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PHYTOCHEMICAL EVALUATION OF ETHANOLIC EXTRACT OF *CAJUNUS CAJAN* PEEL BY USING GC-MS TECHNIQUE

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ABSTRACT

Cajanus cajan Linn's. This shrub, which is a member of the Fabaceae family, is grown in Central India and is used as medicine. However, their peel is often discarded as waste, applications include hepatoprotective, wound healing, and anti-ulcer. The ethanolic extract of *Cajanus cajan* (pigeon pea) peel is the subject of this study's phytochemical evaluation by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Using phytochemical analysis, the presence of flavonoids, alkaloids, tannins, and phenol chemicals was confirmed. Ethanol was used to extract the bioactive components of *Cajanus cajan* peel, an agricultural waste. Numerous phytochemicals, such as phenolic compounds, esters, fatty acids, and other secondary metabolites, were identified by the GC-MS study. Among the prominent substances found were [insert important compounds if known], which have noteworthy pharmacological characteristics like anti-inflammatory, anti-cancer, antioxidant, and antibacterial effects. These bioactive substances promote the use of *Cajanus cajan* peel in traditional medicine and its possible use in the creation of natural medicines by highlighting its therapeutic potential. This work encourages more investigation into the biological activities of the identified constituents and emphasizes the importance of using agricultural waste for the discovery of bioactive compounds.

KEYWORDS: *Cajanus Cajan*, Phytochemicals, GC-MS Technique, Bioactivecompounds, Therapeutic activity, Medicinal plants.

INTRODUCTION

Natural products are extracts of plants or the components and metabolites of insects, marine organisms, and microorganisms, as well as a large number of endogenous compounds in humans and animals (Peng et.al., 2023). For many years, humans have employed plants as a source of food, flavouring and medicines, and various parts of medicinal plants such as seeds, leaves, flowers, fruits, stems and roots are rich sources of bioactive compounds and those are considered as important dietary supplements (Wenli Sun and Mohamad Hesam Shahrajabian, 2023).

Pigeon pea (*Cajanus Cajan*), also known as toor dal, is a perennial legume of the Fabaceae family that is native to the Eastern Hemisphere. Pigeon peas are widely cultivated in tropical and semitropical climates around the world, and are popular in South Asia, Southeast Asia, Africa, Latin America, and the Caribbean. Pigeon pea is a short-lived perennial that is most typically planted as an annual. *Cajanus Cajan* can be grown as a pulse crop (dried seed) or consumed as a vegetable. Grain is widely consumed in India, Asia, and Africa. India is the major importer and producer of dhal (dry split pea).

Extraction is the process of treating plant or animal tissues with a solvent so that the medically active ingredients dissolve while the majority of the inert matter remains undissolved. Menstruum is the solvent used for extraction, while Marc is the inert, insoluble substance that is left behind following extraction. The different extraction techniques are: Maceration is an essential extraction method for separating plant materials' active ingredients. For a set amount of time, usually a few days, the crude medication, normally in powdered form, is soaked at room temperature in an appropriate solvent or menstruum (such as alcohol, water, or a combination). Throughout this period, the solvent dissolves the soluble components, permeates the plant tissues, and promotes their diffusion into the surrounding liquid. To separate the liquid extract—which includes the desired phytochemicals—from the solid residue, the mixture is filtered. Hot Continuous Extraction (SOXHLET**)** In pharmaceutical analysis, Soxhlet extraction is a continuous technique used to extract active ingredients from solid materials. A appropriate solvent is heated to evaporate, condense, and drip onto the sample, which is contained in a thimble inside the Soxhlet device. After extracting the necessary components, the solvent automatically repeats the operation by siphoning back into the boiling flask. This makes it perfect for non-volatile, heat-stable chemicals since it enables effective, repetitive extraction without solvent loss.

GC-MS, or gas chromatography-mass spectrometry, is a potent analytical method for determining the content of complicated mixtures. Mass spectrometry (MS) detects and quantifies the compounds based on their mass-to-charge ratio, while gas chromatography (GC) separates the components of a mixture based on their volatility and interaction with the stationary phase of the column. The sample is vaporized in GC and then transported along a lengthy capillary column by an inert gas, such as helium. Different compounds can be separated because they move through the column at different speeds. The mass spectrometer receives each compound as it leaves the column.

The process of finding, detecting, and assessing the bioactive chemical compounds (phytochemicals) found in plants is known as phytochemical assessment. Alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids are some of the substances that give the plant its therapeutic qualities. Standardization and quality control of herbal medications and formulations are aided by the evaluation, which usually includes qualitative and quantitative tests to ascertain the presence and quantity of these elements.

