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GC-MS based Phytochemical Profiling and Pharmacological Potentials of *Annona muricataand Psidium guajava* Leaves for Novel Drug Development

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ABSTRACT

Annona muricata and Psidium guajava areediblefruitscommonly cultivated in Nigeria and arerecognized for their promising applications in the pharmaceutical, nutraceuticals and cosmetic industries. This present study investigated the bioactive constituents present in theethanolic extract of A. muricata and P. guajavain order to assess their pharmacological significanceusing Gas Chromatography-Mass Spectrometry (GC-MS) techniques. Fresh leaves of A. muricata and P. guajava were collected, authenticated, extracted with 99% ethanol, and subjected to GC–MS analysis. Compounds were identified using the NIST library and grouped by pharmacological class.Sixteen compounds were identified to be present in the ethanolic extractof P. guajavawhile 10 compounds were detected in A. muricata. In P. guajava leaves extaract, the dominant constituents were benzenemethanamine (21.94%), benzenamine (16.60%), and benzamide (8.44%), while A. muricata leaves extract were rich in benzenemethanamine (33.51%), benzenamine (24.55%) and Benzyl alcohol (11.38). The identified compounds belong to classes associated with antioxidant, antimicrobial, antifungal, anti-inflammatory, antidepressants, analgesic, antidiabetic, antipruritic, flavouring agents, preservatives, organic solvent, repellents, fumigants, plant metabolites, insecticides and anticancer properties. The presence of pharmacologically active compounds in A. muricata and P. guajavaethanolicleaves extract highlights their potential as sources of natural therapeutic agents. These findings provide scientific validation for the traditional medicinal uses of these plant leaves and underscore their potential for pharmaceutical applications

Keywords: *Ethanolic extract, Bioactive compounds, Annona muricata, Psidium guajava, GC-MS analysis, Phytochemical Profiling, Pharmacological Potentials.*

1.0 Background of Study

Medicinal plants are a valuable reservoir of bioactive compounds with immense pharmaceutical relevance. Globally, more than 60% of therapeutic agents are directly or indirectly derived from natural sources, highlighting the importance of phytochemical research in drug discovery [1,2, 3]. Among the array of medicinal plants, *Annona muricata* (soursop) and *Psidium guajava* (guava) have attracted considerable scientific attention due to their long history of ethnomedicinal applications and diverse pharmacological activities [4,5,6, 7].

Annona muricata is traditionally used in tropical and subtropical regions for the treatment of cancer, hypertension, diabetes, malaria, and microbial infections. Its leaves and fruit pulp are reported to contain annonaceousacetogenins, alkaloids, flavonoids, and phenolic compounds with cytotoxic, anti-inflammatory, antioxidant, and antimicrobial properties [8,9, 10].

Similarly, Psidium guajava is widely recognized for its therapeutic effects against gastrointestinal disorders, respiratory infections, diabetes, and inflammation. The plant is rich in flavonoids (such as quercetin and kaempferol), tannins, terpenoids, and phenolic acids, which confer antioxidant, antimicrobial, and anti-diabetic activities (4,11, 12,13).

Despite the ethnopharmacological uses of these plants, there is a growing need for systematic chemical profiling of their bioactive constituents using advanced analytical tools. Gas Chromatography–Mass Spectrometry (GC–MS) has emerged as a robust technique for qualitative and quantitative characterization of volatile and semi-volatile phytochemicals [2]. It offers high sensitivity, resolution, and the ability to identify a wide range of bioactive molecules based on their unique fragmentation patterns and mass spectra [2]. Application of GC–MS to *A. muricata* and *P. guajava* provides an opportunity to uncover pharmacologically active compounds that could serve as leads in drug development pipelines.

Furthermore, comparative phytochemical evaluation of these species can provide valuable insights into their therapeutic overlaps, synergistic potentials, and unique metabolites with novel biological activities. Such information is critical in rationalizing their traditional uses, validating their pharmacological importance, and guiding pharmaceutical industries toward bio-prospecting for new drug candidates.

Despite their therapeutic significance, there is a paucity of research on the detailed chemical composition of their leaves. Therefore, this study aims to carry out GC–MS-based chemical profiling of *Annona muricata* and *Psidium guajava* leaves to identify bioactive compounds of pharmaceutical interest. By correlating the identified metabolites with reported pharmacological activities, the research will contribute to the scientific basis for the therapeutic applications of these plants and highlight their relevance in the development of natural product-based pharmaceuticals.

