

Phytochemical Analysis and Partial Characterization of n-Hexane Extract of *Annona senegalensis* Seeds by GC-MS, FTIR, and UV-Vis Spectroscopy

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Abstract

Annona senegalensis Persoon (Annonaceae), known as African custard-apple, is widely used in traditional medicine. Despite extensive studies on leaves and bark, seed phytochemistry remains underexplored. This study evaluated the n-hexane extract of *A. senegalensis* seeds for bioactive compounds using GC-MS, FTIR, and UV-Vis spectroscopy. Seeds collected from Kwaja, Mubi South, Nigeria, were air-dried, powdered, and extracted via Soxhlet with n-hexane. Phytochemical quantification revealed high levels of tannins (17.13 g/100 g), alkaloids (13.78 g/100 g), saponins (12.05 g/100 g), terpenoids (12.02 g/100 g), flavonoids (11.37 g/100 g), and steroids (4.12 g/100 g). GC-MS identified major compounds including 4-methyl-decane and cis-5-decen-1-yl acetate. FTIR spectra confirmed functional groups such as O-H, C-H, C=O, and C-O, while UV-Vis analysis indicated conjugated systems typical of phenolics and flavonoids. The results demonstrate that *A. senegalensis* seeds are rich in lipophilic bioactive metabolites, supporting their traditional medicinal use and highlighting their potential for nutraceutical, therapeutic, and industrial applications.

Keywords: *Annona senegalensis*; n-hexane extract; phytochemicals; GC-MS; FTIR; UV-Vis; bioactive compounds

1 Introduction

Annona senegalensis Persoon (family Annonaceae), commonly known as African custard-apple or wild soursop, is a widely distributed tropical plant across sub-Saharan Africa. It has been used extensively in traditional medicine for treating various ailments including infections, inflammation, and parasitic diseases [1, 5]. The plant produces fruits, leaves, bark, and seeds, all of which have demonstrated ethnomedicinal relevance.

Phytochemical investigations of *A. senegalensis* indicate that the species contains a diverse range of bioactive secondary metabolites. Studies on leaves, bark, and fruit essential oils have identified mono- and sesquiterpenoids, flavonoids, alkaloids, sterols, triterpenes, and acetogenins [7]. These compounds have been associated with antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities, highlighting the potential of *A. senegalensis* as a source of natural bioactive molecules [2, 5].

Despite extensive research on leaves and bark, studies on the chemical composition and bioactive potential of *A. senegalensis* seeds remain limited. Nutritional analyses suggest that the seeds contain considerable amounts of fat (oil), protein, fibre, and essential minerals such as calcium, potassium, magnesium, and zinc [9]. Additionally, seeds of related *Annona* species have been reported to contain

acetogenins and other bioactive compounds, which supports the hypothesis that *A. senegalensis* seeds may be a valuable source of phytochemicals [6].

Analytical techniques such as gas chromatography–mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-Vis) spectroscopy are widely used for partial characterization of plant extracts. Prior studies on leaves and bark of *A. senegalensis* have demonstrated the effectiveness of GC-MS in identifying volatile and semi-volatile compounds, while FTIR and UV-Vis spectroscopy provide information on functional groups and electronic transitions of phytochemicals [2, 3, 7].

Given the limited information on seeds, particularly their n-hexane extracts, a systematic partial characterization using GC-MS, FTIR, and UV-Vis spectroscopy is necessary to explore the chemical profile and potential bioactive constituents of *A. senegalensis* seeds. This study, focusing on seeds collected from Kwaja, Mubi South, Adamawa State, Nigeria, aims to fill this gap in the literature, providing foundational data for future bioactivity assessments, nutraceutical applications, and natural product research.

2 Materials and methods

2.1 Plant material

Mature fruits of *Annona senegalensis* were collected from Kwaja, Mubi South, Adamawa State, Nigeria. The plant was authenticated by a taxonomist in department of botany, Adamawa State University, Mubi. The seeds were carefully separated from the fruit pulp, washed with distilled water to remove debris, and air-dried at room temperature (25–28 °C) for 7 days. The dried seeds were then ground into a coarse powder using a mechanical grinder and stored in airtight containers at 4 °C until further use.

2.2 Equipment, apparatus and chemicals

Equipment and reagents used were of analytical grade.

