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Formulation of Green Zucchini Vaginal Cream and Evaluation of Its Irritation Potential in Rabbits

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ABSTRACT

Cucurbita pepo L. subsp. pepo (green zucchini variety) contains abundant bioactive compounds, positioning it as a potential remedy for vaginal dryness based on principles from Persian medicine. This research sought to develop a vaginal cream incorporating green zucchini extract and to investigate its potential for causing vaginal irritation through an in vivo study. A vaginal cream was prepared with 5% aqueous extract from green zucchini. Evaluations included physicochemical characteristics such as visual appearance, pH level, viscosity, ease of spreading, and gallic acid concentration, alongside microbial contamination checks. The formulation underwent stress testing and a six-month accelerated stability assessment; additionally, its irritancy was tested in rabbits over five days. The optimized cream consisted of green zucchini extract (5%), cetyl palmitate (4.2%), octyl dodecanol (11.7%), polysorbate 60 (7.2%), sorbitan monostearate (2.0%), cetostearyl alcohol (12.5%), benzyl alcohol (0.5%), lactic acid (0.1%), and purified water (56.8%). It exhibited appropriate physicochemical and microbiological qualities, including a pH of 4.0 and viscosity of 39.57 Pa.s. Analysis revealed 23 mg gallic acid per 100 g of cream. The product remained stable under stress conditions and during the six-month accelerated stability evaluation. Irritation testing in rabbits showed no evidence of erythema or edema, confirming its tolerability. The vaginal cream derived from green zucchini demonstrated favorable physicochemical properties and a non-irritating profile, indicating its viability as a safe, standardized option; nevertheless, additional human clinical trials are required.

Keywords: Cucurbita pepo, Irritation test, Rabbit, Vaginal cream, Zucchini

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Introduction

Vaginal dryness is a common issue affecting women of different age groups, especially during menopause, after childbirth, or due to specific medical therapies. It frequently leads to symptoms like discomfort, pruritus, irritation, and pain during intercourse, thereby substantially reducing quality of life and personal well-being [1, 2].

Standard approaches to managing vaginal dryness involve hormonal therapies (e.g., estrogen creams) as well as non-hormonal options such as moisturizers and lubricants. Although these provide relief from symptoms, issues related to long-term use, possible adverse effects, and restrictions on hormonal treatments have driven interest in alternative solutions [3].

In recent years, formulations based on natural plants have attracted growing interest owing to their potential efficacy, good tissue compatibility, and lower risk of side effects. The role of herbal remedies in alleviating vaginal dryness has gained recognition for their capacity to restore hydration, aid tissue repair, and maintain vaginal health [4, 5]. Synthetic options, particularly those involving hormones, may increase risks like higher infection rates, changes in vaginal flora, or systemic endocrine effects. By comparison, herbal extracts, essential oils, and plant-

based products have shown promise in improving moisture levels, supporting healing, and promoting vaginal wellness [4, 5]. Moreover, the rising consumer demand for natural and integrative health strategies highlights the importance of exploring plant-derived treatments. Traditional systems of medicine represent valuable resources for discovering novel therapeutics, with Persian medicine—due to its extensive historical record—serving as a particularly rich foundation for identifying new herbal interventions [6, 7].

Within the scope of Persian medicine, Cucurbita pepo L. subsp. pepo (green zucchini variety) stands out as a noteworthy option because of its high content of beneficial substances, such as vitamins A, C, and E, antioxidants, and mucilaginous components. These elements contribute to moisturizing, anti-inflammatory, and restorative effects on tissues, rendering green zucchini suitable for addressing vaginal dryness. The mucilage in the extract may also help retain moisture and provide a calming action on vaginal tissues [8, 9]. In Persian medicinal theory, it is classified as having a cool and moist nature, employed as a cooling and hydrating remedy for relevant conditions affecting various organs [10].

When creating vaginal products, it is critical to confirm their safety and suitability for vaginal tissues. A key preclinical step is the animal-based vaginal irritation evaluation, which identifies possible irritant or inflammatory responses. This assessment offers essential data on the product's safety prior to human testing, verifying that it avoids harmful reactions or alterations to the vaginal ecosystem. Results from these investigations enhance the reliability and market acceptance of herbal vaginal products [11-13].

This investigation focused on creating a vaginal cream using green zucchini extract as the primary active component for treating vaginal dryness. Emphasis was placed on refining the cream's ingredients, examining its physicochemical attributes and durability, and assessing its potential for irritation in a rabbit model.