2.0 Materials and Methods

2.1 Collection of Plant Sample

Fresh leaves of Soursop (*Annona muricata*) and Guava (*Psidium guajava*) were collected in different locations within Akwa Ibom State, Nigeria. The identification and authentication of the plant's material was done at the Herbarium in the Department of Botany and Ecological studies, University of Uyo.

2.2 Preparation of Plant Material

Following identification, the leaves were thoroughly washed and air-dried under sunlight. They were then cut into smaller pieces, spread on clean cellophane sheets, and left to dry at room temperaturefor 72 hours. Once completely dried, the leaves were ground into a fine powder using a wooden mortar and pestle.

2.3 Preparation of Ethanolic Extract (Maceration and Extraction) of Plant Samples

The study employed the cold extraction (maceration) technique as described in reference [14]. In this process, 240 g of the prepared plant material was soaked in 1000 mL of 99% ethanol inside a sealed container and left at room temperature in the laboratory for 72 hours (3 days). After the maceration period, the mixture was first strained using muslin cloth to obtain the filtrate. Further extraction was carried out using a funnel, whatman filter paper, a conical flask and a vacuum pump. The resulting extracts were collected in 250 mL conical flask, properly labeled, sealed with alumunium foil, and secured with masking tape to ensure airtight storage.

2.4 Extract Concentration

A 200 mLportion of the extract was measured into 250mL beaker and concentrated in a water bath maintained at 80 °C. the solution was evaporated to reduce the filtrate and yield the plant extract.

2.5 GC-MS Analysis of the Plant Extract

Gas chromatography-mass spectrometry (GC-MS) was performed using GCMS-QP2010 Plussystem. Separation was achieved on a Perkin Elmer Elite - 5 capillary column (30m × 0.25mm internal diameter, 0.25 µm film thickness) coated with 95% dimethylpolysiloxane. Helium served as the carrier gas at a constant flow rate of 0.5ml / min, and a 1µl sample injection volume was in introduce into the system. The inlet temperature was set at 250 °C. The oven temperature program began at 80 °C and was held for 4 minutes, then rampedto 200 °C,and subsequently increased to 280 °C at a rate of 20 °C per minute, with a final hold of 5 minutes. The total analysis time was 25 minutes. The MS transfer line temperature was maintained at 200°C, while the ion source temperature was set at 180°C. Mass spectrometricdetection was conducted using electron impact ionization at 70 eV.Compound identification and quantification were based on total ion chromatograms (TIC), and the obtained mass specra were matched with reference spectra available in the instrument's GC-MS librarydatabase[15,16, 17, 18].

3.0 Results

3.1 Bioactive compounds Identified in *Psidium guajava*Extract by GC-MS

The chromatogram presented in Fig.1 displayed 16 distinct peaks,suggesting the presence of sixteen (16) bioactive constituents in the analyzed sample. The GC-MS analysis of the ethanolic extract of *Psidium guajava* identified a total of sixteen (16) bioactive compounds based on their retention time, percentage peak area, percentage peak height, molecular weight and molecular formular (Table 1). The key compounds identified include; Benzenemethanamine (21.94), Benzenamine (16.60), 1-Pyrrolidinecarboxylic acid (8.67), Benzamide (8.44), N-Benzylformamide (8.29), Benzaldehyde (6.97), 3-Buten-2-one (6.03), Z-8-Methyl-9-tetradecenoic acid (3.67), N-Benzylbenzamide (3.35), 9,12-Octadecadienoyl chloride (2.79), Benzyl Benzoate (2.73), Pyrazole-5-carbonitrile (2.56), 2-Pentanone (2.35), Benzylamine (2.25), n-Hexadecanoic acid (1.96) and dl-2-Phenyl-1,2-propanediol (1.40) (Table 1). Major phyto-compounds detected in *P. guajava* extract and their medicinal/therapeutic uses have been tabulated in (Table 2).

