

RESEARCH PAPER

Development, Optimization and Evaluation of a Herbal Pet Collar Containing Essential Oils for Ectoparasite Management in Companion Animals

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Abstract

Health issues related to the consideration of ticks, fleas and lice as ectoparasites are a significant worry among companion animals, which held interest in plant-based alternatives. But there is still little systematic optimization of herbal pet collars made of polymer. The objectives of the current research were to design, optimize, and test a pet collar which contains neem, citronella and cedarwood oils in order to manage ectoparasites. Solvent casting was used to prepare the collar, polyvinyl alcohol (PVA) as polymer matrix-forming agent, and polyethylene glycol 400 (PEG 400) as a plasticizer. To examine the impact of PVA and PEG 400 on the swelling index, folding endurance, and moisture content, an 11-run Central Composite Design (CCD) was used. The answers were adjusted to quadratic formulas and tested on the basis of analysis of variance (ANOVA). Formulation F7 had a thickness of 2.01 ± 0.03 mm, surface pH of 6.51 ± 0.13 , swelling index of 98.14%, folding endurance of 659 folds and a moisture content of 6.89%. The quadratic model of swelling index was not statistically significant ($F = 4.54$, $p = 0.0612$) but PEG 400 ($p = 0.0368$) and quadratic PEG 400 ($p = 0.0258$) had significant individual effects. The models for folding endurance ($F = 5.92$, $p = 0.0366$) and moisture content ($F = 46.29$, $p = 0.0003$) were significant. The formulation chosen had a highest in-vitro tick repellency of 46.67% at 2 h and retained desirable properties over 60 days of stability test. The choice of F7 was due to the general balance of the formulation characteristics. F7 showed good physicochemical properties and potential in-vitro tick-repellent efficacy and has a chance to be used as a plant-based solution in the management of ectoparasites.

Keywords: Companion animals, Herbal pet collar, Ectoparasite management, Central Composite Design, Tick repellency.

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1. INTRODUCTION

Ectoparasites are organisms that are located on the surface of their hosts and feed on blood, skin debris, tissue fluids or other materials of the host. Tick, flea, and lice infestations in companion animals, especially dogs and cats, are significant veterinary health issues which can lead to pruritus, irritation, allergic dermatitis, loss of hair, anemia, discomfort, and decreased animal welfare. Moreover, a number of ectoparasites may harbor infectious pathogens, which highlights the importance of the effective prevention and management policies.^[1-3]

Various approaches have been developed for ectoparasite management, including shampoos, sprays, powders, creams, spot-on preparations, oral

formulations, and medicated collars.^[4] Among these approaches, collars offer a convenient method of application and can facilitate contact between active agents and the animal with comparatively little handling. Medicated collars containing synthetic insecticidal or acaricidal agents have been investigated for the management of ectoparasites.^[12,13] However, resistance to commonly used insecticides and acaricides represents an important challenge in ectoparasite control.^[6,7] These concerns, together with interest in safer and more environmentally acceptable alternatives, have stimulated research into botanical products and essential oils possessing insecticidal, and repellent properties.^[5,8-11]

Neem (*Azadirachta indica*), citronella (*Cymbopogon nardus*), and cedarwood (*Cedrus deodara*) oils are

promising botanical materials for ectoparasite-management applications. Neem contains azadirachtin and other bioactive constituents associated with insecticidal and repellent activity.^[16–19] Citronella oil contains volatile constituents associated with insect-repellent activity,^[20–22] while cedarwood oil contains terpenoid constituents investigated for biological and insect-related applications.^[23–25] Their combination therefore provides a rationale for developing a botanical collar formulation.

For incorporation of these oils into a practical collar, an appropriate polymeric matrix is required to provide adequate flexibility, mechanical integrity, and suitable moisture and swelling characteristics. Previous studies have demonstrated the incorporation of neem oil into PVA-based polymeric systems, supporting the feasibility of PVA as a polymer matrix-forming agent.^[26,27] Accordingly, PVA was selected as the matrix-forming polymer and PEG 400 as the plasticizer to improve the flexibility and handling characteristics of the formulation.^[14,15]

Despite the reported biological potential of botanical oils and the feasibility of incorporating plant-derived materials into PVA-based systems, limited information is available on the systematic optimization of a PVA-based collar containing neem, citronella, and cedarwood oils in combination. In particular, the influence of PVA and PEG 400 concentrations on multiple formulation characteristics remains insufficiently investigated. Therefore, a systematic formulation-development approach is required to identify suitable concentrations while considering swelling index, folding endurance, and moisture content. Design of Experiments (DoE)

provides a systematic approach for evaluating formulation variables and their interactions. Central Composite Design (CCD) enables investigation of linear, interaction, and quadratic effects within a defined experimental region and can be applied for formulation optimization.^[32] In the present study, PVA and PEG 400 were selected as independent variables, while swelling index, folding endurance, and moisture content were selected as response variables. The experimental data were analyzed using response-surface methodology and ANOVA to assess the fitted models and formulation terms.

