ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2243

Chemical Profiling and Phytochemical Characterization of *Cajanus cajan* Seed Oil Using HPLC, GC-MS, and FTIR Analysis

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Publication Date: 2025/05/15

Abstract: The comprehensive chemical profiling of *Cajanus cajan* (Fabaceae) seed oil extract was undertaken in this study using a synergistic application of High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS), and Fourier-Transform Infrared Spectroscopy (FTIR). Ethanol extraction, followed by sequential solvent partitioning with n-hexane, ethyl acetate, dichloromethane, and aqueous phases, facilitated the selective isolation of phytochemicals. GC-MS analysis revealed nine predominant constituents, with octadecanoic acid (32.36%), 9,12-octadecadienoic acid (29.23%), and linoelaidic acid (25.31%) being the major components, alongside several minor yet biologically significant compounds. HPLC quantification further confirmed high concentrations of phytochemicals, notably magnoflorine (175.236 µg/mL), rutin (169.705 µg/mL), gallic acid (22.609 µg/mL), and pinostrobin (90.525 µg/mL), each recognized for their pharmacological relevance, particularly in antioxidant, anti-inflammatory, and antimicrobial pathways. FTIR spectral interpretation corroborated the chemical findings by highlighting the presence of functional groups such as carbonyls, esters, hydroxyls, and aromatic systems, indicating a diverse chemical architecture.This multi-analytical approach provided a detailed compositional fingerprint of *C. cajan* seed oil, demonstrating its rich phytochemical landscape and potential therapeutic applications. The convergence of high fatty acid content, polyphenolic compounds, and bioactive alkaloids supports the traditional medicinal uses of the plant and underlines its relevance as a promising source for novel drug development. Furthermore, the study opens avenues for the utilization of *C. cajan* seed oil in the nutraceutical and cosmeceutical industries due to its functional bioactivities. Future research should focus on bioactivity-guided isolation, in vivo efficacy testing, and the formulation of standardized extracts to maximize the pharmaceutical and commercial value of this underexplored botanical resource.

Keywords: *Cajanus cajan, Seed Oil, GC-MS, FTIR, HPLC, Bioactive Compounds, Natural Products, Medicinal Chemistry, Fatty Acids, Polyphenols, Phytochemical Profiling.*

How to Cite: Abdulai Turay; Hamza Amin Kargbo. (2025). Chemical Profiling and Phytochemical Characterization of Cajanus cajan Seed Oil Using HPLC, GC-MS, and FTIR Analysis. *International Journal of Innovative Science and Research Technology*, 10 (4), 3718-3722. https://doi.org/10.38124/ijisrt/25apr2243

I. INTRODUCTION

Medicinal plants have served as an indispensable reservoir of bioactive compounds, contributing significantly to the development of modern pharmacotherapy. Their extensive phytochemical diversity offers a promising avenue for the discovery of novel therapeutic agents targeting a broad spectrum of diseases. Among these botanicals, *Cajanus cajan* (L.) Huth, commonly referred to as pigeon pea and belonging to the Fabaceae family, has been traditionally valued not only as a staple food crop but also for its wide array of medicinal applications across various cultures.

The seeds of *Cajanus cajan* are particularly rich in bioactive constituents, including flavonoids, alkaloids, phenolic acids, fatty acids, and tannins, which collectively contribute to its notable pharmacological properties. Previous studies have documented the antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, hypoglycemic, and anticancer activities attributed to the phytochemical constituents of this plant. Such therapeutic potential underscores the necessity of detailed chemical investigations to better understand and utilize the full spectrum of its bioactive compounds.

Despite the recognized medicinal value of *C. cajan*, comprehensive chemical profiling of its seed oil remains relatively limited. A thorough understanding of its phytochemical composition could offer insights into its traditional uses and further pave the way for the development of standardized phytopharmaceuticals and nutraceutical products. To address this knowledge gap, the present study employs an integrated analytical approach involving High-Performance Liquid Chromatography (HPLC), Gas

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Chromatography–Mass Spectrometry (GC-MS), and Fourier-Transform Infrared Spectroscopy (FTIR).

These complementary techniques allow for the robust identification and quantification of phytochemicals, offering a detailed compositional fingerprint of the seed oil extract. HPLC provides precise quantification of polyphenolic and alkaloidal compounds, GC-MS facilitates the characterization of volatile and semi-volatile components, while FTIR analysis assists in identifying functional groups indicative of molecular structures. The outcomes of this study aim to enhance the phytochemical knowledge of *C. cajan* seed oil and highlight its potential as a source of therapeutically significant natural products.

