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Research article

Assessment of response surface methodology-optimized polyherbal feminine hygiene wash against *candida albicans*

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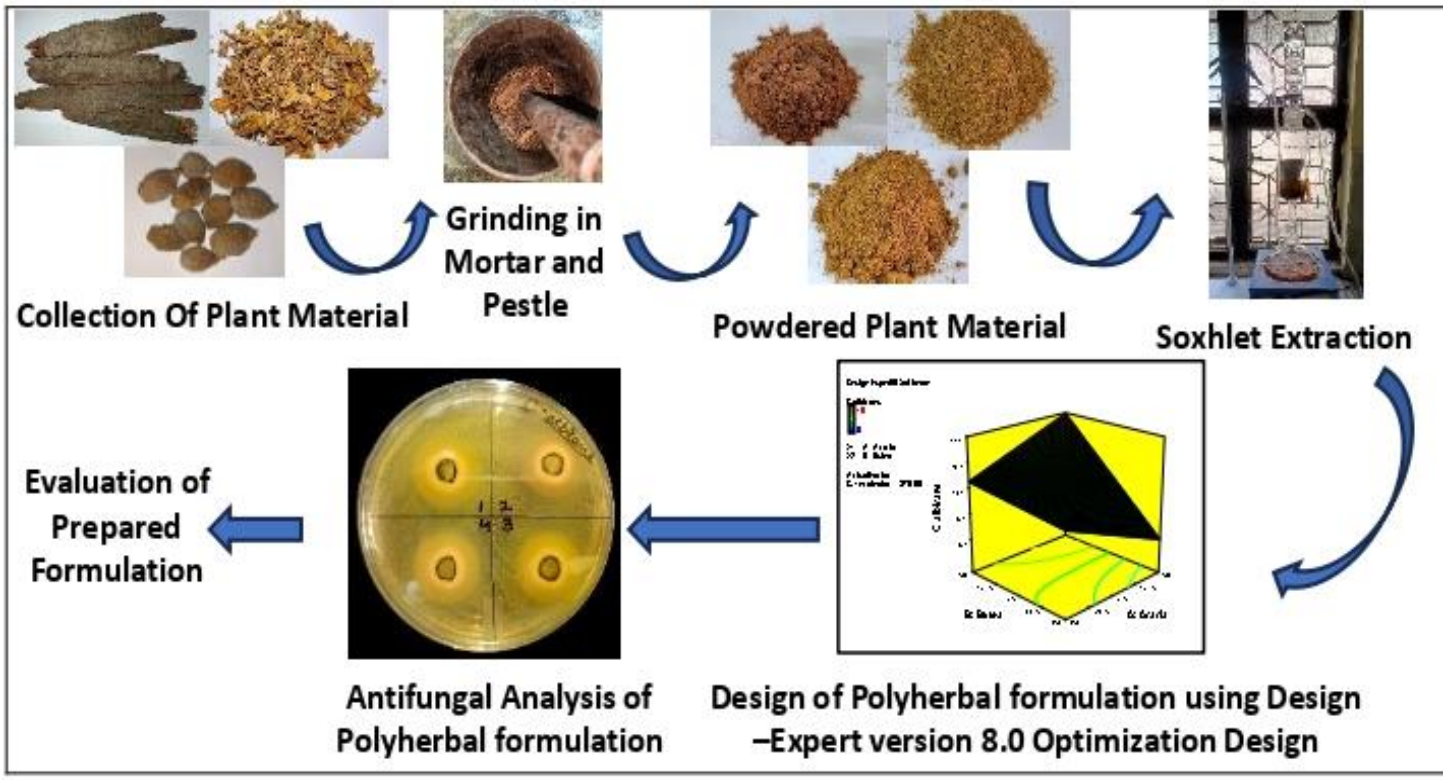
ABSTRACT

Feminine hygiene products are widely used for personal care, yet their antimicrobial properties, particularly against fungal pathogens like *Candida albicans*, require further exploration. Vaginal infections, particularly those caused by *Candida albicans*, are a major health concern among women of all age groups. These infections manifest as itching, burning, vaginal pain, swelling, and abnormal discharge, significantly impacting women's quality of life. The use of polyherbal formulations offers a promising alternative to conventional antifungal treatments due to their synergistic therapeutic effects.



