Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1510-1517 | ISSN:2251-6727

A Study on Clinical and In-Vitro Evaluation of Triphaladi Kashaya in the Management of Kaphaja Yonisrava Vis-A-Vis Vulvovaginal Candidiasis

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(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 24 July 2025)

KEYWORDS

Yonisrava,*Yoni prakshalana*, Vulvovaginal candidiasis, Triphaladi Kashaya, Culture, Sensitivity.

ABSTRACT:

Introduction: *Yonisrava* is a common gynaec complaint in reproductive age group of females. Clinical features of inflammatory discharges present in vaginal infections caused by Bacteria, Fungi and protozoa are similar to *Yonisrava* present in *Doshaja Yonivyapath* mentioned in Ayurvedic literature. The most common lesion causing vaginal discharge is Vulvovaginal Candidiasis. Although *Acharya Charaka* outlines *Chikitsa Sutras* for *Vimshati Yonivyapath*, he particularly emphasizes the treatment for *Yonisrava.* In addressing *Yonisrava*, the traditional Ayurvedic remedy of *Tripahala Kashaya, Gomutra*, and *Haridra* is chosen for *Yoni prakshalana,* offering an alternative perspective to conventional treatments. This article aims at exploring the efficacy of *Triphaladi Kashaya* clinically through *Yoni prakshalana* and also in-vitro on the organisms grown on culture.

Objectives: To identify the microorganism present in *kaphaja yonisrava* and to evaluate the antimicrobial activity of hydroalcoholic extracts of *Triphala* and *Haridra* and to evaluate the efficacy of *Triphaladi Kashaya yoni prakshalana* in the same.

Materials & methods: A 43-year-old female patient complaining of white curdy vaginal discharge for the past 6 months associated with vaginal itching was treated clinically with *Triphaladi Kashaya yoni prakshalana* for 5 days and the ingredients of *Triphaladi Kashaya* were also used to check sensitivity through in-vitro study.

Results: After the completion of treatment protocol, patient got relieved by her symptoms and vaginal swab culture revealed no growth of microorganisms.

Conclusions: *Triphaladi kashaya Yoni prakshalana* has demonstrated significant results in the management of Vulvovaginal candidiasis clinically and the hydroalcoholic extracts of Triphala and Haridra have exhibited in-vitro antifungal activity on Candida albicans.

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JCHR (2025) 15(4), 1510-1517 | ISSN:2251-6727

INTRODUCTION

Women represent the cornerstone of a family's overall health, ensuring they have access to quality care, leads to improvement in health of children as well as the whole family[1]. In different phases of a woman's life from puberty to menopause the concept and importance of a healthy yoni has been described in *Ayurveda* as well as in modern system of medicine. *Yonisrava* is a common feature among *Doshaja yonivyapath* which appears similar to the clinical features of inflammatory discharges present in vaginal infections caused by bacteria, fungi and protozoa. Discharge caused by infections can be mucopurulent or frankly purulent, its color varies from cream to yellow or green. It is often offensive, especially when bacteria are present as primary or secondary invaders[2]. The most common lesions causing a discharge of this kind is Vulvovaginal Candidiasis (VVC) and about 75% women experience Vulvovaginal candidiasis (VVC) at least once in their lifetime[3]. Clinical examination supported by laboratory investigations is the main tool in the diagnosis of vaginitis and vaginal swab culture is used as a tool to identify organisms causing infections. *Acharya Charaka* in *Chikitsa Sthana*, highlights the significance of *Yonisrava* in *Stree*[4]. Although *Acharya Charaka* outlines *Chikitsa Sutra* for all *Vimshati Yonivyapath*, he particularly emphasizes the treatment of *Yonisrava*[5].