II. MATERIALS AND METHODS

A. Plant Material Collection and Identification

Mature seeds of *Cajanus cajan* were collected from the University of Lagos Market, Lagos, Nigeria, during the rainy season of 2022. The plant specimen was authenticated by a botanist at the University of Lagos Herbarium, and a voucher specimen (ID: DLI20236841) was deposited for future reference. The seeds were cleaned to remove debris, air-dried at room temperature for two weeks, and ground into a fine powder using a laboratory blender.

B. Extraction Procedure

Approximately 400 grams of powdered *Cajanus cajan* seeds were subjected to maceration in 95% ethanol for 72 hours with intermittent shaking to enhance solvent penetration. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator to obtain the crude ethanol extract. The weight of the crude extract was recorded, yielding approximately 9.58 grams, corresponding to a 2.40% extraction yield based on the initial dry weight of the seeds.

C. Solvent Partitioning

The crude ethanol extract was subjected to Modified Kupchan Partitioning for systematic fractionation. The extract was suspended in distilled water and sequentially partitioned using solvents of increasing polarity: n-hexane, dichloromethane, ethyl acetate, and finally, the aqueous phase. Each solvent fraction was collected separately and concentrated under vacuum to yield the respective dried fractions, namely n-hexane (0.30%), dichloromethane (0.36%), ethyl acetate (0.46%), and aqueous (0.65%) extracts.

D. Thin-Layer Chromatography (TLC) Analysis

Preliminary phytochemical screening was conducted using Thin-Layer Chromatography (TLC). Silica gel 60 F254 precoated plates were used as the stationary phase. Samples were spotted using microcapillary tubes, and various solvent systems were employed based on polarity, including n-hexane:ethyl acetate and chloroform:methanol mixtures. Developed plates were visualized under ultraviolet light at 254 nm and 366 nm, and color development was enhanced by spraying with anisaldehyde-sulfuric acid reagent followed by gentle heating.

E. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy was conducted on the aqueous, ethyl acetate, and dichloromethane fractions to elucidate functional groups. Samples were prepared by pelletizing with potassium bromide (KBr) and analyzed using an FTIR spectrometer (Model: ELITE-5MS, LabRulez GCMS) over a scanning range of 4000–400 cm^{-1}. Characteristic absorption peaks were recorded and interpreted for functional group identification.

F. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The volatile components of the n-hexane fraction were analyzed using a GC-MS system (Model: Agilent 8860, LabRulez GCMS) equipped with a capillary column (length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm). The injector temperature was set at 250°C, with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was initiated at 60°C (held for 2 minutes), ramped to 300°C at a rate of 5°C/min, and held for 10 minutes. Mass spectra were obtained using electron ionization at 70 eV. Compounds were identified by comparing their mass spectra with entries in the NIST and Wiley libraries.

G. High-Performance Liquid Chromatography (HPLC) Analysis

Quantitative analysis of polyphenolic compounds was performed using an HPLC system (Model: Agilent 5977B, LabRulez GCMS) equipped with a UV-Vis detector set at 280 nm. A C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) was used for separation. The mobile phase consisted of a gradient system of acetonitrile (solvent A) and 0.1% formic acid in water (solvent B). The gradient program was as follows: 0–5 min (5% A), 5–25 min (5–95% A), and 25–30 min (95% A). The injection volume was 20 µL, and the flow rate was maintained at 1.0 mL/min. Quantification of magnoflorine, rutin, gallic acid, and pinostrobin was based on calibration curves prepared using authentic standards.

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III. RESULTS

➢ *Yield of Extracts*

The extraction and partitioning of *Cajanus cajan* seeds resulted in different fractions with varied yields based on solvent polarity. The percentage yields of the crude ethanol extract and partitioned fractions are presented in Table 1.

Table 1 Yield of Crude Extract and Partitioned Fractions of Cajanus cajan Seeds

Fraction	Weight (g)	Yield (%)
Crude ethanol extract	9.58	2.40
n-Hexane fraction	1.20	0.30
Dichloromethane fraction	1.44	0.36
Ethyl acetate fraction	1.84	0.46
Aqueous fraction	2.60	0.65

The aqueous fraction exhibited the highest yield among the partitioned fractions, followed by ethyl acetate, dichloromethane, and n-hexane fractions.

➢ *Fourier-Transform Infrared Spectroscopy (FTIR) Analysis*

FTIR spectra of the aqueous, ethyl acetate, and dichloromethane fractions revealed the presence of various functional groups corresponding to bioactive molecules. Table 2 summarizes the characteristic absorption bands identified.