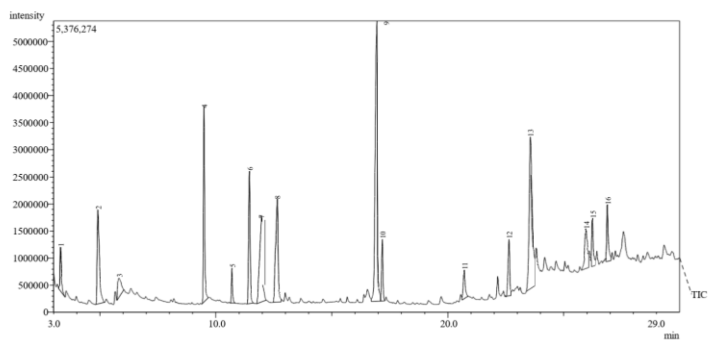

Figure 1: GC-MS analysis of Psidium guajava leaves of Ethanolic Extract: the GC-MS chromatogram showed sixteen peaks, indicating the presence of sixteen compounds

Table 1: Bioactive compounds Identified in Psidium guajava Leaves Extract by GC-MS

Peak	Name of Compound	Retention time	% Peak Area	% Peak Height	Molecular Weight	Molecular formular
1.	2-Pentanone	3.280	2.35	3.00	116	$C_5H_{10}O$
2.	Benzaldehyde	4.906	6.97	6.57	106	C_6H_5CHO
3.	Benzylamine	5.815	2.25	1.35	107	$C_6H_5CH_2NH_B$
4.	1-Pyrrolidinecarboxylic acid	9.480	8.67	13.67	219	$C_{12}H_{13}NO_3$
5.	dl-2-Phenyl-1,2-propanediol	10.691	1.40	2.43	152	$C_9H_{10}O_2$
6.	3-Buten-2-one	11.441	6.03	9.23	146	$C_{10}H_{10}O$
7.	Benzamide	11.961	8.44	6.04	121	C_7H_7NO
8.	N-Benzylformamide	12.639	8.29	7.21	135	C_8H_9NO
9.	Benzenemethanamine	16.942	21.94	19.54	195	$C_{14}H_{13}N$
10.	Benzyl Benzoate	17.179	2.73	4.31	212	$C_{14}H_{12}O_2$
11.	n-Hexadecanoic acid	20.714	1.96	1.97	256	$C_{16}H_{32}O_2$
12.	N-Benzylbenzamide	22.638	3.35	3.94	211	$C_{14}H_{13}NO$
13.	Benzenamine	23.564	16.60	10.62	245	$C_6H_5NH_2$
14.	Z-8-Methyl-9-tetradecenoic acid	25.969	3.67	2.79	240	$C_{15}H_{28}O_2$
15.	Pyrazole-5-carbonitrile	26.249	2.56	3.37	245	$C_{16}H_{11}N_3$
16.	9,12-Octadecadienoyl chloride	26.888	2.79	3.96	298	$C_{18}H_{31}ClO$
	Total		**100.0**	**100.0**		

Table 2: Medicinal Properties/Therapeutic Uses of Phytocomponents Identified in the Ethanolic extract of Psidium guajava

S/N	Name of Compound	Nature of Compound	Medicinal Properties/Therapeutic Uses	References
1.	2-Pentanone	Aliphatic methyl ketone (volatile solvent/plant VOC)	Repellant and fumigant, plant metabolite, organic solvent, antioxidant	[19, 20]
2.	Benzaldehyde	Aromatic aldehydes	antimicrobial, antioxidant, antifungal, anti-inflammatory, antitumor and insecticidal properties	[21, 22]
3.	Benzylamine	Primary Aromatic amine	Antibacterial	[23]
4.	1-Pyrrolidinecarboxylic acid	Amino acid derivative	Antimicrobial, anticancer, antiviral, antidiabetic and anti-inflammatory	[24, 25]
5.	dl-2-Phenyl-1,2-propanediol	Aromatic secondary alcohol	Antioxidant, anti-inflammatory and antimicrobial	[26]
6.	3-Buten-2-one	Methyl vinyl ketone	Not reported	
7.	Benzamide	Simple aromatic amide	Anti-inflammatory, analgesic, antimicrobial, anti-cancer, anticonvulsant, antidepressant and cardiovascular activities	[27].
8.	N-Benzylformamide	Formamide and benzene class	Not reported	
9.	Benzenemethanamine	Primary amine	Antibacterial	[23]
10.	Benzyl Benzoate	Aromatic ester	antiparasitic	[28]
11.	n-Hexadecanoic acid	Saturated long-chain fatty acid	Antioxidant, antifungal, antimicrobial, anti-inflammatory and anti-cancer properties	[29, 30]
12.	N-Benzylbenzamide	Benzamide derivative	antimicrobial, anti-cancer, herbicidal properties	[31, 32]
13.	Benzenamine (Aniline)	Aromatic Primary Amine	Antibacterial and antifungal	[33]
14.	Z-8-Methyl-9-tetradecenoic acid	Fatty acid	Antibacterial and antifungal, antioxidative	[34]
15.	Pyrazole-5-carbonitrile	Heterocyclic nitrile	Antibacterial, anticancer, antioxidant, antidepressant, anti-inflammatory, antiviral and analgesic activity.	[35]
16.	9,12-Octadecadienoicacid	Linolenic acid	Biosynthesis of prostaglandins and cell membranes, ant-inflammatory, hepatoprotective, anti-arthritic and anti-histamine.	[36, 37]

