

CASSIA FISTULA: A TREASURE TROVE OF BIOACTIVE MOLECULES - AN INTEGRATED APPROACH TO ANALYSIS, DOCKING & SYNTHESIS

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ABSTRACT

Cassia fistula, commonly used in traditional medicine systems like Ayurveda and Unani, is rich in bioactive compounds, including anthraquinones, flavonoids, and coumarins, known for their pharmacological properties. This study explores the therapeutic potential of Cassia fistula, focusing on its anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and hepatoprotective effects. Molecular docking techniques were employed to analyze compound interactions with biological targets such as COX-2 and GLUT-3, providing insights into its anti-psoriatic and other therapeutic activities. Coumarin derivatives, prominent in the plant, were synthesized using techniques like the Pechmann condensation. Analytical methods, including HPLC, were used to identify phytochemicals, while in vitro and in vivo studies validated its antimicrobial and antioxidant efficacy. Herb-drug interaction cases highlight the need for cautious use, considering factors

like gender and dietary influences. This integrated approach underlines the significance of Cassia fistula in drug discovery and natural medicine development.

KEYWORDS: Cassia fistula, Molecular docking, bioactive compounds, antimicrobial activity.

INTRODUCTION

Cassia species have a long history of utilization in various traditional medicine systems worldwide, and they are widely consumed for medicinal purposes, particularly the officinal

species. The aim of this review article is to provide an integrated overview of analysis, docking and synthesis of chemically similar molecule. A thorough literature review had been conducted using major scientific databases, publishers, and online websites such as Science Direct, Scopus, PubMed, Springer, among others. The findings of the review indicate that Cassia, have been extensively used for medicinal purposes in various traditional medicine systems, including Traditional Chinese Medicine (TCM), Islamic Traditional Medicine (ITM), Unani medicine, and Ayurveda. These plants have traditionally been employed to treat respiratory and gastrointestinal conditions, as well as skin disorders. In ITM, the reported species include *Cassia acutifolia*, *Cassia fistula*. These plants are rich in phytochemicals such as anthraquinones, alkaloids, and flavonoids, which contribute to their diverse pharmacological activities. These activities include antimicrobial, anti-inflammatory, antioxidant, antidiabetic, antiulcer, hypolipidemic, anti- atherosclerotic, and hepatoprotective effects. Based on the available literature, it can be concluded that the *Cassia Fistula* possesses significant preventive and therapeutic potential. The rich phytochemical composition and wide range of pharmacological activities make *Cassia fistula* as valuable resource in the field of natural medicine.

Coumarins are present naturally in a large number of plants, considerably in high concentration in Coumarounaodorata (tonka bean) (Fabaceae/Leguminosae)]. It is also present in high content in vanilla grass (*Anthoxanthumodoratum*), sweet clover (genus *Melilotus*), cassia cinnamon (*Cinnamomum cassia*), in extracts of *Justiciapectoralis*, and a large number of cherry blossom trees. Some species from the Apiaceae family (*PrangosLindl.*, *Ferula L.*, *Heracleum L.*, *Pachypleurum Hoff.*, *ConioselinumFisch.*, *Libanotis L.*, and *Seseli L.*) were rich with coumarin contents. Many plants contain different concentrations of coumarin. Tonkabean, liquorice, and cassia cinnamon have a high concentration of natural coumarins. Some cherry blossom strawberries and apricots contain coumarin in smaller quantities. Despite its sweet smell, animals tend to avoid plants that contain coumarins due to their bitter taste. Coumarins are natural bioactive compounds recognized for their anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antituberculous, anticonvulsant, antiadipogenic, and antihyperglycemic pharmacological activities, as well as its antioxidant and neuroprotective actions. Over 1300 coumarins have been identified from plant sources. Tonka beans can contain, on average, 1% to 3% coumarin. Animal studies have shown that these grains can be toxic, even in small amounts, causing serious liver damage in just a few weeks.

