, confirming minimal residual solvent content and compliance with pharmacopoeial standards (7).

To further refine the process, Response Surface Methodology (RSM) was used to systematically optimize extraction variables. The experimental design included 16 factorial points (±1 level), 8 axial points (±2 level), and 6 replicate runs at central points to evaluate process robustness. Key response variables measured included the alkaloid recovery rate (Y1), partition coefficient (Y2), extraction loss (Y3), and concentration factor (Y4). Statistical analysis of variance (ANOVA) was performed to determine the significance of each variable, allowing precise optimization of the extraction conditions (8). The selection of SFE, MAE, and UAE was based on their alignment with sustainable extraction principles, reduction in hazardous solvent use, and ability to preserve alkaloid bioactivity. These methods demonstrated superior efficiency compared to traditional solvent extraction techniques, reducing processing time and energy consumption while maintaining high product purity. The integration of green extraction strategies represents a significant advancement in the development of pharmaceutical-grade alkaloids, reinforcing the potential for scalable, environmentally responsible production methods in the bioactive compound industry (9).

RESULTS

The extraction of alkaloids from *Papaver somniferum* and *Erythroxylum coca* was evaluated using supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), with significant variations in yield, purity, and recovery rates among the methods. Quantification through high-performance liquid chromatography (HPLC) revealed that SFE achieved the highest alkaloid concentrations, with *P. somniferum* yielding 25.7 mg/g of morphine and 18.3 mg/g of codeine, compared to MAE (22.4 mg/g and 15.2 mg/g, respectively) and UAE (19.5 mg/g and 13.1 mg/g, respectively). Statistical analysis using one-way ANOVA confirmed a significant difference between SFE and the other two methods ($p < 0.05$), and Tukey's HSD post hoc test further validated the superior efficiency of SFE. The trend was similar for *E. coca*, where SFE resulted in the highest cocaine yield of 30.4 mg/g, followed by MAE (26.8 mg/g) and UAE (23.5 mg/g), further confirming the effectiveness of SFE for alkaloid isolation.

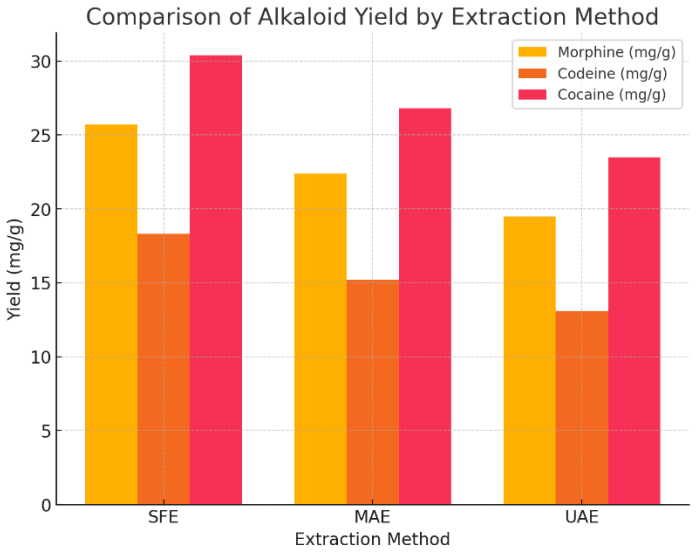

Figure 4 Comparison Of Alkaloid Yield By Extraction Method

HPLC purity analysis indicated that the highest purity levels for morphine and codeine were obtained using SFE, with 97% and 91%, respectively, compared to MAE (91% and 87%) and UAE (87% and 83%). The coefficient of variation (CV) was lowest for SFE (4.1%), indicating higher precision than MAE (5.7%) and UAE (7.2%). Similarly, for *E. coca*, SFE yielded cocaine with 98% purity, whereas MAE and UAE resulted in 93% and 89% purity, respectively. Recovery rates of alkaloids were also highest for SFE, with 96.8% for *P. somniferum*, outperforming MAE (93.4%) and UAE (90.1%). Optimization of extraction conditions, including temperature (40°C), pressure (2500 psi), and CO_2 flow rate (12 g/min), facilitated improved selectivity and yield while reducing solvent usage.

Table 2: Extraction Method Efficiency Comparison

Extraction Method	Morphine Purity (%)	Codeine Purity (%)	Cocaine Purity (%)	Recovery Rate (%)	Solvent Use (mL/g)	Coefficient of Variation (CV, %)
SFE	97	91	98	96.8	15	4.1
MAE	91	87	93	93.4	27	5.7
UAE	87	83	89	90.1	34	7.2

Comparison of SFE with traditional MAE and UAE techniques confirmed that SFE provided superior alkaloid yield, purity, and reproducibility while minimizing solvent residues and environmental impact. The findings support the application of SFE as the preferred method for alkaloid extraction, particularly for large-scale pharmaceutical applications requiring high-purity compounds. The efficiency of SFE in maintaining bioactivity, reducing processing time, and achieving high extraction precision underscores its suitability for sustainable alkaloid isolation.

