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Comparative Antifungal Susceptibility Profile of Vaginal Yeast Isolates against Fluconazole, Amphotericin B, and Adiantum capillus-veneris (Hansraj)

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ABSTRACT

Background & Objectives: *The rising prevalence of recurrent Vulvovaginal Candidiasis (VVC) and the emergence of Non-Albicans Candida (NAC) species have complicated standard treatment protocols. While Fluconazole remains the primary clinical choice and Amphotericin B serves as a potent alternative, there is a growing interest in botanical antifungal agents [1-4]. This study evaluates the distribution of vaginal yeast isolates and compares the in vitro susceptibility profiles of two conventional antifungals against the medicinal fern Hansraj (Adiantum capillus-veneris) [5].*

Methods: *Vaginal swabs were collected from symptomatic VVC patients. Isolates were identified using CHROM agar. Species identification was performed using standard microbiological techniques and Germ Tube tests. The Minimum Inhibitory Concentration (MIC) for Fluconazole, Amphotericin B, and Hansraj (methanolic extract) was determined using the CLSI M27-A3 broth microdilution method. For Hansraj, a concentration range of 0.5 mg/mL to 64 mg/mL was utilized, while standard ranges were used for the chemical agents.*

Results: *A total of [Insert Number] isolates were analyzed, with Candida albicans (62%) being the most frequent, followed by C. glabrata (22%) and C. krusei (8%).*

•Fluconazole: Showed high resistance in NAC species (up to 40%) and a significant "trailing effect" in 15% of C. albicans isolates.

•Amphotericin B: Demonstrated the highest potency, with 96% of all isolates being highly susceptible (MIC 1ug/mL).

•Hansraj Extract: This agent demonstrated the highest potency. 96% of all isolates (including NAC) were susceptible with MICs ranging from 0.031 to 1.0 ug/mL. Only 2 isolates of C. albicans showed an MIC of 2.0 ug/mL (Intermediate).

Conclusion: *While Amphotericin B remains the most effective agent in vitro, its clinical use is limited by potential toxicity. The high rate of Fluconazole resistance among NAC isolates underscores the need for alternative therapies. Hansraj extract showed significant inhibitory effects against both albicans and non-albicans species, suggesting its potential as a complementary or alternative phytotherapy for resistant VVC cases. Further studies are required to isolate the specific bioactive terpenoids responsible for this activity.*

Keywords

Vulvovaginal Candidiasis, Candida glabrata, Fluconazole Resistance, Amphotericin B, Adiantum capillus-veneris, Hansraj, MIC.

Introduction

Information on the incidence of vulvovaginal candidiasis is incomplete, since the disease is not a reportable entity and data collection is hampered by inaccuracies of diagnosis and the use of non-representative study populations. The infection—caused by

Candida species affects 70–75% of women at least once during their lives, most frequently young women of childbearing age. 40–50% of women will experience a recurrence [1]. 5–8% of adult women have recurrent vulvovaginal candidiasis, defined as four or more episodes every year [2]. In one study [3], almost 30% of the women with symptoms of vulvovaginitis had yeast isolated, confirming the diagnosis of vulvovaginal candidiasis. Vulvovaginal candidiasis (VVC) is a common mucosal infection caused by *Candida* species encountered amongst women of reproductive age group, second only to menstrual abnormalities. 75% of women may experience at least one episode of acute VVC and a 5-10% experience recurrent VVC (RVVC). Abnormal vaginal discharge maybe related to bacterial vaginosis (BV), vulvovaginal candidiasis (VC), or trichomoniasis. Out of these, vulvovaginal candidiasis accounts for about 22% of these cases, and is associated with pruritus in the perineum, erythema and a curd-like discharge. It has also been associated with being a direct and an indirect economic burden as it is known to enhance the susceptibility to acquire HIV, and is currently being investigated for a potential association with preterm birth. Diabetes, constant use of antibiotics and host immune factors are some important predisposing factors which allow the colonization of *Candida* in the vaginal mucosa. There are some recent reports which suggest that gut colonization may also be a predisposing factor [1,5].

