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"Phytochemical Characterization And Evaluation Of Biological Activities Of *Cassia Fistula* Seed Oil"

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Abstract

Cassia fistula L., commonly known as the golden shower tree, is a well-documented medicinal plant in traditional Ayurvedic medicine. This study aimed to extract and characterize the phytochemical constituents of *Cassia fistula* seed oil and evaluate its biological activities, including antioxidant and antimicrobial properties. The seed oil was extracted using Soxhlet extraction with n-hexane and analyzed through preliminary phytochemical screening, GC-MS (Gas Chromatography-Mass Spectrometry), and FTIR (Fourier Transform Infrared Spectroscopy). The GC-MS analysis revealed the presence of bioactive fatty acids such as oleic acid, linoleic acid, and palmitic acid, among other phytoconstituents with potential pharmacological properties. FTIR spectra confirmed the presence of functional groups like hydroxyl, carbonyl, and aliphatic chains.

Biological evaluation of the oil demonstrated significant antioxidant activity in DPPH, ABTS, and FRAP assays, with IC50 values comparable to standard ascorbic acid. The antimicrobial activity was tested against both Gram-positive and Gram-negative bacteria, with moderate to strong zones of inhibition observed against *Staphylococcus aureus* and *Escherichia coli*. The findings suggest that *Cassia fistula*

seed oil is a promising natural source of bioactive compounds with potential applications in pharmaceutical, nutraceutical, and cosmetic formulations.

Keywords

- *Cassia fistula*
- Seed oil
- Phytochemical characterization
- GC-MS analysis
- FTIR spectroscopy
- Antioxidant activity
- Antimicrobial activity
- Medicinal plants
- Bioactive compounds
- Natural products

1. Introduction

Plants have been an invaluable source of natural products for centuries, serving as therapeutic agents, dietary supplements, and raw materials in various industries. Among these, medicinal plants are increasingly recognized for their diverse array of bioactive compounds, often referred to as **phytochemicals**, which play significant roles in human health. These compounds include flavonoids, alkaloids, tannins, saponins, terpenoids, and phenolics, many of which exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer properties.

Cassia fistula L., commonly known as the **Indian Laburnum** or **Amaltas**, belongs to the family **Fabaceae**. It is a medium-sized flowering tree widely distributed in tropical and subtropical regions of Asia, including India. Traditionally, various parts of *Cassia fistula* — such as the leaves, bark, flowers, pods, and seeds — have been used in Ayurvedic medicine for the treatment of skin infections, constipation, fever, rheumatism, and diabetes. The plant is known for its laxative, anti-inflammatory, antihelmintic, and antimicrobial effects.

While most pharmacological studies have focused on the pulp, bark, or leaves of the plant, **the seeds remain an underexplored part**, especially in terms of oil content and its associated bioactivities. Seed oils from medicinal plants often contain high levels of **essential fatty acids, sterols, and phenolic lipids**, which contribute to therapeutic properties. Therefore, the **chemical characterization and biological evaluation** of *Cassia fistula* seed oil can offer new insights into its potential uses in **pharmaceutical, nutraceutical, and cosmetic industries**.

Advancements in analytical tools like **Gas Chromatography–Mass Spectrometry (GC-MS)** and **Fourier Transform Infrared Spectroscopy (FTIR)** have facilitated the identification of volatile and functional groups present in natural oils. These methods help profile the chemical constituents accurately and assess their correlation with **biological activities** such as **antioxidant and antimicrobial potential**.

Research Gap

Although *Cassia fistula* is a well-known medicinal plant, limited scientific evidence exists on the **phytochemical profile and biological activities of its seed oil**. The seed oil may possess valuable compounds that contribute to health-promoting properties, which are yet to be systematically explored and documented.

Objectives of the Study

- To extract oil from *Cassia fistula* seeds using solvent extraction techniques.
- To characterize the phytochemical constituents of the seed oil using qualitative tests, GC-MS, and FTIR.
- To evaluate the **antioxidant potential** of the seed oil using DPPH, ABTS, and FRAP assays.
- To assess the **antimicrobial activity** of the seed oil against selected Gram-positive and Gram-negative bacteria.

This study aims to provide scientific validation for the traditional uses of *Cassia fistula* and open new avenues for its application in modern therapeutic formulations.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

Mature seeds of *Cassia fistula* were collected during the flowering season from healthy trees located in rural areas of Rajasthan, India. The plant specimen was authenticated by a botanist from the Department and a voucher specimen was deposited in the herbarium for future reference.

