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Research

Phyto-Centric Formulation Approach for an Antidandruff Shampoo Using Cassia Fistula L. Extract

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Abstract

Investigations into the antimicrobial and antifungal efficacy of Cassia species, particularly Cassia fistula Linn., reveal substantial bioactivity in extracts prepared from leaves, fruit husks, and other plant parts using solvents such as methanol, ethanol, water, n-hexane, and chloroform. Phytochemical analyses indicate the presence of diverse secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, and polyphenols, which are considered responsible for the observed biological effects.(1) In vitro evaluations employing agar well diffusion, disc diffusion, and minimum inhibitory concentration assays demonstrate pronounced inhibitory activity against clinically relevant Gram-positive and Gram-negative bacteria, notably Escherichia coli and Staphylococcus aureus, as well as pathogenic fungi such as Candida albicans, Aspergillus niger(2), and dandruff-associated Malassezia species. Organic solvent extracts, particularly methanolic and n-hexane fractions, consistently exhibit stronger antimicrobial and antifungal activities, with higher concentrations producing greater zones of inhibition, suggesting a concentration-dependent response. Collectively, these findings underscore the significant therapeutic potential of Cassia fistula as a natural source of antimicrobial and anti-dandruff agents and support its traditional medicinal applications,(3) while emphasizing the need for further in vivo investigations and mechanistic studies to validate its role in developing plant-based alternatives to combat microbial resistance.

Keywords: Antifungal Activity, Anti-Dandruff Activity, Aspergillus Niger, Cassia fistula, Candida albicans, Disc diffusion, Fabaceae, Herbal Medicine, Invitro Antibacterial Activity, Secondary Metabolites.

1. INTRODUCTION

Cassia fistula L (Fabaceae), a very common plant known for its medicinal properties is a semi-wild in nature also known as the Golden Shower. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers.[4] It is the national tree of Thailand and its flower is Thailand's national flower. It is also state flower of Kerala in India.[5] *Cassia fistula* is a deciduous, medium-sized tree up to 24 m in height and 1.8 m in girth, cultivated almost throughout India. It is one of the most important trees widely spread in the forest of India. It is usually occurring in deciduous forests throughout the greater part of India, ascending up to an altitude of 1,220 m in the sub-Himalayan areas and the outer Himalayas. It is common throughout Gangetic valley, particularly abundant in Central India and South India. It is planted as an ornamental tree in homesteads and along the roadside. Many of the biologically important compounds were isolated and identified from different parts of the plant. Medicinally it has been various pharmacological activities like antimicrobial, antifungal, antipyretic, analgesic, larvicidal, anti-inflammatory, antioxidant, anti-tumour, hepatoprotective, hypoglycemic activities, anti-diabetic activity, and laxative property. In the traditional medicine, *Cassia fistula* is one of the most commonly used plants in Unani and Ayurvedic medicines, this plant has been described to be useful against skin diseases, liver troubles and is also used as an anti-fungal drug[6]. Traditionally, the plant is also used as an infusion, decoction, or powder, either alone or in combination with other medicinal plants. The antifungal activity of different leaf extracts (petroleum ether, chloroform, ethanol, methanol and aqueous) against human pathogenic fungi and the biological activities of the extracts in terms of MIC and MFC were also determined.[7] Extracts of *Cassia fistula* leaves against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents. The leaves are laxative, antiperiodic, depurative, anti-inflammatory, and are useful in skin diseases, boils, carbuncles, ulcers, intermittent fever, gouty arthritis, and rheumatologic. *Cassia fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds.[8]

2. METHODOLOGY

Authentication were carried out by a qualified botanist from the Botanical Survey of India (TNAU) Coimbatore.

Figure: 2 *Cassia Fistula leaf*

2.1. Chemical Constituents

Cassia fistula (golden shower tree) is rich in bioactive compounds, primarily anthraquinones, flavonoids - quercetin, alongside other polyphenols like kaempferol, catechin, and epicatechin. Quercetin is present in various parts of the plant and tannins, with significant concentrations of rhein, sennosides (A and B), chrysophanol, kaempferol, and catechin found across its leaves, bark, and fruit. The plant is well-known for its laxative, antioxidant, and anti-inflammatory properties.

