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Research Article

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CHEMICAL PROFILING OF *CAJANUS CAJAN* SEED ETHANOL EXTRACT USING POLARITY-BASED FRACTIONATION.

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Background: *Cajanus cajan* (pigeon pea) is a nutritionally important legume with documented phytochemical diversity. Systematic profiling and quantitative analysis of seed extracts are needed to support reproducible phytochemical characterization and downstream isolation work.

Aim: To investigate the chemical profile of *C. cajan* seed extracts by conducting quantitative analysis of the seed extract and isolating and characterising phenolic constituents.

Methods: Ethanol extract from *C. cajan* seeds (400 g; crude extract 9.58 g) was dispersed in water and partitioned sequentially with n-hexane, ethyl acetate, and dichloromethane (100 mL × 3 each), yielding n-hexane (1.2115 g), ethyl acetate (1.8224 g), dichloromethane (1.4314 g), and aqueous (2.6188 g) fractions. TLC profiling was performed using chloroform/methanol (9:1) and hexane/chloroform (9:1) with UV visualization (256–266 nm) and Rf determination. ATR-FTIR spectra were recorded for aqueous, ethyl acetate, and dichloromethane fractions to infer dominant functional groups. GC–MS (EI, 70 eV) was used to profile the n-hexane fraction. HPLC employed external calibration (mixed standards; 20–100 µg/mL) under isocratic (Method A) and gradient (Method B) conditions for identification and quantification.

Results: The crude ethanol extract yield was 2.40% (9.58 g/400 g). Fraction yields relative to seed mass were aqueous 0.65%, ethyl acetate 0.46%, dichloromethane 0.36%, and n-hexane 0.30%. TLC (chloroform/methanol 9:1) showed four UV-active spots for the ethyl acetate fraction (Rf 0.640, 0.681, 0.855, 0.913), two spots for n-hexane (Rf 0.860, 0.887), one spot for crude extract (Rf 0.905), and no detectable spot for the aqueous fraction under the stated conditions. FTIR suggested hydroxyl/carbonyl-associated features in the aqueous fraction, amine/carbonyl-related signals in the ethyl acetate fraction, and aromatic/phenolic features in the dichloromethane fraction. GC–MS of the n-hexane fraction

identified nine compounds dominated by fatty-acid-related constituents; major components were octadecanoic acid (32.36%), 9,12-octadecadienoic acid (Z,Z) (29.23%), and linoelaidic acid (25.31%) (total oil content 98.85%). HPLC quantified gallic acid (221.609 µg/mL), magnoflorine (175.236 µg/mL), rutin (169.705 µg/mL), and pinostrobin (90.525 µg/mL), with calibration linearity ($R^2 \geq 0.9$).

Conclusion: Quantitative profiling using TLC, FTIR, GC–MS, HPLC demonstrated chemical diversity across *C. cajan* seed extract fractions, with a lipophilic fatty-acid-rich n-hexane fraction and detectable polar markers including phenolic-associated constituents. These findings support further work focused on isolation and characterisation of phenolic compounds from the seed extract.

Keywords: *Cajanus cajan*; seed extract; quantitative analysis; solvent–solvent partitioning; TLC; ATR-FTIR; GC–MS; HPLC; phenolics.

Introduction

Medicinal plants remain central to global health care because they are simultaneously therapeutic resources, cultural health technologies, and major starting points for drug discovery. Across many low- and middle-income settings, plant-based traditional medicine continues to be the most accessible and affordable option for primary health needs, and international policy frameworks increasingly emphasize strengthening regulation, quality assurance, and evidence generation for traditional and complementary medicine products and practices [1]. In Africa specifically, dependence on traditional medicine remains high, with WHO regional communication noting that a large proportion of the continent's population relies on it for basic health needs [2]. This sustained reliance is not merely historical; it reflects practical realities of health-system constraints, medicine affordability, and community trust in local therapeutic knowledge, while also highlighting an urgent scientific need for standardised evaluation of safety, quality, and efficacy of herbal materials used for prevention and treatment of disease [1].

From a pharmaceutical sciences perspective, the continuing importance of medicinal plants is reinforced by their proven contribution to modern therapeutics. Natural products offer exceptional chemical diversity and occupy regions of biologically relevant "chemical space" that are often underrepresented in purely synthetic libraries [3, 4]. Comprehensive analyses of approved medicines consistently show that a substantial proportion of clinically used agents are natural products, natural-product derivatives, or inspired by natural scaffolds, particularly in anti-infective and anticancer therapy [4]. Classic success stories from plant-derived antimalarials to anticancer drugs and neuroactive agents demonstrate how plant secondary metabolites can transition from traditional use or ethnobotanical leads to validated pharmaceuticals when supported by rigorous pharmacognostic and pharmacological development pathways [4, 5]. These examples also illustrate

a recurring theme, crude remedies may be effective at the community level, but pharmaceutical translation requires identity confirmation, reproducible preparation, chemical characterisation, and dose-consistent exposure [1, 4].

The medicinal value of plants is strongly linked to their secondary metabolites, which serve ecological roles in defence and signalling but also interact with human biological targets. Major phytochemical classes such as alkaloids, flavonoids, tannins, saponins, phenolic acids, and terpenoids are widely reported in medicinal species and are frequently associated with antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and other bioactivities [6, 7]. Importantly, the presence and relative abundance of these constituents can vary substantially with species or cultivar, plant part, geographical location, season, post-harvest processing, and storage conditions, which contributes to inter-study variability and inconsistent clinical outcomes when herbal medicines are used without standardisation [1, 8]. For this reason, modern herbal research increasingly integrates ethnobotanical relevance with pharmaceutical-quality principles, including authentication, contaminant/adulterant surveillance, and the development of chemical fingerprints or validated marker-based assays [1, 8].

Advances in analytical chemistry and drug-discovery methodology now make it more feasible to characterise complex botanical matrices with depth and speed. Hyphenated analytical platforms such as GC–MS, LC–MS, LC–NMR, and related coupled techniques enable rapid dereplication, chemical fingerprinting, and online or near-online characterisation of constituents prior to full isolation [9]. Contemporary reviews in natural-product drug development further highlight how improvements in extraction or isolation workflows, metabolomics, and bioassay-guided fractionation can accelerate identification of bioactive leads while strengthening reproducibility and traceability of findings [10, 11]. Such technological capability is particularly relevant for medicinal plants used widely in community practice, where scientific validation must address both the chemical complexity of crude extracts and the regulatory requirement for consistent quality [1, 8].

Despite these opportunities, major obstacles persist. Only a minority of plant biodiversity has been systematically explored, leaving extensive chemical and pharmacological potential untapped [12]. At the same time, habitat loss and unsustainable harvesting threaten medicinal plant availability and genetic diversity, creating urgency to document, conserve, and scientifically evaluate valuable species and traditional knowledge systems before further erosion occurs [14]. In parallel, shifts in pharmaceutical R&D priorities especially the rise of high-throughput synthetic and combinatorial approaches historically contributed to a reduced emphasis on natural product screening in some industrial settings, even though interest has re-emerged as the limitations of purely synthetic libraries became clearer [11, 13]. These constraints underscore the need for carefully designed studies that connect traditional relevance with robust phytochemical profiling

and reproducible analytical methods, thereby producing data that are both scientifically credible and practically useful for product development or clinical translation [1, 9, 10].

Within this broader context, *Cajanus cajan* (L.) Millsp. (pigeon pea; Fabaceae) is a nutritionally and medicinally important legume cultivated widely in tropical and subtropical regions. Beyond its food value, pigeon pea has attracted sustained scientific attention because it contains diverse phenolic and flavonoid constituents, including flavone C-glycosides and other polyphenols reported across different plant parts, and these compounds are frequently linked to antioxidant and other biological effects [15, 16]. Reviews focusing on pigeon pea phytochemistry emphasize the richness of its flavonoid profile and the relevance of these metabolites for health-related applications, while also drawing attention to the importance of systematic characterisation to distinguish varietal, geographical, and processing-related differences [15]. In addition, metabolite-focused investigations of pigeon pea extracts report the presence of multiple phenolic/flavonoid-related constituents and reinforce the rationale for deeper profiling of extracts using modern analytical workflows to clarify which compounds dominate particular fractions and may contribute most to observed biological activities [17].

Given the global challenge of therapeutic failure driven by antimicrobial resistance and the continuing demand for safer, affordable, plant-derived interventions, medicinal plants remain a high-value source of chemically diverse candidates that may complement current therapies or provide new lead structures [4, 18]. However, meaningful progress depends on shifting from general claims of "activity" toward well-controlled chemical studies that quantify key phytochemical groups and isolate or characterise representative constituents using validated analytical strategies [1, 9, 10]. Therefore, the aim of this study is to investigate the chemical profile of seed extracts from *Cajanus cajan*, with emphasis on quantitative assessment of extract phytochemical content and isolation and characterisation of phenolic constituents to support further evaluation of their potential pharmaceutical relevance.

