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A Comprehensive Study On Formulation And Standardization Parameters For Polyherbal Intimate Wash

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ABSTRACT

Objectives:
The study aimed to develop and standardize a polyherbal intimate wash as a natural cosmeceutical alternative. Objectives included qualitative and quantitative standardization of herbal extracts, formulation development, stability assessment, user acceptability evaluation, and comparison with commercial products. Additionally, the formulation's anti-inflammatory, antifungal, and antioxidant activities were assessed in vitro.

Methods:
Selected herbs were extracted using suitable techniques and standardized through phytochemical screening and marker compound estimation. A polyherbal intimate wash was formulated using these standardized extracts. The formulation was evaluated for physicochemical properties, stability, and user acceptability. In vitro assays were conducted to determine anti-inflammatory, antifungal, and antioxidant activities. Comparative analysis was performed against commercially available vaginal wash products to assess efficacy.

Results:
The in-house formulation (IHF) demonstrated the highest antimicrobial activity against *Candida albicans* with a 24 mm zone of inhibition, compared to FFVW (18 mm) and marketed VW (10 mm). Anti-inflammatory activity, evaluated via protein denaturation assay, was found to be 82.72 ± 31.52% for IHF, 85.45 ± 1.09% for FFVW, and 84.77 ± 2.11% for VW. Antioxidant activity, measured using the hydrogen peroxide scavenging assay, was 92.80 ± 0.24% for IHF and 92.75 ± 0.40% for FFVW, indicating strong free radical scavenging potential.

Conclusion:
The study successfully developed and standardized a stable and effective polyherbal intimate wash with significant antimicrobial, anti-inflammatory, and antioxidant properties. High user acceptability and comparable or superior performance to commercial products suggest strong potential for market positioning as a safe and effective herbal cosmeceutical product

Keywords: Formulation, Standardization Parameters, Polyherbal Intimate Wash, High- Performance Thin-Layer Chromatography, Medicinal Herbs

1. INTRODUCTION

One of the most important aspects of contemporary personal hygiene and healthcare is the development and standardization of polyherbal intimate wash products [1]. Demand for these items has increased dramatically in recent years due to growing awareness of the significance of intimate cleanliness in preventing various illnesses and preserving general well-being [2]. Intimate washes that are polyherbal, made from a combination of herbal extracts, present a viable substitute for traditional chemical-based solutions, offering both cleansing and maybe medicinal advantages [3].

Due to their apparent safety and effectiveness, polyherbal intimate washes—which draw from the rich history of traditional herbal medicine—have grown in popularity [4]. Combining different plant extracts creates a synergistic effect in which each ingredient enhances the product's total effectiveness [5]. In addition, customers looking for natural alternatives to synthetic chemicals find the use of natural substances appealing, which fits with the growing trend towards sustainable and natural lifestyles [6].

Due to the complex nature of the herbal constituents and the requirement for uniformity and efficacy, these products, despite their popularity, present substantial problems in terms of formulation and standardization [7]. The variety in plant ingredients, extraction processes, and sourcing procedures can affect the quality and performance of polyherbal formulations [8]. Furthermore, maintaining uniformity and stability from batch to batch creates more challenges for product development and commercialization [9].

To guarantee the efficacy, safety, and quality of polyherbal intimate washes, a thorough investigation concentrating on formulation techniques and standardization factors is essential [10]. By combining the best aspects of both paradigms, this research aims to close the knowledge gap between conventional herbal medicine and modern science, creating dependable, empirically supported products [11].

We explore the complex process of creating intimate wash solutions made of polyherbal substances, with a focus on the compatibility and choice of herbs [12]. The selection of herbs, their synergistic effects, extraction techniques, and the addition of additional functional components to improve stability and efficacy are just a few of the many variables that must be carefully considered during the formulation process [13].

The study investigates the significance of standardization in guaranteeing the potency and uniformity of polyherbal formulations from batch to batch [14]. Important issues covered in this study include standardization factors including stability testing, microbiological quality, phytochemical profiling, and marker compound analysis [15]. Manufacturers can satisfy legal standards and preserve product quality by implementing standardized procedures, which gives customers and medical professionals trust [16].

By means of thorough testing and clinical trials, this study seeks to validate the safety and efficacy of polyherbal intimate wash solutions [17]. We want to present empirical evidence in favor of the product's use as a safe and effective replacement for conventional goods by analyzing its performance in real-world circumstances and evaluating its effects on intimate hygiene and general well-being [18].

The creation and standardization of polyherbal personal hygiene products is a complex process that calls for rigorous attention to detail and scientific validation [19]. This thorough investigation aims to decipher the complexity associated with creating these products, providing valuable perspectives on practical approaches and standardization criteria that are critical to guaranteeing their efficacy, safety, and quality [20]. In the end, we hope that our research will open new avenues for the creation of trustworthy and creative polyherbal intimate washes that meet the changing needs of consumers everywhere.

2. AIM AND OBJECTIVE

Aim - To formulate and develop the polyherbal intimate wash as cosmeceutical and to compare its efficacy with marketed formulations.

Objectives

To perform the extraction and standardize the extract qualitatively and quantitatively.

To formulate and evaluate polyherbal intimate wash.

To perform in vitro anti-inflammatory and antifungal activity.

To perform the comparative study of in-house polyherbal formulation with marketed preparations.

3. MATERIAL AND METHODS

Plant Profile

Ocimum sanctum Linn | *Piper betle* Linn | *Aloe vera* Linn

Figure 1: Plant Profile

Collection of plant material:

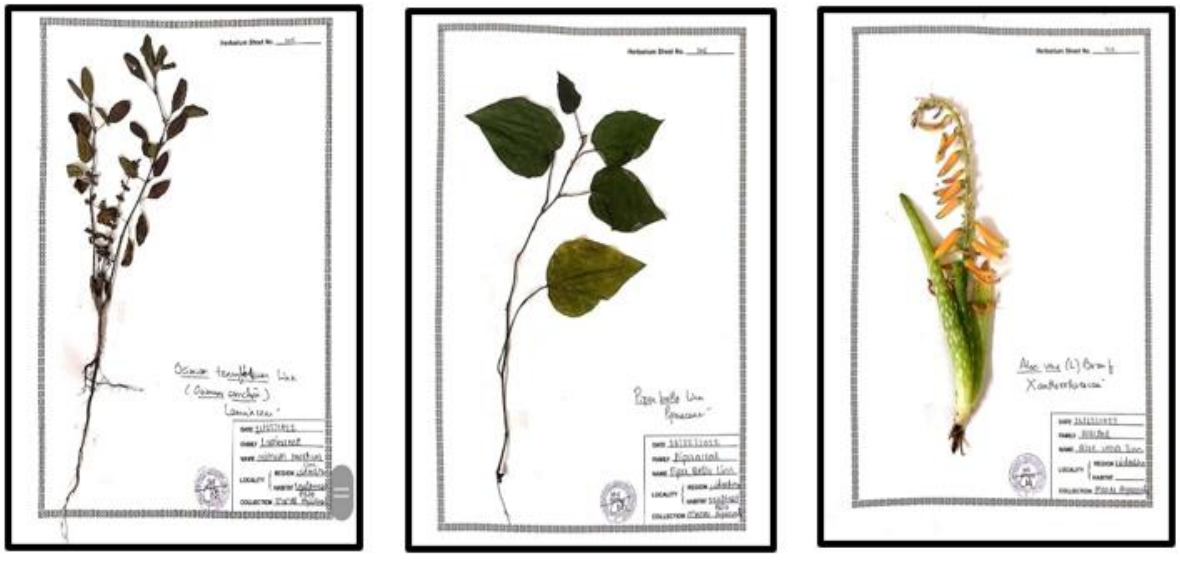
Purchasing raw ingredients from Manas Ayurveda in Nagpur, Maharashtra 440001

Authentication of plant material

Dr. Nitin Dongarwar, a professor in the Botany Department of Rashtrasant Tukadoji Maharaj Nagpur University in Nagpur, verified the authenticity of the collected plant material.

Table 1: Authentication of plant material

Sr. No.	Plants used	Part used	Authentication No.
1	*Ocimum sanctum*	leaves	105
2	*Piper betle*	leaves	106
3	*Aloe vera*	leaves	104



Ocimum sanctum Linn | *Piper betle* Linn | *Aloe vera* Linn

Figure 2: Herbarium Sheets

Physicochemical evaluation [21-23]

Spreading a weighted sample, visually classifying foreign matter, and then sifting through a sieve were the steps involved in

determining the presence of foreign particles in herbal products. A standard limit of no more than 2% was established for foreign particles. Plant material was evenly distributed in a porcelain dish, and the drying process was used to determine the loss on drying, with a standard limit of not more than 10%. A weighed sample was burned until it turned white, with a standard limit of no more than 18%, to calculate the total amount of ash. By incorporating hydrochloric acid into the total ash, up to a specified limit of 6%, the amount of acid-insoluble ash was ascertained. By boiling the entire amount of ash in water and deducting the residue from the total weight of ash, the amount of water-soluble ash was found. Plant material was submerged in a variety of solvents, filtered, concentrated, and dried to ascertain the extractive value, which was then computed as a percentage of the weight of the crude medication.