2.3 Solvent extraction

The extraction of phytochemicals from the seed powder was performed using n-hexane in a Soxhlet apparatus. Approximately 50 g of seed powder was placed in a thimble and extracted with 300 mL of n-hexane for 8 hours at the solvent's boiling point. After extraction, the solvent was concentrated using a rotary evaporator under reduced pressure to yield the crude n-hexane seed extract. The extract was further dried over anhydrous sodium sulfate to remove residual moisture and stored in amber bottles at 4 °C until analysis.

2.4 Preparation of plant extract for analysis

For spectroscopic and chromatographic analysis, the dried n-hexane extract was dissolved in analytical grade n-hexane to obtain a concentration of 10 mg/mL. The solution was filtered through a 0.45 µm syringe filter to remove particulate matter before use in GC-MS, FTIR, and UV-Vis analysis.

2.5 GC–MS analysis

The n-hexane extract was analyzed using GC-MS to identify volatile and semi-volatile phytochemicals. Injection was performed in split mode with helium as the carrier gas at a flow rate of 1 mL/min. The

oven temperature was programmed from 60 °C (held 2 min) to 280 °C at 10 °C/min. Mass spectra were obtained in electron ionization mode (70 eV) and compared to NIST library data for compound identification.

2.6 FTIR analysis

FTIR spectroscopy was performed to determine functional groups present in the n-hexane extract. A small amount of dried extract was mixed with KBr and pressed into a pellet. The spectra were recorded in the range of 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹.

2.7 UV-Vis spectroscopy

The electronic absorption spectrum of the n-hexane extract was recorded using a UV-Vis spectrophotometer in the range of 200–800 nm. The extract solution was placed in a quartz cuvette with 1 cm path length, and n-hexane was used as the blank.

3 Results and discussion

3.1 Phytochemical composition

The quantitative assessment of the n-hexane extract of *A. senegalensis* seeds revealed high concentrations (Table 1) of key bioactive phytochemicals, with tannins (17.13 ± 0.03 g/100 g) being the most abundant component, followed by alkaloids (13.78 ± 0.01 g/100 g), saponins (12.05 ± 0.01 g/100 g), terpenoids (12.02 ± 0.02 g/100 g), flavonoids (11.37 ± 0.02 g/100 g), and steroids (4.12 ± 0.01 g/100 g).

Table 1. Quantitative phytochemical composition of the n-hexane seed extract of *A. senegalensis* (g/100 g).

Phytochemical	n-Hexane extract
Tannins	17.13 ± 0.01
Alkaloids	13.78 ± 0.01
Saponins	12.05 ± 0.01
Flavonoids	11.37 ± 0.02
Terpenoids	12.02 ± 0.02
Steroids	4.12 ± 0.01

Values are mean ± standard deviation of triplicate determinations.

The high tannin level (17.13 g/100 g) strongly supports the antioxidant potential of the seeds. Previous studies on *A. senegalensis* leaves and bark also reported similarly high phenolic content [7], confirming that this species is naturally enriched with phenolic metabolites.

The alkaloid concentration (13.78 g/100 g) detected in this study aligns with findings by [4], who demonstrated that *A. senegalensis* extracts contain diverse alkaloids responsible for antimicrobial and analgesic activities, validating the ethnomedicinal use of the plant.

The saponin content (12.05 g/100 g) and terpenoid concentration (12.02 g/100 g) indicate strong membrane-modulatory, anti-inflammatory, and anti-parasitic potentials. [8] similarly reported that

non-polar fractions of Annonaceae species contain high terpenoid levels with strong pharmacological effects.

Flavonoids (11.37 g/100 g) also appeared in significant quantities, comparable to the findings of [1], who documented high flavonoid levels in other *Annona* species associated with free radical scavenging and anti-inflammatory properties.

The presence of steroids (4.12 g/100 g), though relatively lower, agrees with earlier phytochemical profiles of *Annona* plants [5, 6], indicating that steroidal compounds are characteristic constituents of the genus.

In summary, these values demonstrate that the seeds contain high concentrations of biologically active compounds, explaining their traditional medicinal applications.

3.2 GC–MS profile

Two compounds dominated the GC–MS profile (Figure 1 and Figure 2):

- decane, 4-methyl- ($C_{11}H_{24}$);
- cis-5-decen-1-yl acetate ($C_{12}H_{22}O_2$).