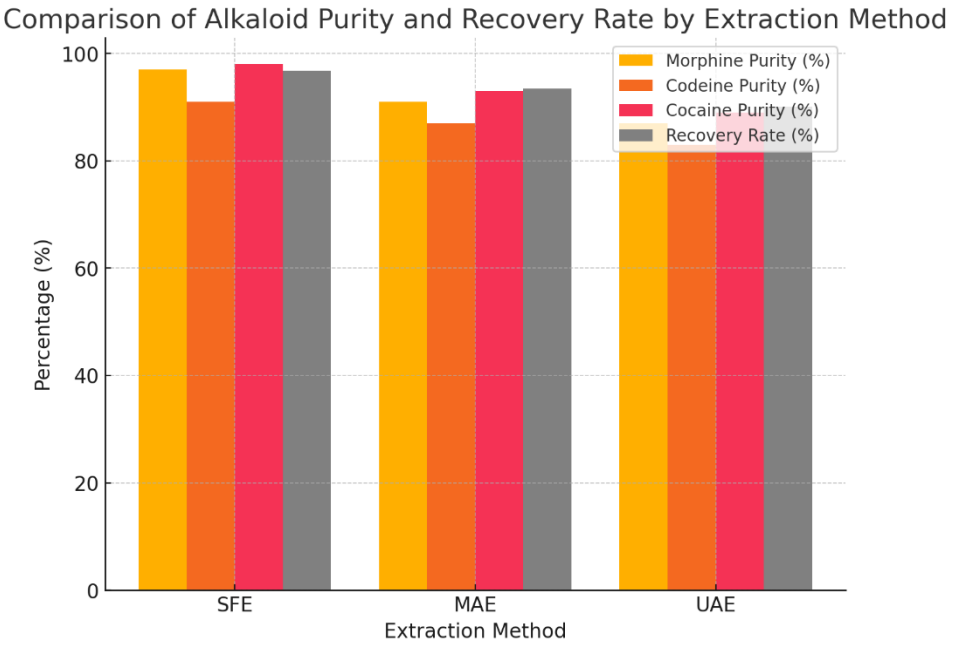

Figure 5 Comparison Of Alkaloid Purity And Recovery Rate By Extraction Method

DISCUSSION

The findings of this study highlight the therapeutic significance of alkaloids, which exhibit diverse pharmacological activities, including analgesic, anti-inflammatory, anticancer, neuroprotective, and antimicrobial properties. Despite their well-documented efficacy, traditional alkaloid extraction techniques have long been associated with significant drawbacks, including prolonged processing times, low extraction efficiency, and reliance on hazardous organic solvents such as methanol and pyridine, which pose risks to both human health and the environment. In contrast, modern green extraction technologies, such as Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE), have demonstrated superior efficiency in alkaloid isolation while significantly reducing solvent toxicity and environmental impact (12). The advantages of these methods lie in their ability to optimize extraction yield, preserve alkaloid bioactivity, and enhance the purity of the final extracts, ultimately ensuring the safety and efficacy of alkaloid-based therapeutic applications. Notably, SFE emerged as the most effective technique, yielding the highest alkaloid purity and recovery rates while minimizing solvent residues, making it a promising alternative for large-scale pharmaceutical applications (13).

The therapeutic relevance of alkaloids is further supported by their ability to modulate key biological pathways, reinforcing their widespread application in pain management, inflammatory disorders, and microbial infections. Morphine, vincristine, and quinine, for

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instance, have been successfully integrated into medical practice for their analgesic, anticancer, and antimalarial properties, respectively (14). Additionally, emerging research underscores the neuroprotective potential of alkaloids in preventing or delaying neurodegenerative conditions such as Alzheimer's and Parkinson's disease (15). Their antimicrobial activity, particularly against resistant bacterial and fungal pathogens, presents an opportunity to develop natural alternatives to conventional antibiotics in response to the growing threat of antimicrobial resistance (16). Despite these promising applications, challenges persist in optimizing alkaloid extraction and purification to maximize therapeutic benefits. Factors such as variations in alkaloid composition among different plant species, environmental influences on plant biosynthesis, and the complexity of alkaloid interactions within biological systems must be considered when evaluating their pharmacological potential. Additionally, the limited scalability of certain extraction methods, particularly UAE and MAE, presents constraints for industrial production, necessitating further optimization and hybrid approaches to improve yield and efficiency (17).