Coumarins belong to the benzopyrone family commonly found in many medicinal plants. Their structure consists of two six-membered rings with lactone carbonyl groups. Most coumarin compounds are thermally stable and have notable optical activity. The coumarin biosynthesis takes part in involving multiple P450 enzymes. Ortho-hydroxylation is a key point for the biosynthesis of natural coumarins in plants.

This study explores *Cassia fistula* as a source of bioactive compounds with medicinal potential, re viewing their properties, using docking to predict interactions with biological targets, herb drug interaction and discussing synthetic approach of coumarin synthesis. Study aims to show how these combined approaches leads to advanced drug discovery discovery. To compile and evaluate existing literature on the bioactive compounds identified in *Cassia fistula* and their potential health benefits.

To review molecular docking studies that provide insights into how *Cassia fistula* compounds may interact with biological targets. To study published synthesis techniques which lead to modified synthetic compounds for improved medicinal efficacy. To study Herb drug interactions of *Cassia fistula* with enzyme involve in skin disease.

PHYTOCHEMISTRY

Anthraquinones, naphthopyrones, naphthalene derivatives, alkaloids, essential oils, triterpenes, and flavonoids are the major components of plant *C. Fistula*. The anthraquinone derivatives such as sennosides A-D are responsible for the laxative effects of the plants. Sennosides A and B, the major anthraquinones in these genera, are optical isomers distinguishable by the configuration of C-10 and C-10'. The total content of sennosides varies in different species ranging between 1.5– 3.0%. Sennosides C and D, palmidin A, aloemodin, dianthronediglucoside, aloemodin-8- glucoside, rhein-8-glucoside, and emodin-8-sophoroside are the less abundant anthraquinone derivatives. 6- Hydroxy musizin glucoside and tinnevelly Glucoside are the two main naphthalene glycosides in these plant. However, other derivatives of naphthalene glycosides have also been reported. Flavonoids constitute one of the major classes of phytochemicals present in these genera. The flavonol kaempferol and its glucoside kaempferin, and isorhamnetin are some examples of flavonoid derivatives isolated from the genus. Several volatile constituents including mono- and sesqui-terpenes, phenylpropanes, fatty acids, and esters have been reported, but they are unlikely to be present in the plant extracts because they may leak during its preparation.

ETHANOMEDICINAL USE

The typical and most recognized representation of Cassia genus is *C. fistula* L., which is a well-known medicinal plant used for the treatment of a broad spectrum of human diseases. In ITM, the pulps of *C. fistula*, commonly known as "Folus" is widely used as a purgative febrifuge and a remedy for jaundice, leishmaniasis, and infantile colic. In Indian folk medicine, *C. fistula* is believed to be beneficial for the treatment of inflammatory diseases, skin problems, rheumatism, ulcers, anorexia, jaundice, and as a laxative. In Brazil, the bark, leaves, and seeds of *C. fistula* have been used as a remedy against malaria parasites. In China, *C. fistula* has been extensively used as an anti-inflammatory, antioxidant, chemo preventive and chemotherapeutic activity.