- **Fruit/Pulp:** Contains rhein, sennosides, fistulic acid, 1,8-dihydroxy-3-anthraquinolone, tannin, and waxy substances.
- **Leaves:** Contain flavonoids – quercetin, sennosides A and B, rhein, rhein glycoside, kaempferol, and various fatty acids.
- **Bark:** Contains lupeol-sitosterol, hexacosanol, and oxyanthraquinone substances, including barbaloin.
- **Seeds:** Comprise carbohydrates (50%), proteins (24%), and amino acids like glutamic acid and lysine.
- **Flowers:** Contain kaempferol, rhein, and a bianthroquinone glycoside

2.2. PREPARATION OF EXTRACT

2.2.1. Material Preparation

The process biggened with collection of fresh Cassia fistula leaves, which was thoroughly cleaned with water to remove any surface contaminants. The leaves were then shade-dried. Once dried, they were ground into a fine powder using mixer to increase the surface area for more efficient solvent penetration.

[1] The Soxhlet apparatus has been fixed up.

[2] The powdered leaf material was placed inside the "thimble" (typically made of thick filter paper) which was then inserted into the main chamber of the Soxhlet extractor. The extractor was placed onto a round-bottom flask containing the solvent (Ethanol).

[3] Extraction Process was proceeded.

[4] **(a) Solvent and Temperature:** Ethanol was used as the solvent. The flask was heated at constant temperature of 70°C.

(b) Cycle Duration: The extraction was a continuous process that was maintained for a total of 72 hours. During the time, the solvent vaporized, condensed and dripped into the thimble, soaking the leaf powder and extracting the phytochemicals. Once the chamber is full, the solvent siphons back into the boiling flask.

(c) Exhaustion: This cycle repeats until the leaf material is completely exhausted of its chemical constituents.

2.2.2. Post-Extraction Refinement

After the 72-hour period, the resulting liquid (the extract) was filtered while still warm to remove any fine particulate matter.

Figure 3: *Soxhlet Apparatus*

2.2.3. Concentration and Storage

To obtain the final crude extract, the ethanol is removed using a rotary flask evaporator under vacuum. This concentrates the extract into a dark greenish, firm filtrate. The final product is stored in a desiccator to ensure it remains completely dry and stable for further antimicrobial testing.

3. PRELIMINARY PHYTOCHEMICAL STUDIES

The present study was carried out to evaluate the phytochemical studies of the Cassia fistula. The details of the material used and methods followed are described below.

3.1. CHEMICALS AND REAGENTS

Dilute sulphuric acid, Benzene, Dilute ammonia, Ferric chloride, Lead acetate, Sodium hydroxide, Concentrated Hydrochloric acid, Distilled water.

3.2. QUANTITATIVE CHEMICAL TEST

Different chemical test for Anthraquinones, Tannins, Flavonoids and Polyphenols was carried out in the study.

3.2.1. Test for Anthraquinones

- **Borntrager's Test:** A small quantity of the plant extract was boiled with dilute sulphuric acid and filtered. The filtrate was shaken with benzene. The benzene layer was separated and treated with dilute ammonia solution.
- **Modified Borntrager's Test:** The extract was treated with ferric chloride and hydrochloric acid, heated, and then filtered. The filtrate was shaken with benzene, followed by the addition of ammonia solution.

3.2.2. Test for Tannins

- **Lead Acetate Test:** To a small volume of the extract, a few drops of lead acetate solution were added.
- **Ferric Chloride Test:** The extract was treated with a few drops of ferric chloride solution.

3.2.3. Tests for Flavonoids

- **Alkaline Reagent Test:** The extract was treated with sodium hydroxide solution.
- **Shinoda Test:** The extract was treated with magnesium ribbon followed by the addition of concentrated hydrochloric acid.

3.2.4. Tests for Polyphenols

- **Ferric Chloride Test:** The extract was treated with a few drops of ferric chloride solution produces a bluish black colour indicating the presence of Phenols.
- **Liebermann's Test:** The extract was treated with a few drops of sodium nitrite and concentrated H_2SO_4 and then added with excess NaOH solution produces a red colour indicating the presence of phenols.