Materials and Methods

Chemicals

Cetyl palmitate, polysorbate 80, gallic acid standard, benzyl alcohol, and lactic acid were sourced from Merck Co., Germany. Octyl dodecanol and cetostearyl alcohol were obtained from Sigma Aldrich Co., Germany. HPLC-grade acetonitrile and methanol were acquired from Dr. Mojallali Co., Iran. Remaining reagents were of analytical purity.

Plant material and extraction

Fresh green zucchini fruits were acquired from a local herbal market in Tehran. Extraction involved boiling the material in water (ratio 1:10 plant to solvent) for 30 minutes, followed by filtration and drying of the extract at 70 °C in an oven.

Vaginal cream formulation

The cream was developed by incorporating 5% aqueous green zucchini extract with varying amounts of excipients as outlined in **Table 1.**

The formulation exhibiting optimal uniformity, texture, absence of separation over time, and no oily residue on application was chosen for further evaluation. This selected version was subjected to physical stress testing, along with assessments of physicochemical and microbial standards, and an accelerated stability study.

Table 1. Different Formulations of Green Zucchini Vaginal Cream

No.	Extract (%)	Octyl Dodecanol (%)	Cetyl Palmitate (%)	Lactic Acid (%)	Polysorbate 60 (%)	Cetostearyl Alcohol (%)	Espan 60 (%)	Benzyl Alcohol (%)	Water (%)
F1	5	11.50	2.90	0.10	1.90	9.60	1.90	0.00	Qs. to 100
F2	5	12.60	3.10	0.10	2.60	10.50	2.10	1.00	Qs. to 100
F3	5	12.00	6.00	0.10	4.00	10.00	2.00	1.00	Qs. to 100
F4	5	12.00	3.00	0.10	4.00	10.00	2.00	1.00	Qs. to 100
F5	5	12.00	4.00	0.20	4.00	10.00	2.00	0.50	Qs. to 100
F6	5	11.40	3.80	0.10	4.90	13.30	1.90	0.50	Qs. to 100
F7	5	11.40	5.70	0.10	4.80	13.30	1.90	0.50	Qs. to 100

F8	5	11.40	4.80	0.10	5.70	13.30	1.90	0.50	Qs. to 100
F9	5	11.60	4.80	0.10	6.70	12.50	1.00	0.50	Qs. to 100
F10	5	11.40	4.80	0.10	7.20	12.40	1.90	0.50	Qs. to 100
F11	5	12.00	4.10	0.10	7.15	12.40	1.90	0.50	Qs. to 100
F12	5	11.70	4.40	0.10	7.15	12.40	1.90	0.50	Qs. to 100
F13	5	11.70	4.40	0.05	7.20	12.40	1.90	0.50	Qs. to 100
F14	5	11.70	4.40	0.00	7.25	12.40	1.90	0.50	Qs. to 100
F15	5	11.70	4.40	0.05	7.40	12.40	1.90	0.50	Qs. to 100
F16	5	11.70	4.40	0.10	7.30	12.40	1.90	0.50	Qs. to 100
F17	5	12.00	4.10	0.10	7.15	12.50	1.90	0.50	Qs. to 100
F18	5	12.00	4.20	0.10	7.15	12.40	2.00	0.50	Qs. to 100
F19	5	11.80	4.20	0.10	7.20	12.50	1.90	0.50	Qs. to 100
F20	5	11.70	4.20	0.10	7.20	12.50	2.00	0.50	Qs. to 100

Assessment of the product under stress conditions

Centrifugation test

The optimized formulation underwent centrifugation at 3750 rpm for 15 minutes, followed by an inspection for any alterations in visual appearance [17].

Thermal cycling test

Three samples of the cream were stored at 4 °C for two weeks, subsequently transferred to 40 °C for an additional two weeks. A separate group of three samples followed the reverse sequence, starting at 40 °C before moving to 4 °C. After completion of the four-week cycle, the samples were examined for any physical modifications [17].

Evaluation of physicochemical and microbiological properties of the product

The physicochemical parameters of the cream—encompassing visual appearance, ease of application, pH value, viscosity, and gallic acid concentration—were thoroughly analyzed. Microbiological examinations were carried out in accordance with the British Pharmacopoeia [18], covering total aerobic microbial count, total combined yeasts and molds count, as well as tests for the absence of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans.