Table 1: Symptomatic co-relation of *Kaphaja yonisrava* and Vulvovaginal candidiasis

***Kaphaja yonisrava*[6]**	**Vulvovaginal candidiasis[7]**
Pandu pichila yonisrava	Curdy white discharge
Yonikandu	Itching
Alpa toda	Dull pain occasionally

In addressing *Yonisrava*, the traditional Ayurvedic remedy of *Triphala kashaya, Gomutra arka* and *Haridra* is chosen for *Yoni prakshalana*[8], offering an alternative perspective to conventional treatments. Notably, the Center for Disease Prevention and Control (CDC) recommends Clotrimazole for Vulvovaginal candidiasis[9]. *Acharya Charaka* has outlined two aspects of *Chikitsa* - *Abhyantara* and *Sthanika chikitsa*, referring to specific local treatments, which are beneficial in addressing localized imbalance and strengthening the respective *Sthana*. These therapies, although primarily local, prove advantageous in many *Stree Roga* when executed accurately.

CASE STUDY:

Presenting features: A 43-year-old married woman resident of Mysuru reported to the *Prasuti Tantra* and *Stree Roga* outpatient department (OPD) of JSS Ayurveda Medical College and Hospital, Mysuru, Karnataka with complaints of white, thick, profuse, curdy discharge per vagina which wets her undergarments since 6 months associated with vaginal itching for 15 days.

The lady is having recurrent episodes of white discharge per vagina (WDPV) on & off for 2 years. In the present episode, initially the discharge was watery for about 2-3 days. As days passed by, vaginal discharge turned thick, curdy and profuse. She consulted gynecologist for the same and on taking prescribed medicines, the severity had decreased, but the symptoms are persistent. In the last 6 months, symptoms have increased and also, she is experiencing itching over the genital area.

Personal history: Her insignificant personal history details with a normal appetite with strict vegetarian diet, eats moderate quantities of food, which is often dominated by sweet and spicy items, minimal usage of ghee and milk products. She has the habit of taking hot water bath daily and cleans her private parts occasionally. Her bowel and bladder habits are regular and have no addictions. She gets a sound sleep at night and also sleeps for about 1-2 hours in daytime, regularly.

Menstrual History: She had her menarche in 13th year, and she is having regular menstrual cycle with an interval of 28-30 days and moderate flow of 4-5 days, associated with mild lower abdominal pain.

Obstetric History: $P_1L_1A_0D_0$ Last childbirth 23 years ago.

Local Pelvic examination:

- Examination of external genitalia:

➢ On inspection:

1. Vaginal wall redness – present.
2. Discharge: Thick white discharge seen over the labia.
3. Sign of pruritus vulvae – present.

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4. Curdy white flakes adherent to walls of vagina.
5. Any visible masses – absent.

➢ On Palpation:
- Pelvic tenderness – absent.

- Per Speculum Examination: Cervix normal in position, inflamed, profuse curdy white discharge seen, motion tenderness absent.

- Per Vaginal Examination: Uterus normal in size & position – anteverted, freely mobile and fornices – non tender.

Diagnosis:

Established based on symptoms of *Kaphaja Yonisrava* / Vulvovaginal candidiasis through clinical signs and symptoms and clinical examination.

***Kaphaja yonisrava lakshana*[10]**

- *Pandu, pichila yonisrava*
- *Yoni kandu*
- *Yoni pichilata*
- *Alpa Vedana / Avedana*

Vulvovaginal candidiasis signs[11]

- Inflammation of the vulva, especially the labia minora and introitus, with excoriation.
- The vaginal discharge is thick, white and has the appearance of cottage cheese.
- The discharge often adheres to the vagina in the form of white patches or plaques, and when removed, multiple petechial hemorrhagic areas are left behind.

Vulvovaginal candidiasis symptoms[12]

- Vaginal and vulvar pruritus
- Dyspareunia
- Extreme discomfort from skin irritation in severe infections.