3.2 Bioactive Compounds Identified in *Soursop* Leaves Extract by GC-MS

The GC-MS analysis of the ethanolic extract of *Annona muricata* leaves identified 11 bioactive compounds, with the chromatogram exhibiting 11 distinct peaks each corresponding to a unique bioactive compound (Fig. 2). The analysis provided data on the retention time, percentage peak area, percentage peak height, molecular weight and molecular formula of the detected compounds. The bioactive compounds identified with respect to their percentage (% peak area) include; Benzenemethanamine, N-(phenylmethylene)- (33.51), Benzenamine, N, N-diphenyl- (24.55), Benzyl Alcohol (11.38), Benzaldehyde (7.78), N-Benzyl-N-(3-phenylprop-2-en-1-yl)-tosylamide (4.61), 2,6,2',6'-Tetramethylazobenzene (3.48), 10-Undecena (3.47), Benzyl chloride (3.38), Pyrazole-5-carbonitrile, 1,3-diphenyl- (3.33), n-Hexadecanoic acid (3.18) and 1-Pyrrolidinecarboxylic acid, 3-oxo-, phenylmethyl ester (1.31) (Table 3).

Medicinal Properties/Therapeutic uses of phytocomponents identified in the ethanolic extract of *A. muricata* is presented in Table 4.

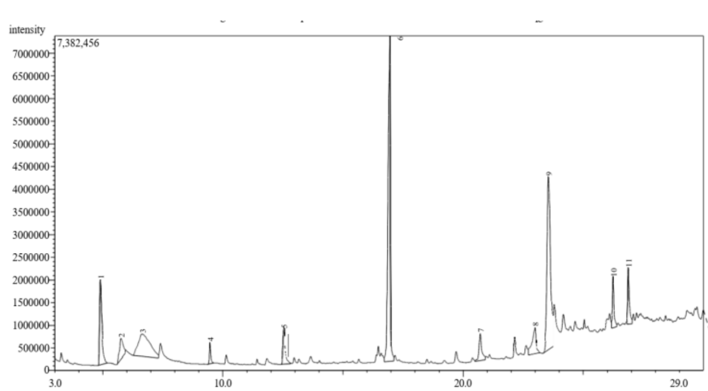

Figure 2: GC-MS analysis of Annona muricata leaves extract of Ethanolic Extract: the GC-MS chromatogram showed eleven peaks, indicating the presence of eleven compounds

Table 3: Bioactive compounds Identified in Annona muricata Leaves Extract by GC-MS

Peak	CompoundAnalysed	Retention time	% Peak Area	% Peak Height	Molecular Weight	Molecular formular
1.	Benzaldehyde	4.893	7.78	10.02	106	C_7H_6O
2.	Benzyl chloride	5.759	3.38	2.49	126	C_7H_7Cl
3.	Benzyl Alcohol	6.653	11.38	2.63	108	C_7H_8O
4.	1-Pyrrolidinecarboxylic acid	9.460	1.31	2.58	219	$C_{12}H_{13}NO_3$
5.	2,6,2',6'-Tetramethylazobenzene	12.541	3.48	4.40	270	$C_{16}H_{18}N_2O_2$
6.	Benzenemethanamine	16.954	33.51	38.44	195	$C_{14}H_{13}N$
7.	n-Hexadecanoic acid	20.714	3.18	3.19	256	$C_{16}H_{32}O_2$
8.	N-Benzyl-N-(3-phenylprop-2-en-1-yl)-tosylamide	22.994	4.61	3.13	377	$C_{23}H_{23}NO_2S$
9.	Benzenamine	23.567	24.55	20.41	245	$C_{18}H_{15}N$
10.	Pyrazole-5-carbonitrile	26.245	3.33	6.07	245	$C_{16}H_{11}N_3$
11.	10-Undecena	26.885	3.47	6.64	168	$C_{11}H_{20}O$
Total			**100.0**	**100.0**		