Macroscopical evaluation

A comparative macroscopic assessment was conducted, considering physical characteristics such leaves size, shape, colour, and odour

Extraction Process

Procedure: Cold maceration

Defatting of dried plant material: The verified plant material was dried beneath the shed, cleaned with tap water, and then roughly ground into a powder. For seven days, acetone maceration was used to defat the powdered plant material. The extract was collected and allowed to dry at room temperature following filtering. A distillation assembly was used to recover the acetone from the extract.

Extraction: 99.9% ethanol was used to macerate the defatted plant material for seven days. The extract was collected and concentrated in a water bath following filtering. The sticky final extract was kept dry in a desiccator.

Phytochemical screening [22]

Plants are natural labs that generate a wide range of molecules that have medicinal effects. These compounds are referred to as secondary metabolites and include alkaloids, glycosides, tannins, saponins, flavonoids, proteins, amino acids, steroids, phenols, and carbohydrates. To determine the main and secondary metabolites present in plant extracts, phytochemical screening procedures were performed. Molisch's test was one of the tests for carbs, and the test for proteins for amino acids, use the Xanthoprotein and Biuret tests. The test for steroids, Ninhydrin Test Salkowski for Glycosides The saponin test, or Keller-Kilani test the alkaloids can be tested with Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, and the flavonoids using the Shinoda test. The ferric chloride test, lead acetate test, potassium dichromate test, and bromine water test were used to test for tannins and phenols. These analyses aided in the identification of the different bioactive substances found in plant extracts

Quantitative estimation:

A gravimetric method was used to determine the total alkaloid content [24]. The drug was first extracted in powder form using 0.1N H2SO4, then the drug was basified, extracted using diethyl ether, and finally dried to determine the percentage of alkaloid. The Folin-Ciocalteau reagent was used to determine the total phenolic content [25], and the blue hue that resulted indicated the content's measurement at 765 nm. Using a spectrophotometer to measure the intensity at 775 nm, the total tannin content [25] was calculated based on the reduction of phosphotungstomolybdic acid by tannin-like compounds. The production of a flavonoid-aluminium combination was used to determine the total flavonoid content [25], and the absorbance was measured at 435 nm. By evaluating the yellow hue that resulted from the reaction of flavanol with aluminium trichloride, with absorbance measured at 440 nm, the total flavanol concentration was ascertained. Using ethanol and vanillin in a sulfuric acid solution, the absorbance was measured at 544 nm to determine the total saponin concentration [26]. By hydrolysing polysaccharides into monomeric units and measuring the green colour complex that results from furfural interacting with Anthrone reagent at 630 nm, the total carbohydrate content [27] was ascertained.

Chromatographic evaluation [28]

Silica gel G plates were made by spreading a homogenized mixture over them, drying them in an oven, and then activating them. This allowed for the use of the plates in Thin Layer Chromatography (TLC). Spots were placed to the plates after samples and standards were prepared in methanol. After developing the chromatograms in particular solvent solutions, the plates were allowed to air dry before being seen. To compare the extracts with standards, the Rf values were computed. The sophisticated technique known as High-Performance Thin-Layer Chromatography (HPTLC) is used to analyse herbal medications both qualitatively and quantitatively. Using HPTLC fingerprinting, phytochemical composition is evaluated by creating digital images of chromatograms. *Ocimum sanctum* and *Piper betle*, for instance, were fingerprinted utilizing chromatographic conditions, such as stationary and mobile phases, sample concentrations, and development parameters. Aloe-emodin was utilized as a standard in a different study that examined the qualitative and quantitative analysis of *Aloe vera* extract under chromatographic settings designed for identification and quantification. These methods provide accurate and effective ways to assess the phytochemical profiles of extracts from herbs

In-house formulation

Vitamins, herbal extracts, and proteins are among the substances used in intimate wash formulations to protect and cleanse the vaginal region while preserving pH equilibrium and enhancing skin health. Unlike other body cleansers, they have a different structure that emphasizes antibacterial qualities, low foam, simple rinsing off, and refreshing benefits. To cover up the smells of the herbal extract, a small amount of perfume is added together with preservatives, ideally free of parabens. For example, hydroxypropyl methylcellulose, plant extracts, surfactants, preservatives, glycerine, and perfume were blended to create a polyherbal intimate wash [29,30]. Next came physical examination, pH testing, viscosity evaluation [31,32], and skin irritancy testing using the HET-CAM method [33], which measures reactions on the chorioallantoic membrane of fertilized eggs.

In-vitro pharmacological activity

Assessing a compound's capacity to prevent or postpone oxidation processes brought on by oxygen or reactive oxygen species is known as in vitro antioxidant activity. This is important for several reasons, such as stabilizing food, medicines, and polymeric materials. Tests for hydrogen peroxide scavenging capacity establish an ethanolic extract's capacity to neutralize hydrogen peroxide [34,35], while assays such as membrane lysis assay, protein denaturation inhibition, and proteinase inhibitory activity are used in in vitro anti-inflammatory studies to evaluate an extract's ability to inhibit mechanisms related to inflammation [36]. Furthermore, the objective of in vitro antimicrobial investigations is to appraise the effectiveness of extracts against bacterial strains such as Candida albicans, Candida glabrata, Staphylococcus aureus, and Escherichia coli by employing methods like sub culturing, the agar well diffusion method, and measuring the inhibition of microbial growth. The study of natural extracts' medicinal potential for a range of health applications is aided by these assays [37,38].

Comparative study

Table 2: Comparative study of formulations

Formulations		
femifair v-wash	**V wash**	**IHF**
		
Ingredients		
Eleteria cardemomum (fruit) extract, *Triphala* (Fruit) extract, *Haridra* (Rhizome) extract, Kumar*i* (Leaf), *Chameli* (Flowers), Anantmul (Root), *Ushira* (Root), Excipients, sodium benzoate, Colours- Erythrosine.	Purified water, Triethanolaminelauryl sulphate, Coco amido propyl betaine, Peg7, Glyceryl cocoate, Lactic acid, Dehydro acetic acid, sorbitol, Hydroxypropyl cellulose, Polyquaternium-7, Fragrance, *Hyppophae rhamnoides* (sea buckthorn) seed oil, Sodium hydroxide, *Melaleuca alternifolia* (Tea tree) leaf oil, Hexyl cinnamal, Alpha-isomethyl Ionone, Coumarin, Linalool, Butyl phenyl Methyl propional.	Hydroxypropyl methylcellulose low viscosity 5, Hydroxy propyl cellulose, *Ocimum sanctum* (Leaf)extract, *Piper betle* (Leaf)extract, Alor vera(Leaf) extract, Sodium lauryl ether sulphate, Sodium benzoate, Glycerin, Rose oil, distilled water.

4. RESULTS

The source of the raw plant materials was Manas Ayurveda, located in Nagpur, Maharashtra 440001. Dr. Nitin Dongarwar, a professor in the Botany Department of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, verified the authenticity of the materials. The macroscopical examination of the plant materials showed that *Ocimum sanctum* (OS) leaves are oval, green, and aromatic. They also have an astringent flavor with hints of pepper. The heart-shaped, green leaves of the *Piper betle* (PB) have a strong flavor that is bitter and spicy. The leaves of *Aloe vera* (AV) are bitter-tasting, lance-shaped, and green with a strong oniony smell.

The plant extracts underwent physicochemical evaluation, with parameters compared to the benchmark limitations established by the Ayurvedic Pharmacopoeia 2015. Values of 0.87% for OS, 1.04% for PB, and 0.07% for AV in the determination of foreign particles were all within the permissible limit of not more than 2%. Each of the three categories saw a drying loss below the 10% threshold: 6.70% for OS, 7.65% for PB, and 3.95% for AV. For OS, PB, and AV, the total ash content was 17.62%, 16.28%, and 4.13%, respectively, all falling within the allowed range of 18%. The percentage of acid-insoluble ash was less than 6% for OS, 2.68% for PB, and 1.71% for AV. For OS, PB, and AV, the water-insoluble ash values were 9.14%, 8.32%, and 9.21%, respectively. Water-soluble extractives were 18.88% for OS, 21.94% for PB, and 60.57% for AV, whereas alcohol-soluble extractives were 6.77% for OS, 10.37% for PB, and 80.6% for AV. These extractive values varied.

Over the course of seven days, the maceration method was used to extract the plant components. After calculating the extracts' percent yields, the results showed that EEOS (110g of crude drug used), EEPB (150g of crude drug used), and EEAV (150g of crude drug used) had yields of 5.85%, 7.78%, and 4.92%, respectively.