Investigations:

- Vaginal pH – 4-5

- Whiff Test: Negative

- Wet mount showed:

➢ 8 epithelial cells / hpf
➢ 6-7 branching hyphae / hpf
➢ Bacteria – Absent
➢ Clue cells – Absent
- Direct gram staining:
➢ 8 Epithelial cells / hpf
➢ 9-10 branching hyphae with budding yeast / candida present / hpf
➢ Lactobacillus – Absent

- Culture on SDA showed creamy white colonies.
- Culture microscopy reveals: Candida albicans, confirmed by germ tube test.

IN – VITRO SENSITIVITY TESTING:

Hydroalcoholic extract of *Triphala churna* and *Haridra churna* was prepared using cold maceration method. Powdered 100gm of each *Triphala churna* and *Haridra churna* was added to 420ml of methanol and 120ml of distilled water. The conical flask was then plugged tightly with cotton and was sealed with tape. The conical flask was shaken manually for 10-15 minutes at an interval of every 3 hours during the daytime continuously for three days. On the 4th day, the contents of the conical flask were filtered, which yielded 180ml and 150ml of *Triphala* and *Haridra* Hydroalcoholic filtrate respectively. The filtrate was then kept over a water bath in a Petri dish at 100°C for four hours. 26.57gm and 9.14gm of hydroalcoholic extract of *Triphala* and *Haridra* were obtained by this process.

Vaginal sample was collected from the patient using sterile cotton swab. One portion of the sample was used for direct microscopic examination to identify the presence of normal Vaginal epithelial cells, presence of Clue cells, WBCs and the characterization of fungi. Another portion of the sample was used for direct gram staining examination to identify the presence of normal vaginal epithelial cells, WBCs, branching hyphae with budding candida and lactobacillus. The sample was also inoculated on Sabouraud dextrose agar by continuous streaking method, and agar plates were placed in incubator at 27°C and cultured for 48 hours. After 48 hours of incubation, the culture characteristics like colony morphology, size, shape, surface, consistency color, opacity and hemolysis were studied. An isolated colony was smeared on a glass slide, heat fixed and stain

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using Gram staining technique and microscopic observation was done. The results that showed positive cultures for Candida albicans were further examined for sensitivity with *Triphala* and *Haridra*.

The sensitivity test was done using Agar well diffusion method. One isolated colony of Candida albicans from 24 hours culture was transformed into the Mueller Hinton Agar plate (one for each extract) with a sterile cotton swab and swabbed over the media (lawn culture). Four equidistant wells were made on both the plates with a sterile cork borer and different concentrations of hydroalcoholic extracts (50µl,75 µl, &100µl) were added into wells on the two plates. Petri plates were incubated at 37°C for 48 hours. After the incubation period the zone of inhibition was measured in mm with a ruler.

- 50µl hydroalcoholic extract of Triphala Sensitivity against Candida showed 9mm inhibition zone.
- 75µl hydroalcoholic extract of Triphala Sensitivity against Candida showed 13mm inhibition zone.
- 100µl hydroalcoholic extract of Triphala Sensitivity against Candida showed 17mm inhibition zone
- 50µl hydroalcoholic extract of Haridra sensitivity against Candida showed 13mm in the inhibition zone
- 75µl hydroalcoholic extract of Haridra sensitivity against Candida showed 15mm in the inhibition zone
- 100µl hydroalcoholic extract of Haridra sensitivity against Candida showed 17mm in the inhibition zone

***Chikitsa* given:** *Triphaladi Kashaya yoni prakshalana* once daily for 5 days followed by *Yoni Pichu* with *Jatyadi taila* for 5 days.

During this period, the patient was advised to follow strict abstinence.