Product standardization

Quantification of gallic acid as a quality marker in the cream was performed via high-performance liquid chromatography (HPLC) under the specified conditions: Agilent 1260 Infinity system; column: C18 Phenomenex (250 × 4.6 mm, 5 µm); column temperature: 25 °C; flow rate: 0.8 mL/min; injection volume: 20 µL; detection wavelength: 272 nm; mobile phase: water:acetonitrile (80:20) in isocratic elution, adjusted to pH 3 with phosphoric acid; diluent: methanol [19].

Preparation of standard and sample solutions

The standard stock solution was prepared by dissolving 10.5 mg of gallic acid in methanol and making up the volume to 50 mL with the same solvent. From this, a working standard of 2.625 µg/mL was obtained.

For the sample, 500 mg of cream was dispersed in 40 mL methanol, agitated for 20 minutes at 70 °C, and then placed in a freezer for 1 hour. The supernatant was filtered, adjusted to 50 mL with methanol, and further passed through a 0.45 µm filter prior to analysis.

Stability assessment

An accelerated stability study was conducted on the formulation at 40 ± 2 °C and 75 ± 5% relative humidity, following International Conference on Harmonisation (ICH) guidelines [20]. Evaluations were performed at three and six months after preparation.

Mucosal irritation assessment via vaginal irritation testing in rabbits

The investigation adhered to current OECD Good Laboratory Practice standards [14-16, 21]. Three healthy young adult female New Zealand White rabbits, each weighing at least 2 kg and sourced from a uniform strain at the Pasteur Institute of Iran, were used. Animals were individually housed in stainless steel wire-mesh cages under controlled conditions: 12-hour light/dark cycle, temperature of 22 ± 2 °C, relative humidity of 50 ± 5%, and unrestricted access to food and water. Prior to initiation, each rabbit was examined for any signs of vaginal discharge, inflammation, or injury.

In the procedure, 1 mL of the green zucchini cream was administered intravaginally to each rabbit. This application was repeated daily at 24-hour intervals for five consecutive days. Following each administration, animals were returned to their cages. No additional test substances were applied. Observations were conducted 24 hours after each treatment and immediately before the next application. The vaginal orifice and surrounding perineal area were inspected for indications of erythema and edema. Findings were scored and classified for toxicity using the Draize scale **(Table 2)** [14]. Clinical signs of systemic toxicity were also monitored during each observation. Individual scores were averaged across observations to calculate the primary irritation index **(Table 3).**

Table 2. Draize Scale for Assessing Irritation

Erythema and Eschar Formation	**Reaction Score**
No erythema	0
Very slight erythema (barely visible)	1
Clearly defined erythema	2
Moderate to severe erythema	3
Severe erythema (deep red) to minor eschar formation (injury into tissue)	4
Edema Formation	**Reaction Score**
No edema	0
Very slight edema (barely noticeable)	1
Slight edema (edges clearly raised)	2
Moderate edema (raised about 1.0 mm)	3
Severe edema (raised >1.0 mm, extending beyond exposure area)	4

Table 3. Interpretation of Mean Primary Irritation Index (PII) Scores

Mean PII Value	**Irritation Category**
0	Negligible
Greater than 0 up to 2 ($0 < PII \leq 2$)	Slight
Greater than 2 up to 5 ($2 < PII \leq 5$)	Moderate
Greater than 5 ($PII > 5$)	Severe

Results and Discussion

This investigation focused on developing and assessing a vaginal cream incorporating an aqueous extract from green zucchini to address vaginal dryness. During the formulation stage **(Table 1),** cetyl palmitate and octyl dodecanol were identified as primary excipients influencing the product's properties. Cetyl palmitate functions as an emollient, emulsifying agent, and thickener. It contributes to a uniform, silky texture, improves emulsion durability, and establishes a protective barrier on mucosal surfaces that minimizes water evaporation while maintaining hydration. Additionally, it is non-oily, helping to mitigate any greasy feel in lipid-rich formulations and refining the product's aesthetic qualities. Octyl dodecanol, in contrast, acts as an emollient, humectant, and solvent. It improves application ease and emulsion integrity while suppressing excessive foaming. It imparts a silky, easily spreadable feel [22, 23]. Given its low potential for dermal irritation [23], octyl dodecanol is appropriate for application in vaginal products. Cetostearyl alcohol, incorporated at levels between 9.6% and 13.3%, serves as an emulsifier and viscosity enhancer. It functions as a structurant, imparting the required semi-solid consistency to creams. Furthermore, it promotes skin softening and smoothness by creating a lipid film that

retains moisture, thereby supporting hydration. Polysorbate 60 is a nonionic hydrophilic surfactant employed mainly for emulsification, solubilization, and stabilization purposes. It facilitates even dispersion of components within creams. It is particularly efficient in producing oil-in-water emulsions and improving both stability and tactile properties. Span 60 (sorbitan stearate) is a nonionic lipophilic surfactant utilized as an emulsifier and stabilizer in topical preparations. It is frequently paired with hydrophilic surfactants such as polysorbate 60 to achieve robust emulsions [22, 23]. This pairing effectively stabilizes both aqueous and oily phases, yielding a homogeneous and pleasant texture.