Thus, the aim of the current study was to design, improve, and test a PVA-based herbal pet collar with neem, citronella, and cedarwood oils to treat ectoparasites in companion animals. The use of CCD was done to determine the impact of concentrations of PVA and PEG 400 on the desired responses, and to determine an optimized formulation that was subsequently measured based on physicochemical properties, in-vitro tick repulsive effects and stability.

2. MATERIALS AND METHODS

2.1 Materials

The materials used for the preparation of the herbal pet collar are presented in Table 1 along with their respective quantities and roles. Neem oil (*Azadirachta indica*), citronella oil (*Cymbopogon nardus*), cedarwood oil (*Cedrus deodara*), Apricot Kernel oil and vitamin E (tocopherol) were procured from YUCCA Enterprises, Mumbai, Maharashtra, India. The other excipients used in the formulation were of analytical grade and were obtained from authenticated sources.

SR. NO.	Ingredients	Quantity	Role
1	Polyvinyl Alcohol	12.5gm	Polymer matrix-forming agent.
2	Polyethylene Glycol 400	2.5ml	Plasticizer
3	Neem Oil (<i>Azadirachta indica</i>)	4ml	Herbal active ingredient
4	Citronella Oil (<i>Cymbopogon nardus</i>)	2.5ml	Herbal active ingredient
5	Cedarwood Oil (<i>Cedrus deodara</i>)	2.5ml	Herbal active ingredient
6	Apricot Kernel Oil	1.25ml	Carrier oil
7	Tween 80	1.5ml	Surfactant
8	Talc	2.5gm	Anti-sticking agent
9	Vitamin E (Tocopherol)	0.25ml	Antioxidant
10	Purified Water	q.s	Solvent

Table 1: Composition of Optimized Herbal Pet Collar Formulation (F7)

2.2 Experimental Design

A Central Composite Design (CCD) was employed using Design-Expert® software version 12.0.3.0 to optimize the herbal pet collar formulation. PVA concentration (X_1) and PEG 400 concentration (X_2) were selected as independent variables, whereas

swelling index, moisture content and folding endurance were selected as dependent responses. A total of eleven experimental runs were generated and evaluated. The experimental data were analyzed using response surface methodology to determine the optimum formulation.^[32]

Table

Independent variables	Symbol	Coded levels		
		-1	0	+1
PVA (g)	X_1	10	12.5	15
PEG 400 (mL)	X_2	2.5	3.75	5

2:

Independent variables and their coded levels used in CCD

2.3 Preparation of Herbal Pet Collar

The herbal pet collar was prepared by the solvent-casting technique. PVA was gradually dispersed in distilled water and heated at 85–90°C with continuous magnetic stirring until a clear and homogeneous polymer solution was obtained. The solution then was left to cool at room temperature, followed by the addition of PEG 400 when it cooled down to room temperature.

Neem oil, citronella oil, cedarwood oil, apricot kernel oil, Tween 80, and vitamin E were put in a separate

beaker and mixed thoroughly to achieve a homogeneous oil phase. Oil phase was then added slowly to the polymeric solution and it was stirred continuous until even distribution of the herbal ingredients was achieved. Talc was put gradually into the formulation and mixed thoroughly. A mixture was then left to settle to eliminate air bubbles entrained, and poured into a designed collar mould. The cast formulation was allowed to dry at room temperature over 24-48 hours. The dried collar was taken off the mould and put in polyethylene airtight pouches until later analysis.^[30,31]



Fig 1: Formulated Herbal Pet Collar

2.4 Evaluation of Herbal Pet Collar

2.4.1 Organoleptic Characteristics

The visually inspection of the prepared herbal pet collar was done in normal daylight on a clean white background. The collar was tested to colour, surface appearance, texture, homogeneity, flexibility, the existence of air bubbles, cracks, stickiness, and typical odour. It was observed and compared with the desired characteristics of a uniform polymeric collar.^[30]