Procedure:

- ***Purvakarma:*** *Preparation of Prakshalana dravya.*
  - Preparation of *Triphaladi Kashaya* was done as per *kwatha* preparation method[13]
  - Ingredients *Haritaki, Vibhitaki* and *Amalaki*, 133 gm of each drugs were taken in coarse powdered form in clean stainless steel decoction vessel along with 6400ml of clean and soft water.
  - The vessel is placed over mild fire, boiled and reduced to 1/8th part and later filtered.
  - The obtained 800ml filtrate, *Gomutra arka* 200ml from Sri Sri tattva and *Haridra churna* 1 pinch are used as Prakshalana *Dravya*
  - All these three are mixed into a homogenous mixture and added to an enema can.
  - Patient is advised to empty the bladder and made to lie in Dorsal Position.
- **Pradhana Karma:**
  - Under all aseptic precautions, 100ml of *Triphala Kashaya* is poured over the external genitalia.
  - *Triphaladi Kashaya* is taken in the enema can and a rubber catheter of size 16 is attached to the can and introduced into the introitus.
  - *Triphaladi Kashaya* is allowed to flow slowly by gravity into the vaginal canal with the catheter being slowly rolled over, so that the medication reaches all around the vagina.
  - This procedure is continued until all the *dravya* enters the vaginal canal.

Pashchat Karma:

- With the help of gauze vaginal canal and external genitalia are cleaned.
- *Yoni pichu* with *Jatyadi taila* is placed in *Yoni marga* for one hour.

Results: Table showing the comparison of study parameters before and after the intervention

Investigation	Before treatment	After treatment
White discharge per vagina	Profuse	Absent
Whiff Test	Negative	Negative
Vaginal pH	4.5	4.5

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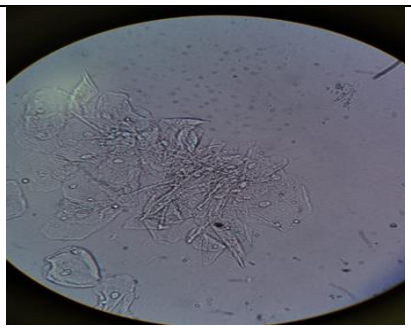
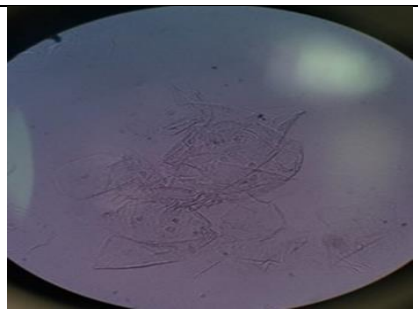
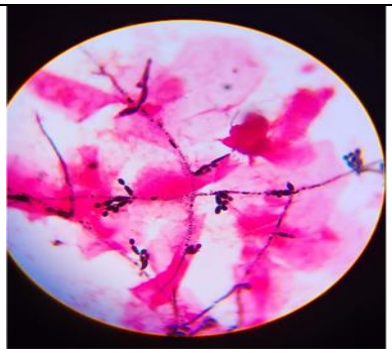
Wet mount	• 8 epithelial cells/hpf • 6-7 Branching hyphae • Bacteria -Absent • Clue cells- Absent	• 7-8 epithelial cells • Hyphae – Absent • Bacteria- Absent • Clue cells- Absent
Direct gram staining	• 8 Epithelial cells/hpf • 9-10 branching hyphae with budding candida -present • Lactobacillus-Absent	• 6 epithelial cells/hpf • branching hyphae with budding candida – Absent • Lactobacillus- present
Growth characteristics of organism on SDA	• Size -1mm • Shape- spherical • Surface- Regular • Colour- Creamy white • Consistency- Sticky	• No growth
Culture microscopy	Gram positive – Candida	-
Local pelvic examination	On Inspection: • Vaginal wall Redness- Present • Discharge- Thick white discharge seen over the labia • Signs of pruritus vulvae-Present • Curdy white flakes adherent to walls of vagina-present	On Inspection: • Vaginal wall redness- Absent • Discharge-absent • No Signs of pruritus vulvae • Curdy white flakes adherent to walls of vagina- Absent