Materials and Methods

This research applied solvent fractionation followed by chromatographic and spectroscopic techniques to characterise the chemical constituents of *Cajanus cajan* seed extract. The crude ethanol extract was first separated into fractions of different polarities by solvent-solvent partitioning (modified Kupchan-type workflow) to simplify the complex mixture and concentrate related compounds into the same fraction, which improves downstream profiling and interpretation [19, 20]. The crude extract and each fraction were then examined using thin layer chromatography (TLC) for rapid comparative separation and fingerprinting [21], ATR-FTIR to identify dominant functional groups [22], GC-MS to profile volatile/semi-volatile constituents

in the non-polar fraction under electron ionisation conditions [23], and HPLC to support targeted profiling or quantification using mixed standards and defined isocratic or gradient conditions [24].

Source of *C. cajan* seed extract

The *C. cajan* seeds (400 g) were extracted with ethanol. A total of 9.58 g of crude ethanol extract of the seeds was obtained from the African Centre of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science (ACEDHARS), University of Lagos. This crude extract (oily in nature) was used as the starting material for partitioning and analytical evaluation.

Solvent–solvent partitioning of *C. cajan* seed extract

The oily ethanol extract was partitioned into solvents of different polarities using a modified Kupchan partitioning approach [19, 20]. In this approach, the crude extract is dispersed into an aqueous phase and then extracted sequentially with immiscible organic solvents. Because phytochemicals distribute between phases according to their relative solubility and polarity, non-polar constituents tend to move into non-polar solvents (e.g., n-hexane), moderately polar constituents distribute into intermediate solvents (e.g., ethyl acetate or dichloromethane), and highly polar constituents remain predominantly in the aqueous fraction [19]. This stepwise separation reduces overlap between compound classes and makes TLC, GC-MS, FTIR, and HPLC results easier to interpret for the purposes of phytochemical characterisation [19, 20].

Preparation of aqueous solution

The oily crude ethanol extract was dispersed in 100 mL of distilled water to obtain an aqueous solution/suspension (mother solution). The solution was then partitioned consecutively using solvents of different polarities. Each obtained fraction was preserved and analysed separately for detection and identification of pharmacologically relevant phytochemicals [19].

Partitioning with n-hexane

The aqueous solution was extracted with 100 mL of n-hexane using a separatory funnel. Two layers formed, with the aqueous layer at the bottom and the organic n-hexane layer at the top. The n-hexane extraction was repeated three times (100 mL × 3) until the upper layer became clear, indicating minimal further transfer of extractable non-polar constituents into the n-hexane phase [19]. The combined n-hexane fractions were retained for subsequent analyses, including GC-MS profiling.

Partitioning with ethyl acetate

The residual aqueous solution was extracted with 100 mL of ethyl acetate in a separatory funnel, producing two layers with the aqueous phase at the bottom and the ethyl acetate phase at the top. The procedure was repeated three times (100 mL × 3) until the organic layer became clear [19]. The pooled ethyl acetate fraction was collected for independent TLC profiling and further chemical assessment.

Partitioning with dichloromethane

The aqueous solution was also extracted with 100 mL of dichloromethane using a separatory funnel. Two layers formed, with the aqueous phase at the bottom and the dichloromethane phase at the top. The extraction was repeated three times (100 mL × 3) until the upper layer became clear [19]. The pooled dichloromethane fraction was collected and stored for further analysis as required.

Thin Layer Chromatography (TLC) analysis

TLC was used as a preliminary qualitative technique to separate and compare constituents in the crude ethanol extract and the various solvent fractions (n-hexane, ethyl acetate, aqueous, and crude). TLC is particularly useful at this stage because it provides a quick "chemical fingerprint" (number of spots, their intensities, and Rf values), allowing comparison of complexity and polarity distribution across fractions [21]. Aluminium-backed TLC plates were used. A start line was drawn lightly with a pencil about 1–2 cm from the bottom of the plate. Each fraction (and the crude extract) was dissolved in a volatile solvent, spotted onto the line using a micro-syringe, and air-dried to prevent streaking.

The TLC plates were developed using two solvent systems: chloroform/methanol (9:1) and hexane/chloroform (9:1). Development was performed in a closed chromatographic tank to allow vapour saturation, which improves reproducibility of migration [21]. The plate edge with the spots was dipped into the mobile phase (with spots above solvent level), and the solvent was allowed to rise by capillary action. Compounds separated based on differential partitioning between the stationary phase and mobile phase, largely influenced by polarity and interactions with the adsorbent [21].

After development, the solvent front was marked immediately. Spots were visualized under UV light at 256 and 266 nm. Retention factor (Rf) values were calculated as the ratio of the distance travelled by the compound to the distance travelled by the solvent front [21].

Fourier-Transform Infrared Spectroscopy (FTIR) of *C. cajan* seed extracts

ATR-FTIR analysis was performed using an Agilent Cary 630 ATR-FTIR spectrometer with a Diamond ATR accessory. ATR-FTIR was used to obtain rapid information on the dominant functional groups in the extracts (e.g., O–H, C=O, C–H, C–O), supporting chemical interpretation alongside chromatographic findings [22].

To load the sample and collect a spectrum, the sample press knob was turned counter-clockwise to open the sample press so that the tip was elevated slightly from the diamond sampling window. A small amount of the material was placed on the diamond crystal (the clear, circular window) and spread to cover the full surface area. The sample press was then engaged to ensure good contact between the sample and the crystal before spectrum acquisition [22].

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the hexane fraction of *Cajanus cajan* was carried out using an Agilent 5977B GC/MSD system coupled with an Agilent 8860 auto-sampler. Separation was performed on an Elite-5MS capillary column (5% diphenyl/95% dimethyl polysiloxane; 30 × 0.25 μm ID × 0.25 μm df, as stated). Detection used an electron ionisation source operating in electron impact mode at 70 eV, which is widely used for generating reproducible fragmentation patterns useful for compound identification [23].

Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. An injection volume of 1 μL was used with a split ratio of 10:1. Injector temperature was maintained at 300 °C, ion-source temperature at 250 °C, and the oven temperature programme was 100 °C (isothermal for 0.5 min), then increased at 20 °C/min to 280 °C (held for 2.5 min). Mass spectra were acquired at 70 eV with a scanning interval of 0.5 s and mass range of 45–450 Da. The solvent delay was set at 0–3 min [23].

High-Performance Liquid Chromatography (HPLC) analysis

HPLC analysis was performed using external calibration with mixed standards and defined chromatographic conditions. Mixed-standard calibration improves efficiency and supports consistent quantification across multiple analytes when appropriately validated [24]. Two HPLC methods (A and B) were used, differing in standard composition and elution mode (isocratic vs gradient).

Preparation of standard solutions (Method A)

Standards used were formononetin, rutin, magnoflorine, luteolin, and quercetin. A 5 mg portion of each standard was weighed and dissolved in 5 mL methanol to obtain 1000 μg/mL solutions (prepared separately). Exactly 2 mL from each standard solution was combined and made up to 10 mL, resulting in a mixed standard where each component was 200 μg/mL (1:5 dilution for each analyte). From this 200 μg/mL mixed standard, graded concentrations of 20, 40, 60, 80, and 100 μg/mL were prepared. Each mixed standard concentration was transferred to HPLC vials, placed in the autosampler tray, and 10 μL was injected in duplicate to support calibration [24].

Preparation of standard solutions (Method B)

Standards used were gallic acid, magnoflorine, luteolin, and pinostrobin. As in Method A, 5 mg of each standard was dissolved in 5 mL methanol to obtain 1000 μg/mL solutions. Exactly 2 mL from each of the four standards (8 mL total) was combined and 2 mL methanol was added to make 10 mL, giving 200 μg/mL for each analyte. Graded concentrations of 20, 40, 60, 80, and 100 μg/mL were prepared from the 200 μg/mL mixed standard. Each concentration was placed into vials and 10 μL was injected in duplicate [24].

Preparation of sample solutions

A total of 0.5 mL of the sample was added to 4.5 mL of 40% methanol to obtain a 1:10 dilution. The sample was ultrasonically extracted for 20 min, cooled, and filtered through a 0.45 mm microporous membrane to remove particulates and protect the column system [24].

HPLC conditions (Method A)

Mobile phase: methanol:acetonitrile:water (40:15:45) containing 1% acetic acid. Column: C18 (150 × 4.6 mm, 5 µm). Detection wavelength: 257 nm. Temperature: ambient. Flow rate: 1 mL/min. Injection volume: 10 µL. Method A was isocratic (constant mobile-phase composition throughout), suitable where target analytes are adequately resolved without changing solvent strength [24].

HPLC conditions (Method B)

Mobile phase A: acetonitrile:TFA (100:0.5). Mobile phase B: acetonitrile:water:TFA (20:80:0.01). Column: C18 (150 × 4.6 mm, 5 µm). Detection wavelength: 260 nm. Temperature: ambient. Flow rate: 1 mL/min. Injection volume: 10 µL. Method B was a gradient method, allowing solvent strength to change over time to improve separation of compounds with wider polarity differences and potentially overlapping retention under isocratic conditions [24]

RESULTS

Percentage yield of *C. cajan*

The percentage yield of the ethanol extract of *Cajanus cajan* was calculated using the weight of the seeds used for extraction (400 g) and the mass of crude extract obtained (9.58 g). This produced a percentage yield of 2.40%, indicating that 2.40 g of crude extract was recovered per 100 g of the starting seed material [25].