Following a preliminary phytochemical screening of the plant extracts, the following is a summary of the findings. Molisch's assay was used to confirm that carbohydrates were present in all extracts (EEOS, EEPB, and EEAV). Using the Xanthoprotic and Biuret assays, proteins were found in every extract. The Ninhydrin test showed that amino acids were present in EEOS and EEAV but not in EEPB. Salkowski test was used to detect steroids in all extracts. The Keller-Killiani test indicated that glycosides were only present in EEAV.

The results of the Foam test indicated that saponins were present in all extracts. The Shinoda test revealed the presence of flavonoids in every extract. The ferric chloride, lead acetate, and potassium dichromate tests verified the presence of tannins and phenolic chemicals in all extracts; however, the bromine water test yielded positive results only for EEOS and EEPB and not for EEAV.

All extracts included alkaloids, as shown by the results of the Dragendorff, Hager, Wagner, and Mayer tests. This thorough screening reveals that there are many different phytoconstituents present in abundance in all of the plant extracts.

Quantitative Estimation of Phytochemicals

Table 3 displays the absorbance values for six distinct chemicals at varying concentrations: diosgenin, dextrose, gallic acid, and quercetin (which is shown twice, but is probably the same substance). Absorbance measurements were made for each material at a range of concentrations, from 20 to 120 units. Gallic acid absorbance data show a proportionate relationship between concentration and absorbance, rising steadily from 0.057 at a concentration of 20 to 0.4912 at 120. The absorbance values of quercetin exhibit a comparable pattern, starting at 0.24 at a concentration of 20 and increasing to 1.12 at 120, indicating a robust linear association. When compared to quercetin and gallic acid, dextrose's absorbance increases less sharply with concentration; it begins at 0.3137 at a concentration of 20 and reaches 0.3512 at 100. Diosgenin has a notable increase in absorbance, rising from 0.1861 at 20 to 1.6 at 100, demonstrating a robust positive connection between absorbance and concentration. According to the data, each chemical appears to have a distinct linear connection between concentration and absorbance. By measuring the absorbance of these substances, one can use this relationship to determine the concentration of these compounds in samples that are unknown.

Table 3: Values of Absorbance for Different Substances at Various Concentrations

Sr. No.	Absorbance values of gallic acid		Absorbance values of quercetin		Absorbance values of quercetin		Absorbance values of gallic acid		Absorbance values of dextrose		Absorbance values of diosgenin	
	Concentrations	Absorbance	Concentrations	Absorbance	Concentrations	Absorbance	Concentrations	Absorbance	Concentrations	Absorbance	Concentrations	Absorbance
1	20	0.057	20	0.24	20	0.24	20	0.057	20	0.3137	20	0.1861

2	40	0.1332	40	0.47	40	0.47	40	0.1202	40	0.3258	40	0.489
3	60	0.2321	60	0.57	60	0.66	60	0.2311	60	0.3397	60	0.83
4	80	0.3153	80	0.80	80	0.81	80	0.3151	80	0.3472	80	1.2
5	100	0.4207	100	0.96	100	0.98	100	0.4105	100	0.3512	100	1.6
6	120	0.4912	120	1.12	120	1.12	120	0.4732				

Chromatographic Evaluation

We used Thin Layer Chromatography (TLC) to analyze EEOS, EEPB, and EEAV qualitatively. The solvent systems utilized for EEOS and EEPB were n-hexane: ethyl acetate (8:2) and toluene: ethyl acetate: glacial acetic acid (8:2:3). Using aloe-emodin as the reference, EEAV was examined in a pet ether, ethyl acetate, and formic acid (7.5:2.5:0.1) solvent solution. Aloe-emodin was found in EEAV by High-Performance Thin Layer Chromatography (HPTLC) study. The Rf value for the standard and the EEAV sample was 0.22, and the mobile phase was Pet. Ether: Ethyl acetate: Formic acid (7.5:2.5:0.1). Aloe-emodin was present in EEAV at a concentration of 41.84 µg/mL. Furthermore, EEOS and EEPB fingerprinting was carried out via HPTLC employing the Toluene: Ethyl acetate: Glacial acetic acid (8:2:3) solvent system.

The in-house formulations were assessed according to their viscosity, pH, homogeneity, color, and odor. Each of the six formulations had good homogeneity, a pleasant odor, and a greenish-brown color. The viscosity varied from 0.0031 P to 1.092 P, and the pH values ranged from 2.09 to 5.96. Formulation F3, which had a greenish-brown hue, a pleasant odor, good homogeneity, a pH range of 3.98–4.06, a viscosity of 1.092 P, and a satisfactory spread ability, was the most consistent of these. As a result, F3 was determined to be the optimal batch and chosen for a thorough comparison analysis to confirm its superiority in vitro research.

3.2.1. Thin Layer Chromatography

Table 4: Qualitative analysis of EEOS, EEPB, EEAV by TLC

Sr. No.	Sample	Standard	Solvent system	Ratio
1	EEOS	-	Toluene: Ethyl acetate: Glacial acetic acid n-hexane: ethyl acetate	8:2:3 8:2
2	EEPB	-	Toluene: Ethyl acetate: Glacial acetic acid n-hexane: ethyl acetate	8:2:3 8:2
3	EEA	Aloe emodin	Pet. Ether: Ethyl acetate: formic acid	7.5:2.5:0.1

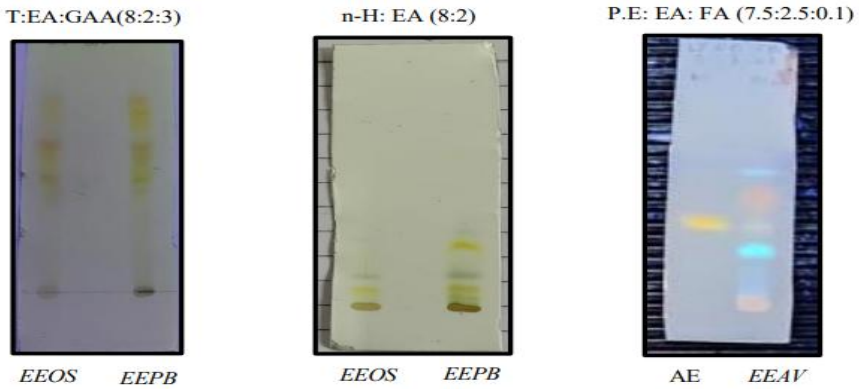


Figure 3: Thin layer chromatography of ethanolic extracts

3.2.2. High performance thin layer chromatography (HPTLC):

Table 5: Identification of Aloe- emodin in EEAV by HPTLC analysis

Sr. No.	Sample	Mobile phase	Rf value	Quantity found
1	Aloe – emodin (std)	Pet. Ether: Ethyl Acetate: Formic acid (7.5:2.5:0.1)	0.22	-
2	EEAV (sample)	Pet. Ether: Ethyl Acetate: Formic acid (7.5:2.5:0.1)	0.22	41.84 ug/mL

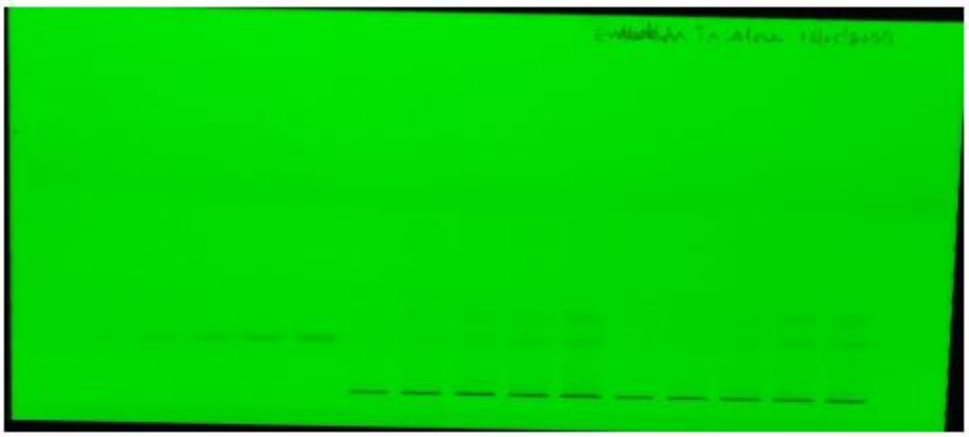


Figure 4: HPTLC chromatogram of aloe- emodin and ethanolic extract of *Aloe vera* Linn 254 nm.