Table 3 – Images of comparison of wet mount, staining, growth and other parameters before and after the intervention

Investigation	Before treatment	After treatment
Wet mount		
	• 8 epithelial cells/hpf • 6-7 Branching hyphae	• 7-8 epithelial cells
Direct gram stain		
	• 8 Epithelial cells/hpf	• 6 epithelial ells/hpf

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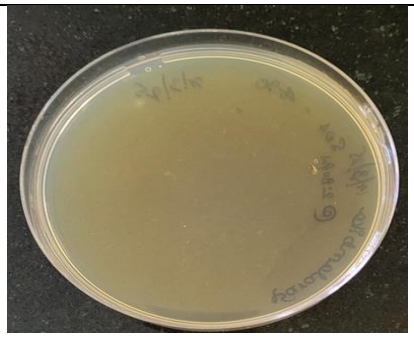
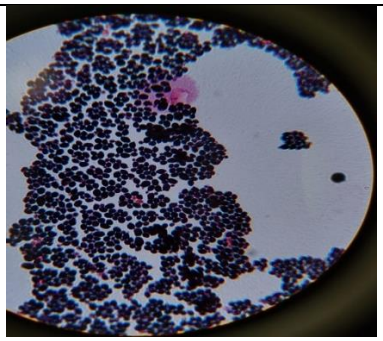
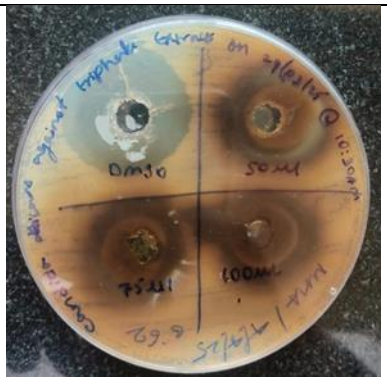
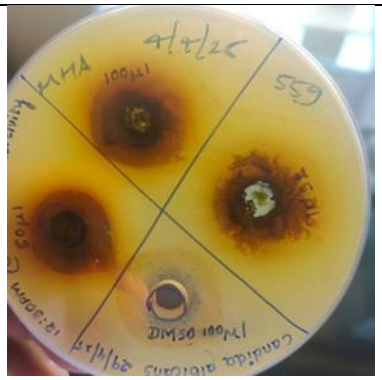
	• 9-10 branching hyphae with budding candida -present	
Culture morphology		
	• Size -1mm • Shape- spherical • Surface- Regular • Colour- Creamy white Consistency- Sticky	No growth
Culture microscopy		
In-vitro sensitivity		
	In-vitro sensitivity of *Triphala*	In-vitro sensitivity of *Haridra*

DISCUSSION: On assessment of *Nidana*, patient has history of indulgence in *Madhura rasa pradhana ahara*, *diwaswapna* which are *kapha prakopaka*, due to which *kapha* attains *sthana samshraya* in *yoni* leading to increased *pichila panduvarna yonisrava* which can be understood as *Kaphaja Yonisrava.*

Clinical assessment revealed significant improvement in the symptoms of *Kaphaja Yonisrava* such as *pandu yonisrava, kandu* and *picchilata*. The symptomatic relief experienced by the patient highlights the potential of *Triphaladi Kashaya* as a safe and effective intervention in VVC too. The mode of action of *Sthanika chikitsa* can be understood as, the vaginal

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epithelium absorbs water soluble / lipophilic active principles, which is provided in the form of kashaya. The drugs may help in restoring the acidic pH of vagina which is a protective shield against infections, which was also identified in post treatment evaluation. The drugs may restore or enhance the growth of natural vaginal flora preventing the growth of pathogens, hence effective in *yonisrava*[14].

In-vitro analysis further substantiated the clinical results. *Triphaladi Kashaya* exhibited notable antifungal activity against *Candida albicans* strains isolated from vaginal swabs. The zone of inhibition values demonstrated its capacity to inhibit fungal growth, supporting its use as a promising antifungal agent.