STRUCTURE OF CHEMICAL CONSTITUENTS

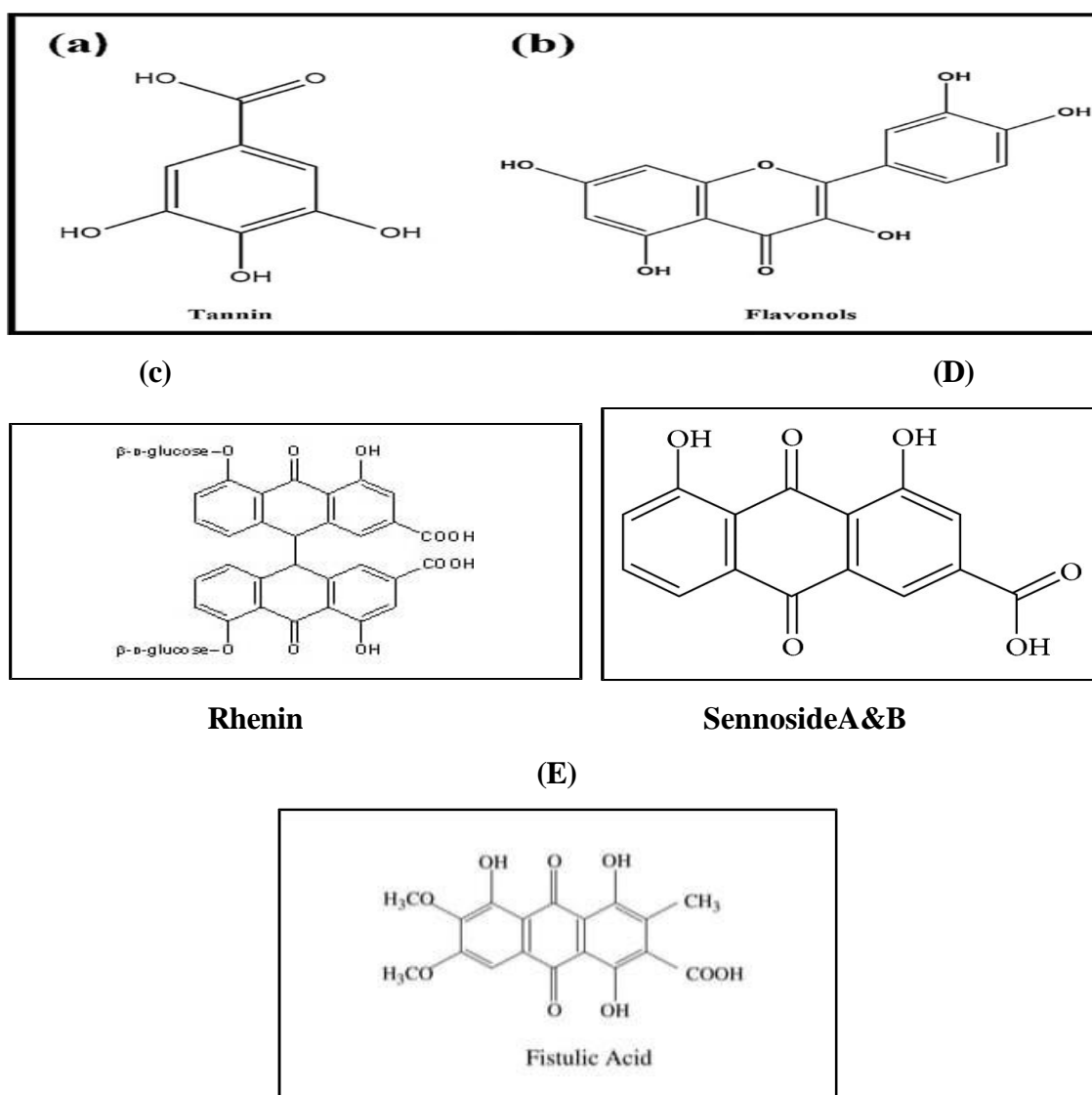




Figure1. *CassiaFistula*Fruit.



Figure2. *Cassiafistula*Flower.



Figure 3: *Cassia Fistula* Seed.



Figure 4: *Cassia Fistula*Leaf.

MOLECULAR DOCKING

Molecular docking is a computational technique used to predict the preferred orientation of one molecule, such as a drug, when bound to a target protein. This method helps in understanding the interactions between the ligand and the receptor, facilitating drug design and discovery. By simulating binding interactions, researchers can identify potential therapeutic candidates and optimize their structures. The accuracy of docking results is

crucial for effective drug development and can significantly reduce the time and cost associated with experimental screening. Overall, molecular docking serves as a vital tool in modern medicinal chemistry and biopharmaceutical research.

Molecular docking is such a structure-based drug design method that simulates the molecular interaction and predicts the binding mode and affinity between receptors and ligands. In recent years, this technology has been widely used in drug design research field. Compounds had been used from databases to screen the potential pharmacophores is not only convenient for researchers to purchase, synthesize and complete follow-up pharmacological tests, but also greatly improves. The efficiency and reduces the research cost.