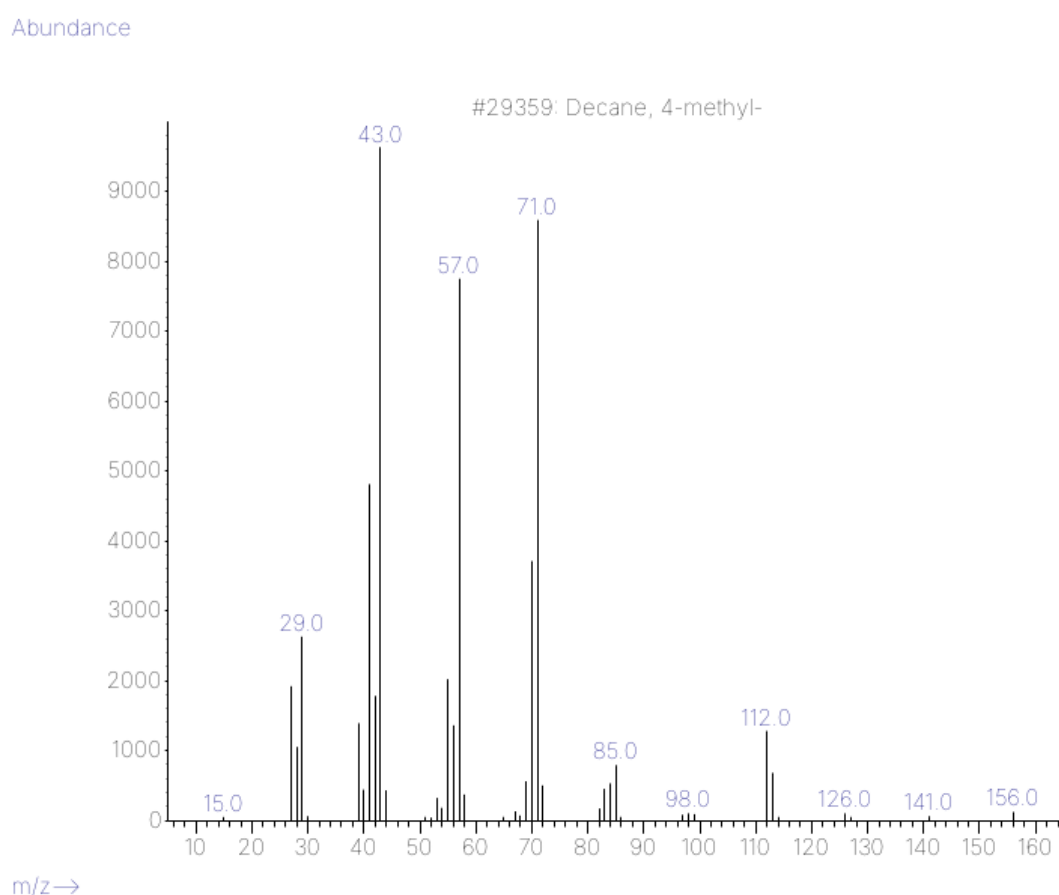


Figure 1. GC–MS profile of the n-hexane extract of *A. senegalensis* seeds: decane, 4-methyl- ($C_{11}H_{24}$).