For the green zucchini vaginal cream, 5% of the dried aqueous plant extract was combined with the excipients described above. A total of twenty different compositions were developed. The physical attributes of these prototypes are outlined in **Table 4.**

The preliminary version, formulated without any preservative, displayed a thin texture and developed fungal contamination within three days. This necessitated the addition of a preservative. After reviewing suitable candidates, benzyl alcohol at 1% was chosen as the most effective option. Lactic acid was included to adjust the cream's pH. To improve thickness, incremental increases were made to the levels of cetyl palmitate, octyl dodecanol, and surfactants. Nevertheless, certain trials resulted in phase separation (F2, F3). Subsequent reduction in cetyl palmitate content resolved the separation but led to persistently low viscosity (F4).

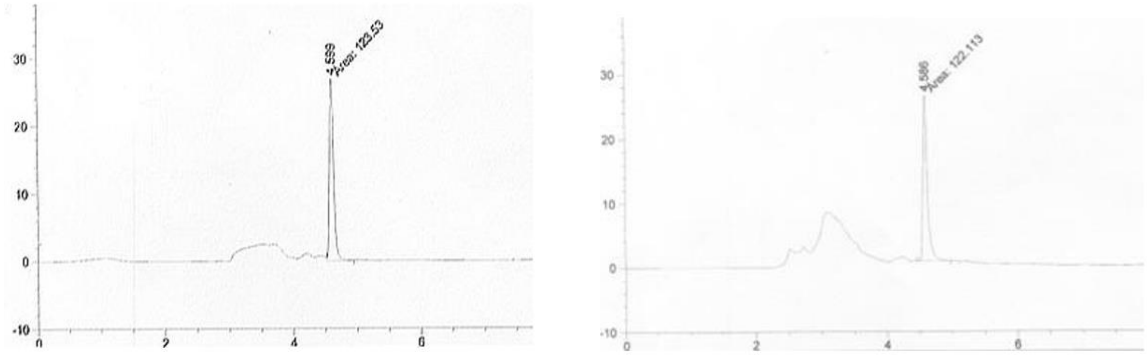
Table 4. Evaluated Physical Properties of Green Zucchini Vaginal Cream Formulations

Formulation	Physical Observation
F1	The cream exhibited a very loose texture and developed mold within three days.
F2	Layers separated within a day, indicating instability.
F3	Cream separation was apparent after 24 hours.
F4	The formulation had a thin and runny consistency.
F5	Texture improved compared to earlier formulations.
F6	Cream consistency showed a noticeable enhancement.
F7	Initially smooth, the cream thickened excessively after 24 hours.
F8	The formulation was too dense and thick in texture.
F9	Cream exhibited phase separation.
F10	Texture was acceptable, though it left an oily residue on application.
F11	Phase separation occurred during mechanical stress testing.
F12	Stress testing revealed instability with phase separation.
F13	The cream separated into distinct layers.
F14	Centrifugation caused the cream to break into phases.
F15	Consistency remained acceptable, but some oil accumulated on the skin surface.
F16	Fat deposition reduced compared to F15, though cream thickened after 24 hours.
F17	Separation of layers occurred after 24 hours.
F18	Centrifugal testing caused phase separation.
F19	Cream broke into phases under centrifugation.
F20	Texture was ideal, stable under stress tests, and spreadability was satisfactory.

Additionally, the cream displayed a strong odor from benzyl alcohol, prompting a reduction of this ingredient to 0.5% alongside an increase in cetyl palmitate content (F5). To improve thickness and prevent emulsion breakdown, the level of cetostearyl alcohol was raised (F6, F7). Nevertheless, within 24 hours, the product became overly firm, requiring a subsequent decrease in cetyl palmitate (F8). In the next iteration, cetostearyl alcohol was lowered while polysorbate 60 was elevated, which unfortunately led to emulsion instability. Raising polysorbate 60 to 7.2% successfully resolved the separation problem, although residual oily particles remained on the skin after application (F10). This issue was addressed by boosting octyl dodecanol and decreasing both cetyl palmitate and polysorbate 60 (F11). However, this version failed stability under stress conditions, leading to further refinements in the proportions of cetyl palmitate, octyl dodecanol, and surfactants (F12, F13). Elevating polysorbate 60 caused renewed oily residue on the skin, limiting major changes to this component. Following

several fine-tuning modifications to the excipients, the optimal composition (F20) was achieved, demonstrating appropriate texture and application ease, along with satisfactory performance in stress evaluations.