This study focuses on the optimization of a polyherbal feminine hygiene wash using Response Surface Methodology (RSM) to enhance its antifungal efficacy against *Candida albicans*. The formulation was prepared using ethyl acetate extracts of *Acacia nilotica*, *Butea monosperma*, and *Terminalia bellirica* in a hydroxypropyl methylcellulose (HPMC) gel base. In the present investigation a comparative analysis was done amongst experimental formulation, V-Wash, Arogita and Ayouthveda. An *in vitro* analysis was conducted to evaluate the inhibitory effects of these products on the growth and viability of *Candida albicans*. The formulation was assessed for its physicochemical properties, including texture, pH, buffer capacity, specific gravity, surface tension, solid contents and total phenolic contents, along with *in vitro* antifungal activity against *C. albicans*. The results indicate that the poly-herbal vaginal wash exhibit acceptable physical characteristics and maintain stability over the short term. *In vitro* studies reveal that the formulation demonstrated strong antifungal activity, with a zone of inhibition (ZOI) of 16.61 ± 0.76 mm, surpassing some commercially available products. These findings suggest that the formulated poly-herbal vaginal wash is a promising alternative for the management and prevention of *Candida albicans* infections, offering a natural and effective solution for improving intimate hygiene.

Keywords: Antifungal, *Candida albicans*, Feminine hygiene, Polyherbal, Vaginal wash rehabilitation.

INTRODUCTION

Vulvovaginal candidiasis is a common fungal infection affecting the mucosal tissues, leading to considerable morbidity among many women of childbearing age globally. As per an estimate about 75% females experience a vaginal fungal infection during their lifetime, with at least half experiencing a second episode. 5% of these women may suffer from recurrent infections. The normal vaginal flora predominantly consists of *Lactobacillus* sp., along with various other bacteria, including both Gram-positive and Gram-negative, facultatively or obligate anaerobic species. They form a microbial ecosystem in dynamic balance with the vaginal epithelium of the host. *Lactobacillus* plays a pivotal role in maintaining this balance by producing lactic acid and peroxide, which limit the growth of other microbial species. Interestingly, conditions favourable for the presence of *Lactobacillus* also support vaginal *Candida* colonization. Local immunity is crucial for defending against vaginitis, more so than systemic immune responses [1].

Candida albicans is the primary cause of mild to moderate candidal vaginitis, particularly in women who are not immunocompromised. This type of infection tends to be infrequent or sporadic. Symptoms include a vaginal rash, watery discharge, pain, and soreness, along with thick, white, odourless discharge resembling cottage cheese. Other common symptoms are irritation, itching, redness, swelling of the vulva, and a burning sensation during urination or intercourse. Key risk factors include recent antibiotic use, which increases the likelihood of developing candidal vaginitis. Additionally, conditions such as HIV, AIDS, or diabetes mellitus can raise the risk, as can frequent douching or prolonged wear of wet clothing [2].

Hundreds of plants from around the world have traditionally been used to treat microbial infections, and some of them have undergone *in vitro* screening. However, the effectiveness of these herbal remedies has rarely been evaluated through rigorous clinical trials. Phytochemical studies of various plant species suggest that these natural compounds may serve as a superior source of medicine as compared to the synthetic drugs. The use of plants for medicinal purposes date back to ancient times, with traditional plant-based remedies being utilized for centuries. As a result, one common method for discovering antimicrobial agents has been the evaluation of plant extracts [3]. Aren and Kumar have discussed the formulation, ingredients, and health implications of polyherbal feminine hygiene washes, highlighting their role in managing vaginal infections and their widespread use among women, while emphasizing the importance of informed decision-making and regulatory oversight [4].

MATERIALS AND METHODS

Design and Evaluation of Formulation Studies

The polyherbal feminine hygiene wash was designed using commercially available software package Design –Expert version 8.0 Optimization Design (2^3 Full Factorial Design). The ethyl acetate extracts of *Acacia nilotica* (L.) P.J.H. Hurter & Mabb., *Butea monosperma* (Lam.) Küntze and *Terminalia bellirica* (Gaertn.) Roxb. Were formulated in water based HPMC gel system. The prepared polyherbal vaginal wash formulation was assessed for its physicochemical properties, including texture evaluation, pH measurement, buffer capacity, specific gravity, solid contents, surface tension, and total phenolic contents.