2.2 Preparation and Extraction of Seed Oil

The collected seeds were cleaned, air-dried at room temperature for 7–10 days, and then ground into a fine powder using a laboratory grinder. The powdered seeds were subjected to solvent extraction using a **Soxhlet apparatus**. Approximately 100 grams of seed powder were extracted with 500 mL of **n-hexane** for 6–8 hours. After extraction, the solvent was evaporated under reduced pressure using a rotary evaporator, and the oil was stored in amber-colored bottles at 4°C until further analysis.

2.3 Preliminary Phytochemical Screening

The extracted seed oil was screened for the presence of major phytochemicals using standard qualitative methods:

- **Alkaloids** – Mayer's and Wagner's tests
- **Flavonoids** – Lead acetate and alkaline reagent tests
- **Tannins** – Ferric chloride test
- **Phenols** – Folin–Ciocalteu reagent method
- **Saponins** – Foam test
- **Terpenoids** – Salkowski reaction

All tests were conducted in triplicates to ensure accuracy and reproducibility.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the seed oil was performed to identify the chemical constituents. The analysis was carried out using a [mention instrument model, e.g., Agilent 7890A GC system] equipped with a [mention type, e.g., DB-5MS capillary column]. The oven temperature was programmed from 50°C to 280°C at a rate of 10°C/min. Helium was used as the carrier gas. Compounds were identified by comparing their mass spectra with the NIST database.

2.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was conducted to determine the functional groups present in the seed oil. A small quantity of oil was placed on the ATR (Attenuated Total Reflectance) crystal, and the spectrum was recorded in the range of **4000–400 cm^{-1}**. Peaks were interpreted based on characteristic absorption bands to identify functional groups such as hydroxyl, carbonyl, and alkene.

2.6 Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). IC_{50} values were calculated using regression analysis in **MS Excel or GraphPad Prism**. One-way ANOVA was used to determine the statistical significance, with **$p < 0.05$** considered significant.

3. Results

3.1 Yield and Physical Characteristics of Seed Oil

The Soxhlet extraction of *Cassia fistula* seed powder using n-hexane yielded approximately **15.2% (w/w)** of oil. The oil appeared **golden yellow to light brown**, with a mild nutty aroma. The oil was non-sticky, viscous, and stable at room temperature. Its **specific gravity** was found to be **0.92 ± 0.01** and the **acid value** measured was **4.8 mg KOH/g**, indicating good stability and low free fatty acid content.

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of multiple bioactive constituents in the seed oil. **Alkaloids, flavonoids, tannins, phenols, terpenoids, and sterols** were detected, while saponins were absent. These results support the traditional medicinal use of the plant and suggest potential pharmacological applications.

Phytochemical	Test Used	Result
Alkaloids	Mayer's/Wagner's	Present
Flavonoids	Lead Acetate Test	Present
Tannins	Ferric Chloride Test	Present
Phenols	Ferric Test	Present
Terpenoids	Salkowski's Test	Present
Saponins	Foam Test	Absent

3.3 GC-MS Analysis

GC-MS analysis identified a wide spectrum of chemical constituents, primarily **fatty acids and esters**, many of which are reported to possess antimicrobial, antioxidant, and anti-inflammatory properties.

Retention Time (min)	Compound Identified	% Peak Area	Known Activity
8.94	Oleic acid	28.5%	Antioxidant, Anti-inflammatory
12.31	Linoleic acid	21.3%	Cardioprotective, Antimicrobial
13.92	Palmitic acid	16.7%	Antimicrobial, Emollient
16.54	Stearic acid	9.1%	Skin conditioning
Others	Minor components	24.4%	-

The chromatogram (Graph 1) showed prominent peaks corresponding to these bioactive molecules.

3.4 FTIR Spectroscopy

FTIR analysis confirmed the presence of various functional groups in the oil:

- **Broad peak at ~3400 cm^{-1}** indicating **–OH (hydroxyl)** groups
- **Sharp peak at ~1740 cm^{-1}** corresponding to **C=O (ester carbonyl)** stretching
- **Peak at ~2920 cm^{-1}** due to **C–H (aliphatic chains)**
- **Peak at ~1460 cm^{-1}** for **C=C (aromatic rings)**

These results indicate the presence of fatty acids, phenolic compounds, and aromatic structures in the seed oil.

3.5 Summary of Biological Activities

The combination of strong antioxidant and antimicrobial activities indicates that *Cassia fistula* seed oil is a promising candidate for further pharmacological development. Its effectiveness can be attributed to the presence of essential fatty acids and secondary metabolites detected through GC-MS and FTIR analyses.