Solvent–solvent partitioning of *C. cajan* seed extract

After solvent removal by evaporation, four fractions were obtained from the crude oily ethanol extract. The masses of the fractions and their percentage yields relative to the starting seed weight (400 g) are summarised in Table 1. Overall, the aqueous fraction gave the highest recovery (2.6188 g; 0.65%), followed by the ethyl acetate fraction (1.8224 g; 0.46%), the dichloromethane fraction (1.4314 g; 0.36%), and the n-hexane fraction (1.2115 g; 0.30%). This distribution shows that a larger proportion of the extractable material remained in the more polar fractions, consistent with the presence of polar phytochemicals in the seed extract [26]

Table 1. Weight of fractions obtained from the crude extract of oily Cajanus cajan seed

Fractions	Weight	% Yield compared to seed used (400 g)
n-Hexane fraction	1.2115 g	0.30%
Ethyl acetate fraction	1.8224 g	0.46%
Dichloromethane fraction	1.4314 g	0.36%
Aqueous fraction	2.6188 g	0.65%

Thin layer chromatography (TLC) analysis of *C. cajan* seed extracts

TLC profiling revealed clear differences in the number of detectable components across fractions when developed in chloroform/methanol (9:1) and visualized under UV light (256 nm). The ethyl acetate fraction produced four (4) spots, the n-hexane fraction produced two (2) spots, the crude extract produced one (1) spot, and no spot was observed for the aqueous fraction under the stated conditions. This suggests that the ethyl acetate fraction contained the greatest diversity of UV-active components among the tested fractions under the chosen solvent system and detection wavelength [21].

No coloured spots were observed when plates were developed using hexane/chloroform (9:1). For the chloroform/methanol (9:1) system, the ethyl acetate fraction showed four spots with Rf values of 0.640, 0.681, 0.855, and 0.913. The n-hexane fraction showed two spots with Rf values of 0.860 and 0.887, while the crude extract showed one spot with an Rf value of 0.905.

Rf = (distance moved by compound) / (distance moved by solvent front) [21].

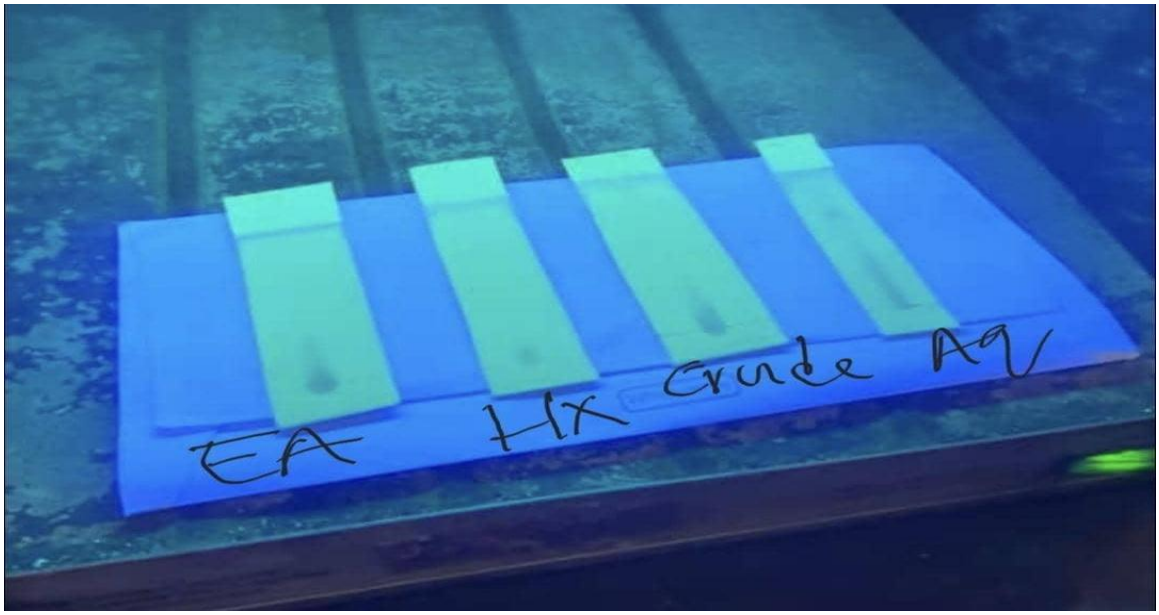

Figure 1: TLC plates showing partitioning of phytochemicals for chloroform/methanol (9:1) viewed under 256 nm (UV).

FTIR analysis results

FTIR spectra were obtained for the aqueous, ethyl acetate, and dichloromethane fractions to support functional group identification. Infrared interpretation was based on characteristic absorption bands associated with common functional groups (e.g., O–H, C=O, C–N, C–O, aromatic C=C), which can provide preliminary chemical-class indications in plant-derived extracts [22, 27].

FTIR results for the aqueous fraction of Cajanus cajan

The aqueous fraction produced seven (7) peaks between 3332.24 cm^{-1} and 1058.56 cm^{-1}. A broad and intense band at 3332.24 cm^{-1} indicated an O–H stretch (hydrogen-bonded), suggesting alcohol/phenolic-type constituents. A band at 2180.49 cm^{-1} suggested C≡C stretching (alkyne) or C≡N stretching (nitrile). A sharp and intense peak at 1636.30 cm^{-1} was interpreted as a carbonyl-related absorption (C=O region) and was supported by the presence of a C–O peak at 1058.56 cm^{-1}, consistent with oxygenated functional groups. A band at 1401.47 cm^{-1} suggested C–H bending (methylene). Overall, the spectrum suggested the presence of oxygenated compounds (including carbonyl/phenolic-type features) and nitrile-related functionality, with the phytochemical-class implication reported as esters/flavonoid-type signals and alkaloid-type signals based on the observed bands [22, 27].

Table 2. IR results for the aqueous fraction of *Cajanus cajan* seed crude extract

Characteristic absorption range (cm^{-1})	Functional group	Functional group name	Phytochemical class suggested
1058.56	C–O stretch	Ester/ether	
1401.47	C–H bend	Methylene	
1636.30	C=O stretch	Carbonyl	Esters, flavonoids
1938.22	Unassigned	Unassigned	
2113.40	Unassigned	Unassigned	
2180.49	C≡C stretch or C≡N stretch	Substituted alkyne / substituted nitrile	Alkaloids
3332.24	O–H stretch	Alcohol	Alcoholic/phenolic compounds

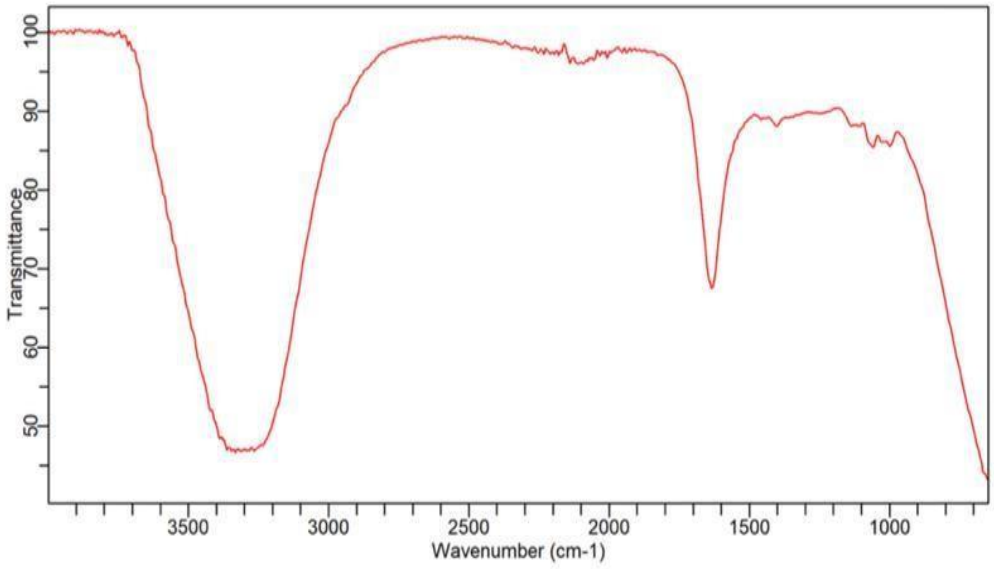

Figure 2: IR spectrum of Cajanus cajan aqueous extract.

FTIR results for the ethyl acetate fraction of *Cajanus cajan*

The ethyl acetate fraction produced sixteen (16) peaks between 3526.06 cm^{-1} and 723.10 cm^{-1}. A broad but non-intense band at 3470.15 cm^{-1} suggested a primary N–H stretch (hydrogen-bonded), supported by a C–N feature at 1162.92 cm^{-1}, consistent with amine-type signals. A sharp and intense band at 2855.14 cm^{-1} was

assigned to C–H stretching (aldehydic) and was discussed alongside carbonyl-related features at 1729.48 cm^{-1} and an overtone band near 3440.33 cm^{-1}. Strong aliphatic features were also observed: 2922.23 cm^{-1} (C–H stretch, alkane), with methyl or methylene bending bands at 1379.11 cm^{-1} and 1461.11 cm^{-1}. Oxygenated functionality was suggested by a C–O stretch around 1271.02 cm^{-1}, and a band at 1073.47 cm^{-1} was assigned to an S=O stretch (sulfoxide or sulfonic-related region). Overall, based on the presence of N–H or C–N features and the stated assignments, the fraction was interpreted as containing alkaloid-type signals and carbonyl-containing compounds [22, 27].