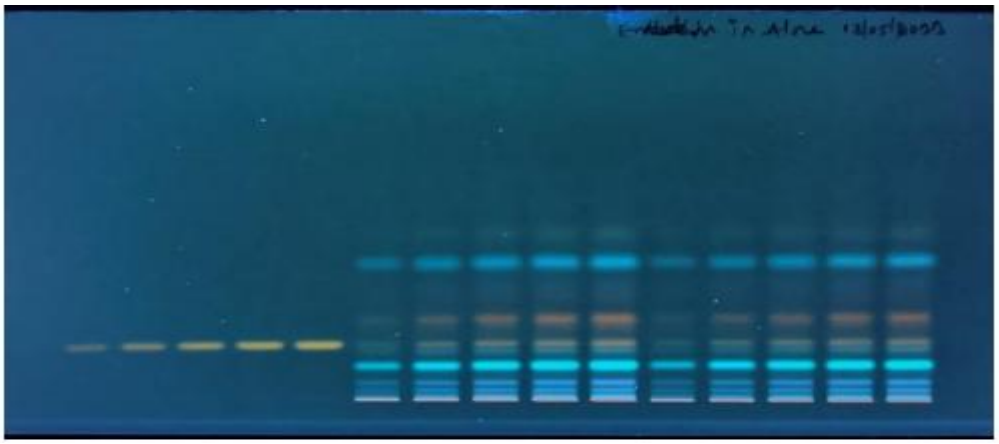


Figure 5: HPTLC chromatogram of aloe- emodin and ethanolic extract of *Aloe vera* Linn 366 nm.

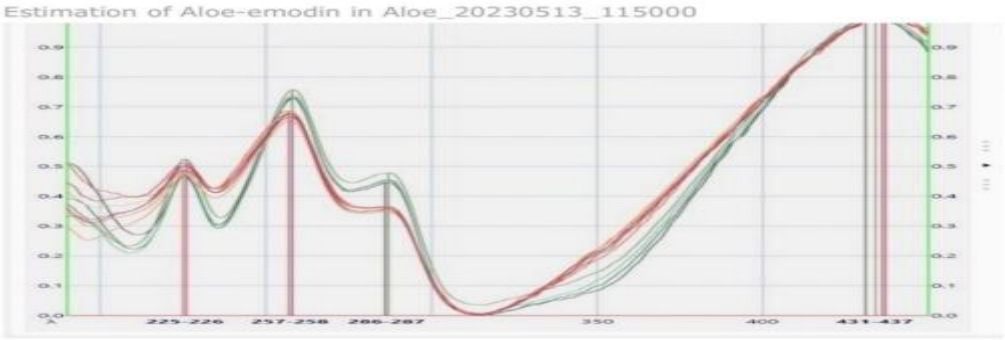


Figure 6: Overlapping spectrum of Aloe- Emodin standard and EEAV

3.2.3. Fingerprinting of EEOS & EEPB

Table 6: Qualitative analysis of EEOS & EEPB by HPTLC

Sr. No.	Sample	Solvent system	Ratio
1	EEOS	Toluene: Ethyl acetate: Glacial acetic acid	8:2:3
2	EEPB	Toluene: Ethyl acetate: Glacial acetic acid	8:2:3

Figure 7: HPTLC fingerprint of ethanolic extract of *Ocimum sanctum* Linn. At visible light

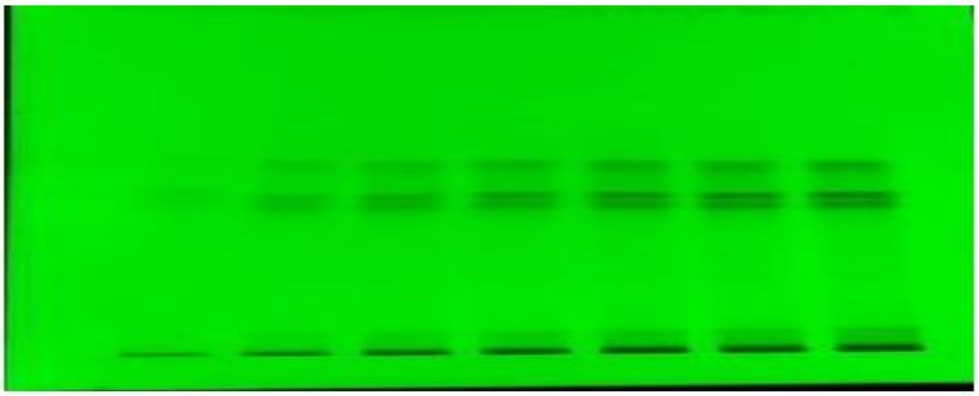


Figure 8: HPTLC fingerprint of ethanolic extract of *Ocimum sanctum* Linn 254 nm.

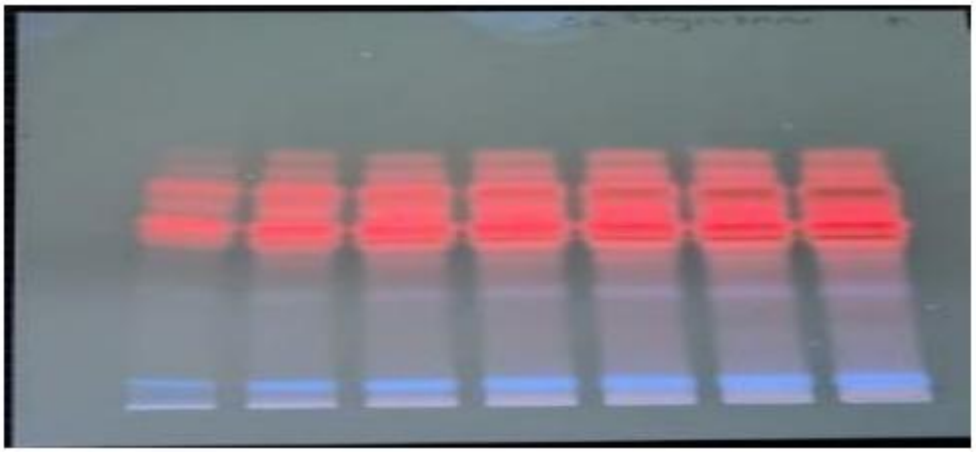


Figure 9: HPTLC fingerprint of ethanolic extract of *Ocimum sanctum* Linn 366 nm.

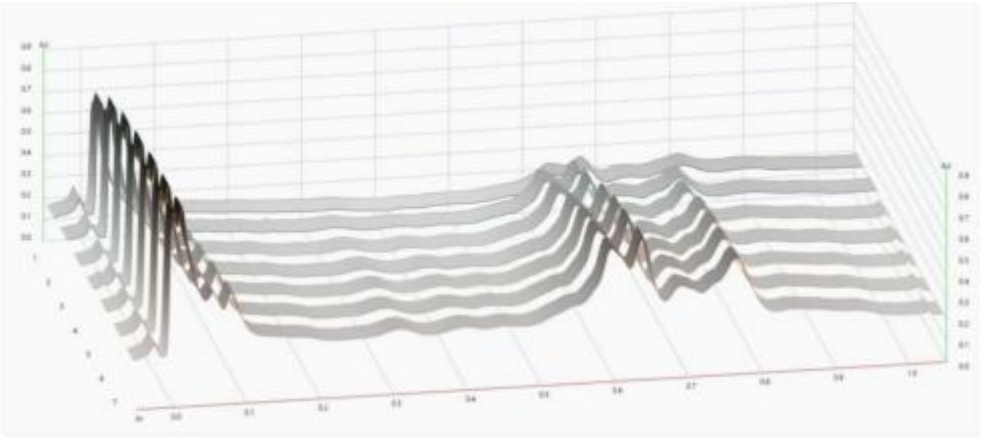


Figure 10: 3D view of scan at 254 nm of HPTLC fingerprint of ethanolic extract of *Ocimum sanctum* Linn.

