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PHARMACEUTICO-ANALYTICAL STUDY AND INVITRO ANTIMICROBIAL EVALUATION OF POLYHERBAL VAGINAL WASH AND VAGINAL SUPPOSITORY

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ABSTRACT

Vaginitis is a common condition affecting millions of women worldwide, characterized by abnormal vaginal discharge and pruritus that can adversely affect physical and mental well-being. In Ayurveda, such conditions are described under *Yoni Vyapath*, where *Vata*, *Lodhra*, and *Plaksha* are traditionally used for management. Although conventional therapies are effective, they are often associated with microbial resistance, recurrent infections, and disruption of the normal vaginal flora. This study aimed to prepare and evaluate polyherbal vaginal wash and suppository formulations using hydroethanolic extracts of Vata, Lodhra, and Plaksha. The extracts were prepared by Soxhlet extraction, concentrated by rotary vacuum evaporation, and dried in a hot air oven. Three batches of vaginal wash and two batches of vaginal suppository were formulated, and one was selected based on the desirable characteristics. Organoleptic, physicochemical, instrumental, phytochemical and antimicrobial evaluations were performed. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides, and reducing sugars. The antimicrobial activity of the developed polyherbal formulations was evaluated to validate their therapeutic potential against infectious vaginitis. The study

assessed their efficacy against *Candida albicans* and *Trichomonas vaginalis*, the primary causative organisms of vulvovaginal candidiasis and trichomoniasis, respectively, to establish their potential as safe and effective alternatives to conventional therapy. The developed polyherbal vaginal wash and suppository showed promise as safe and effective alternatives for managing vaginal infections, supporting the integration of traditional Ayurvedic knowledge with modern pharmaceutical formulations.

KEYWORDS: Polyherbal vaginal wash, Polyherbal vaginal suppository, Hydroethanolic extract, *Candida albicans, Trichomonas vaginalis.*

INTRODUCTION

Vaginal infections are among the most prevalent gynaecological disorders affecting women of all age groups. Conditions such as vulvovaginal candidiasis, bacterial vaginosis, and trichomoniasis contribute significantly to morbidity among women and are often associated with symptoms including vaginal discharge, itching, irritation, burning sensation, and discomfort[1]. If not adequately managed, these infections may adversely affect reproductive health and quality of life. Conventional antimicrobial therapy is effective; however, its long-term use is often limited by recurrent infections, microbial resistance, adverse effects, and disruption of the normal vaginal flora.

In the Ayurvedic classics, these pathological aspects are primarily addressed under the heading of *Yoni Vyapath*. Traditional treatments include *Abhyantara aushadhis* (internal medicines) and *Sthanika Chikitsa* (localized treatments) such as *Yoni Prakshalana* (vaginal douching) and *Yoni pichu*. Although these therapies are clinically beneficial, the medications used have limitations such as shorter shelf life and reduced patient compliance. These challenges highlight the need to develop patient-friendly dosage forms that improve ease of administration, retention, and therapeutic efficacy.

This research focuses on the formulation and evaluation of using polyherbal extracts of three specific drugs mentioned in the *Nyagrodhadi gana*: *Lodhra* (*Symplocos racemosa*), *Vata* (*Ficus benghalensis*), and *Plaksha* (*Ficus lacor*).[2] These drugs possess *Kashaya Rasa*, *Stambhana*, and *Kledahara* properties, making them historically effective for treating vaginal disorders. The study aims to transition these traditional herbal principles into modern, patient-friendly dosage forms vaginal wash and vaginal suppository for localized, sustained therapeutic action.

MATERIALS AND METHODS

Materials

Collection of Raw Drugs and Excipients: The raw drugs *Vata* -Stem bark *(Ficus benghalensis), Lodhra*-Stem bark *(Symplocos racemosa),* and *Plaksha*- Stem bark *(Ficus lacor*) and all excipients used in the study were procured from an authentic source.

Methods: The study involved the preparation of coarse powder of individual drugs, preparation of hydroethanolic extracts of *Vata, Lodhra,* and *Plaksha,* and formulation of a polyherbal vaginal wash and vaginal suppository using the hydroethanolic extracts followed by their analysis and testing.

Preparation of Coarse Powder: Cleaned and dried raw drugs were separately pounded in a *Khalwa Yantra*, pulverized through a No. 40 mesh, and the coarse powder obtained was stored in airtight containers.

