

**FORMULATION & EVALUATION OF MOSQUITO REPELLENT POLYHERBAL  
BODY SPRAY****Bhagyashri Dhakate\* and Nilesh M. Mahajan**

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**ABSTRACT**

The research aims to formulate and evaluate polyherbal body sprays using natural essential oils, such as citronella oil and catnip oil, which are safer and more environmentally friendly alternatives to synthetic mosquito repellents like DEET. The body sprays are formulated in three types: temporary emulsion (F1), alcohol-based (F2), and aqueous-based (F3). Pre-formulation studies include verifying the absorption maxima ( $\lambda$  max) for both oils and preparing calibration curves. To remove the objectionable smell of alcohol in the formulations, pre-fixation and distillation processes are applied. The formulation process involves creating stock solutions of the oils, blending various excipients, and evaluating the physical and chemical properties of the final products. Key materials include propylene glycol, butylated hydroxytoluene, methyl paraben, propyl paraben, isopropyl myristate, and Tween 80. The formulated sprays undergo a series of tests including pH, viscosity, density, drug content analysis, and skin irritation tests. The efficacy of the sprays is determined through a mosquito repellence test using human subjects, and stability studies are conducted in accordance with ICH guidelines. The results demonstrate that the polyherbal body sprays have significant potential as effective mosquito repellents.

**KEYWORDS:** Polyherbal body spray, Citronella oil, Catnip oil, Mosquito repellent.**INTRODUCTION**

Mosquito repellent polyherbal body spray is a type of body spray, which is similar to aerosol deodorant but intended for use on various parts of the body besides the armpits. Body sprays are lighter and less expensive than colognes and can serve as deodorants. Different types of body sprays include insecticidal medicated patterns, deodorants, hair sprays, and facial humectants. The spray's characteristics are determined by the actuator, valve, propellants, and concentrate used, which are selected through trial and error to achieve the desired spray rate and characteristics. Spray formulations, suitable for applying insect repellent, minimize pain and irritation compared to conventional forms.

Mosquitoes belong to the family Culicidae and are known for their blood-feeding behavior, with females transmitting severe diseases like malaria, yellow fever, and dengue. These diseases affect millions globally, with mosquitoes labeled as "public enemy number one" by the WHO. Common mosquito species include Culex, Anopheles, and Aedes, each having unique breeding habits and associated diseases. Mosquito control methods include source reduction, biocontrol, and using insecticides, with natural predators like dragonflies, certain fish, and fungi playing a significant role in

reducing mosquito populations.

Mosquito repellents are substances designed to make surfaces unattractive to mosquitoes, traditionally using natural extracts like citronella oil. The first effective repellent, citronella oil, discovered in 1901, was limited by its quick evaporation. DEET, synthesized in 1955, became the most widely used active ingredient due to its long-lasting effectiveness. Repellents work by masking human scents or using scents that mosquitoes avoid. They can be synthetic, like DEET, or natural, like citronella oil. Natural repellents are often non-toxic and environmentally friendly, providing an alternative to potentially hazardous chemical repellents.

**MATERIALS AND METHODS****Materials**

The materials utilized in the experiment include various plant materials and excipients such as Citronella oil, Catnip oil, Propylene glycol, Butylated Hydroxy Toluene, Methyl Paraben, Propylparaben, Isopropyl Myristate, Tween 80, Ethanol, Isopropyl alcohol, Chemophore, and Water.

**Methods Pre-Formulation study**Verification of  $\lambda$  Max for Citronella and Catnip Oils:

Accurately weighed 0.1 ml of each oil was diluted in a 10 ml volumetric flask with distilled ethanol to prepare stock solutions, which were then scanned in the 200-400 nm range to determine the absorption maxima ( $\lambda$  max).

#### Preparation of calibration curve

Accurately weighed 0.1ml Citronella oil and Catnip oil dissolved on distilled ethanol in 10 ml volumetric flask as a stock solution. Aliquots of 0.2ml,0.4ml,0.6ml,0.8ml and 1ml solution were pipette out from the stock solution(both citronella and catnip oil ) were transferred in 10ml of volumetric flask and dilute with distilled ethanol. The absorbance was measured at  $\lambda$  288 nm for citronella oil and  $\lambda$  241 nm for catnip oil against reference solution (ethanol). All spectral absorbance measurement was made on the Shimadzu 1800 UV-visible spectrophotometer.

**Pre-fixation and Distillation of Ethanol:** To eliminate the objectionable smell of alcohol in the formulations, a process known as pre-fixation was employed. An aromatic resin extract, such as gum benzoin, was added to the ethanol, and the mixture was left to stand for one month. The ethanol was then distilled to remove any remaining odor. **Formulation of Polyherbal Body Spray:** Three types of formulations were prepared, each containing 5% w/v citronella oil and 2% w/v catnip oil. The formulations included a temporary emulsion (F1), an alcohol-based spray (F2), and an aqueous-based spray (F3).

