WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 17, 1971-1978. Research Article ISSN 2277–7105

AN IN VITRO EVALUATION OF ARAGVADHA LEAF EXTRACTS AGAINST COMMON SKIN PATHOGENS TO VALIDATE IT'S CLASSICAL INDICATION IN KUSHTHA (OBSTINATE SKIN DISEASES)

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Article Received on 05 August 2026,
Article Revised on 25 August 2026,
Article Published on 01 Sept. 2026

https://doi.org/10.5281/zenodo.22707305

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How to cite this Article: Dr. Shailesh Deshmukh* (2026). An In Vitro Evaluation of Aragvadha Leaf Extracts Against Common Skin Pathogens To Validate It's Classical Indication In Kushtha (Obstinate Skin Diseases). World Journal of Pharmaceutical Research, 15(17), 1971–1978.

ABSTRACT

Background & Objective: In traditional Ayurvedic medicine, Aragvadha (Cassia fistula Linn.) is classically designated as a premier Kushthaghna (skin disease-destroying) drug. The ancient treatise Charaka Samhita dedicates a specialized chapter (Aragvadhiya Adhyaya) to it's topical efficacy in mitigating obstinate skin conditions grouped under the umbrella term Kushtha. This study aims toper form an in vitro evaluation of Aragvadha leaf extracts using various organic and aqueous solvents against common clinical skin pathogens (Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans) to scientifically validate these classical claims. **Methods:** Dried Aragvadha leaves (Aragvadha Patra) were subjected to sequential solvent extraction utilizing aqueous, methanolic, and ethanolic matrices via a Soxhlet apparatus. Qualitative phytochemical screening was executed to isolate secondary metabolites. Antimicrobial profiling was carried out using the Agar Well Diffusion Assay. Minimum Inhibitory Concentrations (MIC) were determined via microbroth dilution methods. **Results:** The methanolic and ethanolic leaf extracts of Cassia fistula demonstrated potent broad-spectrum antibacterial activity, generating significant zones of inhibition (ZOI) against Staphylococcus aureus (19.4 ± 0.5 mm) and moderate inhibition against Pseudomonas aeruginosa(14.2 ± 0.3 mm) at a concentration of 100 mg/mL. The extracts showed localized efficacy against fungal isolates

of Candida albicans. Phytochemical analysis confirmed an abundance of anthraquinones (such as rhein), flavonoids, and tannins within the active fractions. **Conclusion:** The experimental data provide empirical validation for the ancient Ayurvedic application of Aragvadha Patra in topical pastes (Lepa) for Kushtha. The presence of bioactive polyphenols and anthraquinones validates its therapeutic role as a natural antimicrobial agent for modern dermatological configurations.

KEYWORDS: Treditional, Aragwadha, Skin, Agar, Antibacterial, Kushtha, Bioactive, Antimicrobial.

1. INTRODUCTION

Dermatological disorders represent a significant portion of global healthcare challenges, impacting quality of life and inducing physical discomfort and psycho-social stress. In contemporary clinical practice, the emergence of multi-drug resistant (MDR) bacterial strains, such as Methicillin-Resistant Staphylococcus aureus (MRSA), alongside persistent fungal biofilms, creates an urgent necessity to identify novel, bio compatible antimicrobial scaffolds from plant origins.

Ayurveda, an ancient systemic medical architecture, addresses the entire spectrum of obstinate skin conditions under the single comprehensive category of **Kushtha**. Kushtha is sub-classified into 7 Mahakushtha (major skin pathologies) and 11 Kshudra kushtha (minor dermatoses), capturing conditions comparable to modern psoriasis, eczema, dermatophytosis, and chronic bacterial pyodermas.[1]

Among the primary therapeutic agents indicated for Kushtha, the plant **Aragvadha** (Botanical name:Cassia fistula Linn., Family: Fabaceae/Caesalpiniaceae) occupies an elevated status. Classically known as the 'King of Trees' (Rajavriksha) due to its visually striking golden-yellow inflorescences, it's name literally translates to 'the destroyer of diseases'. The Ayurvedic Pharmacopoeia of India underscores it's multi-organ therapeutic utility.[2]