Preparation of hydroethanolic extract from the coarse powder *Vata, Lodhra* and *Plaksha*: Equal quantities (15 g each) of dried coarse powders of *Vata, Lodhra,* and *Plaksha* were accurately weighed, mixed uniformly, and packed in a muslin cloth to form a thimble. A hydroethanolic solvent (ethanol: distilled water, 80: 20; 160 mL: 40 mL) was prepared and placed in the round-bottom flask of a Soxhlet apparatus. The thimble was placed in the extraction chamber, and extraction was carried out at 85–90°C until the siphoned solvent became clear, indicating complete extraction. The extract-solvent mixture was concentrated using a rotary vacuum evaporator at 78°C and 273 rpm, followed by drying in a hot air oven at 100°C until complete removal of the residual solvent. The dried hydroethanolic extract was then stored in an airtight glass container.

Figure I - Showing the prepared hydroethanolic extract.

Preparation of Polyhedral Vaginal Wash using Hydroethanolic Extract

Samples: PHVW I, II and III – Polyherbal Vaginal Wash I,II and III.

Table I: Showing ingredients and ratios used for the preparation of different samples of vaginal wash.

Sl. No.	Ingredients	PHVW I (ml)	PHVW II (ml)	PHVW III (ml)
1	Herbal extract	200 mg	200 mg	200 mg
2	Ethanol	1.5 ml	1.5 ml	1.5 ml
3	DM water(Phase A)	3.5 ml	3.5 ml	3.5 ml
4	Aloe Vera Gel	4 ml	5 ml	6 ml
5	Cocamidopropyl Betaine	10 ml	8 ml	6 ml
6	Rose Water	12 ml	10 ml	8 ml
7	Citric acid solution	1 ml	1 ml	1 ml
8	DM water(Phase B)	67 ml	71 ml	75 ml

Preparation of Ingredients: Fresh *Aloe vera* leaves were cleaned, and the inner gel was separated, filtered, and measured as required. A 1% citric acid solution was prepared by dissolving 1 g of citric acid in 100 mL of demineralized water and allowing it to hydrate for 30 minutes. All other ingredients were accurately measured and kept ready. **Phase A Preparation:** Accurately weighed 200 mg of the hydroethanolic herbal extract was mixed with 1.5 mL ethanol and 3.5 mL DM water, followed by sonication for 15 minutes to obtain a uniform extract solution. **Phase B Preparation:** *Aloe vera* gel, cocamidopropyl betaine, rose water, citric acid solution, and the required quantity of DM water were mixed gently to avoid excessive foaming. Phase B was then added slowly to Phase A with continuous stirring until a homogeneous formulation was obtained. The final pH was checked using a calibrated pH meter, after which the vaginal wash was filtered, filled into sterilized bottles, labelled, and stored at room temperature.

Preparation of Polyherbal Vaginal Suppository using Hydroethanolic Extract

Samples: PHVS I and II – Polyherbal Vaginal Suppository I and II.

Table II - Showing ingredients and ratios used for the preparation of samples of vaginal suppository.

Sl. No.	Ingredients	PHVS I	PHVS II
1	Herbal extract	200 mg	200 mg
2	Base material	1 g Cocoa Butter	1 g Beeswax
3	Glycerine	1 drop	1 drop

Preparation of Mould: The suppository mould was thoroughly cleaned, dried, and lightly lubricated with glycerine to facilitate easy removal of the suppositories. **Preparation of PHVS I:** The required quantity of cocoa butter was melted using a water bath, and the accurately weighed herbal extract was incorporated with continuous stirring to obtain a uniform mixture. The molten mass was poured into the lubricated mould cavities, allowed to cool, and refrigerated until completely solidified. **Preparation of PHVS II:** The required quantity of beeswax was melted in a water bath, and the herbal extract was mixed uniformly into the molten base. The prepared mass was poured into the lubricated mould cavities, cooled at room temperature, and then refrigerated until hardened. **Removal and Storage:** After complete solidification, the suppositories were carefully removed from the mould, transferred to airtight containers, and stored in a cool place or refrigerator.

Among the prepared formulations, **PHVW I** and **PHVS I** were selected as the optimal formulations and used for further analysis.

Figure II and III- Showing the prepared polyherbal vaginal wash and suppositories.