#### Formulation process

**Temporary Emulsion (F1):** Phase A consisted of butylated hydroxytoluene, methylparaben, and propylparaben dissolved in propylene glycol, with citronella oil and catnip oil added to this mixture. Phase B consisted of isopropyl myristate and Tween 80 dissolved in a mixture of ethanol and isopropyl alcohol, with sufficient water added. Phase A was mixed with Phase B using moderate stirring with an overhead stirrer.

**Alcohol-Based Spray (F2):** Phase A consisted of butylated hydroxytoluene, methylparaben, and propylparaben dissolved in propylene glycol, with citronella oil and catnip oil added. Phase B consisted of isopropyl myristate and Tween 80 dissolved in ethanol and isopropyl alcohol. Phase A was mixed with Phase B using moderate stirring with an overhead stirrer.

**Aqueous-Based Spray (F3):** Phase A consisted of butylated hydroxytoluene, methylparaben, and propylparaben dissolved in propylene glycol, with citronella oil and catnip oil added. Phase B consisted of cremophore mixed with sufficient water. Phase A was added slowly to Phase B with continuous stirring using an overhead stirrer to form an emulsion.

#### Evaluation studies

##### Determination of pH

The pH of each spray formulation was measured using a

digital pH meter calibrated with a standard buffer tablet of pH 7. Formulations were placed in beakers, and the pH was recorded at room temperature.

##### Determination of viscosity

The viscosity was measured using a Brookfield Viscometer. The spindle no. 61 was immersed in each spray sample, and the viscosity was recorded at 2 RPM.

##### Determination of density

The density was determined by filling a density bottle with 10 ml of each formulation and weighing it on an electronic balance.

##### Drug content analysis

Drug content was determined in each of the formulations. About 0.1ml of the sample was transferred in 10ml of a volumetric flask and made up the volume with distilled ethanol, prepared the stock solution. 0.1ml was pipette out from the stock solution and diluted with distilled ethanol. The content of Citronella oil and Catnip oil was determined by using a Shimadzu 1800 UV-visible spectrophotometer at  $\lambda$  288 nm and 241 nm respectively. The analysis was carried out three times.

##### Skin irritation test

The back hairs of albino mice were shaved using hair removal skin cream to expose a skin area of about 5cm. The exposed area of the albino mice was treated with three types of formulations i.e., Temporary emulsion, alcohol-based, and aqueous, and covered with a gauge pad (bandage). The mice were labeled F1, F2, and F3 respectively for the different formulations. After 24 hrs. The tape and gauge were removed and the treated area was evaluated for irritation, and redness of the skin.

##### Mosquito repellence test

The repellency of the formulations was tested on human volunteers by exposing treated hands to caged mosquitoes (*Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*) for 5 hours. The number of mosquito bites was recorded, and the percentage repellency was calculated using the formula:

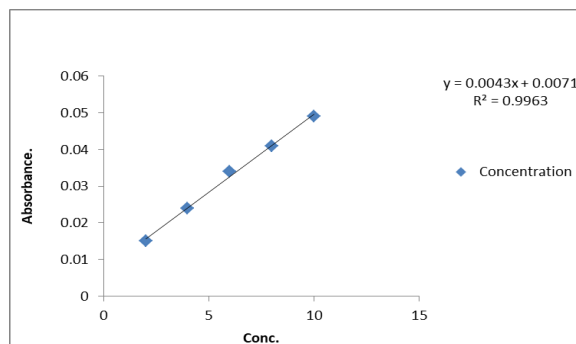
$$\% \text{ Repellency} = \frac{\text{No. of bites on control arm} - \text{No. of bites on treated arm}}{\text{No. of bites on the control arm}} \times 100$$

##### Stability studies

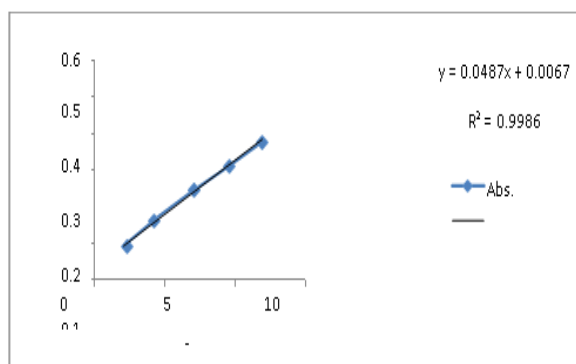
Stability studies were conducted on the formulations by storing them in a REMI SC-6 plus stability chamber at  $30 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH for 30 days. Periodic evaluations were carried out to assess pH, viscosity, drug content, and mosquito repellency.

**RESULT AND DISCUSSION****Table 1: Graded absorbance of citronella oil at 288 nm.**

Sr. no.	Concentrations (mcg/ml)	Absorbance (nm)
1.	2	0.015
2.	4	0.024
3.	6	0.034
4.	8	0.041
5.	10	0.049



The standard curve followed the Beer- Lambert's law in the concentration range 2 to 10  $\mu\text{l} / \text{ml}$  with  $R^2 = 0.9963$ .

**Figure Standard calibration curve of catnip oil at 241 nm.**

The standard curve followed the Beer- Lambert's law in the concentration range 2 to 10  $\mu\text{l} / \text{ml}$  with  $R^2 = 0.9986$ .