2.4.2 Thickness

To determine the thickness of prepared herbal pet collar, a calibrated Vernier caliper was used. Measurements were taken at five different locations (both ends and three intermediate positions) to determine the uniformity of thickness. The measurements were taken in millimeters (mm) and the mean and standard deviation of the thickness was calculated.^[30,31]

2.4.3 Folding Endurance

The herbal pet collar was prepared and a 2 × 2 cm sample was cut. The strip was repeatedly folded manually at the same location through an angle of 180° until visible cracks appeared or the strip broke. The overall count of folds necessary to generate a breakage or a crack was taken as the folding resistance of the formulation.^[30]

2.4.4 Surface pH

A 2 × 2 cm sample was cut on the various parts of the prepared herbal pet collar. The samples were each put into a clean Petri dish and wetted by pouring about 1 mL of distilled water on them. The samples were left to equilibrate at room temperature (30 minutes). Contact with the surface of each sample was made with an electrode of a calibrated digital pH meter and a pH was recorded. The mean pH and standard deviation were calculated.^[30]

2.4.5 Moisture Content

A 2×2 cm sample was cut from the prepared herbal pet collar and accurately weighed using an analytical balance to obtain the initial weight (W_1). The sample was placed in a clean Petri dish and dried in a hot air oven maintained at $105 \pm 2^\circ\text{C}$. The sample was removed at predetermined time intervals, cooled in a desiccator, and weighed until a constant weight (W_2) was obtained. The percentage moisture content was calculated.^[31,32]

2.4.6 Swelling Index

A 2×2 cm sample was cut from the prepared herbal pet collar and accurately weighed to obtain the initial dry weight (W_1). Approximately 80 mL of distilled water was transferred into a clean beaker, and the sample was completely immersed in the water at room temperature. At predetermined intervals of 1, 2, 3, 4, 8, and 24 hours, the sample was removed, gently blotted with filter paper to remove excess surface water, and immediately weighed to obtain the wet weight (W_2) and the percentage water uptake was calculated.^[30,32]

2.4.7 In Vitro Tick Repellency Study

The repellency activity of the optimized herbal pet collar was evaluated using an in vitro Petri dish bioassay. A clean Petri dish of 15 cm diameter was used as the test arena and was divided into three equal zones, namely the treatment zone, neutral zone, and control zone. A 2×2 cm piece of the herbal pet collar was placed in the treatment zone. Healthy adult ticks were collected and acclimatized prior to the experiment. Ten adult ticks were carefully released into the neutral zone of the Petri dish. The Petri dish was placed under a lid as soon as it was released, to avoid the escape of the ticks and avoid interference with the environment to a minimum. The movement and distribution of ticks were observed without disturbing the test arena and recorded at predetermined time intervals of 5 min, 15 min, 30 min, 1 h, and 2 h. The number of ticks present in each zone was recorded at each observation time. The experiment was performed in triplicate, and both the test formulation and standard preparation were evaluated separately under identical experimental conditions. The repellency effect was expressed as the percentage of ticks avoiding the treatment zone and was calculated using the following equation:

Repellency

$$(\%) = \frac{\text{Number of ticks avoiding the treatment zone}}{\text{Total number of ticks released}} \times 100$$

A higher repellency percentage indicated stronger tick-repellent activity of the herbal pet collar formulation.^[28,29]

2.4.8 Stability Study

The optimized formulation (F7) was packed in airtight polyethylene pouches and stored under room temperature for 60 days. Samples were evaluated at 0, 15, 30, and 60 days for physical appearance, thickness, surface pH, moisture content, swelling index, and folding endurance. The study was conducted in accordance with the general principles of stability assessment described in ICH Q1A(R2).^[33]

2.5 Statistical Analysis and ANOVA

The experimental data obtained from the Central Composite Design (CCD) comprising 11 runs (F1–F11) were analyzed using Design-Expert® software version 12.0.3.0. The three responses were fitted to quadratic response-surface models using an inverse square-root transformation, as selected by the software. ANOVA was performed to assess the significance of the fitted models and individual terms, including the linear effects of PVA (A) and PEG 400 (B), their interaction (AB), and quadratic effects (A^2 and B^2). A p-value <0.05 was considered statistically significant. Model adequacy was assessed using R^2 , adjusted R^2 , predicted R^2 , coefficient of variation (CV%), adequate precision, and lack-of-fit. The connection between variables of formulation and the chosen responses were assessed with model diagnostics and response-surface plots.