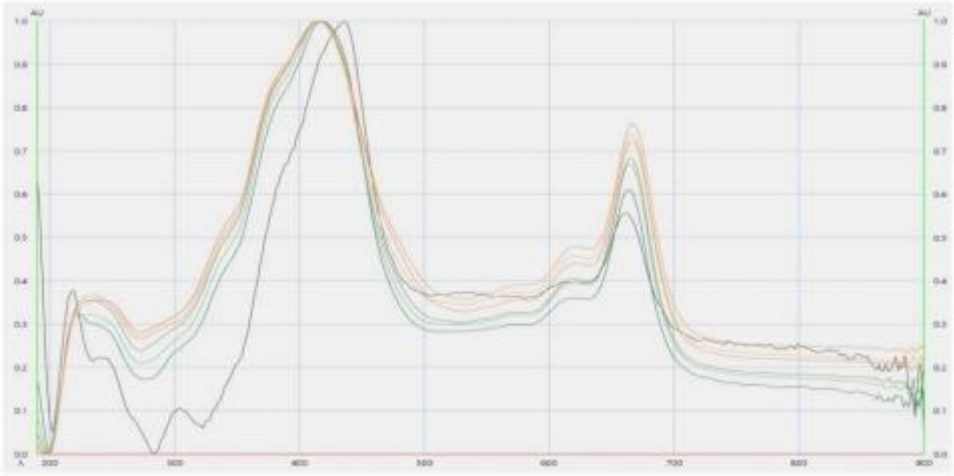
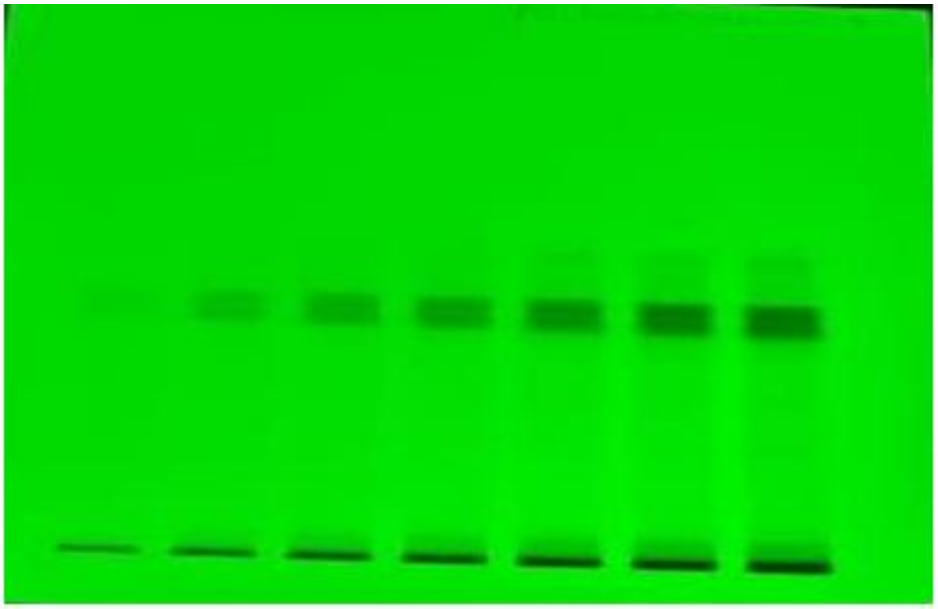


Figure 11: Overlapping spectrum of ethanolic extract of *Ocimum sanctum* Linn.at 190-900 nm

Figure 12: HPTLC fingerprint of ethanolic extract of *Piper betle* Linn. At visible light



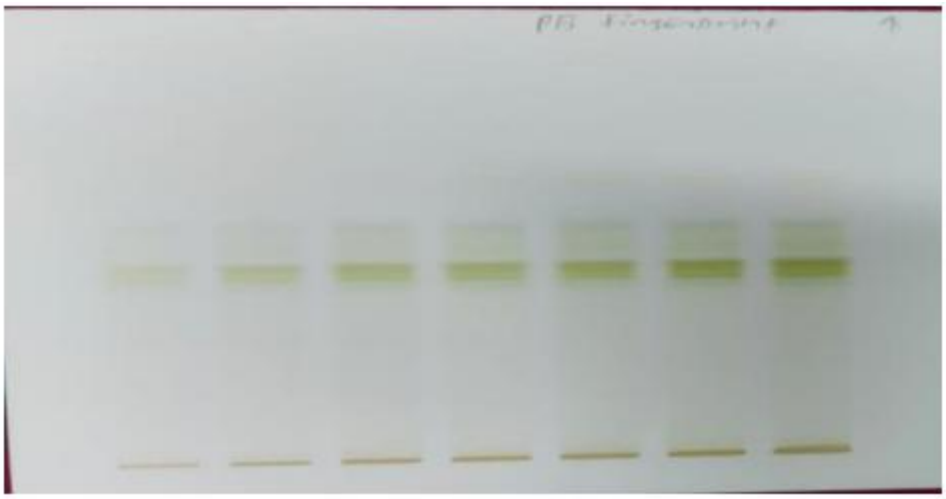


Figure 13: HPTLC fingerprint of ethanolic extract of *Piper betle* Linn. 254 nm.

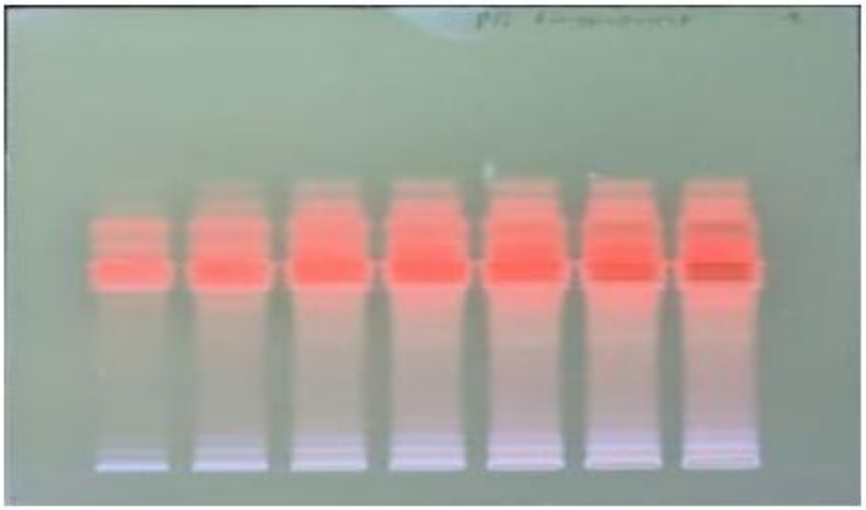


Figure 14: HPTLC fingerprint of ethanolic extract of *Piper betle* Linn 366 nm.

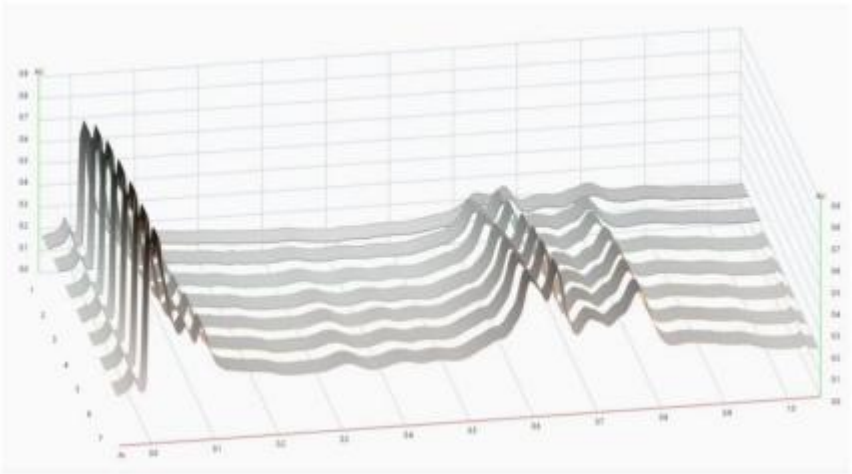


Figure 15: 3D view of scan at 254 nm of HPTLC fingerprint of ethanolic extract *Piper betle* of Linn

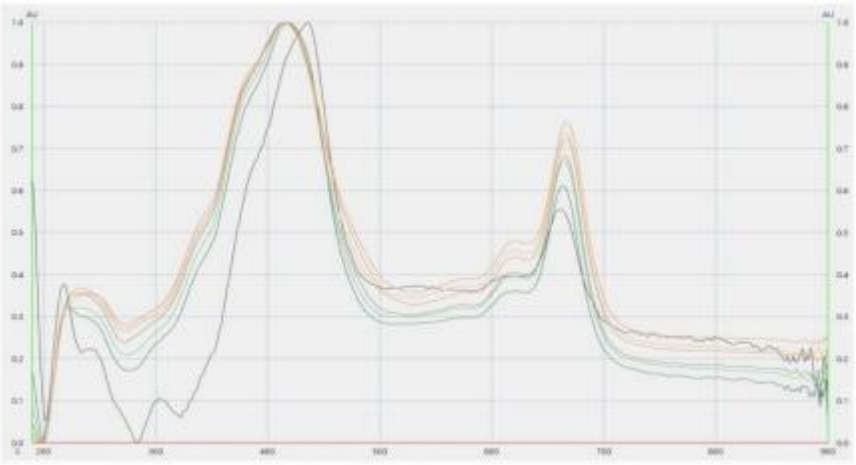


Figure 16: Overlapping spectrum of ethanolic extract of *Piper betle* Linn.at 190-900 nm.