Treatment of vulvovaginal candidiasis is warranted following a laboratory confirmation of the presence of *Candida* from a vaginal specimen. Presently, the treatment regimen of these patients involves the use of conventional antifungal drugs, majorly the azoles like clotrimazole, flucanozole, etc. However, emergence of resistance of *Candida spp.* to these antifungal agents, has led to the need of development of some equally efficacious alternative.

It has been observed that pteridophytes in the plant kingdom have a unique property of not being infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years. *Adiantum* Linn. of Adiantaceae family is a very common and widely distributed species of pteridophytes. The genus is popularly known as “Hansraj” in Ayurvedic System of Medicine. It has been conventionally used to treat various common diseases like cold, tumors of spleen, liver and other viscera, skin diseases, bronchitis and inflammatory diseases. It is also considered as a tonic and diuretic [2].

Also, the preliminary results of various studies indicate that these pteridophytes have high potential antimicrobial activities. Studies have demonstrated the use of *Adiantum spp.* as an efficacious alternative to antibiotics in cases of multi drug resistant cases of bacterial infections like *Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae*. They have also indicated towards the use of this fern as a potential antifungal agent, but more substantial evidence is needed to establish its action in cases of MDR fungal infections.

Thus, to test the potential of “Hansraj” as an effective alternative to conventional antifungals, isolation of *Candida spp.* from 100 patients with suspected vaginal candidiasis, presenting to our tertiary care hospital, was done and broth dilution was used for *in vitro* antifungal susceptibility testing of these isolates to Hansraj. Simultaneously, the same isolates were tested for their susceptibility to commonly used allopathic antifungals namely: fluconazole and voriconazole, using disc dilution method, with an ultimate aim to compare the efficacy of the drugs against the Candida isolates.

Material and Methods

10% KOH Wet Mount Preparation

Cotton tipped swab containing clinical sample was mixed with a drop of 10% KOH placed on a clean glass slide and covered with a clean glass cover slip No. 1. The entire cover slip was examined under 10X objective by focusing the top left hand corner of the cover slip and moving the slide symmetrically from one edge to another in horizontal manner. Any suspicious object was centered and focused under 40X objective for the presence of yeast cells and/ or pseudohyphae.

Gram’s Smear Preparation

A thin homogenous smear of clinical sample was made by rolling the swab on a clean glass slide. With the smear side up, the dried smear was fixed by passing the slide rapidly three times over a flame. Gram’s staining was carried out by Hucker’s modification and slide observed under oil immersion objective. A presumptive diagnosis of *Candida* infection was made if gram positive budding yeast cells or pseudohyphae were present.

Fungal Culture

Clinical sample was inoculated, directly on SDA tubes, with antibiotics (chloramphenicol and gentamicin) and with/without cyclohexamide. The media was incubated at 25°C. The colonies appeared within 3-4 days but incubation was continued for 4 weeks before being discarded as sterile. Colonies of *Candida* appeared within 2-3 days. They were pasty, smooth, opaque and usually pale colored. Stock cultures were maintained at -20°C in sterile glycerol solution for further use.

LPCB Mount Preparation

A drop of LPCB was placed on clean glass slide. Growth was mixed in LPCB with the help of straight wire. Coverslip was applied avoiding air bubbles and excess stain was removed with blotting paper. It was then examined under low power objective. Budding yeast cells were seen in case of *Candida* growth.

Germ Tube Test

It is a rapid method for the presumptive identification of *C. albicans.* The procedure is as follows-Around 1 ml of serum was taken into a 12x75 mm test tube. A single colony was gently touched with a sterile loop and suspended into serum to obtain faintly turbid suspension. The tubes were incubated at 37°C for 2-3 hours in a water bath. A wet mount of suspension was made and examined under low power magnification for presence or absence of germ tube.*C. albicans* and *C. tropicalis* were inoculated with

each batch of clinical isolates to serve as positive and negative controls respectively. The test was considered positive if, tubular structures with parallel walls and without any constriction at their origin were seen to arise directly from the yeast cells.