Factorial Formulations

The effect of formulation variables on the responses was statistically evaluated by applying one way ANOVA, using the commercially available software package Design –Expert version 8.0 Optimization Design (2^3 Full Factorial Design) and the optimization of polyherbal intimate hygiene wash preparation containing three independent (X_1- concentration of *Acacia nilotica*, X_2 –concentration of *Butea monosperma*, X_3 – concentration of *Terminalia bellirica*) and one dependent variable (Y_1 = Zone of inhibition) was done (Table 1).

Table 1: Optimization Design Layout (2^3 Full Factorial Design) for Y_1

Run	Coded Value			Value (mg/ml)		
	X_1	X_2	X_3	X_1	X_2	X_3
1	+1	+1	-1	500	500	250
2	+1	-1	-1	500	250	250
3	-1	+1	+1	250	500	500
4	+1	-1	+1	500	250	500
5	-1	+1	-1	250	500	250
6	-1	-1	+1	250	250	500
7	+1	+1	+1	500	500	500
8	-1	-1	-1	250	250	250

Anti-fungal analysis techniques

Agar well diffusion method was used to screen the antifungal activity of phytoextracts against *Candida albicans* (MTCC No. 227) on Muller-Hinton agar plates [5]. Each plate was inoculated with 0.1 ml of the fungal culture. Then wells of 6 mm diameter were punched from the agar plates. To study this activity 50 µl of the formulation stock was loaded into the well. The formulation was compared with the commercially available products, V-Wash, Arogita and Vagitone. Plates were incubated at 25 ± 2°C for 72 hrs in BOD incubator. The antifungal activity was interpreted from the size of the diameter of zone of inhibition measured in millimetre (mm). All tests were conducted in triplicate, and the mean values along with standard deviations were recorded.

RESULTS

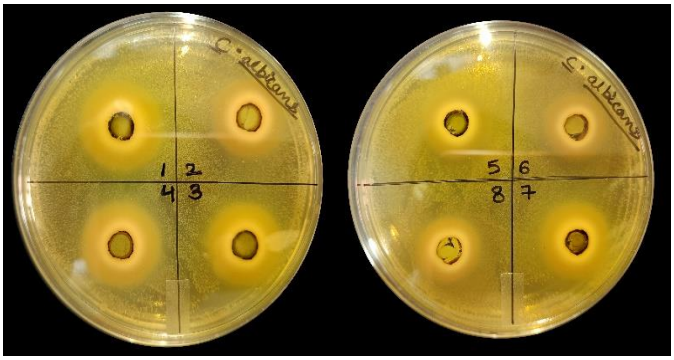
Preparation and Optimization of Polyherbal Formulation

Formulation was designed using factorial design i.e. Optimization Design (2^3 Full Factorial Design). The eight factorial batches were evaluated for antifungal activity. Various combinations of *Acacia nilotica*, *Butea monosperma* and *Terminalia bellirica* extracts were made to achieve a standardized intimate hygiene wash formulation after optimizations of each combination. Each combination was subjected to evaluation studies for consistent effectiveness and characterization used for antifungal intimate hygiene wash. As depicted in table 2, maximum antifungal activity against *C. albicans* was exhibited by batch 3 followed by batch 1.

Table 2: Result of study response Y1 (ZOI) of factorial batches (F1 to F8)

Concentration (mg/ml)			ZOI of *Candida albicans* (mm)
Acacia nilotica	*Butea monopserma*	*Terminalia bellirica*	
500	500	250	17.33 ± 0.57
500	250	250	6.33 ± 0.57
250	500	500	18.67 ± 0.57
500	250	500	6.67 ± 0.57
250	500	250	6.00 ± 0.00
250	250	500	15.33 ± 0.57
500	500	500	13.67 ± 0.57
250	250	250	8.00 ± 1.00

Figure 1a & 1b: Antifungal activity of factorial batches (F1 to F8) against *Candida albicans*



Study of the Response of Optimized Batch

During the present research, the formulated polyherbal formulation underwent rigorous testing and analysis using Design Expert software. Notably, there emerged a slight variation between the predicted values generated by the software and the observed values obtained through practical experimentation. Interestingly, the observed values consistently demonstrated a slight improvement over the predicted ones, indicating an optimistic trend in the formulation's performance. The zone of inhibition (ZOI) of *Candida albicans* was 14.29 and 16.61 mm, for predicted and observed ZOI, respectively. The difference between these values was deemed significant, indicating the effectiveness of the tested formulation in inhibiting the growth of *C. albicans*.