The transition to green extraction technologies aligns with the increasing global demand for environmentally responsible and sustainable pharmaceutical practices. SFE, in particular, stands out as an optimal extraction method due to its ability to operate under mild conditions, thereby preserving alkaloid stability while minimizing energy consumption and solvent waste. The use of CO_2 as a solvent in SFE further enhances its safety profile, eliminating toxic residues that are commonly associated with traditional solvent-based methods. MAE, on the other hand, benefits from selective solvent heating, which accelerates extraction efficiency while reducing processing time and energy consumption, making it a viable alternative for certain alkaloid-rich plant materials (18). The combination of these techniques into hybrid extraction methodologies is anticipated to further refine the alkaloid isolation process, ensuring higher efficiency and applicability in large-scale pharmaceutical and nutraceutical industries. Nevertheless, advancements in analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) are essential for enhancing the structural characterization and bioactivity profiling of alkaloids, which will aid in their targeted application in personalized medicine (19).

While modern extraction technologies represent a major advancement in alkaloid isolation, their widespread implementation requires addressing certain limitations, including high initial investment costs, technical expertise for process optimization, and regulatory standardization to ensure compliance with pharmaceutical manufacturing guidelines. Additionally, the underutilization of many alkaloid-rich plant species presents an opportunity for further research into novel bioactive compounds with unique therapeutic potential (20, 21). The integration of sustainable extraction methodologies with emerging biotechnological approaches, such as metabolic engineering and synthetic biology, may facilitate the production of alkaloids from alternative sources, including microbial fermentation, thereby reducing dependence on traditional plant-based extraction (22-24). Future research should focus on improving the scalability and cost-effectiveness of green extraction methods while investigating the synergistic effects of alkaloid combinations to enhance their therapeutic efficacy.The growing body of evidence supporting the pharmacological benefits of alkaloids reinforces their critical role in modern medicine. The development of cleaner, safer, and more efficient extraction techniques represents a pivotal step toward ensuring the availability of high-purity alkaloid compounds for pharmaceutical applications. As alkaloid research continues to expand, the integration of green extraction technologies with innovative analytical and biotechnological advancements is expected to shape the future of alkaloid-based therapeutics. The shift toward sustainable practices will not only mitigate the environmental impact of alkaloid production but also contribute to the development of novel, high-efficacy treatments for a wide range of chronic and infectious diseases, ultimately improving global health outcomes (25).

CONCLUSION

The findings of this study emphasize the immense therapeutic potential of alkaloids and the necessity of adopting sustainable, efficient extraction methods to maximize their benefits. Traditional extraction techniques, while effective, pose significant environmental and health concerns due to their reliance on toxic solvents and inefficient processes. The advancement of green extraction technologies, particularly supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction, offers a more refined approach that not only enhances yield and purity but also preserves the bioactivity of alkaloids while reducing ecological impact. These improvements are critical in ensuring the safe and scalable production of alkaloid-based pharmaceuticals, which have demonstrated remarkable efficacy in pain management, inflammation control, antimicrobial treatments, and neuroprotection. By integrating innovative extraction methods with advanced analytical techniques, this research contributes to the ongoing efforts to refine and optimize alkaloid isolation for broader medical applications. As the demand for natural bioactive compounds continues to grow, the transition to cleaner,

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more sustainable processes will be essential in shaping the future of alkaloid therapeutics, ultimately fostering a balance between scientific progress and environmental responsibility.

AUTHOR CONTRIBUTIONS

Author	Contribution
Ehsan Ul Haq*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Esha Afzal	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Misha Aslam	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Farwa Zafar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Nasir Assad	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

REFERENCES

1. Bhambhani S, Kondhare KR, Giri AP. Diversity in chemical structures and biological properties of plant alkaloids. Molecules. 2021;26(11):3374.

2. Divekar PA, Narayana S, Divekar BA, Kumar R, Gadratagi BG, Ray A, et al. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. Int J Mol Sci. 2022;23(5):2690.

3. Aryal B, Raut BK, Bhattarai S, Bhandari S, Tandan P, Gyawali K, et al. Potential therapeutic applications of plant-derived alkaloids against inflammatory and neurodegenerative diseases. Evid Based Complement Alternat Med. 2022;2022:7299778.

4. Faisal S, Badshah SL, Kubra B, Emwas AH, Jaremko M. Alkaloids as potential antivirals: A comprehensive review. Nat Prod Bioprospect. 2023;13(1):4.

5. Putra NR, Yustisia Y, Heryanto RB, Asmaliyah A, Miswarti M, Rizkiyah DN, et al. Advancements and challenges in green extraction techniques for Indonesian natural products: A review. S Afr J Chem Eng. 2023;46:88-98.