MATERIALS AND METHODS

Collection

The plant *Cajanus Cajan* was collected from the fields of Nayampalli, Chittoor. They were washed properly with water and dried in the shade for over seven days. The dried peel of Cajanus Cajan and made into fine powder using a mortar and pestle and subjected to extraction. The collected plant was authenticated by botanist Dr. Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India.

Pharmacognostic Studies

The ethanol extract was concentrated using a china dish and a Bunsen burner at a reduced temperature to remove the solvent. The final liquid extract was stored.

Determination Of Total Ash Value

Weigh accurately 1mg of powdered extract in a tared silica crucible. Spread the material evenly and ignite it. Gradually increase the heat to 500- 600 °C until it turns white, which indicates the extract is free from carbon. Allow the residue to cool in a desiccator and weigh. Wait for 30 minutes, then weigh without delay. Calculate the amount of total amount of ash in milligrams per gram of air-dried material.

Determination Of Acid Insoluble Ash

Add 25ml of hydrochloric acid to the above collected ash and cover with a watch glass then boil gently for 5minutes. Rinse the watch glass with 5ml hot water and add this liquid to crucible. Collect the filtrate and transfer the filter paper containing insoluble matter to original crucible, dry on a hot plate and ignite o constant weight. Allow the residue to cool in a suitable desiccator for 30minutes, weigh the content without delay. Calculate the content of acid insoluble ash in milligram per gram of air-dried material.

Determination of Water-Soluble Ash

Add 25ml of water into a crucible containing total ash and boil it for 5mintues. Collect the matter in a sintered-glass crucible or on an ash less filter-paper. Wash with hot water and ignite in cruciable for 15minutes at a temperature not exceeding 450C. Subtract weight of residue in mg from the weight of total ash. Calculate content of water soluble ash in milligram per gram of air-dried material.

Preliminary Phytochemical Screening Tests

1. Test for Alkaloids

The separate extract is filtered after being dissolved in diluted hydrochloric acid. The following reagents were used to further test the filtrate for the presence of alkaloids.

Dragendroff's Test:- Potassium bismuth iodide solution (Dragendroff's reagent) was applied to the filtrate. The presence of alkaloids was revealed by the formation of an orange-red precipitate.

Mayer's Test:- Mayer's reagent, potassium mercuric iodide solution, was applied to the filtrate. Formation of a whitish yellow or cream coloured precipitate indicated the presence of Alkaloids.

Hager's Test:- A saturated aqueous solution of picric acid (Hager's reagent) was applied to the filtrate. A yellow-colored precipitate formed, confirming the presence of alkaloids.

2. Test for Carbohydrates

Molisch's Test: Take 2ml of liquid extract in a clean test tube, add 2 drops of alcoholic a-naphthol. Now add concentric sulphuric acid at inclined position. Observe the formation brown ring at the junction between two layers. Which indicates the presence of carbohydrates.

3. Test for Saponins

Foam Test: Take 4ml of liquid extract in a clean test tube. Add 6ml of distilled water into test tube containing extract. Shake the mixture for at least 1-2 minutes. Allow the test tubeundisturbed for at least 5 minutes. Observe the formation of stable foam lasting for at least 10 minutes. Indicates the presence of saponins in liquid extract.

Forth Test: Take 4ml of liquid extract in a clean test tube. Add 20ml of distilled water into test tube containing extract. Shake test tube for 15 minutes. Observe the formation of 1cm layer of foam. Which indicates presence of saponins.

4. Reducing Sugars

Benedict's Test: Benedict's reagent is used to the presences of reducing sugars. By taking 4ml of the liquid extract in a clean test tube then add 6ml of distilled water and mix thoroughly to ensure dispersion. Now add 4ml of benedict's reagent to the test tube containing sample. Heat the mixture in a water bath for 5 minutes. Then now finally observe any color change formation of green, yellow, orange or red precipitate. Which indicates presence of reducing sugars.

5. Test for Flavonoids

Alkaline Reagent Test: Take 2ml of liquid extract in a clean test tube. Now add a few drops of sodium hydroxide solution. Observe the formation of intense yellow color, which becomes colorless on further addition of dilute acid, which results in presence of flavonoids.

Lead Acetate Test: Take 2ml of liquid extract in a clean test tube. Now add few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids.

6. Test for sterols

Salkowski Test: Take 2ml of liquid extract in a clean test tube and dissolve it in 5ml of chloroform. Now add few drops of concentric sulphuric acid and allow it to stand until formation of brown ring. It indicates the presence of sterols.