Table 4: Medicinal Properties/Therapeutic Uses of Phytocomponents Identified in the Ethanolic extract of Annona muricata

S/N	Name of Compound	Nature of Compound	Medicinal Properties/Therapeutic Uses	References
1.	Benzaldehyde	Aromatic aldehydes	antimicrobial, antioxidant, antifungal, anti-inflammatory, antitumor and insecticidal properties	[21, 22]
2.	Benzyl chloride	Organic compound	Analgesic, antipruritic, antimicrobial, preservative, flavoring agent and solvent	[38]
3.	Benzyl Alcohol	Aromatic alcohol	Analgesic, antipruritic, antimicrobial and preservative	[39]
4.	1-Pyrrolidinecarboxylic acid	Amino acid derivative	Antimicrobial, anticancer, antiviral, antidiabetic and anti-inflammatory	[24, 25]
5.	Benzenemethanamine,	Primary amine	Antibacterial	[23]
6.	n-Hexadecanoic acid	Saturated long-chain fatty acid	Antioxidant, antifungal, antimicrobial, anti-inflammatory and anti-cancer properties	[29,30]
7.	N-Benzyl-N-(3-phenylprop-2-en-1-yl)-tosylamide		Not Reported	
8.	Benzenamine (Aniline)	Aromatic Primary Amine	Antibacterial and antifungal	[33]
9.	Pyrazole-5-carbonitrile	Heterocyclic nitrile	Antibacterial, anticancer, antioxidant, antidepressant, anti-inflammatory, antiviral and analgesic activity.	[35]
10.	10-Undecena	Unsaturated aldehyde	Antimicrobial, antioxidant, antifungal and flavoring.	[40, 41]

4.0 Discussion

Annona muricata and *Psidium guajava* are widely recognized in traditional medicine and have been reported to be useful in the treatments of various bacterial infections. Several studies have documented the antibacterial activity of extracts derived from these plants (Doe, *et.al*., 2019; Abdulhamad, et. *al*., 2014). The antimicrobialeffects of both plants'are larely attributed to the presence of diverse phytochemical constituents [12, 42]. Differences in phytochemicals' constituents and antimicrobial efficacymay occur due to variations in biochemical processes within and between plant species, as well as other influiencing factors[43], geographical location [44], methods of extraction [45] and solvent used for extraction [12]. The present study identified severalphytochemical constituents in the ethanolic extracts of *Annona muricata* and *Psidium guajava*, including but not limited to the following compounds: saturated fatty acids, aromatic acids, volatile aromatic hydrocarbons, alkane, anhydride, monocarboxylic acid and so on. The global rise in antimicrobial resistance and the emergence of newinfectious diseases have intensified the search for novel therapeutic options. In this context,these plantsspecies have demonstrated a wide range of bioactive properties that may be harnessed for applications in the pharmaceuticalsector as well as other industrial fields. Some of the therapeutic activity possessed by these plants include antimicrobial (antibacterial and anti-fungal), anti-viral, anti-inflammatory, antitumor, analgesic, antipruritic, antidiabetic, antidepressant and antioxidant [21, 23, 24, 25,29,39). Other use includesflavouring and preservatives for food [38],organic solvent, insecticides, repellant, fumigant andplant metabolite [19, 20].