3. RESULTS

3.1 Organoleptic and Physical Characteristics of Herbal Pet Collar

The formulated herbal pet collar was light-yellow in colour with a smooth and even surface. It had a flexible collar that was homogeneous and had a characteristic aromatic scent because of the essential oils put in it. The polymeric matrix was successfully formulated as no cracks, air bubbles, or stickiness was detected.

3.2 Evaluation of Formulations

The eleven formulations (F1–F11) of the Central Composite Design were analyzed in terms of thickness, folding endurance, surface pH, swelling index, and moisture content. The results are presented in Table 3.

Batches	Thickness (mm)	Folding endurance	pH	Swelling Index (%)	Moisture Content (%)
F1	2.00 ± 0.12	615	6.50 ± 0.75	91.85	7.82
F2	2.14 ± 0.06	643	6.67 ± 0.34	92.54	7.37
F3	2.02 ± 0.11	735	6.13 ± 0.29	96.72	7.61
F4	2.05 ± 0.08	627	6.42 ± 0.66	98.04	7.58
F5	2.06 ± 0.14	688	6.08 ± 0.35	94.63	7.46
F6	2.09 ± 0.10	635	6.60 ± 0.99	97.21	7.49
F7	2.01 ± 0.03	659	6.51 ± 0.13	98.14	6.89
F8	2.06 ± 0.14	982	6.55 ± 0.57	97.93	6.92
F9	2.06 ± 0.13	810	6.40 ± 0.89	98.56	6.88
F10	2.07 ± 0.06	798	6.22 ± 0.37	98.42	6.91
F11	2.03 ± 0.10	825	6.18 ± 0.20	98.61	6.86

Table 3. Evaluation results of the experimental formulations (F1–F11)

3.3 ANOVA of CCD Responses

The CCD responses were analyzed using quadratic models. The ANOVA results demonstrated different levels of model significance among the three responses. For swelling index, the quadratic model showed a model p-value of 0.0612, indicating that the overall model was not statistically significant at $p < 0.05$. Nevertheless, the individual terms PEG 400 (B) and the quadratic PVA term (A^2) showed p-values of 0.0368 and 0.0258,

respectively. For folding endurance, the quadratic model was statistically significant ($p = 0.0366$). PEG 400 (B) and the quadratic PVA term (A^2) were significant model terms, with p-values of 0.0381 and 0.0094, respectively. For moisture content, the quadratic model was highly significant ($p = 0.0003$). The interaction term between PVA and PEG 400 (AB) was significant ($p = 0.0468$), while the quadratic effect of PVA (A^2) was highly significant ($p < 0.0001$).

Response	Swelling Index	Folding Endurance	Moisture Content
Model F-value	4.54	5.92	46.29
Model p-value	0.0612	0.0366	0.0003
Model significance	Not Significant	Significant	Significant
R ²	0.8195	0.8555	0.9789
Adjusted R ²	0.6390	0.7111	0.9577
Predicted R ²	-0.8921	-0.5118	0.7998

Table 4: ANOVA summary of quadratic models for CCD responses

Response	Significant Factor	p-value
Swelling Index	PEG 400 (B)	0.0368
Swelling Index	PVA ² (A ²)	0.0258
Folding Endurance	PEG 400 (B)	0.0381
Folding Endurance	PVA ² (A ²)	0.0094
Moisture Content	AB Interaction	0.0468
Moisture Content	PVA ² (A ²)	<0.0001

Table 5: Significant Model terms identified by ANOVA

3.4 Response Surface Analysis

The impact of PVA and PEG 400 concentration on swelling index, folding endurance, and water content was demonstrated by the use of response surface and

contour plots. The highest swelling index (98.61%) and lowest moisture content (6.86%) were observed in F11, while the highest folding endurance (982 folds) was observed in F8. Figures 2 and 3 show the corresponding plots.