The HPLC chromatograms for the gallic acid standard and the green zucchini cream revealed a distinct peak at a retention time of 4.6 minutes attributable to gallic acid **(Figure 1).** By comparing the area under the curve (AUC) between the standard and the sample, the gallic acid concentration in the cream was determined to be 23 mg per 100 g of product.

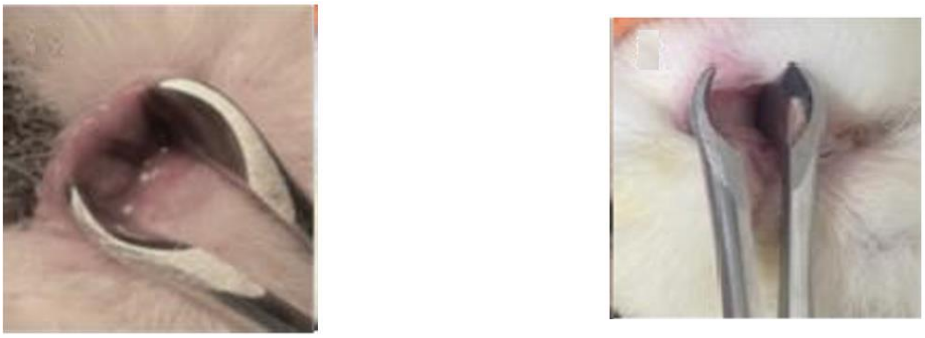


a) b)

Figure 1. High-performance liquid chromatography chromatograms of the gallic acid reference standard (a) and the formulated green zucchini vaginal cream (b).

The outcomes from the accelerated stability evaluation of the green zucchini vaginal cream, conducted at 40 °C with 75% relative humidity, are presented in **Table 5**. Throughout the six-month period, no notable alterations in physicochemical parameters were detected. Microbiological attributes remained compliant with established standards [18].

In the course of the irritation assessment, no evidence of mucosal irritation, erythema, or edema was noted at 24 hours post-application or immediately before subsequent administrations across the five-day testing period in rabbits. Additionally, no systemic clinical abnormalities were recorded **(Figure 2).**



a) b)

Figure 2. Appearance of the vaginal region in rabbits after five consecutive days of green zucchini vaginal cream administration; (a) control group, (b) treated with cream.

The cream was designed leveraging the bioactive components of green zucchini, which is abundant in vital vitamins, antioxidants, and mucilaginous substances recognized for their moisturizing and anti-inflammatory actions [8, 9]. Physicochemical analysis confirmed that the product possessed reliable stability, suitable viscosity, and good application properties, rendering it appropriate for vaginal use. The development process was refined to guarantee long-term integrity of the formulation. Stability assessments revealed that both physicochemical and microbiological attributes remained unchanged under various storage scenarios, underscoring its suitability for potential commercialization. The cream's pH, aligned closely with the physiological vaginal milieu, represents a critical feature that promotes biocompatibility and lowers the likelihood of unwanted reactions.

A central element of this research was the safety profiling through vaginal irritation testing in an animal model. Findings showed that the green zucchini-derived cream induced no signs of irritation, inflammation, or tissue

changes in the vaginal area. These outcomes indicate excellent tolerability and safety for intravaginal application, bolstering its viability as a substitute for traditional vaginal dryness therapies.

Interest in integrating natural botanical ingredients into vaginal products is increasing amid growing concerns over the side effects associated with synthetic or hormone-containing options.