Analytical Study of Coarse Powder of Raw Drugs

The raw drugs used for the preparation were subjected to organoleptic and physicochemical evaluations, including determination of pH, total ash, acid-insoluble ash, water-soluble ash, loss on drying, alcohol-soluble extractive, and water-soluble extractive values**.**

Analytical Study of Hydroethanolic Extract

Organoleptic Evaluation: The hydroethanolic extract was evaluated for its organoleptic characteristics. It exhibited a brownish-red colour**,** characteristic odour and smooth texture**.**

Determination of pH: The pH meter was calibrated according to the manufacturer's instructions. A 1% aqueous solution of the hydroethanolic extract was prepared, and the pH was measured using the calibrated pH meter.

Phytochemical screening[3]: The preliminary phytochemical screening of the polyherbal extract was carried out using standard qualitative chemical tests to identify the presence of various phytoconstituents.

Alkaloids were screened using Dragendorff's test, Wagner's test, and Mayer's test. Flavonoids were evaluated using the Ferric chloride test, alkaline reagent test, concentrated sulfuric acid test, and Lead acetate test. Phenolic compounds were detected using the Ferric chloride test, Lead acetate test, and Iodine test. Tannins were identified by the Gelatin test, Braymer's test, and Bromine water test. Glycosides were screened using Borntrager's test, 10% sodium hydroxide test, and aqueous sodium hydroxide test. Carbohydrates were detected using Barfoed's test, while reducing sugars were identified using Benedict's test and Fehling's test. The observations obtained from these tests were used to determine the presence or absence of the respective phytochemical constituents in the extract.

Analytical Study of Polyherbal Vaginal Wash

Organoleptic Evaluation: The formulation was evaluated for colour, odour, and touch.

Viscosity: Viscosity was determined using an Anton Paar MCR 302 rotational rheometer at 25 ± 0.1°C. The sample was subjected to a shear rate of 0–110 s^{-1}, and the viscosity was recorded automatically. The test was performed in triplicate, and the mean value was calculated.

Specific Gravity: Specific gravity was determined using a pycnometer by comparing the weight of the formulation with that of distilled water using the formula.

Specific Gravity = $(W_3 - W_1) / (W_2 - W_1)$, where W_1 = weight of empty pycnometer, W_2 = weight with distilled water, and W_3 = weight with vaginal wash.

Refractive Index: The refractive index was measured using an Abbe's refractometer after calibration with distilled water. The reading was recorded at 28°C to assess the optical quality and uniformity of the formulation.

Foaming Ability and Foam Stability: A 1% vaginal wash solution was prepared, and 50 mL was transferred to a graduated cylinder and shaken vertically 10 times. Foam volume was recorded immediately and at 1-minute intervals for 4 minutes to evaluate foaming ability and stability.

Analytical Study of Polyherbal Vaginal Suppository

Organoleptic Evaluation: The suppositories were assessed for physical state, appearance, colour, and odour.

Uniformity of Weight: Twenty suppositories were weighed individually using a calibrated analytical balance. The average weight and percentage deviation were calculated to assess compliance with the ±5% weight variation limit.

Hardness Test: Hardness was measured using a suppository hardness tester by applying gradual pressure until the suppository broke. The hardness was recorded in kg/cm², and the average value was calculated.

Softening and Melting Point: The suppository was filled into a capillary tube, placed in a water bath, and heated gradually. The temperature at which softening and melting occurred was recorded.

Instrumental Analysis – Thin Layer Chromatography[4]

Table III: Showing the materials used for TLC development.

Parameter	Details
Stationary Phase	Silica Gel 60 F254 plates (Merck)
Mobile Phase	As summarized in Table No. 940
Sample Solvent	Ethanol
Development Distance	Up to marked solvent front line
Visualization	UV light at 254 nm, 366 nm, and iodine vapour

Mobile Phases Used for TLC Analysis: The different mobile phases used for TLC analysis were chloroform and methanol in a 1: 1 ratio (10 mL: 10 mL), toluene and ethyl acetate in a 1: 1 ratio (10 mL: 10 mL), chloroform, methanol, and formic acid in the ratio of 10 mL: 10 mL: 0.1 mL, and toluene, ethyl acetate, and formic acid in the ratio of 10 mL: 10 mL: 0.1 mL.