We have collected below data from literature survey. Molecular docking study on phyto constituents *Cassia fistula* was reported on different PDB ID.

Table No. 1: PDBID.

| Protein | PDBID |
|--|-------|
| Nucleoside Hydrolase | 5TSQ |
| Sterol24-C-Methyltransferase | 5WP4 |
| Pancreatic Lipase Colipase | 1LPB |
| Epidermal Growth Factor Receptor(EGFR) | 1XKK |
| Dihydro folate Reductase(DHFR) | 4M6J |
| GlucoseTransporter3(GLUT3) | 4ZWB |
| Kappa Opioid Receptor(KOR) | 6VI4 |
| Cyclooxygenase-2(COX-2) | 1CX2 |
| TransientReceptorPotentialVanilloid3 (TRPV3) | 6DVZ |
| Calcium ATPase(SERCA) | 2KNE |
| Calcium Transporter(CAX) | 4K1C |
| AKT1 | 3QKK |
| CTNNB1 | 1JDH |
| ESR1 | 1PCG |
| CASP3 | 3KJF |

Software for Docking

- 1. Auto Dock Vina:** Used for virtual screening of phyto-chemical compounds against L. donovani and Pancreatic Lipase. AutoDock is available from <http://autodock.scripps.edu>
- 2. Auto Dock4.2:** Employed for additional molecular docking studies.
- 3. Discovery Studio:** Provides molecular modeling, simulation, docking, and virtual screening tools. It is available at <https://discover.3ds.com/discovery-studio-visualizer-download>

4. **Chimera:** Used for visualizing, analyzing, and modeling molecular structures. Chimera is available from <https://www.cgl.ucsf.edu/chimera/.2.4>
5. **Dock:** Suitable for docking small molecules into receptor-binding sites. The Dock is available from <http://dock.compbio.ucsf.edu/>.
6. **Glide and MolDock:** Both are widely used for docking in molecular research.

This systematic outline summarizes the information on protein targets, ligand compounds, docking software, methods, toxicity checks, and additional references for molecular docking applications.

SYNTHESIS OF COUMARIN AND ITS DERIVATIVES

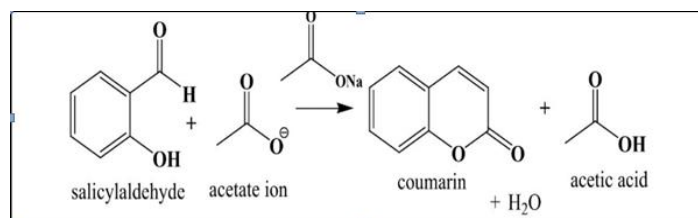
Coumarin is a major bioactive compound found in the *Cassia Fistula* plant, known for its medicinal properties. Various methods used for the synthesis of coumarine derivatives.

Reported Synthesis Methods

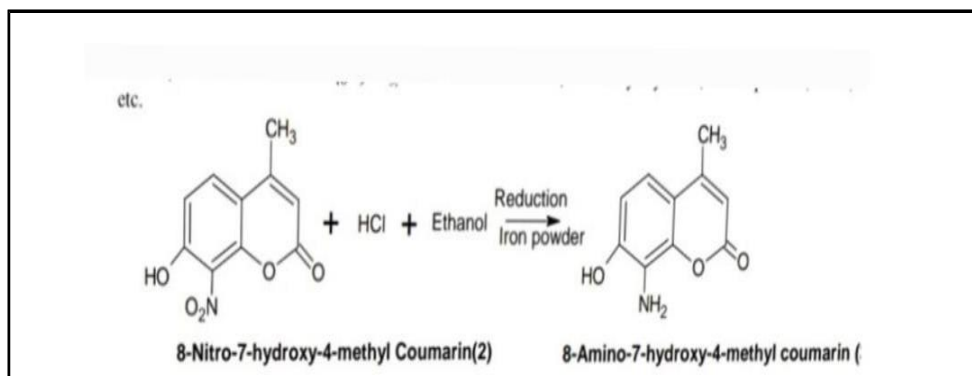
- 1) Perkin Reaction
- 2) Synthesis of 7-Hydroxy-4-Methyl Coumarin
- 3) Synthesis of 8-Amino-7-hydroxy-4-methyl Coumarin:
- 4) One-pot synthesis of coumarin-3-carboxylic acid in water
- 5) Knoevenagel condensation: Synthesis of coumarin-3-carboxylic acid:
- 6) Pechmann reaction
- 7) The synthetic pathway of coumarins developed by Pechmann using a heterogeneous acid catalyst:
- 8) **Pechmann condensation under acidic conditions**
- 9) Synthesis of 4-Chloro-3-[(substituted phenylimino)methyl]-Coumarin