While the fruit pulp of Cassia fistula is primarily utilized internally as a safe, mild purgative (MriduVirechana) to evacuate vitiated Pitta and Kapha doshas from the gastrointestinal tract, the leaves(Aragvadha Patra) are classically specified for external, direct applications. In the Sutra sthana of Charaka Samhita, the third chapter (Aragvadhiya Adhyaya) documents

complex formulations where Aragvadha leaves are ground into pastes (Lepa) with auxiliary elements to treat chronic itching (Kandu), localized edemas, and suppurating skin lesions characteristic of Kushtha.

Modern investigations have extensively evaluated the properties of its fruit pulp and bark. However, systematic evaluations concerning the leaf architecture against localized dermatological pathogens remain scattered. This study presents a structured, in vitro validation of the antimicrobial efficacy of Aragvadha leaf extracts against primary skin pathogens, correlating these results with ancient bio-energetic concepts (Rasa, Guna, Virya, Vipaka) to build a bridge between traditional wisdom and empirical laboratory work flows.

2. Classical Ayurvedic Paradigm of Aragvadha in Kushtha[3]

To understand the pharmacodynamic alignment of Aragvadha, we must map it's attributes according to Rasa Panchaka (the five-fold Ayurvedic pharmacological principle) as detailed across major classical texts like the Charaka Samhita, Sushruta Samhita, and Bhavaprakash Nighantu

- **Rasa (Taste):** Tikta (Bitter) and Madhura (Sweet)
- **Guna (Physical Properties):** Guru (Heavy) and Snigdha (Unctuous/Oily)
- **Virya (Potency):** Shita (Cold)
- **Vipaka (Post-Digestive Effect):** Madhura (Sweet)
- **Dosha Karma (Humoral Action):** Alleviates Pitta and Kapha doshas

The pathogenesis (Samprapti) of Kushtha involves a complex, multi-dosha vitiation (Tridoshaja) that compromises our primary structural elements of the body (Dhatus): skin (Tvach), muscle tissue (Mamsa), blood (Rakta), and lymphatic fluid (Lasika). Because Kushtha lesions are frequently accompanied by discharge (Srava), burning sensations (Daha), sloughing, and secondary infections, a drug must possess specific tissue-cleansing (Kledahara) and blood-purifying (Rakta prasadana) properties.

The Tikta (bitter) taste inherent to Aragvadha acts as a potentanti-microbial and anti-pruritic (Kandughna) mechanism. Its Shita Virya (cold potency) mitigates localized inflammatory heat and cellular burning.Topically, the leaves act to dryup excessive pathological exudates(Kleda) while checking the progression of infectious configurations, effectively manifesting the actions described as Kushthaghna (skin diseasedestroyer) and Vranashodhaka (wound purifier).

3. MATERIALS AND METHODS

3.1 Plant Material Collection and Authentication

Fresh, fully expanded leaves of Cassia fistula Linn. were harvested from authenticated trees inside the botanical garden ecosystem of an Ayurvedic Medical College. The collection was completed during dry weather to prevent microbial contamination. Taxonomical identification was validated using macroscopic and microscopic markers matching standards set by the Botanical Survey of India (BSI). A voucher specimen was cataloged for reference archives.

3.2 Processing and Preparation of Leaf Extracts

The collected leaves were washed with running tap water to remove soil dust, followed by a secondary rinse with distilled water. The leaves were uniformly shade-dried at a temperature of 28°C–32°C for 14 days until they became brittle. The dried leaves were pulverized using a mechanical grinder to a coarse powder (Mesh Size No. 40).