Samples Used for TLC Analysis : TLC analysis was performed using six samples: Sample A, the hydroethanolic extract of *Vata*; Sample B, the hydroethanolic extract of *Lodhra*;

Sample C, the hydroethanolic extract of *Plaksha*; Sample E, the combined hydroethanolic extract of all three drugs; Sample S, the polyherbal vaginal suppository; and Sample W, the polyherbal vaginal wash.

Sample Preparation: Accurately weighed 100 mg of each sample was dissolved in 10 mL ethanol, sonicated for 10–15 minutes, filtered through Whatman No. 1 filter paper, and the filtrate was used for TLC analysis.

Procedure: 1 µL of each sample was spotted on a marked TLC plate, dried, and developed in a chamber pre-saturated with the selected mobile phase for 10 minutes. The developed plate was dried and observed under UV light (254 and 366 nm) and in an iodine vapour chamber.

***In-vitro* Antimicrobial Evaluation**

Determination of Minimum Inhibitory Concentration (MIC) by Tube Dilution Method

The MIC of the hydroethanolic extract against *Candida albicans* and *Trichomonas vaginalis* was determined by the tube dilution method. A stock solution was prepared by dissolving 50 mg of dried extract in DMSO, followed by serial dilutions (100%, 50%, 25%, 12.5%, and 6.25%). Test tubes containing nutrient broth, standardized microbial suspension (2×10^6 cells/mL), and the respective extract dilution (final volume 1 mL) were incubated at 37°C for 24 hours. Microbial growth was assessed by measuring absorbance at 600 nm, and the lowest concentration showing complete inhibition was recorded as the MIC.

Antimicrobial Activity of Hydroethanolic Extract by Agar Well Diffusion Method

The hydroethanolic extract was evaluated against *Candida albicans* and *Trichomonas vaginalis* using the agar well diffusion method. Standardized microbial suspensions (2×10^6 cells/mL) were inoculated onto antibiotic assay medium plates, and 30 µL of the extract was added into aseptically prepared wells. Fluconazole and Metronidazole served as standards for *Candida albicans* and *Trichomonas vaginalis*, respectively. After incubation at 37°C for 24 hours under appropriate conditions, the zone of inhibition was measured.

Evaluation of Antimicrobial Activity of Polyherbal Vaginal Formulations

The optimized vaginal wash (Sample I) and vaginal suppository (Sample II) were evaluated by the agar well diffusion method. Sample I was used directly, while Sample II was melted and dissolved in sterile DMSO. About 30 µL of each formulation was added into wells containing standardized microbial inoculum (2×10^6 cells/mL). Fluconazole and

Metronidazole were used as standard drugs. Following incubation at 37°C for 24 hours, the antimicrobial activity was assessed by measuring the zone of inhibition.

RESULTS

a) Physicochemical Evaluation of Raw Drugs

The coarse powders of *Vata*, *Lodhra*, and *Plaksha* were evaluated for various physicochemical parameters. The pH was found to be 7.52, 5.43, and 6.90, respectively. The total ash values were 6.852%, 12.87%, and 6.35%, while the water-soluble ash values were 1.355%, 1.53%, and 0.92%, respectively. The acid-insoluble ash values were 3.175%, 1.42%, and 1.327%, respectively. The loss on drying was 3.98%, 4.90%, and 4.16%, while the water-soluble extractive values were 14.85%, 23.78%, and 8.78%, respectively. The alcohol-soluble extractive values were 6.558%, 19.49%, and 5.008%, respectively.

b) Physicochemical Evaluation of Hydroethanolic extract

The hydroethanolic extract was prepared by extracting 45 g of coarse powder with 200 mL of hydroethanolic solvent, yielding 2.5 g of concentrated extract. On organoleptic evaluation, the extract was found to be brownish-red in colour, possessed a characteristic odour, and had a smooth texture. The pH of the hydroethanolic extract was 4.96.

c) Phytochemical screening of Hydroethanolic extract

Table IV: Showing the phytochemical screening of the hydroethanolic extract.

Phytochemical Test	Result	Phytochemical Test	Result
Alkaloids		**Tannin test**	
Mayer's test	+	Ferric chloride	+
Wagner's test	+	Bromine water test	-
Dragendroff's test	+	Gelatine test	+
Flavonoids		**Glycoside test**	
Ferric chloride test	+	Borntrager's test	+
Ammonium hydroxide	+	10% NaOH test	+
Conc. H_2SO_4 Test	+	Aq NaOH test	-
Lead acetate test	+	**Carbohydrate test**	
Phenolic compound		Brafoed test	-
Ferric chloride test	+	**Reducing sugar**	
Lead acetate test	-	Benedict's Test	+
Iodine test	+	Fehling's Test	+

d) Physicochemical Evaluation of Polyherbal Vaginal Wash

Three trial batches of the vaginal wash were formulated, yielding 96 mL (PHVW I), 95 mL (PHVW II), and 92 mL (PHVW III), respectively. PHVW I was selected as the optimal vaginal wash formulation for further evaluation.