Response Surface Plot Analysis

In this study, Response Surface Plot Analysis was used to visualize the interaction effects of key formulation variables on the antifungal activity of the polyherbal vaginal wash. The plots illustrate the relationship between the independent variables and their combined effect on the zone of inhibition (ZOI) against *Candida albicans* (Figure 2). The analysis of the response surfaces indicates the optimal conditions for maximizing the antifungal efficacy. The plots also helped in understanding how changes in formulation parameters influenced the antifungal performance, providing insights into the formulation's overall effectiveness.

Desirability of Study Response

The observed desirability of study response was found to be close to one which indicates that the formulation is desirable. Desirability study response was observed to be 0.90 for antifungal activity (Figure 3). This value also suggests that the formulation is promising and could be considered successful.

Physico-Chemical Characteristics

A comparative analysis of the physico-chemical characteristics of the experimental polyherbal vaginal wash formulation and three commercial products—V-Wash, Arogita, and Ayouthveda was conducted as shown in Table 3. In terms of organoleptic properties, the experimental formulation had a brown colour, while V-Wash was transparent, Arogita was pink, and Ayouthveda was orange. All formulations were found to have an acceptable odour and smooth texture. The pH of the experimental formulation was 3.6, slightly more acidic as compared to V-Wash and Ayouthveda (4.3), and Arogita (5.0). The buffer capacity of the experimental formulation (1.50) was similar to that of V-Wash (1.49) and Arogita (1.51), while Ayouthveda had a lower value (1.42).

For specific gravity, the experimental formulation represented a value of 1.12 which is higher than the other commercial products i.e., V-Wash (0.54), Arogita (0.88) and Ayouthveda (0.65). The surface tension of the experimental product matched to that of V-Wash (0.044 N/m), whereas the values of Arogita and Ayouthveda were 0.084 N/m and 0.027 N/m, respectively. The experimental formulation had a solid content of 17.46%, lower than V-Wash (19.91%) but higher than Arogita (7.57%) and Ayouthveda (16.35%).

The total phenolic content of the experimental formulation exhibited a significantly higher value (98.82 mg/g GAE) as compared to V-Wash (95.65 mg/g GAE), Arogita (16.47 mg/g GAE), and Ayouthveda (41.06 mg/g GAE).

Figure 2: Response Surface Plot of ZOI against *C. albicans vs* Conc. of ethyl acetate extracts of *Butea monosperma* flower and *Acacia nilotica* bark.