From the literature survey, the Pechmann condensation method is most commonly used for synthesizing coumarins, especially under acidic conditions. This is because it is effective, simple, and provides high yields.

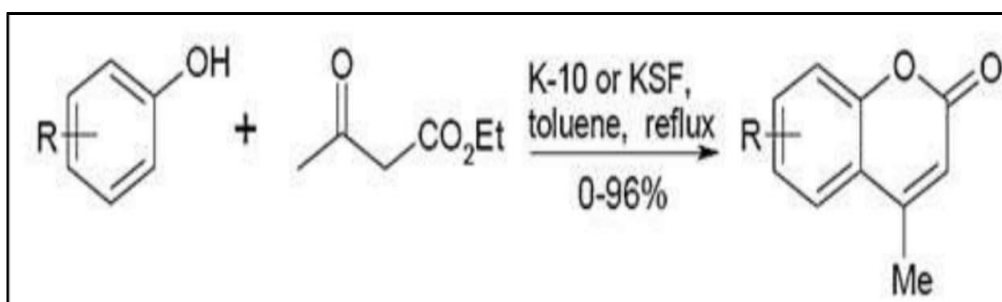
1) Perkin Reaction



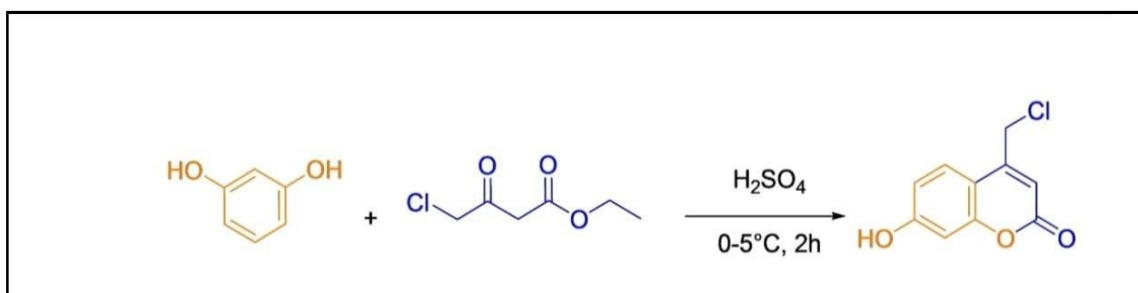
2) Synthesis of 8-Amino-7-hydroxy-4-methyl Coumarin