In-Vitro Pharmacological Evaluation

Antioxidant activity

Table 7 presents information on the antioxidant activity (% inhibition of absorbance) of three plant extracts (*Ocimum sanctum* Linn., *Piper betle* Linn., and *Aloe vera* Linn.) at different concentrations (100, 200, 300, and 400 ppm) in relation to a standard (ascorbic acid). As a baseline, the control sample has an absorbance of 1.237 and 0% inhibition. Strong dose-dependent antioxidant action is exhibited by ascorbic acid, with percentage inhibition rising from 33.42% at 100 ppm to 94.18% at 400 ppm. The percentage of inhibition for *Ocimum sanctum* Linn. increases from 16.02% at 100 ppm to 82.54% at 400 ppm, indicating moderate antioxidant activity. A comparable pattern is seen in *Piper betle* Linn., which exhibits 14.76% inhibition at 100 ppm and 89.19% at 400 ppm. At lower concentrations, *Aloe vera* Linn. exhibits the least efficient antioxidant activity, showing just 11.66% inhibition at 100 ppm; but, at 400 ppm, it reaches a considerable 82.49% inhibition. Overall, the results show that all plant extracts have strong antioxidant qualities, and that these qualities increase with concentration. However, none of the extracts are more effective than ascorbic acid at % inhibition at comparable concentrations.

Table 7: Ascorbic acid, EEOS, EEPB, and EEAV's capacity to scavenge hydrogen

Samples	Concentration(ppm)	Absorbance	%Inhibition
Control	-	1.237	
Standard (Ascorbic acid)	100	0.824	33.42±1.14
	200	0.313	74.63±0.28
	300	0.160	87.02±0.08
	400	0.071	94.18±0.12
Ocimum sanctum Linn.	100	1.039	16.02±0.74
	200	0.836	32.45±2.31
	300	0.541	56.28±2.11
	400	0.216	82.54±1.25
Piper betle Linn	100	1.054	14.76±0.45
	200	0.708	42.54±1.90
	300	0.343	72.25±1.63
	400	0.133	89.19±0.34

Aloe vera Linn.	100	1.072	11.66±2.84
	200	0.904	26.90±0.42
	300	0.634	48.77±1.86
	400	0.216	82.49±0.86

Anti-inflammatory activity

Table 8 shows the results of tests on protein denaturation, proteinase inhibition, and membrane stabilization at varying doses (1000 ppm) of extracts from *Ocimum sanctum* Linn., *Piper betle* Linn., and *Aloe vera* Linn. in comparison to a standard (Diclofenac). Baseline measurements are represented by the control values. The standard, diclofenac, shows significant effects in all three experiments, with corresponding absorbance values of 0.0058, 0.3623, and 0.1068, and percentage inhibitions of 86.16%, 85.71%, and 84.5 percent. With absorbance values of 0.0233, 0.4682, and 0.269 and percentage inhibitions of 47.04%, 75.53%, and 61.02%, respectively, *Ocimum sanctum* Linn. exhibits modest effects in all assays. With absorbance values of 0.0227, 0.8832, and 0.310 and percentage inhibitions of 48.41%, 53.83%, and 55.07%, respectively, *Piper betle* Linn. has variable effectiveness. *Aloe vera* Linn. exhibits strong effects in the assays for membrane stabilization and protein denaturation, with absorbance values of 0.0061 and 0.194, respectively, and percentage inhibitions of 86.14% and 71.88%. However, the proteinase inhibition assay shows much weaker inhibition, with an absorbance of 0.6450 and a percentage inhibition of 66.28%. All in all, the data point to the potential therapeutic effects of plant extracts, with differences in effectiveness observed in several biological experiments, underscoring the complexity of their pharmacological profiles.

Table 8: Comparison of the Biological Impacts of Standard (Diclofenac) and Plant Extracts

Samples	Protein Denaturation Method			Proteinase Inhibition Method			Membrane Stabilization Assay		
	Concentration (ppm)	Absorbance	%Inhibition	Concentration (ppm)	Absorbance	%Inhibition	Concentration (ppm)	Absorbance	%Inhibition
Control	-	0.044		-	1.9131		-	0.6901	-
Standard (Diclofenac)	1000	0.0058	86.81±2.303	1000	0.3623	81.06±1.91	1000	0.1068	84.5±1.08
Ocimum sanctum Linn.	1000	0.0233	47.04±2.1	1000	0.4682	75.53±1.11	1000	0.269	61.02±1.97
*Piper betle*Linn.	1000	0.0227	48.41±0.912	1000	0.8832	53.83±2.03	1000	0.310	55.07±0.97
*Aloe vera*Linn.	1000	0.0061	86.14±1.58	1000	0.6450	66.28±1.83	1000	0.194	71.88±1.54

Antimicrobial activity

Table 9 shows the zone of inhibition, expressed in millimetres, for a variety of samples, including standards (Fluconazole and Ciprofloxacin), a control (Ethanol), single plant extracts (*Ocimum sanctum* Linn., *Piper betle* Linn., and *Aloe vera* Linn.), and three distinct combinations of these extracts. The organisms include S. aureus, E. coli, C. albicans, and C. glabrata. With zones of 17 mm and 29 mm, respectively, Ciprofloxacin has strong inhibitory effects against S. aureus and E. coli, while Fluconazole shows significant inhibition against C. albicans and C. glabrata, with zones of 30 mm and 28 mm, respectively. *Aloe vera* Linn. showed substantial inhibition against E. coli, while *Piper betle* Linn. has the best inhibition against S. aureus and C. albicans among the various plant extracts. However, the efficacy varies depending on the ratios in which they are

combined; the combination that exhibits the highest inhibition against E. coli and C. glabrata is 2:2:1. These results imply that mixtures of plant extracts may provide improved antimicrobial action against various diseases, hence indicating the need for additional research into their synergistic effects.

Table 9: Zone of inhibition for S. aureus, E. coli, C. albicans, C. glabrata, EEPB, EEAV, Standard, and three distinct combinations of extracts.

Samples (50uL)	Zone of inhibition of organisms (mm)			
	S. aureus	E. coli	C. albicans	C. glabrata
Standard (Ciprofloxacin1mg/ml)	17	29	-	-
Standard (Fluconazole1mg/ml)	-	-	30	28
Control(Ethanol)	-	-	-	-
*Ocimum sanctum*Linn.	11	11	10	11
Piper betle Linn.	16	13	15	13
Aloe vera Linn.	14	14	10	8
1:1:1(Os: Pb: Av)	10	9	10	9
1:2:1(Os: Pb: Av)	13	13	12	11
2:2:1(Os: Pb: Av)	16	18	16	16

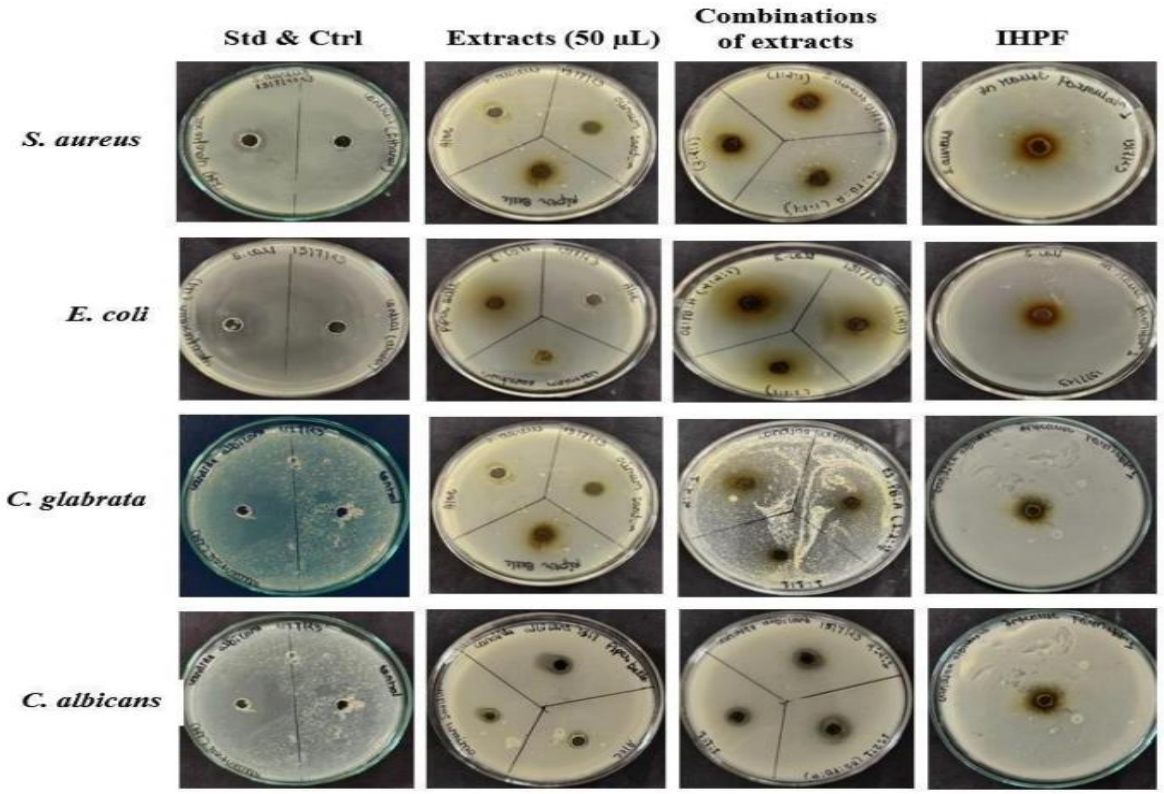


Figure 17: Zone of inhibition of EEOS, EEPB, EEAV, Standard, control, and three different combination of extracts for S. aureus, E.coli, C. albicans, C. glabrata.