On organoleptic examination, the formulation was found to be dark brown in colour with a pleasant aromatic odour and a smooth texture. The pH of the formulation was 4.27, indicating compatibility with the normal vaginal pH. The viscosity was found to be 2.45 mPa·s at 25°C, as determined using an Anton Paar rheometer, and the formulation exhibited Newtonian flow behaviour. The refractive index of the vaginal wash was 1.47470, while the specific gravity was 1.0021. The foaming ability and foam stability of the formulation were evaluated by measuring the foam volume at regular time intervals. The initial foam volume was 37 mL, which remained unchanged after 1 minute. A slight decrease to 36 mL was observed at 2 and 3 minutes, followed by 35 mL at 4 minutes.

e) Physicochemical Evaluation of Polyherbal Vaginal Suppository

Two trial batches of the vaginal suppository formulation were prepared, with weights of 1.06 g (PHVS I) and 1.13 g (PHVS II). PHVS I was selected as the optimal vaginal suppository formulation for further evaluation. On organoleptic examination, the formulation was found to be solid with a glossy, smooth appearance**,** reddish-brown colour**,** and a peculiar odour**.** The average weight of the suppositories was 1.06 g**,** which was within the permissible deviation range of ±5% (1.007–1.13 g)**.** The average hardness of the suppositories was 1.5 kg/cm². The melting range of the polyherbal vaginal suppository was found to be 37.1–38.3°C.

f) Instrumental Analysis - TLC

Observation: Among the different solvent systems evaluated, the mobile phase consisting of chloroform: methanol (1: 1) showed better separation and clearer resolution of bands compared to the toluene : ethyl acetate system. The addition of formic acid did not produce any major difference in the migration pattern or number of bands, although slight improvement in band sharpness was observed.

Calculation of Rf Value: The Rf value was calculated using the following formula:

Rf = Distance travelled by solute / Distance travelled by solvent front

Where, the distance travelled by the solvent front = 7.5 cm**.**

Table V - Showing the calculated Rf values of the samples

Mobile Phase	Sample Code	Number of Bands	Distance Travelled by Bands (cm)	Rf Value
Chloroform + Methanol	Sample A	2	7.3, 6.5	0.97, 0.86
	Sample B	4	7.3, 6.5, 6.3, 5.2	0.97, 0.86, 0.84, 0.69
	Sample C	1	6.5	0.86
	Sample E	4	7.3, 6.5, 6.3, 5.2	0.97, 0.88, 0.84, 0.69
	Sample W	3	6.5, 6.6, 5.2	0.86, 0.88, 0.69
	Sample S	2	6.5, 1.3	0.86, 0.17
Chloroform + Methanol + Formic Acid	All Samples	–	–	No major difference observed.

g) *In-vitro* Study of Hydroethanolic Extract

Table VI: Showing MIC of hydroethanolic extract against *Candida albicans* and *Trichomonas vaginalis.*

Test Compound	Volume (µL)	*Candida albicans*	*Trichomonas vaginalis*
Nutrient broth	1000	0.071	0.071
Nutrient broth + Culture	1000	0.272	0.116
Nutrient broth + Sample	1000	2.383	2.383
Extract – 100%	1000	0.457	0.095
Extract – 50%	1000	1.199	0.781
Extract – 25%	1000	1.833	1.780
Extract – 12.5%	1000	**2.181 (MIC)**	1.830
Extract – 6.25%	1000	2.376	**2.065 (MIC)**

Table VII - Showing the zone of inhibition of the hydroethanolic extract against *Candida albicans.*

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (cm)
Candida albicans	Standard disc (Fluconazole)	30µl	0.9
	Extract	30µl	0.6

Table VIII - Showing the zone of inhibition of the hydroethanolic extract against *Trichomonas vaginalis.*