Liebermann-Burchard Test: Mix 2 mL of chloroform with 5 mL of extract, then carefully add 3 mL of concentrated sulfuric acid. A reddish-brown color at the interface indicates the presence of terpenoids.

7. Test for Tannins and phenols

Ferric chloride Test: Take 2ml of liquid extract in a clean test tube and add 2-3 drops of 1% ferric chloride solution and mix well. Observe the color change to bluish black color

formation. It indicates the presence of phenols Take 0.5g of powdered plant, add 20ml of distilled water in a test tube and mix well. Now add few drops of 1% fecl3 solution. Observe the formation of brownish green or blue black colorization. It indicates the presence of tannins.

8. Test for Proteins

Biuret Test: Take 2ml of liquid extract in a clean test tube, add few drops of % of copper sulphate solution. Then now add 1ml of ethanol followed by excess of potassium hydroxide pellets. Observe the formation of pink color, which indicates the presence of proteins.

Ninhydrin Test: Take 2ml of liquid extract in a clean test tube, add few drops of ninhydrin reagent and boil for few minutes. Observe the formation of blue color, which indicates the presence of proteins.

9. Test for Terpenoids

Liebermann-Bur chard Test: Mix 2 mL of chloroform with 5 mL of extract, then carefully add 3 mL of concentrated sulphuric acid. A reddish-brown color at the interface indicates the presence of terpenoids.

GC-MS Analysis

The Clarus 680 GC was used in the analysis employing a fused silica column, packed with Elite- 5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df), and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample was injected into the instrument, the oven temperature was as follows: 60 °C (2min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan of 0.1 sec. The fragments from 40 to 600 Da. The spectra of the components were compared with the database of spectra of known components stored in the GC-MS NIST (2008) library.

RESULTS AND DISCUSSION

Physiochemical Analysis

Different physicochemical parameters such as ash values, water soluble ash, acid soluble ash, and loss on drying were shown in the table.

Table 1: Physicochemical Properties.

S.No.	Parmeter	Method Used	Observed Value (%)	Standard Range/ Limit (if any)
1	ForeignMatter	Visual Inspection	22.31	NMT2%
2	Moisture Content (Loss on Drying)	Hot air oven/ Desiccator	0.98	NMT8%
3	Total Ash	Mufflefurnance (550°C)	1.92	NMT10%
4	Acid -Insoluble Ash	Ash + HCl	0.97	NMT-2%
5	Water-Soluble Ash	Ash + distilled water	0.95	
6	Extractive Value (Water)	Cold maceration		NLT10%

Extraction Methods for *Cajanus Cajan* peel

The extraction studies using two methods—maceration and Soxhlet extraction—revealed differences in yield. Maceration with 70% ethanol over 72 hours at room temperature yielded 12.4%.• Soxhlet extraction with 95% ethanol over 6–8 hours yielded 10.8%. • The slightly higher yield from maceration could be attributed to the mild extraction conditions, which better preserve heat-sensitive phytoconstituents. This finding highlights the importance of choosing extraction methods based on the nature of targeted compounds, especially for medicinal plant research.

Table 2: Extract Values.

Extraction Method	Intial Weight of Peel (g)	Solvent Used	Duration	Weight of Extraction	% Yield
Maceration	50	70% Ethanol	72 hours (RT)	6.2	1.24%
Soxhlet Extraction	50	95% Ethanol	6-8 hours (reflex)	5.4	10.8%

Preliminary phytochemical screening

Table 3: Preliminary Phytochemical tests.

S.No	Phytochemical	Test Used	Observation	Results
1.	Alkaloids	Wagner's/Mayer's/ Dragendorff's	Reddish-brown ppt (Wagner's)	Present
2.	Flavonoids	Alkaline reagent test / Shinoda test	Yellow → colorless (alkaline test)	Present
3.	Tannins	Ferric chloride test	Blue-black or greenish ppt	Present
4.	Saponins	Foam test	Persistent foam for >10 minutes	Present
5.	Phenols	Ferric chloride test	Deep blue/green coloration	Present
6.	Glycosides	Keller-Killiani test	Reddish-brown	Present

			ring at the interface	
7.	Steroids	Liebermann Burchard test	Green coloration	Present
8.	Terpenoids	Salkowski test	Reddish-brown at the interface	Present
9.	Carbohydrates	Molisch'stest	Purple ring at the junction	Trace
10.	Proteins & Amino acids	Biuret/Ninhydrin test	Weak or no color change	Absent

Phytochemical investigations showed that Cajanus cajan peel is rich in several secondary metabolites: Alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids, and terpenoids were all present. Carbohydrates were detected only in trace amounts. Proteins and amino acids were absent. The detection of flavonoids and phenols suggests strong antioxidant potential, while tannins and saponins are associated with antimicrobial, anti-inflammatory, and anticancer activities. The presence of steroids and terpenoids further supports possible anti-inflammatory and cytotoxic effects. This preliminary screening underscores the medicinal value of the peel and suggests avenues for its pharmacological exploration.