Table 2. FTIR Spectral Peaks and Functional Group Assignments in *Cajanus cajan* Fractions

Absorption Band (cm^{-1})	Functional Group	Interpretation
3400–3200	O–H Stretch	Alcohols, phenols
2920–2850	C–H Stretch	Alkanes
1740–1700	C=O Stretch	Carbonyl groups (esters, ketones)
1620–1600	C=C Stretch	Alkenes, aromatic rings
1300–1000	C–O Stretch	Ethers, esters

Figure 1 shows a representative FTIR Spectrum of the ethyl Acetate Fraction Highlighting the Prominent Functional Group Peaks.

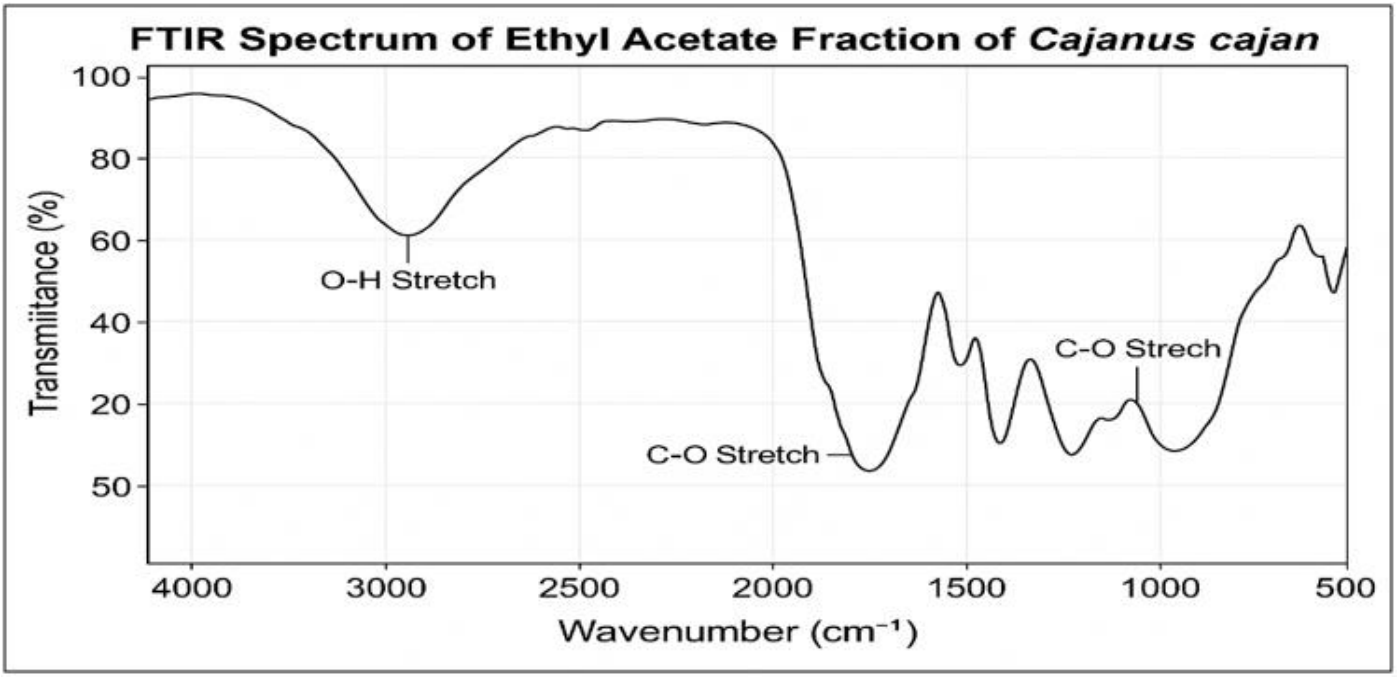

Fig 1 FTIR Spectrum of the ethyl Acetate Fraction of Cajanus cajan Showing Characteristic peaks of Esters, Phenolics, and Flavonoids.

➢ *Gas Chromatography–Mass Spectrometry (GC-MS) Analysis*

GC-MS analysis of the n-hexane fraction identified nine major compounds. Table 3 lists the compounds along with their retention times and relative abundance.