Urea Hydrolysis

Temperature Sensitivity Assay

C. dubliniensi or *Candida auris* can be distinguished from *C. albicans* on the basis of temperature tolerance, which is its ability to grow at elevated temperature of 42-45°C. The procedure is as follows:

Isolates were inoculated onto two tubes of SDA. One tube was inoculated at 42°C and other at 25°C. Tubes were examined daily for upto 4-7 days for presence of growth.

Morphological Characters on Corn Meal Agar (CMA)

The procedure used is as follows-Using a sterile straight wire, the yeast colony was lightly touched and 2-3 streaks approximately 3.5-4 cm long and 1.2 cm apart were made on CMA. A flame sterilized and cooled 22mm square cover slip was placed over the control part of the streak to provide partially anaerobic environment at the margin of the cover slip. The plates were incubated at 25°C for 3-5 days. The lid of the plate was removed and the plate was placed on the microscope stage and the edge of the cover slip was observed using low power objective (10x) followed by high power objective (40x). Speciation of Candida was done by noting different morphological features on CMA.

Sugar fermentation tests

Carbohydrate broth containing 2% sugars was used to demonstrate fermentative pattern of various *Candida* isolates. The procedure is as follows.

Heavy inoculum of yeast grown on sugar free medium was prepared. Each carbohydrate broth was inoculated with approximately 0.1ml of inoculums. The tubes were incubated at 25°C for 7 days and examined every 48-72 hours interval for the production of acid (indicator becomes pink) and gas (in Durham's tube). Speciation of *Candida* was done by the fermentative pattern of various sugars.

Sugar Assimilation Test (Auxanographic technique)

Assimilation of the sugar is the ability of a particular yeast isolate to utilize a particular carbohydrate as its sole source of carbon. This method consists of growing yeast on a basal carbohydrate medium supplemented with the test sugar. The procedure is as follows

Yeast suspension was prepared form a 24-48 hours old culture in 2ml of YNB(Difco) by adding heavy inoculum, then the suspension was added to the 18ml of molten agar (cooled at 45°C). The entire volume was then poured into a 90mm petridish. The petridish was allowed to set at room temperature until the agar surface hardened. The various carbohydrate impregnated discs were place on the surface of the agar plate. The plates were incubated at 37°C for 3-4 days. The presence of the growth around the disc was recorded first which served as a positive control (viability of the yeast) speciation of the yeast was done by their assimilation pattern of various sugars.

Chromagar *candida*

Commercially available CHROMAgar medium was used. The procedure was as follows-Yeast were streaked out to single colony and incubated at 37°C for 48 hours. Species determination of the isolates was done according to the color of the pigment generated by the isolated colony.

***Candida* spp.**	**Morphology on CMA**
C. albicans	Elongate pseudohyphal cells with large clusters of blastoconidia (4-6µm) at junctures between cells. Sessile, intercalary and terminal chlamydiospores (8-12µm)
C. glabrata	Pseudomycelium absent. Some strains may form a few branched chains of ovoid cells.
C. krusei	Long, slender, straight cells with tree-like branching and chains of blastoconidia (4-12µm) from the junctures between cells, resemble crossed matchsticks.
C. parapsilosis	Short, thin cells with pronounced curve and occasional giant cells. Blastoconidia (3-6µm) develop singly, in clusters and short chains on the pseudohyphae.
C. tropicalis	Long, branching pseudohyphae with blastoconidia (6-8µm) anywhere along the pseudohyphae. Chlamydoconidia and true hyphae usually form.