GC-MS Analysis

The GC-MS analysis of *Cajanus cajan* (pigeon pea) peel extract identified 20 major compounds, mainly nitrogen-containing bioactives. The dominant constituents were triazine and aziridine derivatives, known for **anticancer, antimicrobial, and anti-inflammatory** properties. These findings support the medicinal potential of the peel and align with earlier phytochemical results, suggesting it as a promising source for pharmacological research.

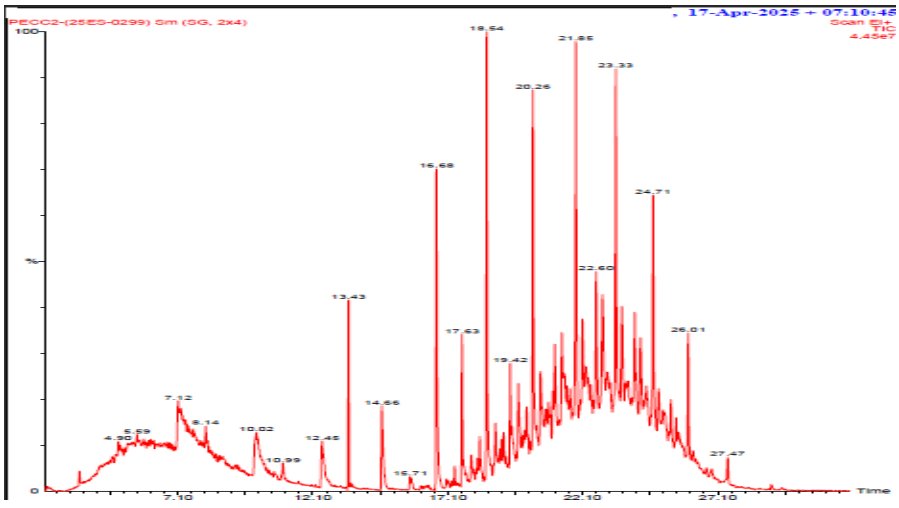
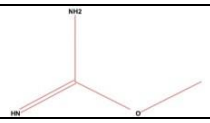
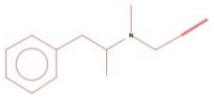
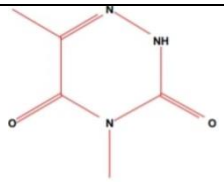


Figure 1: Chromatogram for Phytochemicals.

Table 4: Phytochemical by GC-MS analysis.

S.No	Compound	Retension Time (min)	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Structure
1.	O Methylisour Ea Hydrogen Sulfate	10.012	C2H6ON2	74	589,081.8	
2.	3-Azonia-5-Hexene-1 Ol, N,N-Dimethyl-, Carbamate Ster, Bromide	12.447	C8H1702N2	173	318,335.2	
3.	1,N Dimethyl N-Propargyl-2-Phenylethy Lami Ne	13.428	C13H17N	187	617,989.1	
4.	4,6 Dimethyl 3,5-Dioxo 2,3,4,5-Tetrahydr O- 1,2,4-Triazine	14.658	C5H7O2N3	141	596,047.7	
5.	N-[3 Methy L Amino Propyl] Aziri Din E	20.256	C6H14N2	114	1,746,984.8	

CONCLUSION

The thorough examination of the peel of *Cajanus cajan*, or pigeon peas, indicates that it has the potential to be a significant source of bioactive substances. The raw material's quality, legitimacy, and stability were validated by the macroscopic and physicochemical analyses. According to extraction experiments, ethanol maceration provides a better yield while successfully maintaining key components.

Alkaloids, flavonoids, tannins, saponins, and phenolic chemicals are among the therapeutically relevant secondary metabolites that were found by phytochemical screening and all contribute to the pharmacological potential of the peel. These results were corroborated by GC-MS analysis, which identified phytol as a significant component with antibacterial and antioxidant properties.

All things considered, the results support the traditional medical application of *Cajanus cajan* peel and offer a solid scientific foundation for future research into its potential in the creation of pharmaceutical or herbal formulations.

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