GC-MS technique detected sixteen (16) bioactive compounds in *Psidium guajava*. The percentage peak area represents the relative abundance of a compound in the sample. It is proportional to the amount of the compound presentin the sample. These compounds identified are; 2-Pentanone (2.35), Benzaldehyde (6.97), Benzylamine (2.25), 1-Pyrrolidine carboxylic acid (8.67), dl-2-Phenyl-1,2-propanediol (1.40), 3-Buten-2-one (6.03), Benzamide (8.44), N-Benzylformamide (8.29), Benzenemethanamine (21.94), Benzyl Benzoate (2.73), n-Hexadecanoic acid (1.96), N-Benzylbenzamide (3.35), Benzenamine (16.60), Z-8-Methyl-9-tetradecenoic acid (3.67), Pyrazole-5-carbonitrile (2.56), 9,12-Octadecadienoyl chloride (2.79). Consistent with other studies, *Psidium guajava* extracts show a high abundance of Benzenemethanamine and Benzenamine, indicating their central role in the plant's pharmacological activity. The presence of phenolic compounds like Di-2-Phenyl-1,2-Propanediol supports its reputation for antioxidant activity [26, 46].

The results of this research do not agree with the findings of [47] who reported 33 bioactive compounds in *P. guajava* using GC-MS technique. The variation might be due to differences in climatic conditions, soil type, altitude, availability of nutrients, extraction technique and genetic variability. However, differences in GC-MS detectors and their sensitivity can influence the number of compounds identified, especially if some compounds are present in trace amounts. The results confirm the presence of constituents which are known to exhibit antibacterial, anti-inflammatory, antioxidant, andneuroprotective potential as well as physiological effects [47].

The GC-MS analysis of *Annona muricata* (soursop) leaf extract identified 10 bioactive compounds, with the most abundant being Benzenemethanamine (33.51 %) and Benzenamine (24.55%). The least abundant compound was 1-pyrrolidine carboxylic acid (1.31 %). This profile highlights the plants potential pharmacological activities, which align with its traditional medicinal use. The presence of Benzenemethanamine in *A. muricata* extract confirms earlier studies conducted by [48, 49] who also reported Benzenemethanamine as a major component, albeit in slightly lower concentrations (28 % peak area) compared to the current study. The study also asserted that Benzenemethanamine has both antibacterial and anticancer properties. The variation in Benzenemethanamine abundance may be due to differences in geographical location and environmental factors affecting plants metabolism. Again, extraction method and GC-MS parameters, such as temperature gradient or solvent polarity may also contribute to the variation observed.

In a related study conducted by Adeyemi, *et. al*., (2008), the authors reported the presence of Benzenamine in moderate to high abundance across various GC-MS analysis of *A. muricata.* They also make reference to the significant contribution made by Benzenamine to the bioactivity observed in *A. muricata.* Compounds such as 1-pyrrolidinecarboxylic acid and trace fatty acids (e.g palmitic acid) were also identified in lower concentrations in similar studies [50] confirming their consistency in *A. muricata* extract.

It is well recognized that the Annona plant has medicinal properties, including antidiarrheal [42], antiparasitic [51], antiulcer [52], antioxidant [53], antidiabetic [50], antidepressant [54], antiviral, Dengue vector control [55], anticancer [56], and anti-inflammatory [57] activities.

The ethanolic extract of these plants shows that they are rich in saturated long chai fatty acids, aromatic amines, aromatic amide, amino acid derivatives, methyl vinyl ketone, benzene class, aromatic ester acid and linolenic acid all of which possess both pharmacologicaland therapeutic activities as well as great and varied use in textile, paint, food and cosmetic industries.

However, the presence of some bioactive components of toxicological implications have been confirmed in an In Vivo studies involving the exposure of fingerlings of *C. gariepinus* to ethanolic extract of *A. muricata* and *P. guajava*[58, 59]. This implies that despite the therapeutic potentials derived from plant extracts, there is a need to understand its toxicological effects. Similar toxicological studies involving plant extract confirmed this assertion [60, 61, 62, 63, 64, 65, 66, 67, 68, 69].

4.1 Conclusion

The comprehensive GC-MS analysis of the leaves of *Annona muricata* and *Psidium guajava* has revealed a diverse array of bioactive compounds, each contributing to the plant's pharmacological profiles. Notably compounds such as Benzenemethanamine and Benzenamine were consistently identified across these species, albeit in varying concentrations. These findings align with existing literature, underscoring the therapeutic potential of these plants. However, discrepancies in compound abundance and identification across different studies highlights the influence of factors such as extraction methods, environmental conditions and analytical techniques.

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