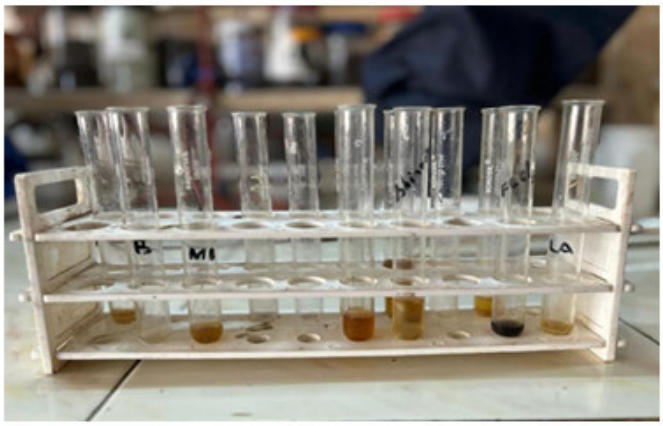

Figure 4: *Chemical Test*

3.3. Formulation of Herbal Shampoo

For formulation of herbal shampoo, herbal extract was mixed with soap nut extract solution, Xanthan gum solution, Glycerine, Benzoic acid and distilled water as per the formula given in Table 1. Distilled water was added to make up the final capacity. Rosemary oil was added to improve its aroma. Then vitamin E and lemon juice was added.

Table 1. Ingredients and Uses

S.NO	INGREDIENTS	F1 50ml	F2 50ml	F3 50ml	USES
1.	*Cassia fistula* extract	10ml	12ml	14ml	Anti-Fungal agent
2.	Soap nut extract	10ml	12ml	14ml	Surfactant, Foaming agent
3.	Aloe vera extract	10ml	10ml	10ml	Soothing agent
4.	Benzoic Acid	0.1gm	0.1gm	0.1gm	Preservative
5.	Glycerin	1ml	1ml	1ml	Humectant (Prevents dryness)
6.	Lemon juice	1ml	1ml	1ml	Anti-fungal agent
7.	Xanthan gum	2ml	2ml	2ml	Thickening agent
8.	Rosemary oil	2drops	2drops	2drops	Fragrance
9.	Vitamin E	1ml	1ml	1ml	Anti-oxidant, Nourishes scalp
10.	Water	14ml	10ml	6ml	Vehicle

3.4. ANTI-FUNGAL STUDIES

Antifungal screening was performed against dandruff-causing organisms, including Aspergillus niger and Candida albicans, using agar well diffusion techniques. The shampoo exhibited significant antifungal activity when compared with commonly available commercial formulations, demonstrating the therapeutic potential of Cassia fistula leaf extract in dandruff management.(9) The study supports the use of plant-based constituents as effective alternatives to synthetic antidandruff agents, providing a safer and eco-friendly approach for scalp care.

The antifungal activity of the prepared shampoo was evaluated using the agar well diffusion method. Different concentrations of the extract were prepared in DMSO loaded with 25 µl of each concentration. Pure fungal cultures of Aspergillus niger and Candida albicans were grown in nutrient broth and then uniformly spread on Sabouraud Dextrose Agar plates using a sterile swab. The extract-impregnated discs were placed on the inoculated agar surface and the plates were incubated at 25–30°C for 24–72 hours. After incubation, the antifungal activity was assessed by measuring the diameter of the clear zone of inhibition around each agar well in millimetres for identifying the concentration that prevented visible fungal growth.(10)