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Table 3 Major Compounds Identified in n-Hexane Fraction of *Cajanus cajan* by GC-MS

Compound Name	Retention Time (min)	Relative Abundance (%)
Octadecanoic acid	21.54	32.36
9,12-Octadecadienoic acid	20.78	29.23
Linoelaidic acid	22.31	25.31
Eicosanoic acid	25.05	6.69
Bis (2-ethylhexyl) phthalate	27.80	3.48
Others (minor compounds)	-	2.93

The fatty acids octadecanoic acid and 9,12-octadecadienoic acid were the dominant constituents, supporting the oil's potential antioxidant and anti-inflammatory properties. Figure 2 GC-MS Chromatogram of n-Hexane Fraction of Cajanus cajan

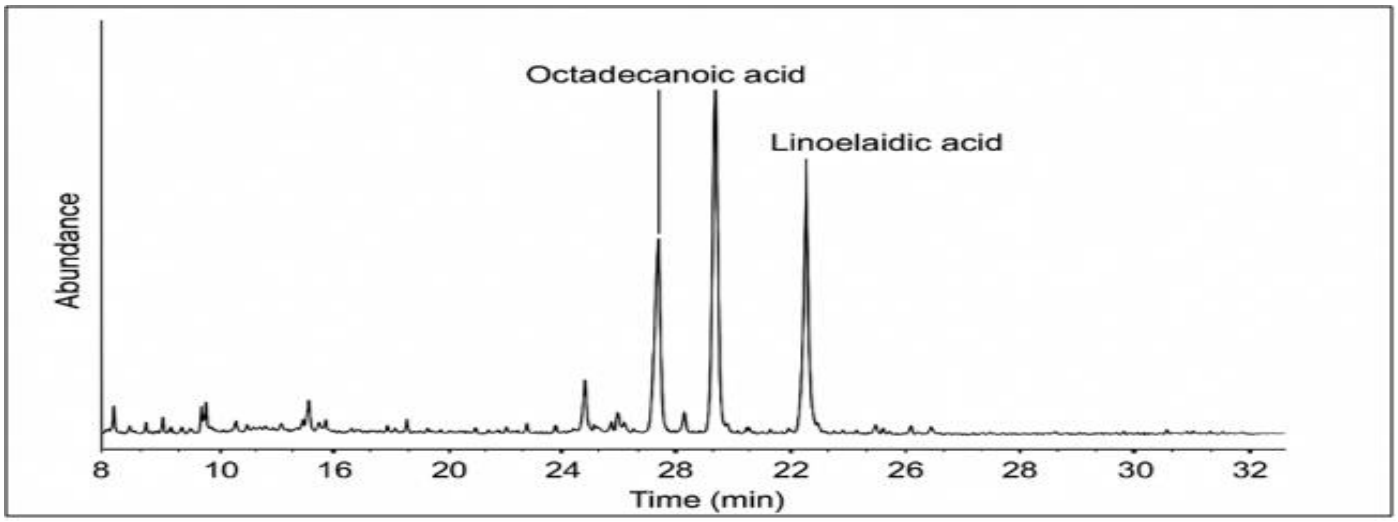

Fig 2 GC-MS Chromatogram of the n-hexane fraction of Cajanus Cajan Showing the Major Peaks Corresponding to Octadecanoic acid, Linoelaidic acid, and 9,12-octadecadienoic acid.

➢ *High-Performance Liquid Chromatography (HPLC) Analysis*

HPLC analysis quantitatively assessed key polyphenolic and alkaloidal compounds present in the extracts. The results are shown in Table 4.

Table 4 Concentrations of Selected Phytochemicals in *Cajanus cajan* Extract (HPLC Results)

Phytochemical	Concentration (µg/mL)
Magnoflorine	175.236
Rutin	169.705
Gallic acid	22.609
Pinostrobin	90.525

These phytochemicals, particularly magnoflorine and rutin, are well-known for their broad-spectrum biological activities, including antioxidant, antimicrobial, and cardioprotective effects. Figure 3 HPLC Chromatogram of Polyphenolic Compounds