Table 3. IR results for the ethyl acetate fraction of Cajanus cajan seed crude extract

Characteristic absorption range (cm^{-1})	Functional group	Functional group name	Phytochemical class suggested
1073.47	S=O stretch	Sulfoxide	
1121.92	C–O stretch	Ether	
1162.92	C–N bend	Amine	Alkaloids
1271.02	C–O stretch		
1379.11	C–H bend	Methyl	
1461.11	C–H bend	Methylene	
1729.48	C=O stretch	Ketone	
2855.14	C–H stretch	Aldehyde	
2922.23	C–H stretch	Alkane	
3440.33	C=O overtone	Ketone	
3470.15	N–H stretch	Primary amine	Alkaloid
3526.06	Weak N–H	2° or 3° amine	Alkaloid

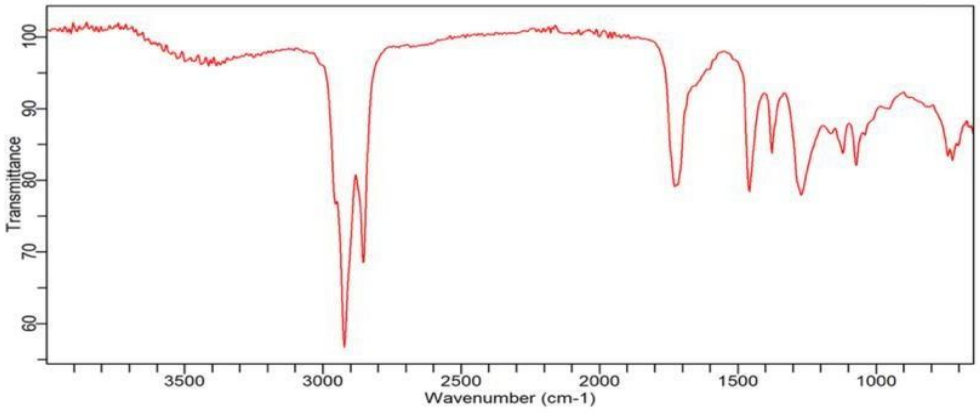

Figure 3: IR spectrum of Cajanus cajan ethyl acetate seed extract.

FTIR results for the dichloromethane fraction of Cajanus cajan

The dichloromethane fraction produced eighteen (18) peaks between 3332.24 cm^{-1} and 708.19 cm^{-1}. A broad, non-intense band at 3332.24 cm^{-1} suggested O–H stretching (hydrogen-bonded). Strong aliphatic signals were observed at 2925.96 cm^{-1} (C–H stretch, alkane) with supporting methyl/methylene bending at 1371.66 cm^{-1} and 1446.20 cm^{-1}. A band at 2855.14 cm^{-1} was described as C–H stretching (alkene-related assignment) with a supporting C=C stretch at 1688.48 cm^{-1}. A band at 2109.67 cm^{-1} suggested C–N stretching (substituted nitrile region). Evidence of aromatic or phenolic character was reported through bands typical of aromatic rings in the 1500–1600 cm^{-1} region (e.g., 1513.29 cm^{-1} and 1602.75 cm^{-1}) and a C–O stretch at 1252.38 cm^{-1} assigned to phenolic C–O. Based on these features, the spectrum was interpreted as indicating the presence of phenolic-type compounds in this fraction [22, 27].

Table 4. FTIR results for the dichloromethane fraction of Cajanus cajan seed crude extract

Characteristic absorption range (cm^{-1})	**Functional group**	**Functional group name**	**Phytochemical class suggested**
826 to 761	Aromatic rings	Benzene/phenol	Phenolic compounds
1252.38	C–O stretch	Phenol	Phenolic compounds
1371.66	C–H bend	Methyl	
1446.20	C–H bend	Methylene	

1513.29	C=C	Aromatic band	Phenolic compounds
1602.75	C=C	Aromatic band	
1688.48	C=C stretch	Alkene	
1990.39	Unassigned	Unassigned	
2109.67	C–N stretch	Substituted nitrile	
2855.14	C–H	Alkene	
2925.96	C–H stretch	Alkane	
3332.24	O–H stretch	Alcohol	
3500	O–H	Phenol	Phenolic compounds

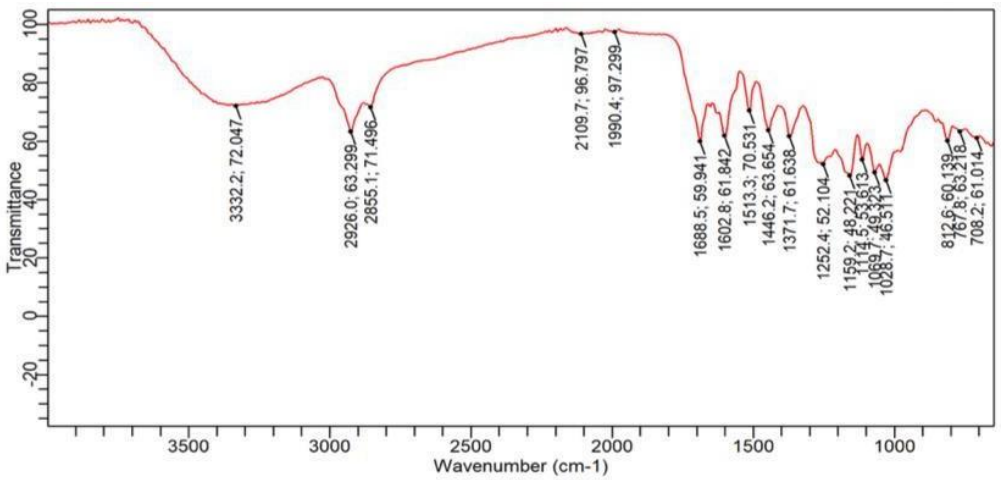

Figure 4: IR spectrum of Cajanus cajan dichloromethane seed extract.

GC–MS analysis results

GC–MS profiling of the n-hexane fraction identified nine (9) compounds (Table 5), with a reported total oil content of 98.85%. The hexane fraction yield was reported as 17.10% from crude oil, and the major compounds detected were octadecanoic acid (32.36%), 9,12-octadecadienoic acid (Z,Z) (29.23%), and linoelaidic acid (25.31%). The high "% quality" values (mostly 97–99) indicate strong library matching confidence for most identifications under the applied method conditions [23, 28]. The total ion chromatogram is presented as Figure 5, and the mass spectra or structures of selected major compounds were presented in Figures 6–15.

Table 5. Chemical composition of essential oils of Cajanus cajan n-hexane seed extract

Peak No	Compound	Retention time (min)	Composition (%)	% Quality
1	1-hexadecyne	12.57	0.42	70
2	Octadecanoic acid	13.56	32.36	99
3	9,12-Octadecadienoic acid, methyl	14.31	0.53	99
4	Linoelaidic acid	14.87	25.31	99
5	9,12-Octadecadienoic acid (Z, Z)	14.90	29.23	99
6	Eicosanoic acid	15.08	6.69	97
7	Hexadecanoic acid, ethyl ester	16.68	0.47	89
8	Bis(2-ethylhexyl) phthalate	18.57	3.48	91
9	Docosanoic acid, ethyl ester	19.14	0.36	98