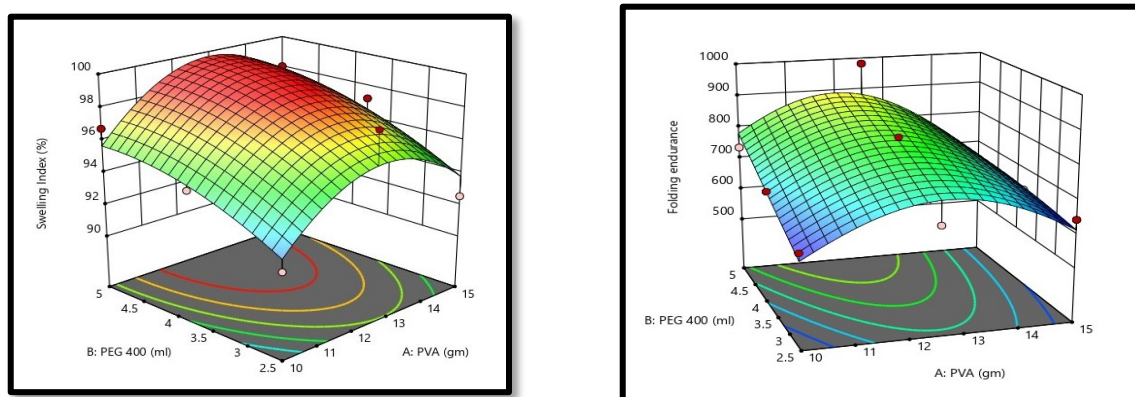


Fig 2: 3D Graph of Response Surface Methodology for Swelling Index and Folding Endurance

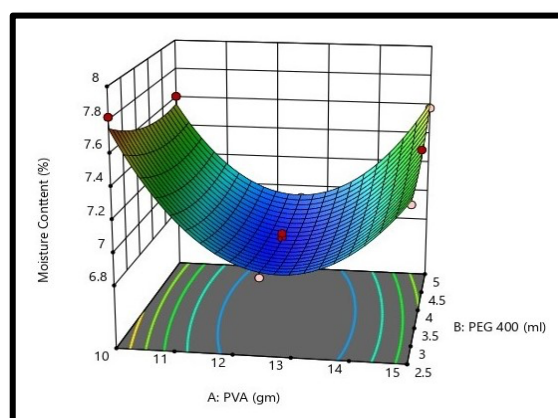


Fig 3: 3D Graph of Response Surface Methodology for Moisture Content

3.5 Selection of Optimized Formulation

Based on the experimental results obtained from the Central Composite Design, formulation F7 was selected for further evaluation and stability studies. Although certain formulations exhibited superior individual response values, F7 demonstrated a desirable balance among the selected response variables, while also maintaining acceptable thickness and surface pH.

3.6 In-Vitro Tick Repellency Activity

The herbal pet collar demonstrated variable tick-repellent activity throughout the 2 h observation period. The repellency was generally higher than that of the standard preparation at most observation intervals, with the maximum repellency of 46.67% observed at 2 h, compared with 30.00% for the standard preparation. The comparative repellency results are presented in Table 6.

Time Interval	Standard Drug (%)	Test Drug (%)
5 min	23.33	40.00
15 min	30.00	16.67
30 min	20.00	43.33
1 h	23.33	33.33
2 h	30.00	46.67

Table 6: Comparative Tick Repellency Activity of Standard Drug and Test Drug



Fig 4: Standard Drug

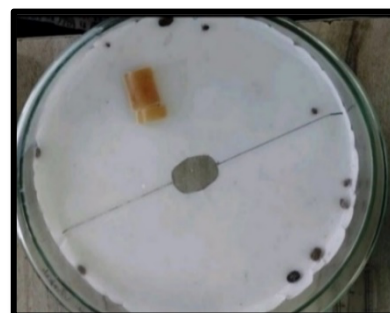


Fig 5: Test Drug

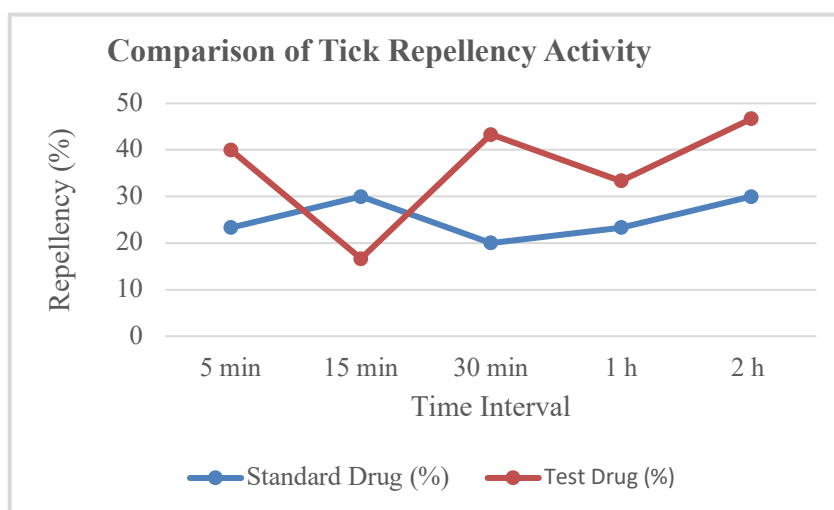


Fig 6: Tick Repellency Activity of Herbal pet Collar Compared with Standard Drug

3.7 Stability Study (Batch- F7)

“The optimized formulation remained physically stable during 60 days of storage. No significant changes in appearance, colour, or odour were observed. The stability data are presented in Table 7.