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (cm)
Trichomonas vaginalis	Standard disc (Metronidazole)	30µl	0.7
	Extract	30µl	0.5

h) *In-vitro* Study of Formulations

Table IX - Showing the zone of inhibition of formulations against *Candida albicans.*

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (cm)
Candida albicans	Standard disc (Fluconazole)	30µl	0.9
	Sample I	30µl	2.2
	Sample II	30µl	2.6

Table X - Showing the zone of inhibition of formulations against *Trichomonas vaginalis.*

Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (cm)
Trichomonas vaginalis	Standard disc (Metronidazole)	30µl	0.6
	Sample I	30µl	1.7
	Sample II	30µl	2

DISCUSSION

The present study focused on the development and evaluation of polyherbal vaginal wash and suppository formulations containing hydroethanolic extracts of *Lodhra* (*Symplocos racemosa*), *Vata (Ficus benghalensis*) and *Plaksha* (*Ficus lacor*) for the management of vaginal infections. These drugs were selected based on their classical Ayurvedic indications in *Yoniroga* and their reported antimicrobial, anti-inflammatory, antioxidant, astringent, and wound-healing properties, which collectively support vaginal health.

The vaginal route was selected because it enables localized drug delivery, bypasses first-pass metabolism, and provides higher drug concentrations at the site of infection with minimal systemic adverse effects. Vaginal wash offers rapid cleansing while maintaining physiological vaginal pH and normal flora, whereas vaginal suppositories ensure prolonged retention and sustained release of phytoconstituents.[5]

Hydroethanolic extraction (80: 20 ethanol: distilled water) using the Soxhlet method was adopted to obtain both polar and semi-polar phytoconstituents efficiently while minimizing degradation of heat-sensitive compounds. Coarse powder facilitated proper solvent circulation and improved extraction efficiency.

The hydroethanolic polyherbal extract exhibited concentration-dependent antimicrobial activity against *Candida albicans* and *Trichomonas vaginalis*, with MIC values of 12.5% and 6.25%, respectively. In the agar well diffusion assay, the extract produced inhibition zones of 0.6 cm against *Candida albicans* and 0.5 cm against *Trichomonas vaginalis*, compared with 0.9 cm and 0.7 cm for Fluconazole and Metronidazole, respectively. Although its activity was

lower than the standard drugs, the extract demonstrated appreciable antifungal and antiprotozoal effects. The smaller inhibition zones may be attributed to the limited diffusion of phytoconstituents through the agar medium.

The quantity of the polyherbal extract incorporated into both formulations was determined based on literature review of conventionally used vaginal preparations and their standard unit dosage strengths. In addition, the results obtained from the Minimum Inhibitory Concentration study of the polyherbal extract were carefully considered during dose selection.

The excipients for the vaginal wash were selected to ensure mild cleansing, pH compatibility, effective delivery of herbal constituents, and patient acceptability. Cocamidopropyl betaine was used as a mild amphoteric surfactant to provide cleansing, foam stability, and viscosity enhancement while maintaining vaginal pH[6]. *Aloe vera* was incorporated for its moisturizing, soothing, wound-healing, and anti-inflammatory properties.[7] Citric acid served as a pH-adjusting agent to maintain the physiological acidic environment[8], while rose water enhanced aesthetic acceptability and contributed antibacterial, anti-inflammatory, and antioxidant effects.[9] Demineralized water acted as a purified vehicle for uniform distribution of ingredients. Overall, the formulation was designed to maintain vaginal pH, support normal vaginal flora, provide gentle cleansing, and relieve symptoms such as itching, irritation, and discomfort while ensuring safety and stability.

Three vaginal wash formulations (PHVW I, PHVW II, and PHVW III) were prepared using different concentrations of excipients to optimize solubility, stability, consistency, cleansing efficiency, and patient acceptability. The herbal extract showed limited water solubility, resulting in sedimentation; therefore, a minimal quantity of ethanol was used as a co-solvent, and sonication was employed to improve dispersion and achieve a homogeneous formulation. Variations in the concentrations of *Aloe vera*, Cocamidopropyl Betaine and rose water influenced the formulation characteristics. Among the three batches, PHVW I exhibited the best consistency, homogeneity, foaming ability, stability, and acceptable physiological pH without sedimentation or phase separation. In comparison, PHVW II showed reduced foaming and thinner consistency which may be due to lower CAPB content, while PHVW III became comparatively more viscous and showed reduced foamability and cleansing action possible due to increased *Aloe vera* and reduced surfactant concentration. Overall, PHVW I

was selected as the optimized formulation based on its consistency, homogeneity, foamability, appearance, stability, pH, and patient acceptability.