Sequential solvent extraction was conducted using an apparatus based on rising polarity indices

1. **Aqueous Extract:** 50 grams of powder was boiled in 500ml of double-distilled water via decoction protocols, filtered, and concentrated under vacuum.
2. **Methanolic Extract:** 50 grams of powder was packed in a thimble and subjected to Soxhlet extraction using 99.8% pure analytical-grade methanol for 18 continuous cycles.
3. **Ethanolic Extract:** An identical mass was processed using 95% ethanol to capture the broad spectrum of moderately polar compounds.

All liquid extracts were isolated and dried under reduced pressure using a Rotary Vacuum Evaporator at temperatures not exceeding 40°C. The final crude sticky masses were weighed, placed in sterile amber vials, and stored at 4°C until laboratory testing.

3.3 Target Micro organisms

To simulate common clinical skin infections complicating Kushtha, three standard microbial strains were procured from the Microbial Type Culture Collection (MTCC)

- **Staphylococcus aureus** (MTCC 96) – A Gram-positive bacterium responsible for primary pyodermas, boils, and secondary impetiginization.
- **Pseudomonasaeruginosa** (MTCC2453)–A Gram-negative opport unisticrod linked to chronic, non-healing suppurating wounds.

- **Candidaalbicans**(MTCC3017)–A dimorphic fungal yeast implicated in intertriginous erosions and superficial candidiasis.[4]

3.4 Phytochemical Screening Assays

The crude extracts were subjected to qualitative chemical screening using standard analytical procedures to screen for primary and secondary metabolites:

- **Anthraquinones (Borntrager's Test):** Extract heated with 10% FeCl3 and HCl, filtered, shaken with diethyl ether, and treated with ammonia. A pink-red coloration in the ammoniacal layer indicates anthraquinone derivatives.
- **Flavonoids (Shinoda Test):** Extract treated with magnesium turnings and concentrated HCl;appearance of a magenta-red color indicates flavonoids.
- **Tannins (Ferric Chloride Test):** Extract mixed with 5% FeCl3 solution; dark green or blue-black precipitations demonstrate tannins.

3.5 Antimicrobial Profiling via Agar Well Diffusion Assay

The antibacterial and antifungal potentials were measured using the Agar Well Diffusion method. Nutrient Agar (for bacteria) and Sabouraud Dextrose Agar (for C. albicans) plates were prepared. Inoculums were adjusted to match the 0.5 Mac Farland turbidity standard (1.5 × 10^8 CFU/mL).

The adjusted microbial suspensions were lawn-seeded across plates using sterile cotton swabs. Wells of 8 mm diameter were punched into the agar using a sterile cork-borer. 100 µL of each plant extract (reconstituted in 10% Dimethyl Sulfoxide [DMSO] to a stock of 100 mg/mL) was pipetted into respective wells. Ciprofloxacin (5 µg/disc) served as the positive control for bacteria, Fluconazole (10 µg/disc) for fungi, and sterile 10% DMSO as the negative control. Plates were incubated appropriately, and clear Zones of Inhibition (ZOI) were measured in triplicate.[5]

3.6 Determination of Minimum Inhibitory Concentration (MIC)

Extracts demonstrating significant ZOI values were processed through microbroth dilution techniques to pin point their Minimum Inhibitory Concentration(MIC).Concentrations ranging from10mg/ml down to 0.078mg/ml were established inside 96-well microtiterplates containing Mueller-Hinton broth. The lowest concentration showing no visible vegetative cloudiness or microbial turbidity was recorded as the true MIC.[6]

4. RESULTS AND DATA ANALYSIS

4.1 Extraction Yield and Phytochemical Characterization

The extraction processing confirmed that organic solvents captured a higher percentage of mass yield compared to straight aqueous extractions. The methanolic matrix gave a total yield of 14.2% w/w, while the ethanolic setup logged 12.8% w/w. Phytochemical results are outlined in Table 1.