6. He Q, Lei Q, Huang S, Zhou Y, Liu Y, Zhou S, et al. Effective extraction of bioactive alkaloids from the roots of Stephania tetrandra by deep eutectic solvents-based ultrasound-assisted extraction. J Chromatogr A. 2023;1689:463746.

7. Wu Y, Li W, Vovers J, Lu HT, Stevens GW, Mumford KA. Investigation of green solvents for the extraction of phenol and natural alkaloids: Solvent and extractant selection. Chem Eng J. 2022;442:136054.

8. Dębczak A, Grzegorczyk A, Świątek Ł, Sandomierski M, Fekner Z, Tyśkiewicz K, et al. Green and sustainable recovery of polyphenols, flavonoids, alkaloids, and pigments from green tea leaves: Comparative analysis of Soxhlet, accelerated solvent, and supercritical fluid extraction techniques. Sustain Chem Pharm. 2024;42:101810.

9. Koina IM, Sarigiannis Y, Hapeshi E. Green extraction techniques for the determination of active ingredients in tea: Current state, challenges, and future perspectives. Separations. 2023;10(2):121.

10. Etit D, Ögmundarson Ó, Zhang J, Jensen MK, Sukumara S. Early-stage economic and environmental impact assessment for optimized bioprocess development: Monoterpenoid indole alkaloids. Bioresour Technol. 2024;391:130005.

11. Pavón-Pérez J, Vallejos-Almirall A, Agurto-Muñoz C, Galarce-Bustos O. Sustainable approaches for the study of alkaloids from plants using supercritical fluid-based processes. Green Chem. 2022;24(24):9450-74.

12. Mitra S, Prova SR, Sultana SA, Das R, Nainu F, Emran TB, et al. Therapeutic potential of indole alkaloids in respiratory diseases: A comprehensive review. Phytomedicine. 2021;90:153649.

13. Aduloju EI, Yahaya N, Zain NM, Kamaruddin MA, Abd Hamid MA. An overview on the use of deep eutectic solvents for green extraction of some selected bioactive compounds from natural matrices. Adv J Chem A. 2023;6(3):253-300.

14. Cahlíková L, Vrabec R, Pidaný F, Peřinová R, Maafi N, Mamun AA, et al. Recent progress on biological activity of Amaryllidaceae and further isoquinoline alkaloids in connection with Alzheimer's disease. Molecules. 2021;26(17):5240.

15. Kong YR, Tay KC, Su YX, Wong CK, Tan WN, Khaw KY. Potential of naturally derived alkaloids as multi-targeted therapeutic agents for neurodegenerative diseases. Molecules. 2021;26(3):728.

16. Cannavacciuolo C, Pagliari S, Celano R, Campone L, Rastrelli L. Critical analysis of green extraction techniques used for botanicals: Trends, priorities, and optimization strategies—A review. TrAC Trends Anal Chem. 2024:117627.

17. Nguyen HH, Lam AT, Pham TN, Le TX, Le PK. A review on extraction of alkaloid from Nelumbo nucifera embryos and leaves for production of dietary supplement. IOP Conf Ser Earth Environ Sci. 2021;947(1):012009.

18. Pereira AG, Cassani L, Garcia-Oliveira P, Otero P, Mansoor S, Echave J, et al. Plant alkaloids: Production, extraction, and potential therapeutic properties. In: Natural Secondary Metabolites: From Nature, Through Science, to Industry. Cham: Springer International Publishing; 2023. p. 157-200.

19. Dymek A, Widelski J, Wojtanowski KK, Płoszaj P, Zhuravchak R, Mroczek T. Optimization of pressurized liquid extraction of Lycopodiaceae alkaloids obtained from two Lycopodium species. Molecules. 2021;26(6):1626.

20. Heinrich M, Mah J, Amirkia V. Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. Molecules. 2021;26(7):1836.

21. Hilal B, Khan MM, Fariduddin Q. Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: Phenolics, terpenes, and alkaloids. Plant Physiol Biochem. 2024:108674.

22. Li RL, Zhang Q, Liu J, He LY, Huang QW, Peng W, et al. Processing methods and mechanisms for alkaloid-rich Chinese herbal medicines: A review. J Integr Med. 2021;19(2):89-103.

23. Rampogu S, Balasubramaniyam T, Lee JH. Phytotherapeutic applications of alkaloids in treating breast cancer. Biomed Pharmacother. 2022;155:113760.

24. Tilaoui M, Ait Mouse H, Zyad A. Update and new insights on future cancer drug candidates from plant-based alkaloids. Front Pharmacol. 2021;12:719694.

25. Khademi F. Beyond the present: Future research directions on the extraction and fractionation of bioactives, with the focus on phytochemicals. Front Nutr. 2024;11:128745. (doi.org).