3.5. Test Microorganisms

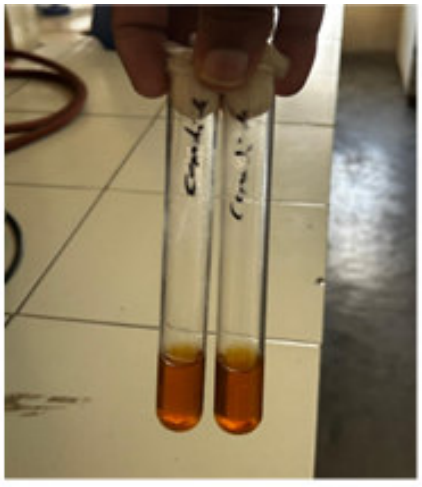

Figure 5: *Sabouraud Dextrose medium for the growth of Candida albicans*

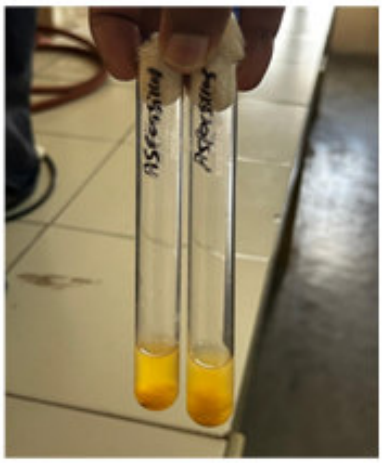

Figure 6: *Sabouraud Dextrose medium for the growth of Aspergillus niger*

The fungal strains used are:
Candida albicans (MTCC 227) and Aspergillus niger (MTCC 282)

3.6. Preparation of Extract Concentrations

The shampoo is dissolved in dimethyl sulfoxide (DMSO). Serial dilutions are prepared to obtain concentrations of 100, 75 and 50mg/ml.

3.7. Preparation of Inoculum

Figure 7: *Preparation of Sabouraud Broth*

A few fungal colonies are emulsified in nutrient broth to form a uniform suspension. This suspension was swabbed evenly across the surface of freshly prepared SDA plates to create a confluent lawn of fungal growth.

3.8. Agar Well Diffusion Procedure

- After inoculation, a sterile cork borer is used to punch wells of 6 mm diameter in the agar.
- Each well is filled with 50, 75 and 100 µL of the plant extract at the desired concentrations.
- A well with DMSO serves as a negative control.
- A standard antifungal drug (Voriconazole) is loaded into a separate well as a positive control as used in the reference study.
- Plates are kept at room temperature for 30 minutes to allow pre-diffusion of the extract.
- Plates are incubated at 25 - 30°C for 24 - 72 hours, depending on the growth rate of the fungal species.
- After incubation, the zone of inhibition (mm) around each well is measured to assess antifungal activity.
- Activity Index (AI) Calculation

- The activity index compares the extract's inhibition zone with that of a standard drug (Voriconazole for *C. albicans* and *A. niger*).
- Formula for Activity Index (AI):

$$AI = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard drug}}$$

4. EVALUATION PARAMETER OF HERBAL SHAMPOO

To evaluate the prepared formulations, quality control tests including visual assessment and physicochemical controls such as pH, density and viscosity were performed.

4.1. Physical Appearance / Visual Inspection

Purpose: To assess initial quality, homogeneity, clarity, colour, odour, and foam characteristics.

Procedure:

- Small quantity of the shampoo was transferred into a clean glass container.
- Observed under adequate lighting for colour uniformity, clarity and presence of suspended particles.
- Shaken gently and checked the type and stability of foam produced.
- Note texture, flow properties and overall appearance.

4.2. Determination of pH

Purpose: To ensure the safety of Shampoo for scalp and hair by maintaining mild acidity.

Procedure:

- **10% w/v shampoo solution** was prepared using distilled water.
- Calibrated by pH meter at room temperature.
- The electrode was immersed in the prepared solution and allowed the reading to stabilize.
- The pH value was recorded.

4.3. Percent of Solid Content

Purpose: Determines the amount of residue remaining after water evaporation, indicating total solids.

Procedure:

- A clean, dry evaporating dish was weighed.
- Exactly **4 g** of shampoo was added into the dish and combined weight was recorded.
- Heated on a hot plate until water evaporates completely.
- Cooled and dry residue was weighed.
- Calculation:

$$\% \text{ Solids} = (\text{Weight of residue} / \text{Weight of shampoo sample}) \times 100$$

4.4. Foam Stability / Foaming Ability (Cylinder Shake Method)

Purpose: To evaluate the amount and stability of foam produced.

Procedure:

- Prepared **1% shampoo solution** (50 mL) was transferred to a 250 mL graduated cylinder.
- The opening was covered and shaken **10 times vigorously**.
- Foam volume was measured immediately (0 min), then at **1 min** and **4 min**.
- Higher foam stability indicates better performance.

4.5. Viscosity Measurement

Purpose: Determines consistency and flow behaviour of the shampoo.