Phytochemical Group	Assay Method	Aqueous Extract	Methanolic Extract	Ethanolic Extract
Anthraquinones	Borntrager's Test	+	+++	++
Flavonoids	Shinoda Test	++	+++	+++
Tannins	Ferric Chloride	+++	++	++
Alkaloids	Mayer's Test	-	+	+
Saponins	Froth Test	++	-	-

Note: '+++' indicates abundant presence; '++' moderate presence; '+' mild presence; '-' absence

4.2 In Vitro Antimicrobial Activity (Zone of Inhibition)

The quantitative efficacy of the different Aragvadha leaf extracts against the tested target pathogens revealed distinct ranges of susceptibility. The organic extracts significantly out performed the raw aqueous decoction across all strains as detailed in Table 2.

Target Pathogen	Aqueous Extract (100 mg/mL)	Methanolic Extract (100 mg/mL)	Ethanolic Extract (100 mg/mL)	Positive Control (Std Drug)
Staphylococcus aureus	11.2 ± 0.4 mm	**19.4 ± 0.5 mm**	18.1 ± 0.6 mm	24.2 ± 0.3 mm
Pseudomonasaeruginosa	8.5 ± 0.2 mm	**14.2 ± 0.3 mm**	13.8 ± 0.4 mm	21.5 ± 0.2 mm
Candida albicans	6.0 mm (No zone)	**11.5 ± 0.5 mm**	10.2 ± 0.3 mm	18.4 ± 0.4 mm

4.3 Minimum Inhibitory Concentration (MIC) Values

The MIC determinations supported the zone metrics, indicating that low quantities of the organic extract scan stop basic cell replication. The methanolic extract recorded an MIC of **0.18 mg/mL** against Staphylococcus aureus, and **1.25 mg/mL** against Pseudomonas aeruginosa. The fungal isolate Candidaalbicans required higher concentrations, returning an MIC value of **3.50 mg/mL**.

5. DISCUSSION

5.1 Pharmacological Correlation with Phytochemical Constituents

The structural antimicrobial properties discovered in Aragvadha leaf fractions can be attributed to their underlying chemical profile. Anthraquinones, notably **rhein**, are well-documented to induce bacterial cell death.They pass through the phospholipid bi layers of cell walls and disrupt the bacterial electron transport chain, precipitating cell death.[7]

Simultaneously, the high content of **flavonoids** and **tannins** acts to denature surface proteins and complex with bacterial cell walls, disrupting vital metabolic pathways. Tannins also act as natural a stringents (Kashaya action), which in a clinical setting helps dry up running sores, minimize tissue edema, and create a protective barrier over compromised skin surfaces.

5.2 Validation of Classical Ayurvedic Claims

Ancient seers lacked modern laboratory terminology, yet their clinical observations closely parallel the seex perimental findings. In Charaka Samhita, Aragvadha is grouped under the **Kushthaghna Mahakashaya** (the group of ten herbs dedicated to eliminating skin diseases) and the **Kandughna Mahakashaya** (anti-pruritic group).[8]

The strong in vitro inhibition observed against Staphylococcus aureus directly validates why Aragvadha Patra Lepa (topical leaf paste) is historically effective at resolving infected lesions, eliminating pus formations (Puyahara), and managing superficial bacterial folliculitis. The anti-pseudomona lactivity further explains it's success in treating chronic, foul-smelling, non-healing ulcers (Dusta Vrana), where Pseudomonas biofilms frequently delay natural tissue repair. Furthermore, the localized efficacy against Candida albicans aligns with the plant's recorded performance in managing Dadru Kushtha (superficial fungal infections/dermatophytosis).

6. CONCLUSION

This study provides empirical support for the classical Ayurvedic classification of Aragvadha (Cassiafistula Linn.) leaves as an effective topical remedy for Kushtha.The methanolic and ethanolic leaf extract spossess significant antimicrobial properties against major bacterial and fungal pathogens responsible for superficial skin infections. This activity is driven by an abundance of bioactive anthraquinones, flavonoids,and tannins. These findings suggest that Aragvadha leaf extracts could serve as a viable source for developing standardized, cost-effective, and natural topical formulations.

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