Parameters	Day 0	Day 15	After 1 month	After 2 months
Appearance	Uniform, flexible	No change	No change	No change
Weight (g)	30.52	30.46	30.33	30.29
Thickness(mm)	2.01 ± 0.03	1.99 ± 0.01	1.98 ± 0.01	1.97 ± 0.01
pH	6.51 ± 0.13	6.42 ± 0.16	6.25 ± 0.09	6.07 ± 0.16
Colour	Light yellow	No change	No change	No change
Odour	Aromatic odour	No change	No change	No change
Moisture Content (%)	6.89	6.76	6.65	6.42
Folding Endurance	659	655	653	653
Swelling Index (%)	98.14	94.33	93.17	91.64

Table 7: Stability Study of Optimized Herbal Pet Collar Formulation

4. DISCUSSION

The present study demonstrated the successful development of a PVA-based herbal pet collar containing neem, citronella, and cedarwood oils using a solvent-casting approach. PVA served as the polymer matrix-forming agent, while PEG 400 acted as a plasticizer. The collar exhibited satisfactory physical characteristics, including uniformity, flexibility, and absence of visible cracks and stickiness. Previous studies reporting the incorporation of neem oil into PVA-based polymeric systems support the suitability of PVA for incorporating plant-derived active ingredients.^[26,27]

The Central Composite Design demonstrated that PVA and PEG 400 influenced the investigated responses to different extents. ANOVA showed statistically significant quadratic models for folding endurance ($p = 0.0366$) and moisture content ($p = 0.0003$), whereas the overall model for swelling index was not significant ($p = 0.0612$). However, PEG 400 (B) and the quadratic term of PVA (A^2) showed significant individual effects on swelling index ($p = 0.0368$ and 0.0258 , respectively). Based on the combined performance of swelling index, folding endurance, and moisture content, F7 was selected as the optimized formulation, exhibiting a swelling index of 98.14%, folding endurance of 659 folds,” and moisture content of 6.89%. It also exhibited thickness of 2.01 ± 0.03 mm and surface pH of 6.51 ± 0.13 further indicated a favourable physicochemical

profile. The incorporation of neem, citronella, and cedarwood oils was supported by their reported biological and insect-repellent properties. Neem oil has been investigated for ectoparasite management,^[16–19] while citronella and cedarwood oils have been associated with insect-repellent activity,^[20,21] providing a rationale for their incorporation into the herbal collar.

The optimized collar exhibited a quantifiable in-vitro tick-repellent activity up to 2 h and had elevated repellency as compared to the standard at four of five locations of observation. “The highest repellency was detected at 2 h (46.67%), and it was 30.00% at 15 min, and the highest repellency was observed with the collar (16.67%) at 15 min compared to the standard (30.00%). This means that the repulsive response was time dependent with the greatest values during the normative period as compared to the standard.

In the 60 days stability experiment, F7 did not change its physical appearance, colour and odor with slight adjustments in the measured physicochemical parameters. These findings indicate that the optimized collar maintained its physicochemical characteristics under the investigated storage conditions throughout the study period. Further in-vivo efficacy and safety evaluation are required to establish its practical applicability.

5. CONCLUSION

The present study successfully developed a PVA-based herbal pet collar containing neem, citronella, and cedarwood oils using the solvent-casting technique. Central Composite Design was used to investigate the effects of PVA and PEG 400 on swelling index, folding endurance, and moisture content. ANOVA indicated significant effects on folding endurance and moisture content, while the overall quadratic model for swelling index was not statistically significant. Based on the combined performance of the predefined responses, F7 was selected as the optimized formulation, exhibiting a swelling index of 98.14%, folding endurance of 659 folds, moisture content of 6.89%, thickness of 2.01 ± 0.03 mm, and surface pH of 6.51 ± 0.13 . F7 demonstrated maximum in-vitro tick repellency of 46.67% at 2 h and maintained its overall physical characteristics during the 60-day stability study.” Overall, F7 showed an acceptable physicochemical and mechanical profile and represents a feasible formulation platform for potential ectoparasite management. Further in-vivo efficacy, safety, and field studies are necessary to confirm its practical applicability in dogs and cats.

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