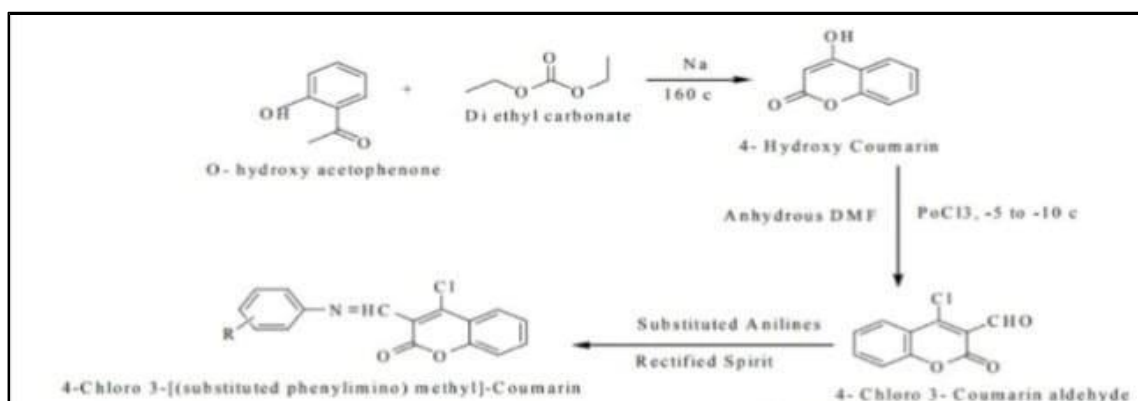
3) The synthetic pathway of coumarins developed by Pechmann using a heterogeneous acid catalyst.



4) Pechmann condensation under acidic conditions.



5) Synthesis of 4-Chloro 3-[(substituted phenylimino)methyl]-Coumarin.



ANALYTICAL STUDY

In analytical studies of *Cassia fistula* extracts using High-Performance Liquid Chromatography (HPLC) and thin layer Chromatography(TLC). The mobile phase is carefully chosen based on the nature of the bioactive compounds present in the extract.

The plant contains a variety of chemical classes, including flavonoids, anthraquinones, phenolic acids, and glycosides, all of which require an appropriate mobile phase for efficient separation and analysis.

For the analysis of *Cassia fistula* extracts by HPLC, a common mobile phase consists of water with 0.1% phosphoric acid or formic acid (aqueous phase) and acetonitrile or methanol (organic phase). This combination with a gradient elution, effectively separates and quantifies the various bioactive compounds found in *Cassia fistula* extract, flavonoids, anthraquinones, glycosides, phenolic acids. These mobile phases are often combinations of aqueous and organic solvents that help separate bioactive compounds based on their polarity and chemical properties.

Table No. 2: Stationary & Mobile Phase.

| Stationary Phase | Mobile Phase |
|---|---|
| Silica gel (e.g.,silicagel60) | Solvent mixture:Chloroform, methanol, hexane, ethyl acetate (86:6:3:5,) |
| C18reversephasecolumn | Water, Methanol, Acetonitrile (80:20to50:50) |
| Silica gel or Alumina(Al_2O_3) | Hexane, acetate, chloroform(30:70 to 20:80) |
| Non-polar(chemically modified silica,e.g.,C8,C12,C18groups) | Polar solvent mixture :Water + miscible organic solvent (acetonitrile, ethanol, methanol)(70:15:15) |
| Silica-based column(e.g.,C18) | Liquid solvent or solvent mixture :Water, acetonitrile, methanol(80:20) |

In vitro - In vivo study of Cassia Fistula: Till date various methods/ assays used for the study of antimicrobial, antioxidant, antifungal study of *Cassia Fistula* plant. This is enlisted in below table:

Table No. 3: *In vitro-In vivo* study of *Cassia Fistula*.

| Species | Parts Used / Extract Type | Activity | Study Design | Result(s) |
|-----------------------|----------------------------------|---------------|----------------------|--|
| <i>Cassia fistula</i> | Fruit / Ethanol, Aqueous Extract | Antioxidant | DPPH Assay | Methanol>Other extracts; IC50: 195–230 μ g/ml. |
| <i>Cassia fistula</i> | Stem Bark/ Alcoholic | Antioxidant | Antioxidant Activity | Effective in DPPH,NO, hydroxyl radical tests |
| <i>Cassia</i> | Flowers, Leaves, | Antimicrobial | Pathogen | Active against ear pathogens; |

| | | | | |
|----------------|------------------------------------|--|------------|---|
| <i>fistula</i> | Bark/Methanol, Ethanol, Acetone | | Inhibition | strongest: acetone flower extract against <i>S. aureus</i> . |
|----------------|------------------------------------|--|------------|---|