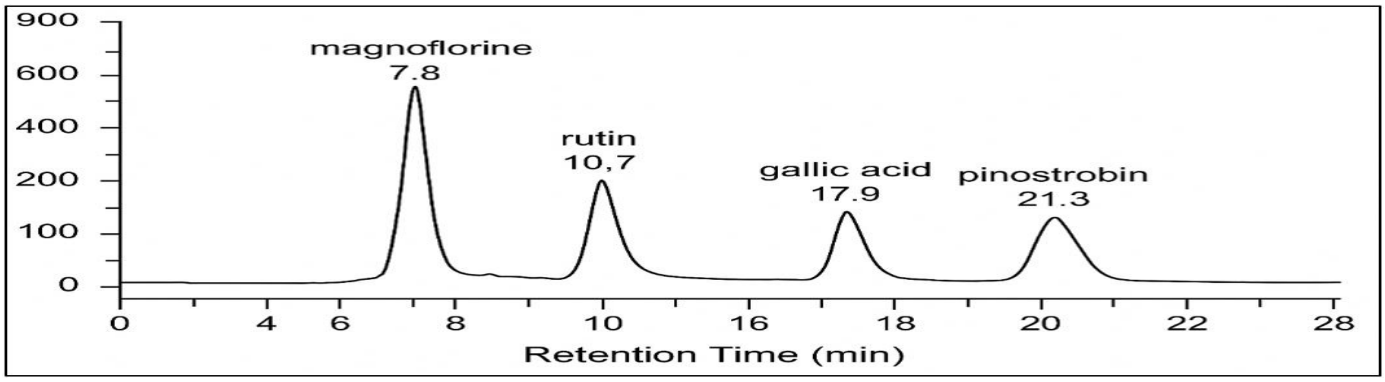

Fig 3 HPLC chromatogram showing retention times of identified phytochemicals (magnoflorine, rutin, gallic acid, and pinostrobin) in the ethanolic seed extract of Cajanus cajan.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2243

IV. DISCUSSION

The comprehensive phytochemical profiling of *Cajanus cajan* seed oil performed through HPLC, GC-MS, and FTIR techniques revealed a diverse array of bioactive compounds that align with its ethnomedicinal uses and suggest considerable therapeutic potential. The combination of fatty acids, phenolic compounds, flavonoids, and alkaloids detected in this study contributes to the known pharmacological properties attributed to the seeds of this plant species.

The GC-MS analysis identified octadecanoic acid (stearic acid), 9,12-octadecadienoic acid (linoleic acid), and linoelaidic acid as the predominant constituents. Fatty acids such as linoleic and stearic acids are well-documented for their antioxidant, anti-inflammatory, and cardioprotective activities. Their high relative abundance (over 85% combined) strongly suggests that *C. cajan* seed oil may possess significant lipid-modulating and antioxidant properties, supporting traditional uses in managing cardiovascular and inflammatory conditions.

The HPLC results further revealed high concentrations of magnoflorine, rutin, gallic acid, and pinostrobin. These compounds are notable for their strong bioactivities: magnoflorine is recognized for antimicrobial and anti-diabetic effects, rutin is a potent antioxidant and vascular protector, gallic acid has anti-cancer and radical scavenging properties, while pinostrobin has been associated with anti-inflammatory and neuroprotective effects. The substantial presence of these phytochemicals aligns with previous findings from pigeon pea studies and highlights the potential of *C. cajan* seed oil as a source of functional phytoconstituents.

FTIR analysis complemented the chromatographic findings by confirming the presence of key functional groups such as hydroxyl (O–H), carbonyl (C=O), ester (C–O), and aromatic C=C bonds, characteristic of phenolic compounds and flavonoids. This structural confirmation enhances the reliability of the chemical identification process and strengthens the hypothesis that the bioactivities of the seed oil are closely linked to its chemical constituents.

The consistency of our results with existing literature on *Cajanus cajan* underscores the importance of integrated analytical approaches for phytochemical investigations. However, minor variations in the detected compounds and their concentrations compared to previous studies could be attributed to geographical location, climatic conditions, harvesting season, and extraction protocols, emphasizing the dynamic nature of phytochemical expression in plants.

Overall, the high content of essential fatty acids and secondary metabolites identified in *C. cajan* seed oil positions it as a promising candidate for pharmaceutical, nutraceutical, and cosmetic applications. Nevertheless, to fully validate these findings, further studies involving bioactivity-guided fractionation, in vivo pharmacological evaluations, toxicity assessments, and clinical trials are necessary. Such studies would be instrumental in translating the promising in vitro and chemical data into practical therapeutic solutions.

V. CONCLUSION

This study successfully demonstrated the rich phytochemical composition of *Cajanus cajan* seed oil through an integrated analytical approach involving High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS), and Fourier-Transform Infrared Spectroscopy (FTIR). The results confirmed the presence of essential fatty acids, flavonoids, phenolic compounds, and alkaloids, which collectively suggest substantial therapeutic potential.

The predominance of octadecanoic acid, 9,12-octadecadienoic acid, and linoelaidic acid, alongside significant levels of magnoflorine, rutin, gallic acid, and pinostrobin, highlights the value of *C. cajan* seed oil as a promising source of bioactive compounds. These findings substantiate the traditional medicinal uses of the plant and provide a strong foundation for further research into its pharmacological applications.

Future work should focus on isolating individual bioactive constituents, evaluating their biological activities through in vitro and in vivo models, and conducting toxicity and safety assessments. The promising chemical profile also opens new avenues for the commercial exploitation of *C. cajan* seed oil in pharmaceutical, nutraceutical, and cosmeceutical industries.In summary, *Cajanus cajan* seed oil represents an underexplored but highly valuable natural resource with significant potential for the development of novel therapeutic agents.

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