Comparative Study

Evaluation parameter of formulations

Table 10 presents a comparison of different evaluation parameters between femifair v-wash, V Wash, and an internal formulation (F3). Color-wise, V Wash is colorless, femifair v-wash is pink, and F3 looks greenish brown. The pleasant odor of all three formulations suggests that people may find them acceptable. For all formulations, homogeneity is said to be good, indicating consistency in composition. But there are noticeable variations in viscosity and pH. femifair v-wash has a pH range of 5.32 to 5.90, while V Wash has a pH range of 2.5-3.36. F3 has a pH range of 3.98-4.06. This suggests that femifair v-wash is less acidic than F3 and V Wash. Furthermore, compared to femifair v-wash (0.0446 Poise) and V Wash (1.630 Poise), F3 has a higher viscosity (1.092 Poise), which may indicate variations in texture and/or application. All things considered, these comparisons offer insights into the unique qualities of each formulation, which can help customers and healthcare professionals choose the best product depending on their needs and preferences.

Table 10: Comparative evaluation metric for femifair v-wash, V wash, and in-house formulation

Formulation / Evaluation Parameter	In-house formulation(F3)	femifair v-wash	Vwash
Colour	Greenish brown	Pink	Colourless
Odour	Pleasant	Pleasant	Pleasant
Homogeneity	Good	Good	Good
pH	3.98-4.06	5.32-5.90	2.5-3.36
Viscosity (Poise)	1.092	0.0446	1.630

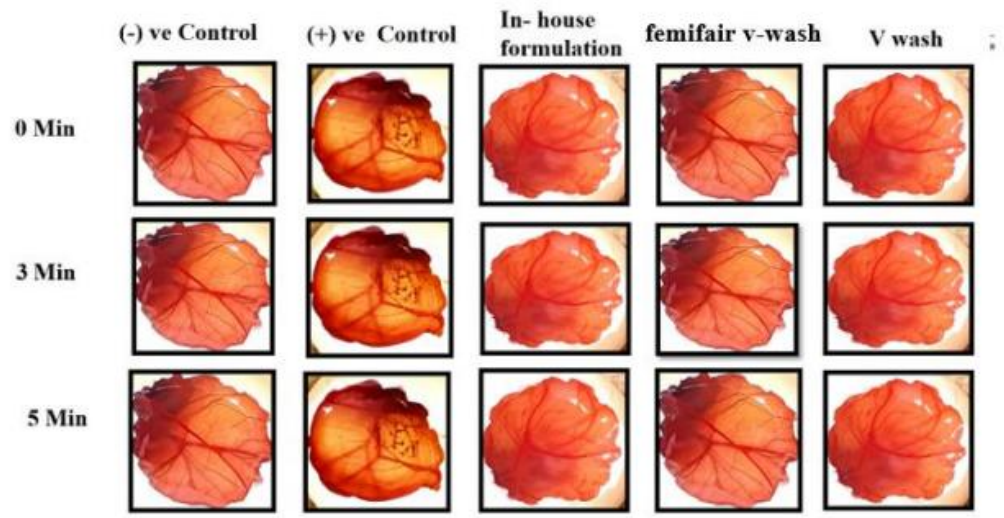


Figure 18: Images showing the vascular effects of different substances applied on the Chorio-allantoic membrane over a period of 5 min. (1) Sodium chloride (0.9 %w/v) (2) 0.1 N sodium hydroxide (3) In-house herbal formulation (4) femifair v-wash (5) V wash

In-vitro pharmacological evaluation

Anti-oxidant study

As evaluated by absorbance and percentage inhibition, Table 11 displays the hydrogen scavenging capacity of many materials

at varying concentrations (100, 200, 300, and 400 ppm), including ascorbic acid (the standard), an in-house formulation (IHF), femifair v-wash (FFVW), and V Wash (VW). The control acts as a reference point. The standard, ascorbic acid, demonstrates a potent scavenging ability; the percentage inhibition rises with concentration, from 33.42% at 100 ppm to 94.18% at 400 ppm, suggesting a dose-dependent impact. IHF has a moderate amount of hydrogen scavenging action; at 400 ppm, the percentage inhibition increased from 30.13% at 100 ppm to 92.80%. FFVW and VW exhibit comparable patterns, with the percentage of inhibition rising with concentration; however, their effectiveness is marginally lower than that of IHF and ascorbic acid. All examined formulations appear to have antioxidant qualities overall, with differences in efficacy and dose-dependent effects. These findings may help to explain the formulations' potential use in scavenging dangerous free radicals and oxidative stress.

Table 11: Ascorbic acid's ability to scavenge hydrogen; in-house formulation; femifair v-wash and v wash.

Samples	Concentration(ppm)	Absorbance	%Inhibition
Control	-	1.237	
Standard	100	0.824	33.42±1.14
	200	0.313	74.63±0.28
	300	0.160	87.02±0.08
	400	0.071	94.18±0.12
IHF	100	0.864	30.13±3.27
	200	0.519	58.06±1.53
	300	0.228	81.52±0.80
	400	0.089	92.80±0.24
FFVW	100	0.828	33.09±1.50
	200	0.605	51.08±0.37
	300	0.237	80.79±2.36
	400	0.089	92.75±0.40
VW	100	1.042	15.78±1.90
	200	0.806	34.84±0.68
	300	0.370	70.03±3.24
	400	0.13	89.51±1.34

Anti-inflammatory study

The assessment of membrane stabilization, protein denaturation, and proteinase inhibition assays for various samples—including a control, a standard (Diclofenac), an internal polyherbal formulation, femifair v-wash, and V Wash—is shown in Table 12 and is conducted at a concentration of 1000 ppm. Lower results in the protein denaturation assay indicate better inhibition, while the absorbance values show the degree of denaturation of the protein. The standard, diclofenac, exhibits considerable inhibition in all assays; absorbance values range from 0.0058 to 0.1068, and the percentage of inhibitions falls between 81.06% and 86.81%. Comparable efficacy is shown by the proprietary polyherbal formulation, which has absorbance values between 0.0076 and 0.132 and percentage inhibitions between 75.02% and 82.72%. In a similar vein, femifair v-wash and V Wash exhibit inhibition in every experiment, suggesting possible therapeutic benefits. These results emphasize the studied formulations' potential as therapeutic choices for disorders involving membrane damage and protein denaturation by indicating that they have anti-inflammatory and membrane-stabilizing qualities.

Table 12: Comparative Analysis of Membrane Stabilization, Protein Denaturation, and Proteinase Inhibition Assays for Various Formulations

	Protein denaturation method	Proteinase inhibition assay	Membrane stabilization assay

Samples									
	Concentration(ppm)	Absorbance	%Inhibition	Concentration(ppm)	Absorbance	%Inhibition	Concentration(ppm)	Absorbance	%Inhibition
Control	-	0.044		-	1.9131		-	0.6901	-
Standard (Diclofenac)	1000	0.0058	86.81±2.303	1000	0.3623	81.06±1.91	1000	0.1068	84.5±1.08
In-house polyherbal formulation	1000	0.0076	82.72±31.52	1000	0.478	75.02±0.96	1000	0.132	80.87±1.67
femifair v-wash	1000	0.0064	85.45±1.090	1000	0.416	78.25±1.33	1000	0.168	75.65±0.84
V wash	1000	0.0067	84.77±2.11	1000	0.459	76.00±2.13	1000	0.119	82.75±2.15

Anti- microbial study

S. aureus, E. coli, C. albicans, and *C. glabrata* are among the organisms for which Table 13 shows the zone of inhibition, measured in millimeters, in response to a variety of samples, including standards (Fluconazole and Ciprofloxacin), a control (Ethanol), an in-house formulation (IHF), femifair v-wash (FFVW), and V Wash (VW). Significant inhibition is shown by ciprofloxacin against *S. aureus* and *E. coli*, with zones measuring 21 mm and 29 mm, respectively, and by fluconazole against *C. albicans* and *C. glabrata*, with zones measuring 30 mm and 28 mm, respectively. All investigated species exhibit moderate inhibition from IHF, with zones ranging from 17 to 24 mm. With significant effects against C. albicans (zone: 28 mm) and moderate effects against S. aureus and E. coli, FFVW exhibits a range of inhibition. VW exhibits comparatively lesser inhibition against other species but substantial inhibition against *C. albicans* (zone: 29 mm). These findings demonstrate the studied formulations' antimicrobial efficacy, with differences in efficacy against various pathogens indicating their potential use in the fight against microbial illnesses.