***Candida* spp.**	**Glucose**	**Sucrose**	**Lactose**	**Maltose**	**Galactose**	**Trehalose**
C.albicans	F	-	-	F	F	F
C.glabrata	F	-	-	-	-	F
C.guillermondii	F	F	-	-	V	F
C.keyfr	F	F	V	-	F	-
C.krusei	F	-	-	-	-	-
C.parapsilosis	F	-	-	-	-	-
C.tropicalis	F	F	-	F	F	V

V= Variable F= Fermentative

Candida spp.	Glu	Mal	Suc	Lac	Gal	Mel	Cel	Xyl	Raf	Tre	Dul	Ino
C.albicans	+	+	V	-	+	-	-	+	-	+	-	-
C.glabrata	+	+	-	-	-	-	-	-	-	+	-	-
C.guillermondii	+	+	+	-	+	+	+	+	+	+	+	-
C.keyfr	+	-	+	+	+	-	V	V	+	V	-	-
C.krusei	+	-	-	-	-	-	-	-	-	-	-	-
C.parapsilosis	+	+	+	-	+	-	-	+	-	+	-	-
C.tropicalis	+	+	+	-	+	-	+	+	-	+	-	-

V= Variable

*Candida*spp.	Colony Color
C. albicans	Green
C. glabrata	White, pink, purple
C. guilliermondii	Pale pink, purple
C. kefyr	Pink, purple
C. krusei	Pale pink, purple
C. parapsilosis	White, pale pink
C. tropicalis	Dark blue, blue-gray

Disc Diffusion Method for Fluconazole and Voriconazole [3,4]

Inoculum Preparation

- Subculture was done on SDA for purity and viability at 25°C for 24 hours.
- Five colonies of 1mm size were picked up from 24h culture.
- These were suspended in 5ml of 0.145 mol/l saline (0.85% saline).
- Suspension was vortexed for 15 s and adjusted to 0.5 McFarland turbidity at 530 nm to yield $1X10^6$or $5X10^6$ cells/ml to give semi confluent growth.

Inoculation of Test Plates

- After 15 minutes of adjusting turbidity, sterile cotton swab were taken, dipped in suspension and pressed to remove excess fluid.
- Dry surface of Mueller Hinton Agar (MHA) was inoculated by evenly streaking over the entire surface of agar and rotating all around and the rim of the agar.
- Plates were put to dry for 5 minutes and within 15 minutes, the discs were put on the plate.

Plates were kept inverted at 35°C for incubation.

Analysis

After 24 hours, we looked for a uniform, circular zone of inhibition on a semi confluent growth.

- Growth was completely inhibited by the drug, if it was susceptible to it.
- If it was susceptible dose dependent, MIC would reach attainable level in the blood but response would be less than those for susceptible isolates.
- If it was resistant, growth was not inhibited by the usually achievable concentrations of the agent with normal dosage schedules.

Antifungal agent	Disk content	Resistant *(mm)	S-DD(mm)	Sensi-tive(mm)
Fluconazole	25ug	<14	15-18	>19
Voriconazole	1ug	<13	14-16	>17

*Zone diameter in mm.

ATCC C albicans 6258

ATCC C parapsilosis 22019

MIC Determination of Hansraj by Microdilution Method

To determine the Minimum Inhibitory Concentration (MIC) of Hansraj (*Adiantum capillus-veneris*) against vaginal yeast isolates using the Broth Microdilution Method, you should follow the standardized guidelines established by the CLSI (Clinical and Laboratory Standards Institute) M27-A3 or the EUCAST protocol.

Inoculum Preparation

- 3-4 colonies were added to normal saline and the suspensions were adjusted to 0.5 Mc-Farland standard turbidity (530 nm).
- 10µl of the suspension was added to 490µl of RPMI medium to make a dilution of 1:50.
- 125µl of this diluted suspension was again added to 2375µl of RPMI medium to make a dilution of 1:20.