Total oil content: 98.85%

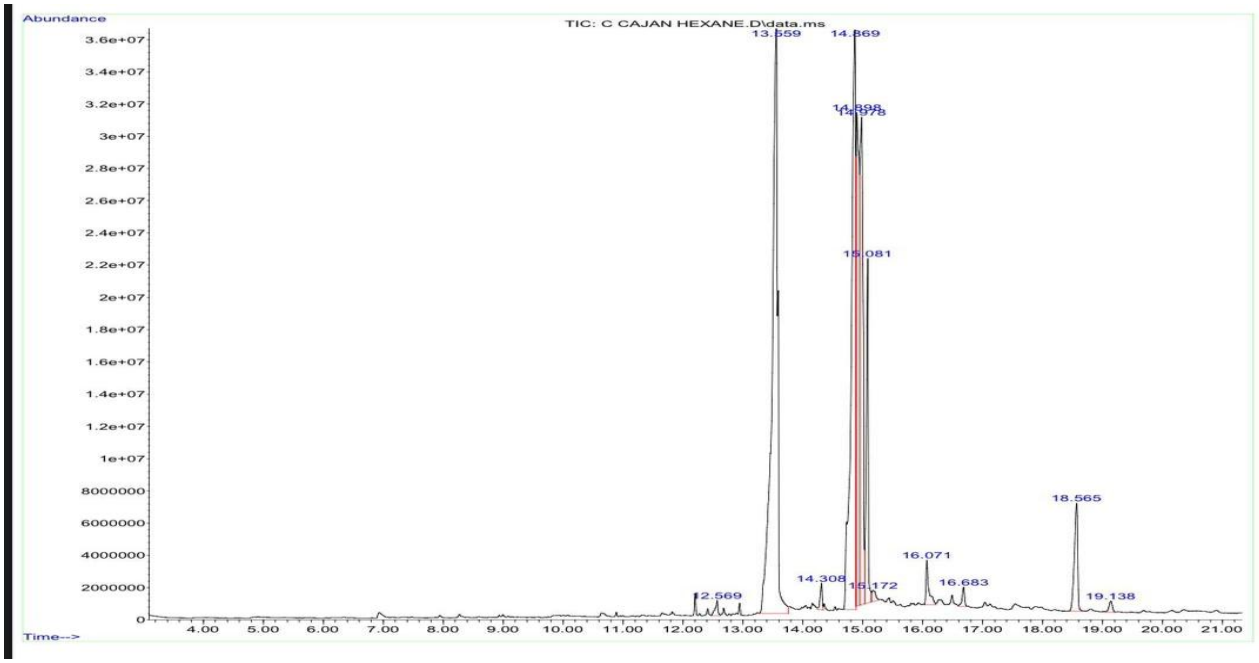

Figure 5: Total ion chromatogram of Cajanus cajan n-hexane seed extracts

Mass Spectra of some major compounds from Total Ion Chromatogram of *Cajanus cajan* n-hexane seed extracts

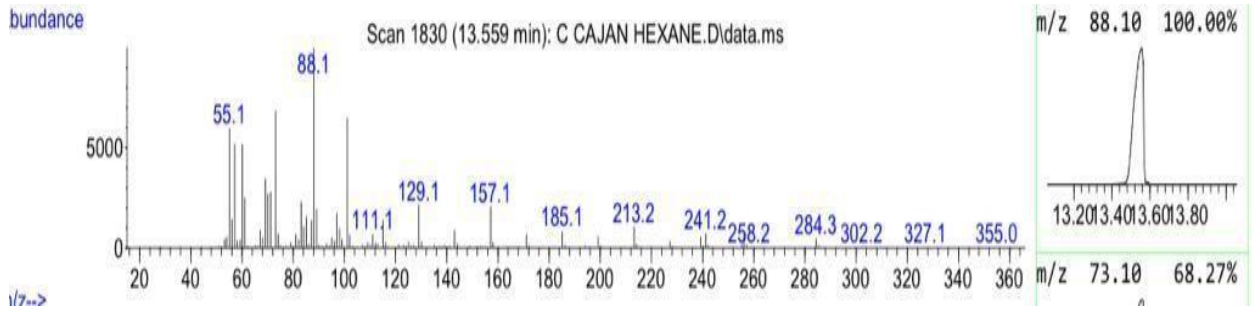

Figure 6: Mass spectrum of Octadecanoic acid, 32.36 % (Peak 2)

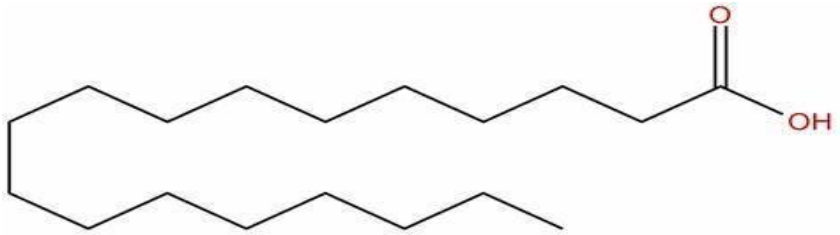

Figure 7: Structure of Octadecanoic acid

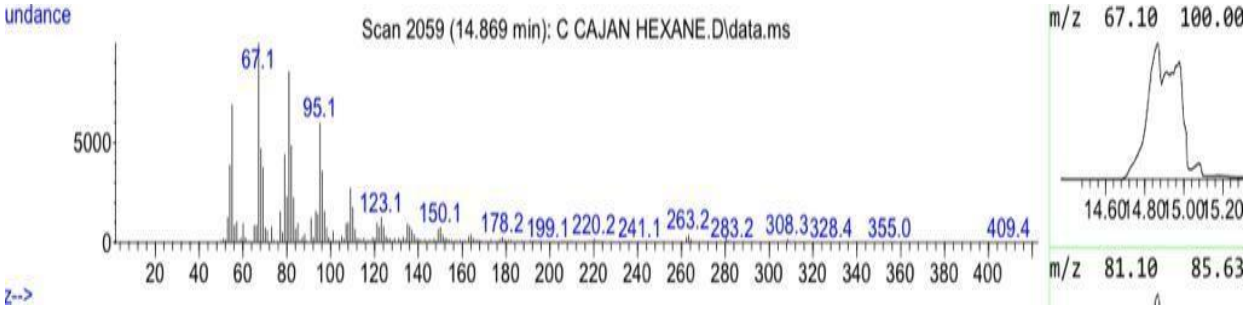

Figure 8: Mass spectrum of Linoelaidic acid, 25.31% (Peak 4)

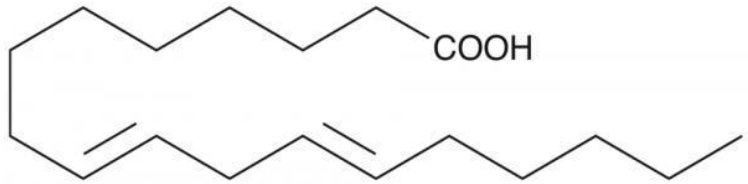

Figure 9: Structure of Linoelaidic acid

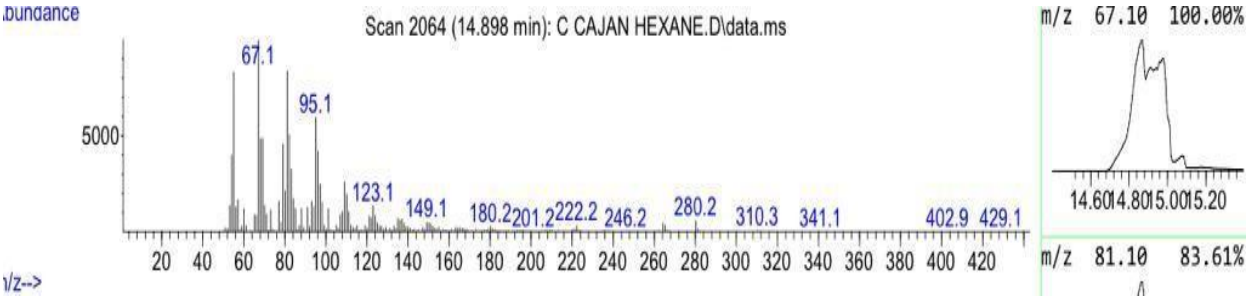

Figure 10: Mass spectrum of 9, 12-Octadecadienoic acid, 14.78% (Peak 5)

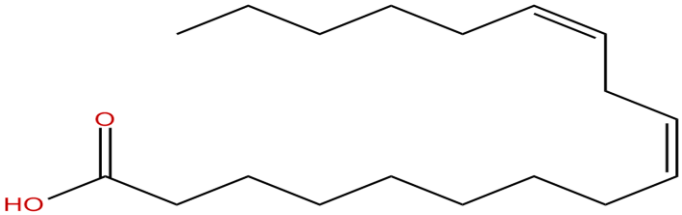

Figure 11: Structure of 9, 12-Octadecadienoic acid

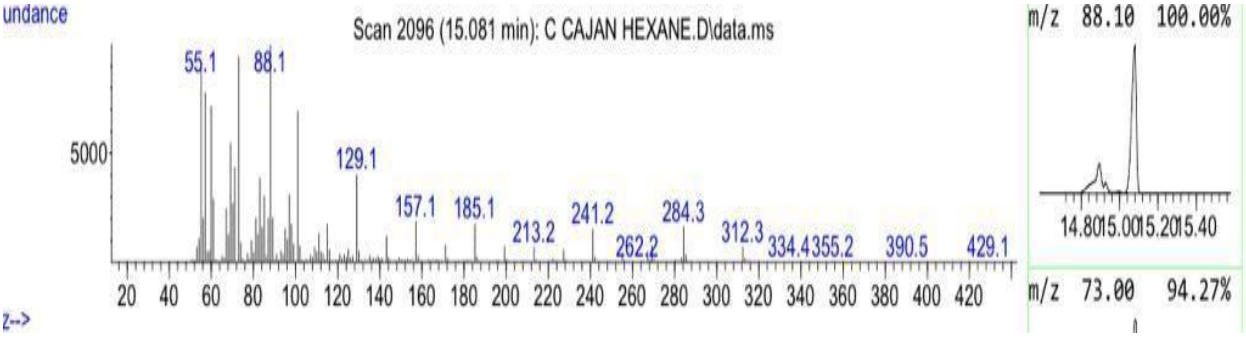

Figure 12: Mass spectrum of Eicosanoic acid, 6.69% (Peak 6)