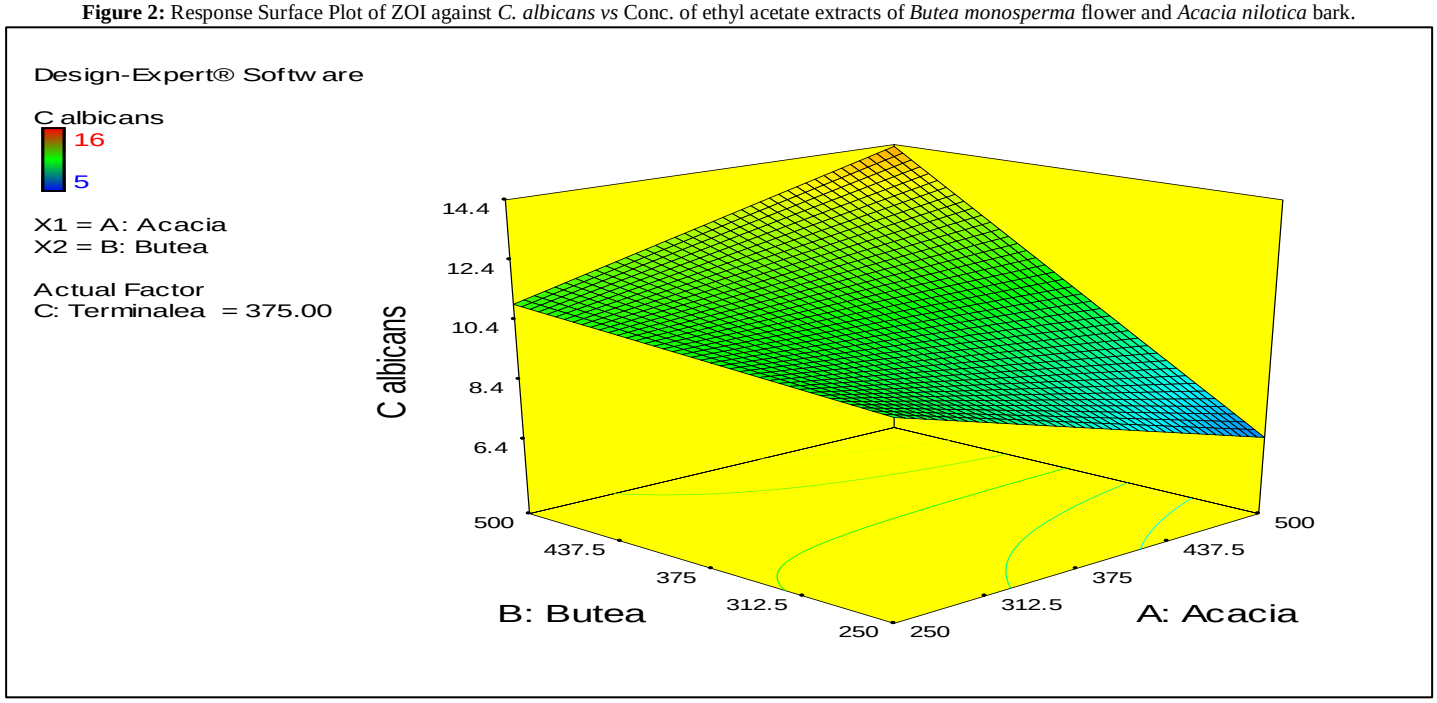


Figure 3: Desirability of Study Response for anti-fungal activity

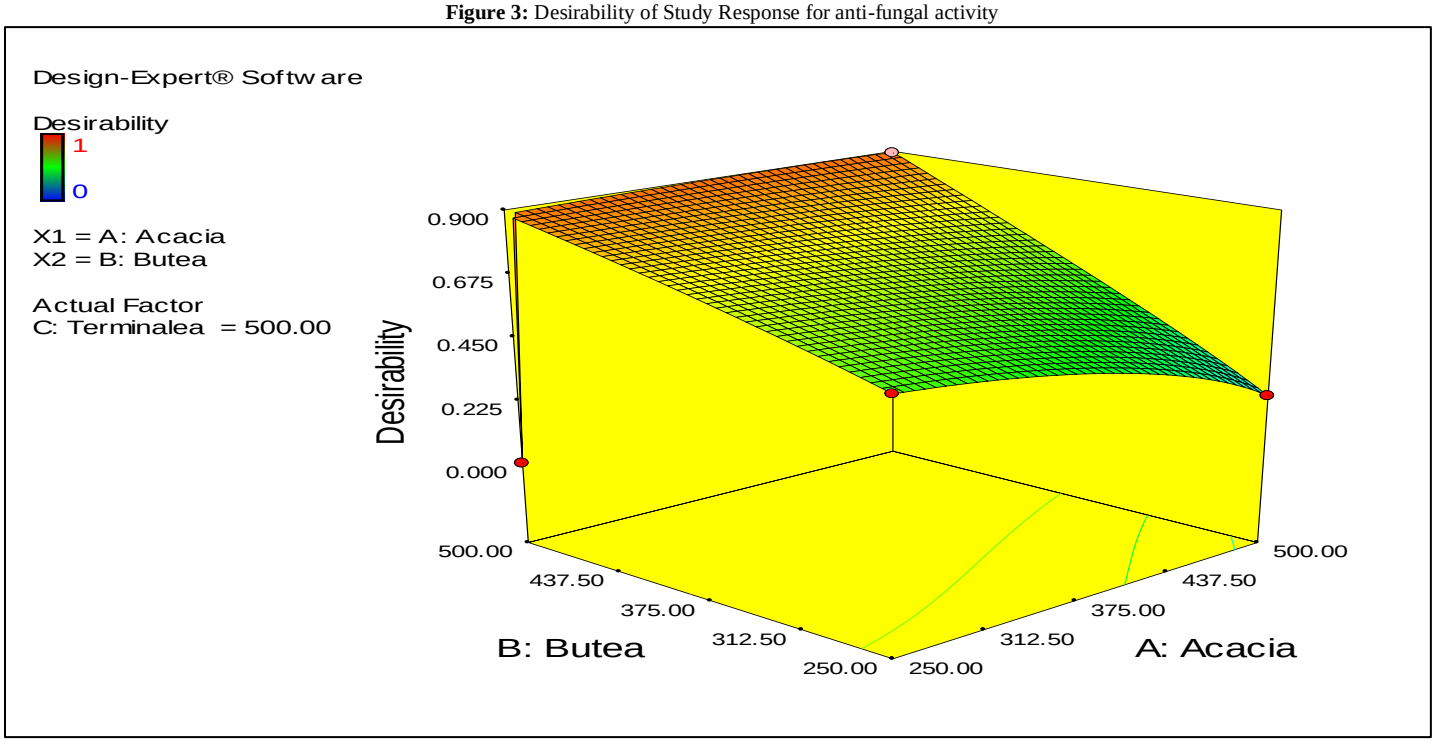


Table 3: Comparative study of the physico-chemical characteristics of the experimental formulation and selected commercial products

Physico-Chemical Characteristic	*Experimental Formulation*	*V-Wash*	*Arogita*	*Ayouthveda*
Organoleptic properties:				
Colour	Brown	Transparent	Pink	Orange
Odour	Acceptable	Acceptable	Acceptable	Acceptable
Texture	Smooth	Smooth	Smooth	Smooth
pH	3.6	4.3	5.0	4.3
Buffer capacity	1.50	1.49	1.51	1.42
Specific gravity	1.12	0.54	0.88	0.65
Surface tension	0.044 N/m	0.044 N/m	0.084 N/m	0.027 N/m
Solid Contents	17.46 %	19.91 %	7.57 %	16.35 %
Total Phenolic Contents	98.82 mg/g GAE	95.65 mg/g GAE	16.47 mg/g GAE	41.06 mg/g GAE
ZOI of C. albicans (mm)	16.61 ± 0.76	15.00 ± 1.00	12.33 ± 0.57	11.33 ± 0.57

Antifungal Activity of Feminine Hygiene Wash

The antifungal activities of the experimental polyherbal vaginal wash formulation, V-Wash, Arogita, and Ayouthveda were assessed through the zone of inhibition (ZOI) observed against *Candida albicans*. The experimental formulation showed a ZOI of 16.61 ± 0.76 mm, which was higher than the V-Wash (15.00 ± 1.00 mm). Arogita and Ayouthveda displayed lower ZOI values of 12.33 ± 0.57 mm and 11.33 ± 0.57 mm, respectively (Table 3). These results suggest that the experimental formulation has a stronger antifungal effect as compared to the commercial products tested in the laboratory.

DISCUSSION