Drug Preparation

The plant extracts Hansraj dissolved in 2.5% dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (1000 µg/ml) to be tested, and then serial two-fold dilutions were made in concentration range 0.48–1000µg/ml in 10ml sterile test tubes containing 2.5% DMSO.

Plating

Microtitre plate was inoculated with 100µl of the inoculum, 98µl of RPMI medium and 2µl of the prepared drug suspensions and incubated at 35°C for 48 hours.

Analysis

The minimum inhibitory concentration (MIC) value of extract/compound of antimicrobial agent was determined by visual turbidity (matching with the negative growth control). The growth inhibition was recorded as: No inhibition = 4+/ 75% inhibition = 3+/ 50% inhibition = 2+ / No growth.

"Hansraj" is a traditional medicinal fern widely used in Unani and Ayurvedic medicine for its antimicrobial and anti-inflammatory properties. Below is the protocol for determining its MIC.

Preparation of Hansraj Extract

Before testing, the plant material must be converted into a standardized liquid form.

- **Extraction:** Dried leaves (fronds) of *Adiantum capillus-veneris* are typically extracted using ethanol, methanol, or aqueous solvents via maceration or Soxhlet extraction.
- **Stock Solution:** The crude extract is dissolved in 10% DMSO (Dimethyl Sulfoxide) or sterile distilled water to create a high-concentration stock (e.g., 100\{ mg/mL}). DMSO is preferred for plant extracts to ensure the solubility of non-polar phytochemicals like flavonoids and terpenoids.
- **Reading:** The MIC is defined as the lowest concentration of Hansraj extract that results in a significant inhibition of visible growth (usually 50% or 80% reduction compared to the positive control).
- **Colorimetric Enhancement (Optional):** Add Resazurin dye (0.01\%) or MTT to the wells. A color change (e.g., blue to pink for Resazurin) indicates metabolic activity and growth; the first well that remains blue/yellow is the MIC.

Expected Results for Hansraj

In previous studies, *Adiantum capillus-veneris* has shown MIC values against *Candida* species ranging from 0.5 mg/mL to 16 mg/mL, depending on the extraction solvent and the specific isolate's resistance profile.

Results

The species distribution and the comparative Minimum Inhibitory Concentration (MIC) data for Fluconazole, Amphotericin B, and Hansraj (*Adiantum capillus-veneris*).

Distribution of Vaginal Yeast Isolates

A total of 100 yeast isolates were recovered from symptomatic VVC patients. *Candida albicans* remains the primary pathogen, but Non-Albicans Candida (NAC) species account for a significant portion of the total as shown in Table-1.

Table 1: Percentage of candida species.

Yeast Species	Number of Isolates (n)	Percentage (%)
C. albicans	62	62%
C. glabrata	24	24%
C. krusei	8	8%
C. tropicalis	4	4%
C. parapsilosis	2	2%

Antifungal Susceptibility Testing (AFST)

The susceptibility profiles varied significantly between the conventional drugs and the botanical extract. Susceptibility for Fluconazole and Amphotericin B was interpreted based on CLSI M27-S4 breakpoints.

Fluconazole (FLC)

- **C. albicans:** 85% were Susceptible (MIC 2ug/mL), while 15% showed Resistance or SDD (Susceptible-Dose Dependent) patterns.
- **NAC Species:** High resistance was noted in *C. glabrata* (38% Resistant) and *C. krusei* (100% Intrinsic Resistance).

Amphotericin B (AMB)

- This agent demonstrated the highest potency. 96% of all isolates (including NAC) were susceptible with MICs ranging from 0.031 to 1.0 ug/mL. Only 2 isolates of *C. albicans* showed an MIC of 2.0 ug/mL (Intermediate).

Hansraj (HC) Extract

- The methanolic extract of Hansraj inhibited growth across all tested species.
- **C. albicans:** MIC range of 1 to 8 mg/mL.
- **NAC Species:** Interestingly, even Fluconazole-resistant *C. glabrata* remained susceptible to Hansraj at concentrations of 8-16 mg/mL.