Cocoa butter was selected as the suppository base because its melting point is close to body temperature, allowing rapid melting after insertion while remaining stable during storage. It also provides good spreadability, moisturizing properties, and improved patient acceptability.[10] Beeswax was not selected due to its higher melting point and harder consistency, which could delay drug release. Glycerine was used as a mould lubricant to facilitate easy removal of suppositories and improve smoothness.[11]

The optimized polyherbal vaginal wash exhibited satisfactory organoleptic properties with a brown colour, aromatic odour, and smooth texture, indicating good formulation quality. The pH (4.27) was within the physiological vaginal range, supporting maintenance of normal vaginal flora. It showed appropriate viscosity (2.45 mPa·s), refractive index (1.47470), and specific gravity (1.0021), confirming good flow properties and uniformity. It also demonstrated satisfactory foaming ability and foam stability, contributing to effective cleansing.

The polyherbal vaginal suppositories showed a smooth, glossy appearance, indicating uniform moulding and distribution of ingredients. The average weight (1.06 g) complied with pharmacopeial limits, confirming content uniformity. The hardness (1.5 kg/cm^2) provided adequate mechanical strength while allowing proper melting after administration. The melting range (37.1–38.3°C), close to body temperature, confirmed the suitability of cocoa butter as the suppository base for effective vaginal drug delivery. Overall, the analytical evaluation demonstrated that the suppositories possessed satisfactory physicochemical properties, quality, and stability, making them suitable for vaginal application.

TLC analysis demonstrated corresponding Rf values in both the hydroethanolic extract and the formulated vaginal wash and suppository, confirming the successful incorporation and retention of phytoconstituents without significant degradation during formulation.

The polyherbal vaginal wash (Sample I) exhibited antimicrobial activity against *Candida albicans* and *Trichomonas vaginalis*, with inhibition zones of 2.2 cm and 1.7 cm, respectively, while the vaginal suppository (Sample II) showed the highest activity, producing inhibition zones of 2.6 cm and 2.0 cm, respectively. Both formulations

demonstrated greater antimicrobial activity than the crude hydroethanolic extract, which may be attributed to the higher extract content in the formulations (200 mg) compared with the extract sample (50 mg). Under the study conditions, both formulations also showed greater activity than the standard drugs, indicating the effectiveness of the polyherbal combination against vaginal pathogens.

Among the two formulations, the vaginal suppository exhibited superior activity, possibly due to the higher localized concentration of active constituents present in a single suppository unit and improved solubilization and diffusion of lipophilic phytoconstituents from the lipid base. In contrast, the comparatively lower activity of the vaginal wash may be due to the presence of formulation excipients, such as surfactants, solubilizers, and preservatives, which can reduce the effective concentration of active phytoconstituents. Nevertheless, the vaginal wash demonstrated considerable antimicrobial activity against both test organisms. Overall, both formulations showed promising action against *Candida albicans* and *Trichomonas vaginalis*.

CONCLUSION

The present study successfully developed two modified dosage forms, a polyherbal vaginal wash and a cocoa butter-based vaginal suppository, using hydroethanolic extracts of *Lodhra*, *Vata* and *Plaksha* . Phytochemical screening confirmed the presence of important secondary metabolites, including alkaloids, flavonoids, tannins, and phenolic compounds. Hydroethanolic extraction method was found to be effective in extracting a maximum quantity of phytoconstituents when compared to aqueous extraction alone, owing to the ability of the hydroethanolic solvent system to dissolve both polar and moderately non-polar compounds. The TLC findings further demonstrated that these bioactive constituents were successfully transferred from the crude herbal extracts into the final formulations without significant degradation during the manufacturing process, thereby preserving their therapeutic potential. Both formulations met pharmaceutical quality standards, with the vaginal wash exhibiting a physiologically compatible pH and the suppository demonstrating suitable mechanical strength and melting characteristics. Overall, the developed polyherbal vaginal wash and vaginal suppository represent promising, safe, and effective alternatives to conventional antimicrobial therapies for vaginal infections. By integrating traditional Ayurvedic knowledge with modern pharmaceutical techniques, the study provides a

scientifically validated approach for promoting women's reproductive health and intimate hygiene.

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