The formulation was developed using a factorial design, specifically a 23 full factorial design, followed by a 3D response surface plot analysis for Candida albicans. The results showed minimal variation between the predicted and observed values across the batches. The desirability index for the study responses was found to be less than one. The medicinal plants incorporated into the polyherbal feminine hygiene wash have been traditionally recognized for their antifungal properties. The plants utilized for the formulation of the feminine wash include Acacia nilotica, Butea monosperma and Terminalia bellirica. This is a novel formulation.

The comparative analysis of the experimental polyherbal vaginal wash formulation with commercially available products, V-Wash, Arogita and Ayouthveda provides valuable insights into the effectiveness and quality of the herbal formulation. The organoleptic properties, including colour, odour and texture, indicate that while the experimental formulation however differs in appearance (brown in colour) but its odour and texture are satisfactory as compared to the market products, making it acceptable for consumer use.

The pH analysis is an important aspect of vaginal health because an acidic environment is crucial for preventing infections. The experimental formulation, with a pH of 3.6, is slightly more acidic than V-Wash and Ayouthveda (4.3) and Arogita (5.0). Its lower pH aligns more closely with the natural acidic environment of the vagina, potentially offering enhanced protection against microbial growth. Buffer capacity is another key factor for vaginal products. The experimental formulation displayed similar values to commercially accepted feminine washes like V-Wash and Arogita which indicates its ability to maintain a stable pH, even in the presence of external changes. However, Ayouthveda exhibited a slightly lower buffer capacity, which may impact its effectiveness in maintaining vaginal pH stability.