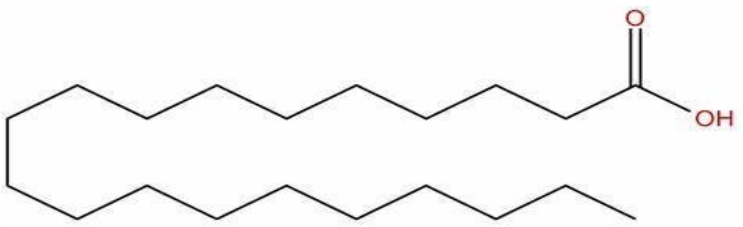

Figure 13: Structure of Eicosanoic acid

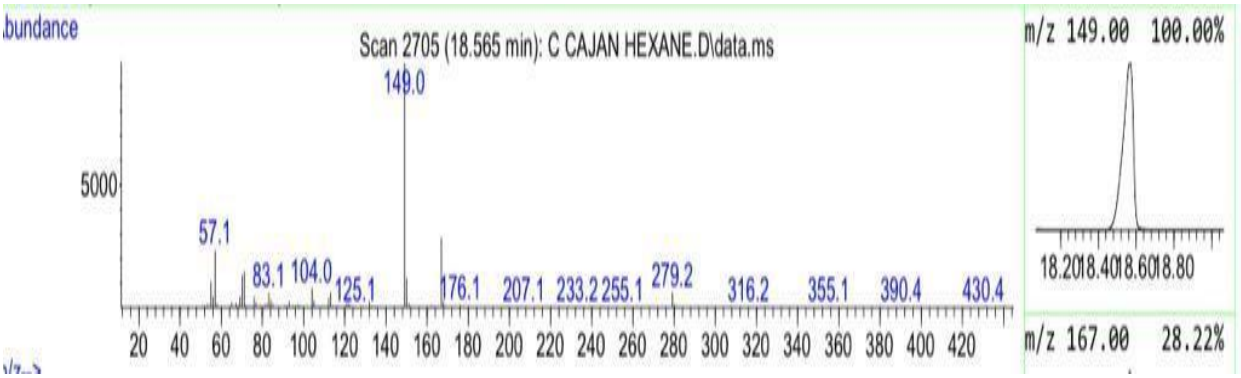

Figure 14: Mass spectrum of Bis (2-ethylhexyl) phthalate, 3.48% (Peak 8)

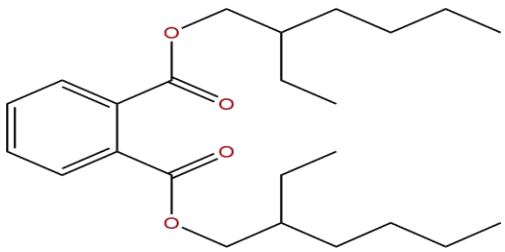

Figure 15: Structure of Bis (2-ethylhexyl) phthalate

HPLC analysis results

HPLC profiling indicated four (4) prominent phytochemicals that were identified and quantified using peak areas and retention times. The chromatograms (Figures 16–17) show the separation of magnoflorine and rutin in one run and gallic acid and pinostrobin in another run, demonstrating that the applied HPLC conditions enabled detection of these target compounds in the ethanol extract. Quantification was supported by external calibration, and acceptable linearity was considered at correlation coefficients (R^2) of 0.9

or greater, consistent with common practice for linear response evaluation in analytical method validation [29].

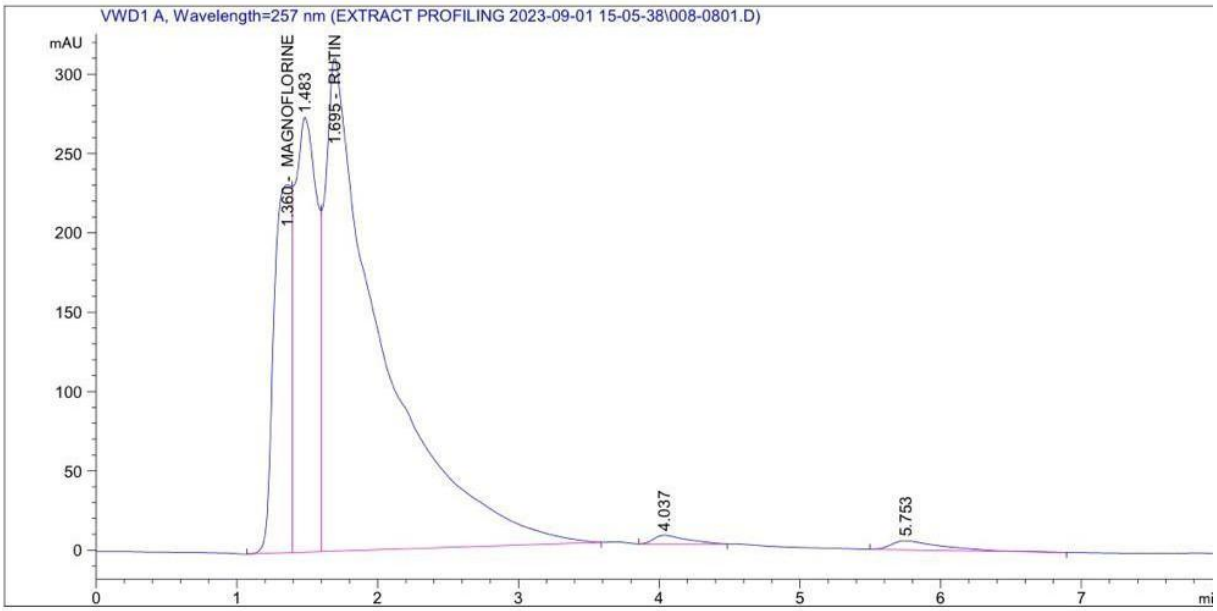

Figure 16: Chromatogram of magnoflorine and rutin versus retention time.

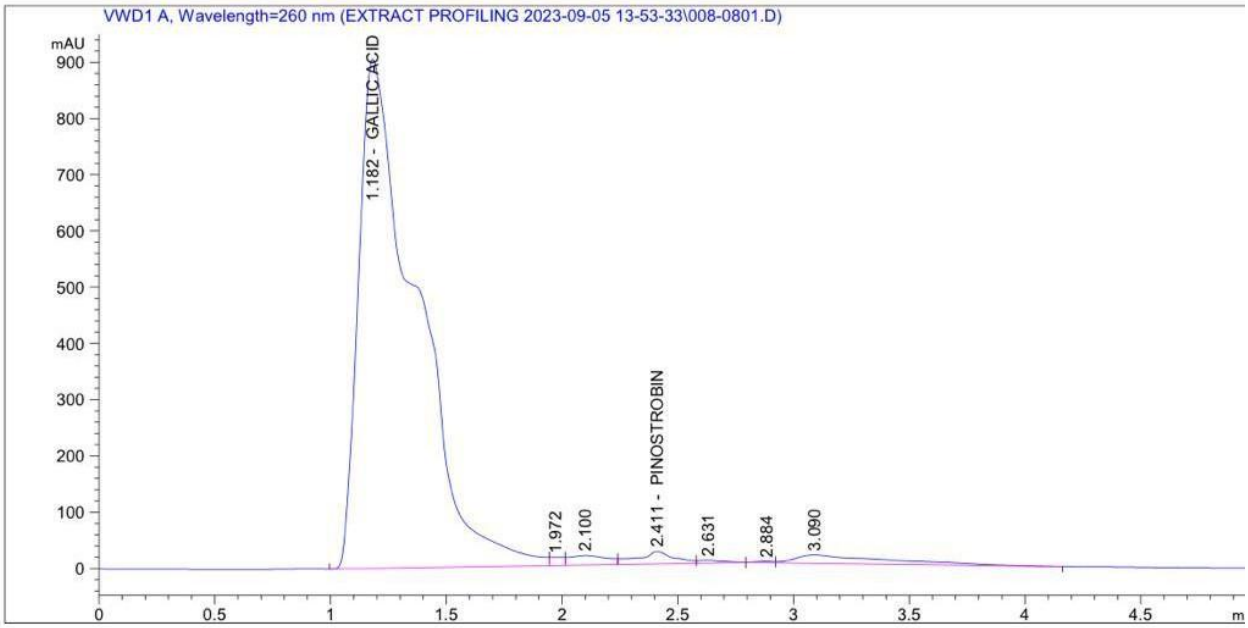

Figure 17: Chromatogram of gallic acid and pinostrobin versus retention time.

Calibration curve determination

Magnoflorine standard

Table 6 presents the magnoflorine standard concentrations and corresponding peak areas used to generate the calibration curve (Figure 18).