Table 13: Zone of inhibition of *S. aureus, E. coli, C. albicans*, and *C. glabrata* for Standard, control, IHF, FFVW, and VW.

Samples (50uL)	Zone of inhibition of organisms (mm)			
	S. aureus	*E. coli*	*C. albicans*	*C. glabrata*
Standard (Ciprofloxacin1mg/ml)	21	29	-	-
Standard (Fluconazole1mg/ml)	-	-	30	28
Control (Ethanol)	-	-	-	-
IHF	17	24	22	22
FFVW	19	18	28	17
VW	11	10	29	11

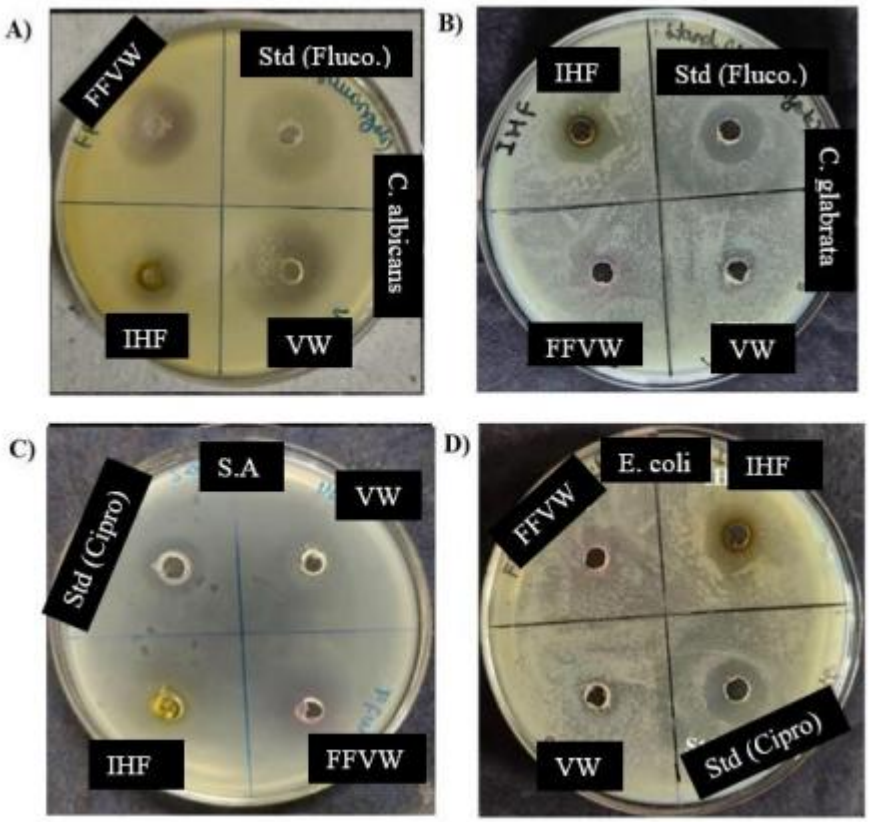


Figure 19: Zone of inhibition of micro-organism A) Candida albicans B) Candida glabrata C) Staphylococcus aureus D) Escherichia coli whereas, Std – Standard, Cipro- Ciprofloxacin, Flu-Fluconazole, IHF- In-house formulation, FFVW- femifair v-wash , VW- v wash

Stability study

After three months, the stability study's findings, which are displayed in Table 14, indicated that the in-house formulation was still stable. When these were proven for physical appearance and pH at two distinct temperature settings (25 ± 2 ºC with a relative humidity of 60 ± 5% RH and 40 ± 2 ºC with a relative humidity of 75% RH), significant alterations were not observed. More than 90% of the medicines suggested that the in-house formulation was stable enough.

Table 14: Results of the internal formulation stability studies

Parameters	In-house formulation (Low viscosity gel)		
	Initial	After3monthsat25ºC	After3monthsat40 ºC
Physical appearance	Greenish- brown	No change	No change
pH	3.98±0.056	3.88±0.23	3.95±0.28
Viscosity (Poise)	1.092±0.04	1.013±0.20	1.036±0.06

5. DISCUSSION

The purpose of the study was to create and standardize a polyherbal intimate wash while analyzing its qualities and possible advantages using a range of tests. Macroscopic analysis, phytochemical analysis, physicochemical testing, and pharmacological investigations concerning antioxidant, antibacterial, and anti-inflammatory activity were all included in these evaluations. Shape, colour, odour, and taste were the first physical attributes of important plant leaves, such as those of *Ocimum sanctum*, *Piper betle*, and *Aloe vera*, that were evaluated. In addition to this analysis, physicochemical parameters including ash value, water-soluble ash, acid-insoluble ash, and loss on drying were determined to define the fundamental characteristics of the materials and guarantee compliance with quality standards.

Using ethanol as the solvent during the maceration procedure, considerable volumes of extracts from *Ocimum sanctum*, *Piper betle*, and *Aloe vera* were produced. *Ocimum sanctum* and *Piper betle* extracts were subjected to a preliminary phytochemical screening process that revealed the presence of many active ingredients such as carbohydrates, proteins, amino acids, steroids, saponins, flavonoids, tannins, phenols, and alkaloids. A thorough presence of these phytoconstituents was demonstrated by *Aloe vera* extract. The formulation's composition was further validated using quantitative estimations, which indicated the presence of polyphenols, flavonoids, tannins, alkaloids, saponins, and flavonols. These constituents are primarily responsible for the formulation's antibacterial, antioxidant, and anti-inflammatory properties.

Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were used in the identification of aloe-emodin, a major component of *Aloe vera* that is recognized for its medicinal potential. The antioxidant activity of the formulation was evaluated by measuring its hydrogen peroxide scavenging capacity. The results showed a noteworthy antioxidant activity that was on par with normal ascorbic acid, especially in the extracts of *Ocimum sanctum* and *Aloe vera*. Additionally, in-vitro anti-inflammatory efficacy demonstrated *Aloe vera*'s superiority over *Ocimum sanctum* and *Piper betle*.

Potential antibacterial qualities were suggested by the extracts' ability to combat pathogens such as S. aureus, E. coli, C. albicans, and C. glabrata, as shown by microbiological investigations. The F3 batch was identified throughout the formulation development process through optimization. This batch possessed ideal attributes including colour, odour, homogeneity, pH, and viscosity. The chorioallantoic membrane of the chick egg was not disrupted by the formulation, according to a safety evaluation conducted using HET CAM testing.

The formulation's antioxidant and anti-inflammatory properties were found to be either equivalent or superior to those of commercial goods when compared. These results were further supported by experiments for membrane stability, protein denaturation, and proteinase inhibition. The formulation's robustness was tested over the course of three months at various temperatures. No discernible alterations were seen at 25 ºC or 40 ºC, suggesting that the formulation has the potential to be a stable and useful polyherbal intimate wash formulation.

Among the formulations tested, femifair V-Wash exhibited the best overall activity, showing superior results in various parameters including pH balance and antimicrobial efficacy, particularly against pathogens like C. albicans. The in-house formulation (F3) demonstrated good activity, with notable antioxidant and anti-inflammatory properties, as well as stability over time. V Wash, while effective, showed lower overall activity compared to femifair V-Wash and the in-house formulation, particularly in its pH range and antimicrobial tests. Thus, in terms of efficacy, the order from best to good is femifair V-Wash, in-house formulation (F3), and V Wash.

6. CONCLUSION

A polyherbal intimate wash that was produced and standardized in this study has a lot of potential to be a cosmeceutical product. The qualities and advantages of the formulation were extensively confirmed by extensive evaluations that included macroscopic analysis, phytochemical screening, physicochemical testing, and pharmacological studies. The investigation guaranteed that the botanical components followed strict guidelines and that the physicochemical properties stayed inside predetermined bounds. Significant extracts were produced, and the active ingredients were found, especially in the *Aloe vera* extract, where the concentration of polyphenols, flavonoids, tannins, alkaloids, saponins, and flavonols was quantified. Prominent antioxidative, anti-inflammatory, and antibacterial properties were noted, and after optimization, the ideal batch containing key characteristics was found. In vitro tests validated the product's safety, antioxidative potential, and antibacterial efficacy, and comparisons with commercial treatments showed similar or better antioxidative and anti-inflammatory properties. The stability tests conducted at different temperatures and for a duration of three months confirmed the formulation's robustness. Overall, these results demonstrate the potential advantages and effectiveness of the polyherbal intimate wash as a cosmeceutical choice for intimate hygiene.

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