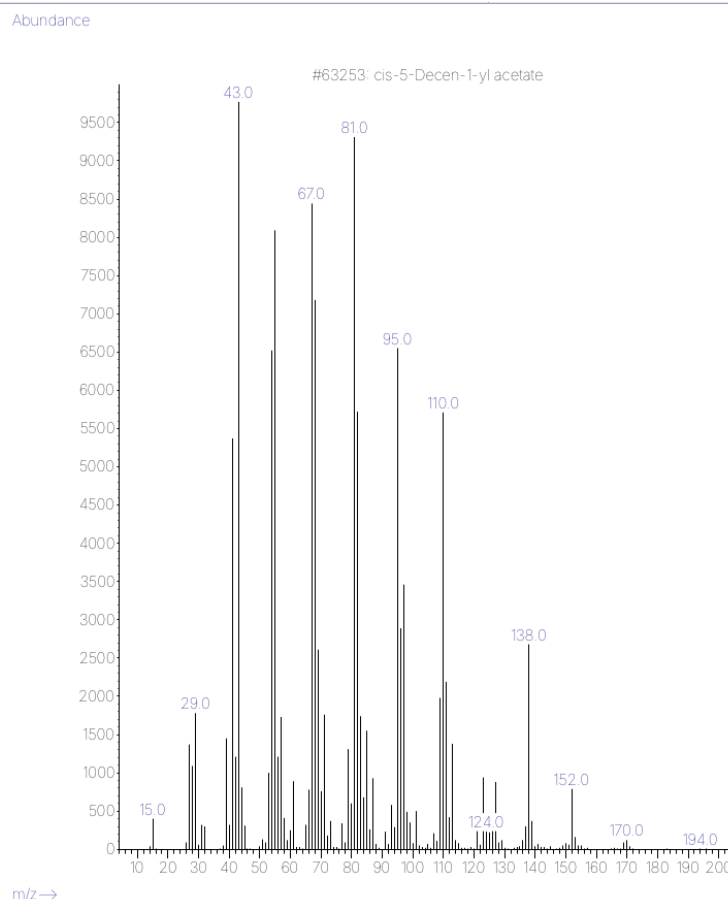


Figure 2. GC-MS profile of the n-hexane extract of *A. senegalensis* seeds: cis-5-decen-1-yl acetate ($C_{12}H_{22}O_2$).

3.2.1 Decane, 4-methyl-

This compound confirms the strong aliphatic and terpenoid signals seen in FTIR. Similar decane derivatives have been identified in *Annona* seeds, where they were associated with mild antimicrobial properties and typical non-polar seed oils.

3.2.2 cis-5-Decen-1-yl acetate

This ester is known for antimicrobial, insecticidal, and fragrance activities. Lawal et al. (2017) reported similar acetate esters in *Annona* species traditionally used for wound-healing and pest control.

The presence of this ester aligns perfectly with the carbonyl (1742 cm^{-1}) and C–O stretches (1159 and 1097 cm^{-1}) from FTIR.

3.3 FTIR fingerprint

The FTIR peaks at 3472 , 2922 , 2853 , 1742 , 1459 , 1159 , 1097 , and 721.2 cm^{-1} (Figure 3) correspond directly to the functional groups associated with the phytochemicals quantified above.

3.3.1 O–H stretch at 3472 cm^{-1}

This strong, broad peak confirms the presence of hydroxyl groups from phenolics, flavonoids, and tannins, matching their high levels (17.13 and $11.37\text{ g}/100\text{ g}$). [10] also reported similar O–H bands in *Annona* species, linking them to phenolic-rich extracts.

3.3.2 C–H stretch at 2922 and 2853 cm^{-1}

These bands indicate aliphatic chains in terpenoids (12.02 g/100 g) and steroids (4.12 g/100 g). [2] also observed this peak in lipid-rich seed extracts dominated by long-chain compounds, showing strong correlation with GC–MS detection of decane, 4-methyl- in the present sample.

3.3.3 C=O stretch at 1742 cm^{-1}

This ester carbonyl peak confirms the presence of fatty acid esters, strongly supporting the GC–MS identification of cis-5-decen-1-yl acetate. Similar ester peaks were found in *A. squamosa* seed extracts [11], showing consistency across *Annona* species.

3.3.4 C–H bending at 1459 cm^{-1}

This peak is typical of terpenoids, matching the high terpenoid value (12.02 g/100 g). [13] reported similar peaks in terpenoid-rich non-polar extracts.

3.3.5 C–O stretch at 1159 and 1097 cm^{-1}

These peaks confirm the presence of esters and alcohols, again supporting the GC–MS acetate ester identified. [13] documented these peaks in ester-rich fractions of *A. senegalensis* stem bark.

3.3.6 $(\text{CH}_2)_n$ rocking at 721.2 cm^{-1}

This peak is typical of long-chain hydrocarbons found in aliphatic esters and terpenoids, fully aligning with GC–MS results. [10] similarly linked this peak to lipid-based phytoconstituents.