Table 6. Concentration and peak area of magnoflorine standard

Concentration (µg/mL)	Peak area (mAU)
20	218.6
40	435.5
60	631.8
80	932.8
100	1103.9

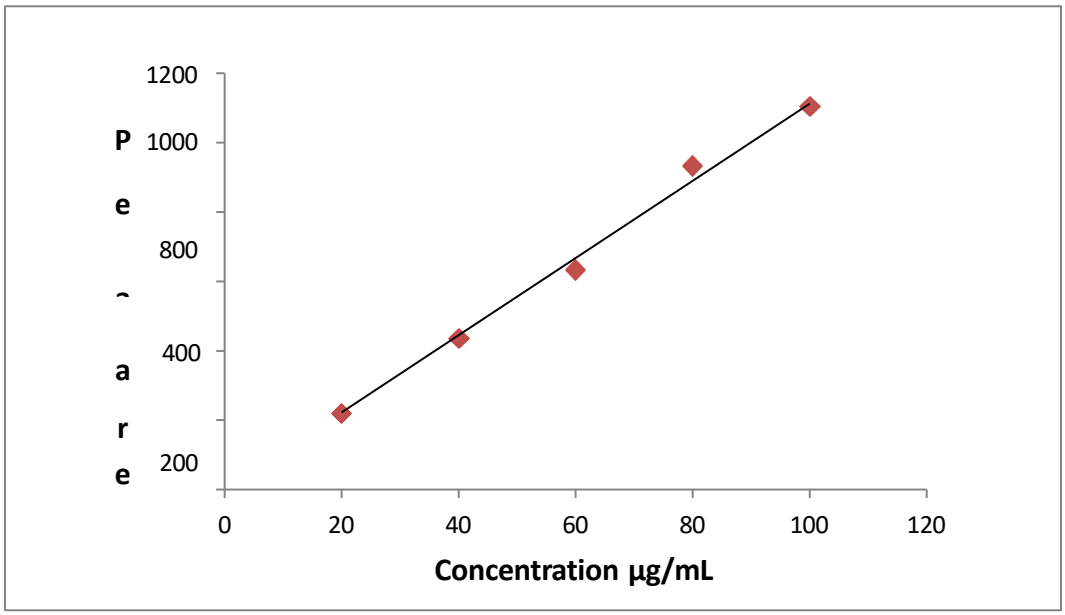

Figure 18: Calibration curve of magnoflorine standard.

Rutin standard

Table 7. Concentration and peak area of rutin standard

Concentration (µg/mL)	Peak area
20	842.7
40	1839.9
60	2697.4
80	4355.9
100	5436.4

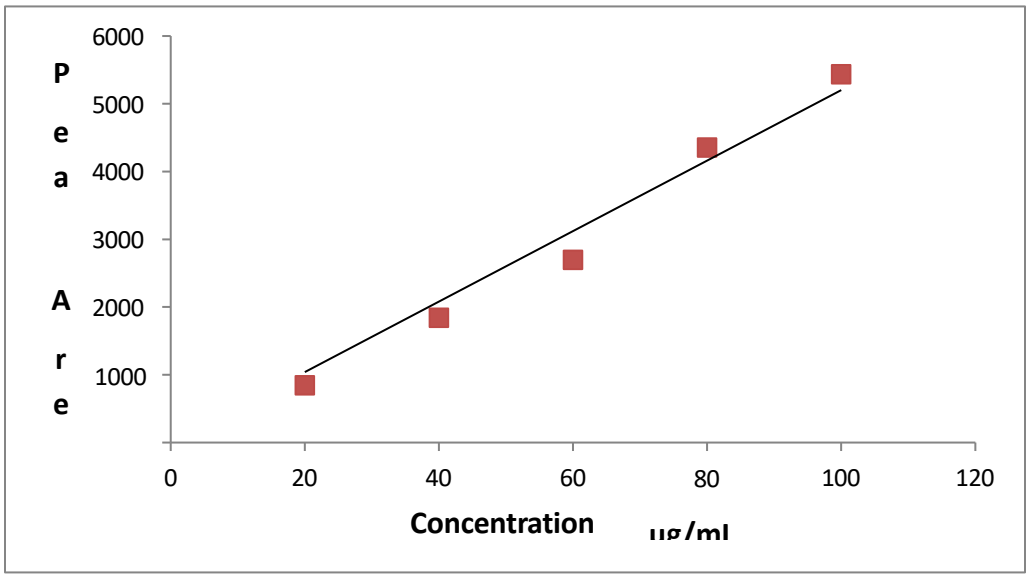

Figure 19: Calibration curve of rutin standard.

Gallic acid standard

Table 8. Concentration and peak area of gallic acid standard

Concentration (µg/mL)	Peak area (mAU)
20	134.6
40	311.1
60	483.1
80	599.7
100	622.1

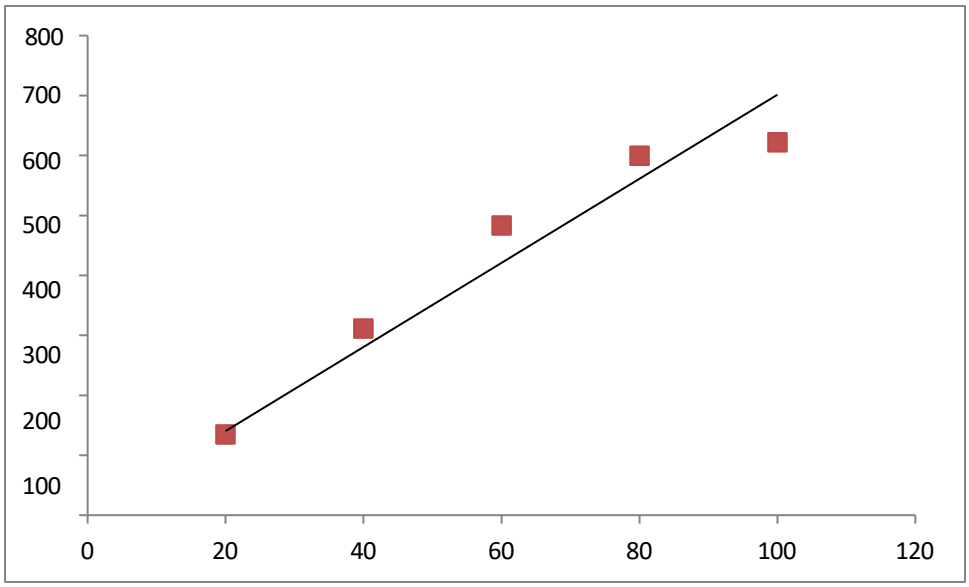

Figure 20: Calibration curve of gallic acid standard.

Pinostrobin standard

Table 9. Concentration and peak area of pinostrobin standard

Concentration (µg/mL)	Peak area
20	51.5
40	120.8
60	170.2
80	221.4
100	237.1

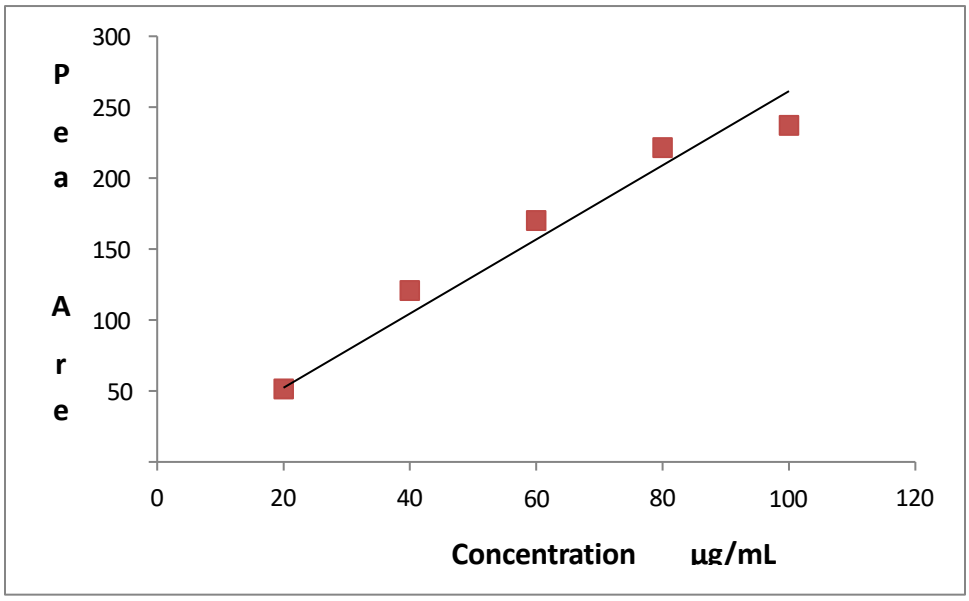

Figure 21: Calibration curve of pinostrobin standard.

A summary of the linear responses for the four phytochemicals is shown in Table 10. The retention times, regression equations, correlation coefficients (R^2), and calculated concentrations (from peak areas) are provided.

Table 10. Statistical summaries of linear response (concentration vs peak area) for four phytochemicals

Phytochemical	Retention time (min)	Regression equation (Y)	Correlation coefficient (R^2)	Peak area (mAU)	Concentration (µg/mL)
Gallic acid	1.182	7.014x	0.9257	1554.37	221.609
Magnoflorine	1.338	11.123x	0.9935	1949.15	175.236
Rutin	1.695	52.018x	0.9733	8827.75	169.705
Pinostrobin	2.411	2.613x	0.9488	236.58	90.525

DISCUSSION

The present study successfully profiled the chemical constituents of *Cajanus cajan* seed ethanol extract using solvent–solvent partitioning, TLC, FTIR, GC–MS, and HPLC. The crude ethanol extract yield was 2.40%, which is within the range typically seen for medicinal-plant extraction where yield depends strongly on solvent polarity, extraction time/temperature, particle size, and post-harvest handling (drying and storage) [25]. In addition, seed extracts of *C. cajan* have been reported to show notable variation in extract recovery between studies, which is commonly attributed to differences in geographical origin, environmental conditions, and processing methods [30]. The moderate crude yield obtained here therefore aligns with published observations that extraction efficiency is method- and matrix-dependent rather than constant for a species [25,30].