**Table 2: Graded absorbance of Catnip oil at 241 nm.**

Sr. no.	Concentration (mcg/ml)	Absorbance (nm)
1.	2	0.097
2.	4	0.207
3.	6	0.305
4.	8	0.394
5.	10	0.490

**Determination of pH (GLOBAL pH DPH-507)****Table 3: pH data of polyherbal-based spray formulation.**

Sr. no.	Formulations	pH*
1.	Temporary emulsion (F1)	5.71
2.	Alcohol-based (F2)	7.76
3.	Aqueous based (F3)	5.76

The pH of the formulation was found to be satisfactory and was in the range of 5.7-7.7.

**Determination of viscosity****Table 4: Viscosity of three formulations (F1, F2 and F3).**

Sr. no.	Formulations	RPM	Torque	Cps.*
1.	Temporary Emulsion(F1)	2	0.8	24
2.	Alcohol based(F2)	2	0.6	18
3.	Aqueous based(F3)	2	0.8	24

Formulations F1 and F2 were aqueous-based emulsion-type of formulations, their viscosity was found to be

higher i.e. 24 Cps as compared to formulation F3 which shows lower viscosity of 18 Cps at 2 rpm.

**Determination of density****Table 5: Density data of polyherbal-based formulation.**

Sr. no	Formulations	Density(g/10ml)
1.	Temporary emulsion (F1)	0.939
2.	Alcohol-based (F2)	0.746
3.	Aqueous based (F3)	0.969

**Drug content of citronella Oil and Catnip oil in formulation****Table no. 6: Drug Content data of polyherbal based Formulations.**

Sr. no.	Formulations	Drug Content of Citronella oil	Drug Content of Catnip oil
1.	Temporary Emulsion(F1)	95.91%	99.79%
2.	Alcohol Based (F2)	93.87%	100%
3.	aqueous Based (F3)	93.87%	98.97%

The all three formulations F1, F2 and F3 were found to content satisfactory drug content that ranges from 93-100% of citronella and catnip oil.

**Skin irritation test****Table 7: Skin irritation data of polyherbal-based Formulation.**

Sr. no.	Formulations	Skin Irritation
1.	Temporary Emulsion (F1)	No
2.	Alcohol Based (F2)	No
3.	Aqueous Based (F3)	No

**Mosquito repellency testing****Table no. 8: Drug Content data of polyherbal based Formulations.**

Sr No	Composition	% Repellency				
		1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.
1	Temporary emulsion	93%	92%	89%	85%	81%
2	Alcohol based spray	85%	82%	81%	78%	75%
3	Aqueous based spray	87%	85%	83%	80%	76%

**Stability studies of formulations****Table 9: pH of polyherbal based formulations after stability studies.**

Sr. no.	Parameter	Formulations	Storage period	
			0 days	30 days
1.	pH	Temporary Emulsion (F1)	5.71	4.59
2.		Alcohol Based(F2)	7.76	5.46
3.		Aqueous Based(F3)	5.76	5.32

**Table 10: Viscosity of formulations after stability studies.**

Sr. no.	Parameter	Formulations	Storage period					
			0 days			30 days		
			RPM	Torque	Cps	RPM	Torque	Cps
1	Viscosity	Temporary Emulsion(F1)	2	0.8%	24	2	0.8%	24
2		Alcohol Based (F3)	2	0.6%	18	2	0.6%	18
3		Aqueous Based (F3)	2	0.8%	24	2	0.8%	19

**Table 11: Drug content data of formulations after stability studies.**

Sr. no	Parameter	Formulations	Drug content(0days)		Dru Drug content (30days)	
			Citronela oil	Catnip oil	Citronell oil	Catnip oil
1	Drug content analysis	Temporary emulsion(F1)	95.91%	99.79%	96.17%	95.45%
2		Alcohol-based(F2)	93.87%	100%	97.56%	93.86%
3		Aqueous based(F3)	93.87%	98.97%	94.05%	93.88%

**Table no. 12: Mosquito repellency test of formulations after stability studies.**

Sr. no.	Parameter	Formulations	Storage period (% repellency)	
			0 days	30days
1.	Mosquito repellencytest	Temporary Emulsion (F1)	93%	91%
2.		Alcohol-based (F2)	84.84%	82.3%
3.		Aqueous based (F3)	87.12%	78.15%

The stability studies were carried out on optimized formulations (F1, F2 and F3) at 30±2oC temperature and 65±5% RH for 30 days. The formulation was showed good stability with no remarkable change in drug content, pH, viscosity, Rf values, drug content and mosquito repellency test profile.

### CONCLUSION

The present investigation revealed that three different formulations [Temporary emulsion (F1), Alcohol based (F2) and Aqueous based (F3)] from the combination of Citronella and Catnip oil are having excellent mosquito repellent properties. Three formulations of Polyherbal body spray are safe and effective. pH, viscosity, density and Thin Layer chromatography having excellent result.

Investigation of skin irritation test on 3 mice of the three formulations has been conducted for a period of 24 hrs. and 48 hrs. after experiments, shows no irritation was experienced. The 1 hr. repellency activity of the three formulations is high. Formulations also retained good stability condition over a period of 30 days.

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