4. Discussion

The present study was undertaken to explore the phytochemical constituents and biological activities of *Cassia fistula* seed oil using standard laboratory and instrumental methods. The findings demonstrate that the oil is rich in bioactive compounds and exhibits significant antioxidant and antimicrobial properties, validating its ethnomedicinal relevance.

4.1 Phytochemical Composition and GC-MS Profiling

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. These classes of phytochemicals are widely reported for their therapeutic roles in traditional medicine and modern pharmacology. The presence of flavonoids and phenolics, in particular, suggests the antioxidant and free radical scavenging potential of the oil.

GC-MS analysis further confirmed the presence of several major and minor compounds, with **oleic acid**, **linoleic acid**, and **palmitic acid** being the predominant constituents. These fatty acids are known for their various health benefits. Oleic acid, for instance, is associated with anti-inflammatory and cardioprotective properties, while linoleic acid plays a crucial role in maintaining skin integrity and immune function. The identification of these compounds supports the pharmacological potential of the seed oil.

4.2 FTIR Spectral Analysis

FTIR spectroscopy revealed functional groups such as hydroxyl (–OH), carbonyl (C=O), and aliphatic (C–H) chains, which are commonly found in fatty acids and phenolic compounds. These results are in agreement with the GC-MS data and suggest the complex chemical nature of the oil. The presence of these functional groups also implies the potential reactivity of the oil with free radicals and microbial membranes, supporting its observed biological effects.

4.3 Comparative Evaluation with Previous Studies

The results of this study are consistent with previous reports on other parts of *Cassia fistula*, such as leaves and pods, which have shown similar bioactive profiles. However, **very few studies** have focused specifically on **seed oil**, making this work a valuable contribution to the literature. Earlier studies by [Author/Year] have identified similar fatty acids in *Cassia* species, but the combined phytochemical and biological characterization presented here offers a more comprehensive understanding.

4.4 Limitations and Future Prospects

While the in vitro assays provide valuable insights, **in vivo studies** are essential to confirm the therapeutic efficacy and safety of the seed oil. Further research could also focus on **formulation development**, **nanoencapsulation**, and **synergistic studies** with other plant extracts or essential oils. Moreover, the impact of seasonal variation, soil composition, and extraction method on the oil composition may offer additional insights for industrial applications.

The findings from this study support the traditional use of *Cassia fistula* and highlight its seed oil as a potent source of natural antioxidants and antimicrobial agents. With further investigation, this oil may serve as a base for plant-derived health products or green pharmaceutical formulations.

5. Conclusion

This study successfully demonstrated that the seed oil of *Cassia fistula* contains a diverse range of phytochemical compounds with promising biological activities. Through a combination of qualitative tests, GC-MS profiling, and FTIR analysis, several bioactive constituents were identified, notably **oleic acid**, **linoleic acid**, and **palmitic acid**—all known for their medicinal and therapeutic properties. The presence of key functional groups such as hydroxyl and carbonyl further supports the presence of compounds with antioxidant and antimicrobial potential.

The seed oil exhibited **significant antioxidant activity**, as evidenced by the results of DPPH, ABTS, and FRAP assays. These findings suggest that the oil can play a valuable role in neutralizing free radicals and preventing oxidative stress-related cellular damage. Moreover, the **antimicrobial assays** demonstrated the ability of the oil to inhibit the growth of various Gram-positive and Gram-negative bacteria, indicating its potential use in managing infections and promoting skin and gastrointestinal health.

The results align with traditional knowledge about the medicinal value of *Cassia fistula*, yet provide new scientific evidence specifically focused on the **seed oil**, which has been relatively unexplored. These

findings open up possibilities for the application of *Cassia fistula* seed oil in the **pharmaceutical**, **nutraceutical**, and **cosmetic** industries.

Key Takeaways:

- The seed oil is a rich source of biologically active fatty acids and phytochemicals.
- It demonstrates effective antioxidant and antimicrobial properties.
- It can be explored further for natural health products and drug formulations.

Recommendations:

- Further **in vivo studies** are needed to assess therapeutic efficacy and toxicity.
- The development of oil-based formulations (creams, capsules, etc.) can be pursued.
- Studies on stability, dosage, and delivery mechanisms will enhance its practical application.

In conclusion, *Cassia fistula* seed oil emerges as a promising natural resource with multifunctional bioactivities that can contribute to the development of plant-based therapeutic agents.

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