Procedure:

- Sample cup was filled with shampoo.
- Brookfield viscometer was with appropriate spindle.
- Viscosity values was recorded.

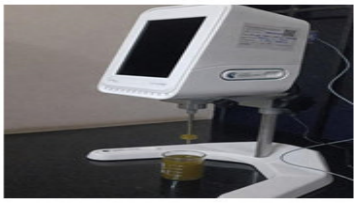

Figure 8: *Viscometer*

5. RESULTS AND DISCUSSION

5.1. Organoleptic evaluation

Organoleptic analysis recorded in Pharmacognostical evaluations describes the leaves as green with a mild odour and slightly bitter taste, whereas the flowers exhibit a sweet aroma. The pod pulp is reported to have a sweet, sticky consistency and a characteristic taste, forming the basis for its use in formulations.

TABLE 2. *Organoleptic Evaluations*

Parameter	Leaf	Flower	Pulp
Colour	Green	Yellow	Brownish-black
Odour	Mild	Sweet	Characteristic
Taste	Slightly bitter	Sweet	Sweet & Mucilaginous

5.2. Chemical Constituents

Leaf Leaves: Contain flavonoids – quercetin, sennosides A and B, rhein, rhein glycoside, kaempferol, and various fatty acids.

Preparation of Extract:

The Ethanolic extract was prepared by Soxhlet Extraction and used for the studies.

Preliminary phytochemical studies:

Preliminary phytochemical analysis indicates the presence of Anthraquinones, Tannins, Flavonoids.

Table 3: *Phytochemical Test*

S.NO	CHEMICAL TEST	RESULTS
	TEST FOR ANTHRAQUINONES	
1.	Borntrager's test	A+++
2.	Modified Bontrager test	A++
	TEST FOR TANNINS	
1.	Lead acetate test	A++
2.	Ferric chloride test	A+++
	TEST FOR FLAVANOIDS	
1.	Alkaline test	A++
2.	Ammonia test	A+++
	TEST FOR POLYPHENOLS	
1.	Ferric chloride test	A+++
2.	Liebermann's test	A++

High: A+++; Intermediate: A++; Low: A+; Negative: A-

5.3. Herbal Shampoo

Formulation of Herbal Shampoo was formulated and Evaluation parameters was done evaluation parameters was performed.

5.4. Evaluation parameters

To evaluate the prepared formulations, quality control tests including visual assessment and physicochemical controls such as pH, percentage of solid content, foam stability and viscosity were performed.

5.4.1. Physical Appearance / Visual Inspection

The physical properties of a shampoo determine its shelf-life, ease of use, and consumer acceptance. The shampoo was visually clear and greenish brown in colour, featuring a pleasant aroma due to the inclusion of rosemary oil.

5.4.2. Determination of pH

The pH of all three formulations was measured using a calibrated digital pH meter, and the values were recorded within the mildly acidic range suitable for scalp application. The results showed a gradual decrease from F1 to F3, indicating increased acidity with higher herbal extract concentration.

Table 4.*PH*

S.No	Formulation	pH
1	F1	5.7
2	F2	5.4
3	F3	5.2

5.4.3. Percent of Solid Content

The percentage of solid content was determined by drying 4 g of shampoo to a constant weight, and the final residue was recorded for each formulation. The results showed an increase from F1 to F3, confirming that formulations with higher extract levels exhibited greater solid content

Table 5: *Percentage Solid Contents*

S.No	Formulation	% Solid Content
1	F1	19.5%
2	F2	21.2%
3	F3	23.0%

6. CALCULATION

- For F1 $\frac{0.78}{4} \times 100 = 19.5\%$
- For F2 $\frac{0.85}{4} \times 100 = 21.2\%$
- ForF3 $\frac{0.92}{4} \times 100 = 23.0\%$

6.1. Foam Stability / Foaming Ability (Cylinder Shake Method)

Foam stability for all formulations was measured using the cylinder shake method, and the foam height was recorded at 0, 1 and 4 minutes. The results showed that F3 retained the highest foam stability, followed by F2 and F1, indicating better surfactant activity with increased surfactant concentration

Table6. *Percentage Foam Stability*

Formulation	Foam Height @ 0 min (cm)	Foam Height @ 4 min (cm)	Foam Stability (%)
F1	7	6	85.7%
F2	8	7	87.5%
F3	9	8	88.8%

6.2. Viscosity Measurement

The viscosity of all three formulations was measured at 10 rpm using a Brookfield viscometer and the values were recorded accordingly. The results showed an increase from F1 to F3, indicating higher viscosity with increasing herbal concentration.