| <i>Cassia fistula</i> | Leaves / Hydro alcohol | Antimicrobial | Agar Disc Diffusion | Effective against <i>S. aureus</i> > <i>E.coli</i> ; dose- dependent |
|-----------------------|---|---------------|---------------------------|--|
| <i>Cassia fistula</i> | Leaves/Pet. Ether, Chloroform, Methanol, Water | Anti-acne | Agar Disc Diffusion | Methanol most effective; MIC: 10 µg/ml |
| <i>Cassia fistula</i> | Stem Bark/ Alcoholic | Antifungal | Hyphae, Zoospores Test | Inhibits growth stages of pathogens |
| <i>Cassia fistula</i> | Bark/Ethanol, Ethyl Acetate Fraction | Antibacterial | Diffusion Agar | Strong activity: <i>S.aureus</i> (MIC: 0.625%), <i>E. coli</i> (MIC: 1.25%) |
| <i>Cassia fistula</i> | Roots / Petroleum Ether, Chloroform, Ethanol, Aqueous | Antibacterial | Broth Micro dilution | Alcoholic extract effective against <i>B. subtilis</i> (MIC: 1.6), moderate against <i>E.coli</i> (MIC: 3.125) |

PSORIASIS

Psoriasis is a commonly immune-mediated disease which is more likely to develop chronic skin disorder characterized by the recurring localized, widespread well-demarcated red plaques often topped by silvery scales over the skin lesions. This disease also diversely damages nails, mucous membranes and joints, but not hair. This health problem strongly influents on patient's psychological aspect because of having to cope with the reactions of others, one of the biggest challenges of living with skin disease. Speculations about the cause of psoriasis are many, such the disease as a result of interplay between genetic and environmental factors. The etiology of psoriasis is believed to be multi factorial; however, this question has not yet been fully resolved and therapeutic intervention for patients with psoriasis is therefore still not standardized.

ANTI-PSORIATIC ACTIVITY

From literature survey weals found that plant is having anti-psoriatic activity. Very less study reported on anti-psoriatic activity.

Reported Materials and methods from literature survey

Materials

They purchased methanol and Di-n-Propyl Disulfide (PPD) from Sigma chemicals. The other ingredients which they used for cream base include stearic acid, cetyl alcohol, isopropyl myristate, glycerin, potassium hydroxide, sodium hydroxide and methyl paraben (Kanto Chemical Co., Inc). They also used distilled water throughout the study.

Animals

For this method they used Adult Swiss albino mice weighing 23 ± 2 g and aging 10 weeks which obtained from Pasteur Institute of Ho Chi Minh City. They then acclimated animals to test conditions for 1 week. The mice were supplied standard pellet diet, water and libitum, and kept at room temperature under 12 h light-dark cycles. Handling and treatment of experimental animals research.

HERB DRUG INTERACTIONS

Common Interactions with Other Herbs: *Cassia fistula* is often used with herbs like Senna for its laxative properties. Combined use might amplify effects, leading to stronger laxative action or gastrointestinal distress.

Mechanisms of Herbal Interactions: Because *Cassia fistula* contains similar compounds to other laxative herbs, combining them can intensify effects. Moreover, its antimicrobial properties might reduce the efficacy of other medications by altering gut florae complied with the ethical guidelines for animal research.

Clinical Case Study: A case involving a patient taking *Cassia fistula* along with a Senna-based laxative showed enhanced laxative effects, resulting in dehydration. This highlights the importance of careful dosing and monitoring when combining herbal treatments.

Factors Affecting *Cassia fistula* Activity

Genetic Factors: Variability in enzymes like cytochrome P450 among individuals affects how *Cassia fistula*'s compounds are metabolized. For instance, certain genetic polymorphisms could lead to higher or lower metabolism of anthraquinones, impacting efficacy and safety.

Gender Differences: Research indicates that male and female bodies may process *Cassia fistula* differently, potentially due to hormonal influences on enzyme activity, though more research is needed here.