Studies have validated a number of potential uses of *Triphala*, which include free radical scavenging, antioxidant, anti-inflammatory, immunomodulating, appetite stimulation, gastric hyperacidity reduction, dental caries prevention, antipyretic, analgesic, antibacterial, antimutagenic, wound healing, anticariogenic, antistress, adaptogenic, hypoglycemic, anticancer, hepatoprotective, chemoprotective, radioprotective, and chemo preventive effects. *Triphala* is also known to exhibit antifungal properties due to the presence of the major constituents like tannins, gallic acid, ellagic acid, and chebulinic acid[15]. *Triphala* includes *Haritaki* (*Terminalia chebula*), *Bibitaka (Terminalia belerica*) and *Amalaki (Emblica officinalis). Terminalia chebula* is a deciduous small or middle-sized tree having *shophaghna* and *shodhana* properties[16], whereas *Terminalia belerica* and *Emblica officinalis* are moderate to large sized deciduous tree having *krimighna* and *kandughna* properties[17].*Triphala Kwatha's* acidic pH potentially aids in preserving the typical vaginal pH level, thereby supporting the equilibrium of healthy vaginal flora. Its primary chemical component, tannin, plays a role in protein precipitation, potentially reducing inflammation and vaginal discharge. Tannins have been demonstrated to possess inhibitory effects on the growth of numerous fungi, yeasts, bacteria, and viruses. Additionally, the immune-modulating properties of ascorbic acid found in *Amalaki* have been established in maintaining the equilibrium of healthy vaginal flora. *Haridra* also showed antibacterial, antifungal and anti-inflammatory effects[18]. *Haridra* (*Curcuma longa*) is a tall herb having *krimighna* and *kandughna* properties[19]. Methanolic extracts of turmeric exhibited different ranges of activity against Candida albicans. The antimicrobial activity of turmeric is reported to be due to the presence of essential oil, curcumins, curcuminoids, turmeric oil, turmerol and valeric acid[20]. Cow urine, also known as *Gomutra*, is traditionally believed to possess antifungal properties and is used in some cultures as a remedy for fungal infections. Studies suggest that gomutra contains compounds like phenolic acids (gallic, caffeic, ferulic, and salicylic acids) that may contribute to its antifungal activity. Specifically, *Gomutra arka*, the distilled form of cow urine, is believed to have enhanced antifungal properties due to the concentration of these active principles[21].

The mentioned medicine combination exhibits potent local effects in infection control, possibly attributed to its antimicrobial and anti-inflammatory properties. Moreover, *Triphala* being *Tridoshahara, Shodhana, Ropana, and Rasayana*[22], it best suits the need and also facilitates the regeneration of healthy cells.

CONCLUSION:

Overall, the study provides evidence that *Triphaladi kashaya* is effective in managing *Kaphaja Yonisrava* vis-à-vis VVC in both in-vitro and clinical parameters. Various studies have reestablished the antimicrobial activities of various drugs mentioned in Ayurveda and in this study, it is proven both clinically and in-vitro methods. *Sthanika Chikitsa*, which plays a crucial role in addressing women's health issues, facilitates the absorption of medication through the vaginal walls due to their high vascularity. The vagina has the capacity to absorb water, electrolytes, and substances with low molecular weights, primarily in the lateral recesses of the lower vagina. This study adds to the growing body of evidence advocating the scientific validation and clinical application of *Ayurvedic* formulations in managing common gynaecological infections.

ACKNOWLEDGEMENT

We, the authors, express our sincere gratitude to JSS Mahavidyapeetha and JSS Ayurveda Medical College and Hospital, Mysuru, Karnataka, India for the support during the conduct of this study.

Journal of Chemical Health Risks

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JCHR (2025) 15(4), 1510-1517 | ISSN:2251-6727

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