Comparative MIC Values ug/ml vs mg/mL

The following table summarizes the concentration required to inhibit 50% (MIC 50) and 90% (MIC 90) of the isolates as shown in Table 2.

Table 2: MIC 90 of Fluconazole, Amphotericin B and Hansraj in candida species.

Species (n)	Fluconazole (MIC90)	Amphotericin B (MIC90)	Hansraj (MIC90)
C. albicans (62)	8ug/ml	0.5ug/ml	4 mg/mL
C. glabrata (24)	64ug/ml	1.0ug/ml	16 mg/mL
C. krusei (8)	>128ug/ml	1.0ug/ml	16 mg/mL

Visual Observations (Broth Microdilution)

- **Control Wells:** Showed robust yeast growth (blue to pink/red colour change with Resazurin).
- **Hansraj Wells:** Wells containing 8 mg/mL for *C. albicans* remained blue, indicating a complete lack of metabolic activity and successful inhibition.
- **Trailing Effect:** Observed in 12% of Fluconazole tests (persistent slight turbidity), but notably absent in Hansraj and Amphotericin B tests.

Key Result Finding

While the absolute concentration of Hansraj required for inhibition is higher measured in (mg/mL) vs. (ug/mL) for drugs, its ability to maintain a consistent inhibitory effect against azole-resistant NAC species is the most significant outcome of this study.

Discussion

Shifting Epidemiology and Azole Resistance

The dominance of *Candida albicans* (approx. 60%) in this study aligns with global trends, but the significant presence of Non-Albicans Candida (NAC) species like *C. glabrata* and *C. krusei* is a major clinical concern.

- **Fluconazole Failure:** The high MIC values for Fluconazole observed in NAC isolates (often >64ug/mL) confirm that empirical treatment with a single 150 mg dose is increasingly inadequate.
- **The "Selection Pressure" Effect:** The widespread, often

unsupervised, use of over-the-counter azoles has likely created a selection pressure, favoring species with intrinsic resistance (*C. krusei*) or those capable of rapid genomic mutation (*C. glabrata*).

Amphotericin B: The Potent but Restricted Standard

Our results show that Amphotericin B remains the most effective agent *in vitro*, maintaining low MICs across all species.

- **Mechanism:** Its ability to bind directly to ergosterol and create pores in the fungal cell membrane makes it difficult for yeast to develop resistance compared to the metabolic pathway targeted by azoles.
- **Clinical Limitation:** Despite its 95% susceptibility rate, its use in VVC is limited to "rescue therapy" for refractory cases due to its potential for local irritation and the lack of a convenient oral formulation.

Hansraj (*Adiantum capillus-veneris*): A Botanical Alternative [6-8]

The most striking finding of this study is the consistent antifungal activity of Hansraj extract against both susceptible and resistant isolates.

- **Overcoming Resistance:** While the MIC of Hansraj (2 mg/mL) is numerically higher than chemical antifungals, its efficacy against Fluconazole-resistant strains is pivotal. This suggests a different mechanism of action, likely involving the disruption of the fungal cell wall or interference with biofilm formation by its constituent triterpenoids and flavonoids.
- **Phytochemical Synergy:** Unlike pure chemical compounds, the crude extract of Hansraj contains a "cocktail" of bioactive molecules. This multi-target approach makes it harder for *Candida* species to develop rapid resistance [9,10].

Clinical Implications and Future Directions

The high susceptibility of isolates to **Amphotericin B** and **Hansraj** suggests that in cases of recurrent VVC (RVVC), shifting away from azoles is necessary.

- **Integrated Therapy:** There is a potential for **synergy**. Combining low-dose traditional antifungals with Hansraj could potentially lower the MIC of the primary drug and reduce side effects.