Although estrogen therapies can be efficacious, they are contraindicated for certain women, especially those with specific health restrictions [3]. Employing green zucchini extract offers an attractive herbal option that not only provides hydration to the vaginal lining but also supports broader vaginal wellness through its antioxidant and tissue-regenerative qualities. Green zucchini is a rich source of bioactive agents, including vitamins A, C, and E, each contributing significantly to mucosal preservation and moisture balance [24]. Vitamin A facilitates epithelial renewal, aiding in the repair of dryness-compromised vaginal tissue. Vitamin C supports collagen production and improves tissue flexibility, whereas vitamin E serves as a strong antioxidant, protecting the vaginal lining from oxidative damage [25]. Furthermore, the mucilage present in green zucchini—a water-attracting polysaccharide—creates a soothing barrier on the mucosal surface, promoting water retention and alleviating discomfort from friction [26]. The prospective benefits of gallic acid and other phenolic constituents for managing vaginal dryness highlight opportunities for non-hormonal approaches. While specific investigations on green zucchini-based products are limited, supporting data from analogous plant-derived therapies indicate effectiveness via diverse pathways. Phenolics have shown notable advantages in alleviating vaginal dryness, primarily through moisturizing and inflammation-modulating effects. For example, a clinical study involving Alcea angulata suppositories at 5% concentration reported improvements in dryness, irritation, itching, and dyspareunia symptoms in postmenopausal participants. The mucilaginous components in such phenolic-enriched preparations establish a hydrating gel-like structure that sustains epithelial moisture, while their anti-inflammatory effects mitigate discomfort and foster mucosal maturation [27].

Table 5. Changes in Physicochemical Properties of Green Zucchini Vaginal Cream during Accelerated Stability Testing

Parameter	Initial Measurement	After 3 Months	After 6 Months
Appearance	Light brown cream with a benzyl alcohol scent	Light brown cream with a benzyl alcohol scent	Light brown cream with a benzyl alcohol scent
Homogeneity	Uniform consistency	Uniform consistency	Uniform consistency
Spreadability	Easily spreadable	Easily spreadable	Easily spreadable
pH	4.00 ± 0.09	4.10 ± 0.07	4.06 ± 0.05
Viscosity	39.57 ± 1.53 Pa·s	40.01 ± 2.61 Pa·s	41.11 ± 1.85 Pa·s
Gallic Acid Content	23.0 ± 1.40 mg/100 g	22.6 ± 1.95 mg/100 g	21.9 ± 0.93 mg/100 g

These observations imply that phenolic constituents may exert comparable effects in formulations derived from green zucchini. Gallic acid, a prominent compound in green zucchini, offers antimicrobial activity that indirectly supports vaginal moisture levels. It preferentially targets pathogenic organisms like Candida albicans, Gardnerella vaginalis, and Trichomonas vaginalis while preserving the beneficial lactobacilli population, thus helping to sustain a healthy vaginal pH [28]. This equilibrium in the microbiome is essential for preserving epithelial structure and water retention. Moreover, the antioxidant capabilities of gallic acid shield mucosal tissues from oxidative damage, promoting overall tissue integrity.

Numerous investigations have examined herbal options for managing vaginal dryness. For example, research involving Aloe vera-containing vaginal gels has documented marked enhancements in moisture levels and mucosal repair [29]. Relative to such products, the green zucchini vaginal cream delivers a distinctive blend of mucilage-driven hydration and inflammation-reducing actions, supplemented by the accessibility and affordability of the source material. Additional plant-derived agents, including soy isoflavones and chamomile, have been evaluated for their influence on vaginal wellness. Evidence indicates that soy-derived preparations can display phytoestrogenic effects; nevertheless, their ability to mitigate vaginal atrophy is still debated [30]. Chamomile extracts, valued for their calming and antibacterial qualities, have been integrated into vaginal products to ease symptoms and maintain microbial harmony [31]. The green zucchini cream developed here

exhibits parallels with these herbal approaches yet provides superior moisturizing and mucoprotective advantages owing to its abundant polysaccharide composition.

Despite the encouraging outcomes of this research, certain constraints should be noted. The evaluation relied on an animal model, and although it yields important safety data, human clinical studies are required to establish therapeutic benefits and patient acceptance. Future work should also investigate prolonged application and any effects on the vaginal microbiome to fully verify the product's safety and performance.

Conclusion

The positive outcomes from the vaginal irritation assessments underscore the tolerability of the green zucchini vaginal cream. Combined with its optimal uniformity, texture, pH, robustness in stress and accelerated stability evaluations, and the inclusion of phenolic compounds, these attributes point to effective moisturizing and mucosal safeguarding potential. Subsequent investigations should prioritize human clinical trials to substantiate these preclinical results and assess the sustained advantages of this herbal product in clinical settings.

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Conflict of Interest: None

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Ethics Statement: The study received approval from the ethics committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran (approval code: IR.SBMU.AEC.1402.114). It adhered to OECD guidelines [14, 15] and ISO 10993-10:2010 [16].

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