Following partitioning, the distribution of the extract across fractions showed the highest recovery in the aqueous fraction (0.65% relative to seed weight), followed by ethyl acetate (0.46%), dichloromethane (0.36%), and n-hexane (0.30%). This pattern is consistent with the expectation that a substantial proportion of ethanol-extractable constituents from seeds will include polar or moderately polar compounds that preferentially remain in aqueous or mid-polar fractions during stepwise partitioning [26]. The use of a modified Kupchan-type partition approach is well established as a practical strategy for simplifying crude extracts by polarity and enriching distinct chemical classes prior to detailed analysis [26]. Thus, the predominance of the aqueous and ethyl acetate fractions in this study agrees with the general outcome reported for similar liquid–liquid fractionation workflows applied to plant extracts [26].

TLC profiling further supported differences in fraction complexity. Under chloroform/methanol (9:1) and UV visualization (256 nm), the ethyl acetate fraction produced four spots (Rf 0.640, 0.681, 0.855, 0.913), the n-hexane fraction produced two spots (Rf 0.860, 0.887), and the crude extract produced one spot (Rf 0.905), while the aqueous fraction showed no detectable spot under the stated conditions. TLC is widely used for rapid qualitative comparison of fractions and for selecting fractions for deeper analysis, with spot number and Rf patterns reflecting differences in component polarity and UV activity [26]. The larger number of UV-active spots in the ethyl acetate fraction in the present work is therefore consistent with TLC being particularly sensitive to mid-polar aromatic and conjugated compounds that absorb 254 or 256 nm, while highly polar constituents in aqueous fractions may require alternative detection methods (e.g., derivatization reagents) to be visualized [26].

FTIR analysis provided functional-group-level evidence that complemented TLC. For the aqueous fraction, the broad O–H stretch (3332 cm^{-1}) indicated hydrogen-bonded hydroxyl groups, while bands in the carbonyl region and C–O region supported the presence of oxygenated constituents such as carbonyl-containing compounds and phenolic/alcoholic features [27]. These assignments are consistent with standard IR interpretation guidance for common organic functional groups [27]. The aqueous spectrum also included a band in the triple-bond region (C≡C/C≡N), which—when considered alongside other bands was interpreted as suggestive of nitrile/unsaturation-related functionality and possibly alkaloid-associated features [27]. Overall, the functional-group pattern observed here is consistent with reports that *C. cajan* extracts contain mixed phytochemical classes, including oxygenated compounds and alkaloid-related constituents [31].
For the ethyl acetate fraction, FTIR bands suggesting N–H stretching and C–N features supported amine-type functionality, while strong aliphatic C–H bands and a carbonyl band (around 1729 cm^{-1}) indicated the presence of carbonyl-containing constituents alongside nitrogen-containing components [27]. The co-occurrence of these features is typical for partially purified plant fractions and supports the interpretation that the ethyl acetate fraction concentrated multiple chemical classes rather than a single compound group [27]. This agrees with the general expectation for mid-polar fractions and also aligns with phytochemical reports of *C. cajan* extracts containing diverse secondary metabolites [31].

The dichloromethane fraction exhibited O–H stretching, aromatic-region bands (1500–1600 cm^{-1}), and phenolic C–O stretching, supporting the presence of phenolic-type constituents [27]. The aromatic or phenolic features observed are consistent with the broader literature on plant extracts where phenolic constituents may partition into moderately non-polar solvents depending on molecular size and substitution patterns [27]. In the context of *C. cajan*, prior phytochemical investigations similarly report multiple phenolic- and flavonoid-associated constituents across extracts, and the present findings are therefore directionally consistent with those reports [31].

GC–MS profiling of the n-hexane fraction identified nine compounds and showed a profile dominated by fatty-acid-related constituents, with octadecanoic acid (32.36%), 9,12-octadecadienoic acid (Z,Z) (29.23%), and linoelaidic acid (25.31%) as major components. Interpretation of this profile is consistent with the known behavior of non-polar solvents, which preferentially enrich lipophilic constituents such as fatty acids and related esters from oily seed

matrices [28]. When compared with published work that reported *C. cajan* oils as being rich in sesquiterpenes (particularly when "essential oils" are discussed), an important methodological distinction arises volatile essential oils are commonly obtained by distillation and are terpene-rich, whereas solvent extraction of seed oil/fractions is more likely to yield fixed-oil components such as fatty acids [33]. Therefore, the absence of sesquiterpenes in the present GC–MS profile is plausibly explained by the extraction target (lipophilic seed fraction) and methodological differences rather than a true contradiction in plant chemistry [33]. Additionally, differences in geography, environment, and time of collection are widely recognized to influence plant metabolite accumulation and may further contribute to inter-study differences [32].

HPLC profiling detected four prominent phytochemicals gallic acid, magnoflorine, rutin, and pinostrobin and quantification was supported by calibration curves with $R^2 \geq 0.9$ [29]. Using an R^2 threshold as an indicator of acceptable linear response is consistent with routine analytical practice, and formal validation frameworks emphasize evaluating linearity as part of method performance [29]. The quantified presence of these markers is consistent with the established chemical diversity of *C. cajan* and supports the interpretation that the ethanol seed extract contains phenolic acids, flavonoid glycosides, and alkaloid-type constituents. Gallic acid is widely reported as a polyphenol with multiple biological activities, including cardiovascular-relevant mechanisms in experimental contexts [37]. Magnoflorine has been reviewed as a bioactive aporphine alkaloid reported across plant taxa and linked to diverse pharmacological effects [38]. Rutin has extensive literature describing its antioxidant and health-related properties and is often discussed as a nutraceutical flavonoid [39]. Pinostrobin has been reported in the literature with metabolic relevance, including adipogenesis-related effects in experimental models [40]. Taken together, the HPLC findings provide compound-level evidence that complements the broader FTIR functional-group indications and the polarity-driven fractionation outcomes observed in this study [26,27,29].

Limitations of the study.

This work provides an analytical profile but has limitations that should be considered when interpreting the findings. First, extraction and fractionation were conducted on a single batch, and replicate extractions/fractionations were not reported, limiting assessment of variability and reproducibility. Second, TLC visualization was limited to UV detection under the chosen solvent systems; highly polar or non-UV-active constituents may have been underestimated without

derivatization reagents [26]. Third, several FTIR peaks were unassigned, and functional-group interpretation alone cannot confirm structures without complementary structural elucidation [27]. Fourth, GC–MS identifications depend on spectral-library matching and were not confirmed by authentic standards [28]. Finally, although calibration linearity was reported for HPLC, a full validation package (e.g., accuracy, precision, LOD/LOQ, robustness) and confirmatory structure elucidation (e.g., NMR) were not presented, which limits the strength of quantitative and identification claims beyond the reported analytical conditions [29].

CONCLUSION

This study demonstrates that ethanol extraction of *Cajanus cajan* seeds followed by solvent–solvent partitioning yields fractions enriched with different chemical classes. FTIR functional-group patterns across aqueous, ethyl acetate, and dichloromethane fractions support the presence of oxygenated constituents (including phenolic or carbonyl-associated features) and nitrogen-containing functionality consistent with alkaloid-related signals [27]. GC–MS of the n-hexane fraction showed a profile dominated by fatty-acid-related constituents, with octadecanoic acid, 9,12-octadecadienoic acid (Z,Z), and linoelaidic acid as major components [28]. HPLC profiling confirmed and quantified four prominent markers gallic acid, magnoflorine, rutin, and pinostrobin using calibration relationships with acceptable linearity [29]. Overall, the combined analytical evidence supports the conclusion that *C. cajan* seed ethanol extract contains chemically diverse constituents spanning lipophilic and more polar phytochemical classes, providing a basis for further isolation and bioactivity-directed investigations [25,26,29].

List of abbreviations

ACEDHARS: African Centre of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science

ATR-FTIR: Attenuated total reflectance–Fourier transform infrared spectroscopy

C=O: Carbonyl

C≡C: Alkyne

C≡N: Nitrile

Da: Dalton

EI: Electron ionisation

GC–MS: Gas chromatography–mass spectrometry

HPLC: High-performance liquid chromatography

mAU: Milli-absorbance units

Rf: Retention factor

R^2: Coefficient of determination

TFA: Trifluoroacetic acid

TLC: Thin layer chromatography

UV: Ultraviolet

Declarations

Ethics approval and consent to participate

Not applicable. No animal model or human participants were involved; therefore, ethical approval was not required.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.T Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing original draft, Writing review and editing.

O.T.A Supervision, Methodology oversight, Writing review & editing.

M.O.A Resources, Technical support, Writing review and editing.

All authors read and approved the final manuscript.

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