Table 7: *Viscosity*

S.No	Formulation	Viscosity
1	F3	2100 cP
2	F2	2280 cP
3	F3	2400 cP

6.3. Anti-fungal activity

The antifungal activity of the shampoo formulations prepared using Cassia fistula extract and other herbal ingredients was evaluated against Candida albicans and Aspergillus niger using the agar well diffusion method, and the zone of inhibition was recorded for each sample and reported.

Table8. *Anti-Fungal Study of Formulation Using Aspergillus Niger*

Sample	Zone of Inhibition (mm)	Activity index
Aspergillus niger		
Control D	0	0
Standard	22±0.57	-
Sample A	19.66±0.33	0.86
Sample B	17.66±0.66	0.77
Sample C	10±0.57	0.47
Candida albicans		
Control D	0	0
Standard	20.33±0.88	-
Sample A	19±1.0	0.90
Sample B	16.01±1.0	0.75
Sample C	7.66±0.88	0.45

Values are expressed as mean ± SEM of triplicate observations.
(-) denotes no activity
Sample A: Cassia fistula ethanolic extract of 100 µl
Sample B: Cassia fistula ethanolic extract of 75µl
Sample C: Cassia fistula ethanolic extract of 50 µl
Sample D: Control
Standard: Voriconazole

7. DISCUSSIONS

The formulation of personal care products, such as polyherbal shampoos, represents a practical application of *Cassia fistula* research. By combining *C. fistula* with *Aloe vera* leaf extracts, a synergistic effect is achieved that enhances hair health. *Aloe vera* provides essential conditioning and soothing properties while other ingredients like Soap nut extract produced foaming and surfactant effect, Benzoic Acid used as preservative, Glycerin as humectant, Lemon juice as antifungal agent, Xanthan gum as thickening agent, Rosemary oil to increase the aroma and Vitamin E as antioxidant and nourishes scalp while *C. fistula* contributes antimicrobial and anti-dandruff benefits as *Cassia alata* showed anti dandruff property[11] Which is a related species of *C. fistula* in the Evaluations of such enriched shampoo showed that they can maintain an ideal pH and provide effective cleaning without the harsh side effects of synthetic detergents[11]

Quercetin, a well-known flavonoid, has shown potential as a natural antifungal agent against *Malassezia* species, including *Malassezia globosa*, *Malassezia furfur* which are primarily responsible for dandruff and seborrheic dermatitis. Research indicates that quercetin and related phenolic compounds can inhibit the growth of these fungi by targeting essential virulence factors, specifically by inhibiting lipase enzymes that *Malassezia* uses to thrive on skin sebum.

Quercetin is identified as a component in herbal extracts (such as from *C. fistula* that exhibit anti-*Malassezia* activity.[12] Quercetin, a natural flavonoid found in many plants, shows promise as an anti-dandruff agent by reducing scalp inflammation and inhibiting dandruff-causing microorganisms.

8. CONCLUSION

The final evaluation of Phyto-Centric anti-dandruff shampoo involved the rigorous testing against common scalp fungi. Formulations incorporating Cassia fistula have shown excellent results in reducing scalp flaking and itching. By standardizing these products according to UGC-approved research guidelines, the pharmaceutical industry can offer "Phyto cosmetics" that provide professional-grade results while adhering to the principles of sustainable and safe personal care.(17) Research highlights the high susceptibility of Candida albicans and Aspergillus niger to modern triazole therapies. While C. albicans remains largely responsive to fluconazole and Ravuconazole investigational agents like Voriconazole demonstrate significantly greater potency, achieving much lower minimum inhibitory concentrations Similarly, for filamentous fungi like Aspergillus niger, Voriconazole provided the broadest spectrum activity whereas Candida showed less activity. On performing traditional treatments such as Itraconazole and Amphotericin B in inhibiting the majority of clinical strains.(13)

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