The specific gravity of the experimental formulation was found to be higher than that of the other products, indicating a denser formulation. Experimental formulations may contribute to better coating and adherence to vaginal tissues, it may also be easy in application. Surface tension measurements, analogous to V-Wash, suggest that the experimental product has comparable spreading properties. Arogita has higher surface tension which indicates that the product might not spread as easily, potentially affecting its effectiveness in thoroughly cleaning and covering the area. Ayouthveda has less surface tension which is an indication of its runny consistency. Vaginal washes with optimal surface tension are formulated to balance effective spreading and ease of rinsing, ensuring comfort and cleanliness without causing irritation.

The higher total phenolic contents in the experimental formulation (98.82 mg/g GAE) as compared to the commercial products reflects its strong antioxidant potential, which is important in combating oxidative stress and supporting overall vaginal health. This significant difference highlights the formulation's potential superiority in terms of delivering beneficial phytochemicals.

The ZOI indicates that the experimental polyherbal vaginal wash formulation outperformed the commercially available products in terms of antifungal efficacy against *Candida albicans*. With a ZOI of 16.61 ± 0.76 mm, it demonstrated superior antifungal activity as compared to V-Wash (15.00 ± 1.00 mm), Arogita (12.33 ± 0.57 mm) and Ayouthveda (11.33 ± 0.57 mm). These results suggest that the phytochemicals present in the experimental formulation may offer better antifungal properties, making it a promising alternative to synthetic chemical-based products like V-Wash. The higher antifungal activity also highlights the potential of polyherbal formulations to be more effective in managing *Candida albicans* infections, providing a natural, cost-effective and safer option for feminine hygiene.

Mariappan and coworkers formulated a feminine hygiene wash utilising extracts of *Citrus aurantifolia* juice, *Rosmarinus officinalis* leaf oil, *Azadirachta indica* leaf extract, *Salvia officinalis* oil, *Aloe barbadensis* leaf juice powder, *Thymus zygis* oil, *Vetiveria zizanoides* root oil. The formulation was found more effective against *C. albicans* then the marketed samples- V wash and Fair Beat[6]. The study of Aliyu and cowokers emphasized the importance of the selection of effective feminine washes for managing infections caused by *C. albicans*. The experimental polyherbal formulation, which showed strong antifungal activity, could be considered a viable alternative to commercial products. Their findings show that all feminine washes are not equally effective, particularly against fungal infections like that of *Candida albicans*[7]. Leo and Benvenuti evaluated the antibacterial, antifungal, anti-inflammatory, and antioxidant effects of the extracts from *Salvia officinalis*, *Thymus vulgaris*, *Chamomilla recutita*, and *Calendula officinalis* when used in intimate hygiene products. These findings demonstrated significant improvement in vaginal health and symptom reduction across different age groups. Their findings support the use of these products for tailored daily intimate hygiene[8]. Powar and Kanade developed a poly-herbal

vaginal wash from the extracts of *Azadirachta indica*, *Ocimum sanctum* and *Sapindus emarginatus* in a carbopol-940 gel base. The formulation demonstrated strong antifungal activity, acceptable physical properties, and stability, concluding that the poly-herbal vaginal wash is effective in preventing *C. albicans* infections[9].

CONCLUSION

The experimental polyherbal vaginal wash formulation shows promising results in terms of pH balance, buffer capacity, phenolic contents and antifungal activity. Overall, the experimental formulation demonstrates superior antioxidant and antimicrobial properties, along with a distinctive physico-chemical profile that could offer advantages in specific applications. The formulation developed by using a novel combination of plant extracts demonstrated enhanced antifungal activity. It can therefore be effective for prevention of vaginal infections. The experimental polyherbal vaginal wash formulation demonstrated superior antifungal efficacy against *Candida albicans* when compared to the commercial products available in the market viz. V-Wash, Arogita, and Ayouthveda. With the highest zone of inhibition among the tested formulations, it represented great potential as an effective natural alternative for managing *Candida* infections. The strong antifungal activity, combined with the benefits of using a plant-based formulation, suggests that the experimental product could be a valuable addition to the feminine hygiene solution range, offering both efficacy, safety and cost effectiveness.

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Conflict of Interest

The authors declare no competing interests.

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