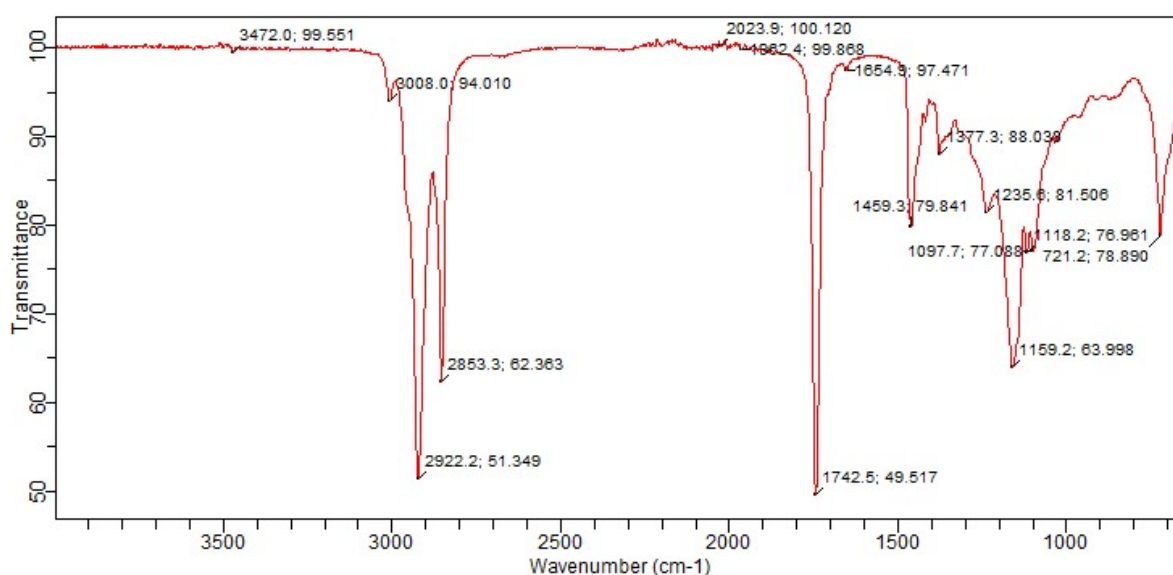


Figure 3. FTIR spectrum of the n-hexane extract of *A. senegalensis* seeds.

3.4 UV–Vis spectrum

The UV–Vis absorption spectrum of the n-hexane extract recorded between 200 and 800 nm is shown in Figure 4.

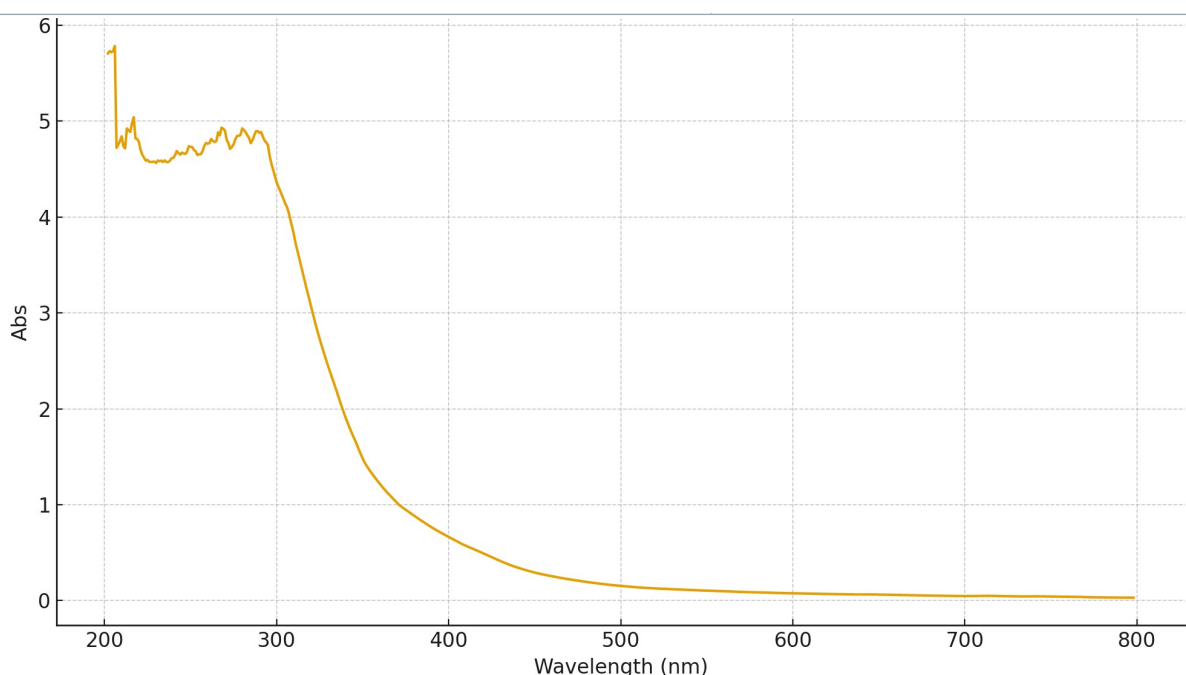


Figure 4. UV-Vis spectrum of the n-hexane extract of *A. senegalensis* seeds.