Case Study on Gender Differences: A study on male and female responses to *Cassia fistula* Laxative effects revealed slightly faster action in females, possibly due to hormonal differences.

Case Study 1: *Cassia fistula* and Calcium-Rich Foods-Reduced Laxative Effect

Background: A 40-year-old man with constipation was prescribed *Cassia fistula* extract by a traditional medicine practitioner. He noticed minimal improvement in his symptoms after a week, despite taking it as directed.

Observed Interaction: Upon dietary review, it was discovered that the patient consumed a diet high in calcium rich foods (dairy products) immediately after taking *Cassia fistula*.

Mechanism of Interaction: Calcium has a binding effect on anthraquinones, the active compounds in *Cassia fistula*, potentially reducing their absorption and efficacy. Calcium-rich foods can delay or diminish the laxative effects of anthraquinones.

Clinical Implications: Patients using *Cassia fistula* for its laxative effects should be advised to avoid calcium-rich foods close to its ingestion time. Allowing a gap between consuming the herbal supplement and such foods could help improve therapeutic outcomes.

Case Study 2: *Cassia fistula* and CYP3A4-Metabolized Drug-Increased Drug Levels

Background: A 60-year-old patient with hypertension and diabetes was prescribed *Cassia fistula* tea for its potential antioxidant and antimicrobial benefits. The patient was concurrently taking Amlodipine, a medication metabolized primarily by the CYP3A4 enzyme, for hypertension.

Observed Interaction: After a few weeks of using *Cassia fistula*, the patient reported dizziness and symptoms of hypotension, indicating an unintended increase in Amlodipine levels.

Mechanism of Interaction: Research suggests that compounds in *Cassia fistula* may inhibit CYP3A4 enzyme activity, slowing the metabolism of drugs processed by this enzyme. In this case, the slower breakdown of Amlodipine resulted in higher-than-expected drug levels, intensifying its hypotensive effects.

Case Study 3: *Cassia fistula* and Gender-Specific Response-Enhanced Effect in Female Patients

Background: A study with male and female participants investigated the laxative effects of *Cassia fistula*. Both groups received a standardized dose, yet female participants reported a more rapid onset and stronger effect compared to males.

Observed Interaction: Females experienced a stronger laxative effect, resulting in some mild abdominal discomfort. No such effects were noted in male participants at the same dose.

Mechanism of Interaction: Gender-based pharmacokinetics may account for different metabolic rates. In this case, hormonal differences in females (e.g., estrogen) could influence gastrointestinal motility or enzyme activity, leading to a faster or more pronounced response.

Clinical Implications: Gender may impact *Cassia fistula* efficacy, particularly for effects like laxation. Dosing adjustments might be considered for female patients, or they could be advised to start with lower doses and monitor their response to prevent adverse effects.

CONCLUSION

From the literature review, we conclude that *Cassia fistula* possesses significant medicinal properties including anti-microbial, anti-fungal, and antioxidant activities. The plant has shown promising anti-psoriatic effects, because of major constituent, chrysophanol. Chrysophanol's mechanism appears to involve interaction with enzymes such as cyclooxygenase (COX), which is vital in the inflammatory pathways associated with psoriasis. This interaction suggests a potential role for *Cassia fistula* in managing psoriasis and other inflammatory skin conditions. Moreover, the plant's rich phytochemical profile—including coumarins, flavonoids, and tannins underlines its therapeutic potential and encourages further exploration in pharmacology, especially in the development of natural anti-inflammatory and skin-protective agents. The future perspectives of the above study to find out novel use Study *Cassia fistula* phyto chemicals to create new, effective medicine, to Find More Targets for Treatment Use of molecular docking studies to discover more ways to identify novel use of existing molecules. Understand Herb-Drug Interactions, to find out how *Cassia fistula* interacts with other drugs to ensure safe use. Make Better Synthetic Compounds Improve synthetic versions of existing Coumarine compounds for stronger health benefits. Explore Disease Prevention Look into *Cassia fistula*'s potential in preventing or treating skin and inflammatory diseases.

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