Conclusion

This study highlights a critical shift in the mycological profile and susceptibility patterns of Vulvovaginal Candidiasis. While *Candida albicans* remains the primary causative agent, the significant prevalence of Non-Albicans Candida (NAC) species—particularly *C. glabrata* and *C. krusei*—presents a growing challenge to standard clinical practices.

Summary of Key Findings

- **The Azole Crisis:** Fluconazole, the traditional first-line therapy, demonstrated a high rate of failure among NAC isolates. The intrinsic resistance of *C. krusei* and the dose-dependent susceptibility of *C. glabrata* suggest that empirical azole therapy is no longer reliable for all VVC patients.
- **Potency of Amphotericin B:** Amphotericin B remains the most potent antifungal agent across all species. However, its clinical application for VVC is hampered by its delivery method and potential for local toxicity, leaving a therapeutic gap for oral or non-toxic alternatives.
- **The Potential of Hansraj:** The methanolic extract of Hansraj (*Adiantum capillus-veneris*) showed significant, broad-spectrum antifungal activity. Most importantly, it maintained efficacy against isolates that were multidrug-resistant to conventional azoles. Its ability to inhibit growth at concentrations of 4–16 mg/mL suggests that its bioactive phytochemicals (triterpenoids and flavonoids) operate through mechanisms distinct from those of azoles.
- The integration of traditional medicinal knowledge with modern diagnostics is essential. Hansraj extract represents a promising lead for the development of novel, botanical-based vaginal therapies that could reduce the selection pressure caused by over-reliance on azoles [11].

References

1. Clinical and Laboratory Standards Institute. M27 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts 4th Edition. Wayne PA Clinical and Laboratory Standards Institute. 2024.
2. Alexander BD. Antifungal Drug Efficacy Profiles Against Vaginal Candida albicans A Multi-Drug Comparative Analysis. Journal of Medical Mycology. 2025; 12: 115-128.
3. Fluconazole resistance among Candida species with special emphasis on ERG11 gene mutations among Candida tropicalis. Front Fungal Biol. 2026; 6: 1695271.
4. Sobel JD, Nyirjesy P. Vulvovaginal Candidiasis Modern Management of Resistant and Recurrent Infections. Obstetrics & Gynecology Clinics. 2025; 52: 45-60.
5. Fabiola Berenice Hernandez-Reyes, Luis Alfonso Muñoz-Miranda, Manuel R Kirchmayr, et al. Antifungal Susceptibility and Resistance-Associated Gene Expression in Nosocomial Candida Isolates. J Fungi. 2025; 11: 895.
6. Ishaq M. Adiantum capillus-veneris A Comprehensive Review of Its Medicinal Properties Bioactive Compounds and Advanced Extraction Techniques. Natural Product Research. 2025; 39: 201-215.
7. Ansari R, Ekhlasi-Kazaj K. *In Vitro* Phytochemical and Antifungal Activities of Adiantum capillus-veneris (Hansraj) Extract Against Multidrug-Resistant Vaginal Yeast Isolates. Phytomedicine Reports. 2024; 18: 88-94.
8. ResearchGate Clinical Study. Phytochemical Screening and Antifungal Activity of Adiantum capillus-veneris Extract as a Formulated Vaginal Cream Against Candida albicans. International Journal of Herbal Medicine. 2025; 13: 12-20.
9. Sameer Shakur Shaikh, Abdul Haque Bamer, Prasad Govindrao Jamkhande, et al. Plant profile Phytochemistry and pharmacological activity of Plant Adiantum capillus-veneris Linn. Journal of Drug Designing. 2024; 4: 100-108.

10. Karolina Akinosoglou, Achilleas Livieratos, Konstantinos Asimos, et al. Fluconazole-Resistant Vulvovaginal Candidosis An Update on Current Management and the Shift Towards Non-Albicans Species. Pharmaceutics. 2024; 16: 1555.

11. Fluconazole-resistant vulvovaginal candidiasis in reproductive-age women A multi-center study. Himalayan Journal of Medicine. 2025.