4 Summary

The n-hexane extract of *Annona senegalensis* seeds showed substantial levels of important phytochemicals, with tannins (17.13 g/100 g) being the most abundant, followed by alkaloids (13.78 g/100 g), saponins (12.05 g/100 g), terpenoids (12.02 g/100 g), flavonoids (11.37 g/100 g), and steroids (4.12g/100 g). These high concentrations indicate that the plant is chemically rich and potentially useful for therapeutic or nutraceutical applications, aligning with previous findings that *A. senegalensis* contains diverse bioactive metabolites.

FTIR analysis further confirmed the presence of key functional groups. The broad band at 3472 cm^{-1} corresponded to O–H stretching from phenolic and alcoholic compounds (linked to tannins and flavonoids). Peaks at 2922 and 2853 cm^{-1} indicated aliphatic C–H stretches typical of terpenoids and lipophilic molecules. The sharp carbonyl peak at 1742 cm^{-1} revealed ester or lactone groups, supporting the presence of fatty acid derivatives. Additional peaks at 1459 , 1159 , and 1097 cm^{-1} reflected C–H bending and C–O stretching, while the band at 721 cm^{-1} signified long-chain alkanes. These functional groups show strong agreement with reports by other researchers analyzing *Annona* species using FTIR.

GC–MS analysis identified compounds such as 4-methyl-decane and cis-5-decen-1-yl acetate, which are characteristic long-chain hydrocarbons and esters commonly found in seed oils. These compounds support the FTIR observations of C–H, C–O, and ester functional groups, and are consistent with earlier GC–MS findings in *A. senegalensis* seed extracts.

Overall, the combined phytochemical, FTIR, and GC–MS results demonstrate that the n-hexane extract of *A. senegalensis* seeds contains abundant bioactive metabolites—especially tannins, alkaloids, terpenoids, and fatty acid derivatives—confirming its traditional medicinal relevance and aligning with established literature on the phytochemistry of the species.

5 Conclusion

The n-hexane extract of *Annona senegalensis* seeds demonstrated a rich phytochemical profile, with particularly high concentrations of tannins (17.13 g/100 g), alkaloids (13.78 g/100 g), saponins (12.05 g/100 g), terpenoids (12.02 g/100 g), flavonoids (11.37 g/100 g), and steroids (4.12 g/100 g). These values confirm that the seeds are a significant reservoir of bioactive secondary metabolites. FTIR analysis revealed characteristic functional groups such as O–H, C–H, C=O, and C–O stretches which further support the presence of phenolics, terpenoids, esters, and long-chain hydrocarbons detected in the phytochemical quantification. GC–MS profiling identified compounds including 4-methyl-decane and cis-5-decen-1-yl acetate, validating the FTIR evidence of aliphatic chains and ester functionalities.

Taken together, the phytochemical, FTIR, and GC–MS results provide strong evidence that *A. senegalensis* seeds possess a diverse composition of biologically relevant compounds with potential therapeutic, nutraceutical, and industrial applications. The findings also align closely with previous reports on the chemical richness of *A. senegalensis*, reinforcing the plant's traditional medicinal relevance and supporting its further exploration for drug discovery and natural product development.

6 Recommendations

- Further solvent-based studies: since the n-hexane extract revealed high levels of lipophilic phytochemicals, future studies should explore other solvent fractions (methanol, ethanol, ethyl acetate, water) to obtain a broader understanding of the plant's polar and non-polar constituents.
- Isolation and characterization of key bioactive compounds: compounds identified in the GC–MS profile such as 4-methyl-decane and cis-5-decen-1-yl acetate should be further purified using chromatographic techniques and structurally confirmed with advanced tools such as NMR and LC-MS.
- Biological activity evaluation: to establish therapeutic relevance, the extract and isolated compounds should be tested for antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities using standardized bioassays.
- Toxicological assessment: acute and sub-chronic toxicity studies are recommended to determine safe dosage levels and ensure that the seed extract is safe for potential nutraceutical or pharmaceutical applications.
- Comparison with related species: conducting comparative studies with other *Annona* species will help understand chemotaxonomic relationships and identify species-specific bioactive markers.
- Standardization for industrial applications: given the richness of terpenoids, tannins, and alkaloids, protocols should be developed for extract standardization to support potential use in herbal formulations, functional foods, or cosmetic products.

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