HERBAL MOSQUITO REPELLENT DHOOP STICK: FORMULATION AND EVALUATION OF EUPHORBIA TIRUCALLI AND CALOTROPIS GIGANTEA LATEX

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Abstract:

Background: *Worldwide, mosquito-borne illness continues to be a serious public health concern. Dengue, chikungunya, and malaria are serious public health issues. The WHO estimates that the mosquito-borne illness affects 14.1 million people in India. There is a need for a natural substitute because synthetic repellents can include dangerous chemicals. The purpose of this study was to use plant latex to create and assess a natural mosquito repellent.*
Methodology: *Fresh latex from Euphorbia tirucalli and Calotropis gigantea was combined with other excipients known to have mosquito-repelling qualities to create the repellent. Following conventional procedures, the Dhoop stick's physico-chemical characteristics, moisture content, ash value, flammability, larvicidal activity, mosquito repellence, and safety profile were assessed. A cage test approach was used to measure repellence against different mosquitoes.*
Result: *For up to three hours, the Dhoop Stick's formulation demonstrated strong insect repellent protection. During safety testing, no skin irritation or toxicity was noted, and physiochemical analysis verified the stability and satisfactory burning qualities. Compared to synthetic repellents, the Herbal Dhoop stick, which is made with latex from E. tirucalli and C. gigantea, is safe, effective, and environmentally friendly.*

KEYWORDS: *Euphorbia tiurcalli, Calotropis gigantea, Mosquito Repellent, Hand roll method.*

INTRODUCTION

Given the increasing prevalence of mosquito-borne illnesses in the current environment, controlling mosquitoes is one of the most crucial challenges [1]. Due to favorable ecological circumstances, mosquito-borne diseases are common throughout all of India's states [2]. Mosquitoes are small, two-winged flies (family Culicidae, order Diptera) that are easily identified by their scaly legs and wings, as well as their lengthy proboscis. Mosquitoes are little, bloodsucking insects that breed in standing water. In order to produce eggs, female mosquitoes must consume blood. They quickly draw blood by puncturing an animal's skin with their mouthparts. They frequently carry viruses that can infect humans while they are feeding [3]. Dengue fever, malaria, yellow fever, and other diseases are spread by mosquito species in the Culex Aedes genera [4]. Due to their potential to spread illness, mosquito-borne diseases pose a serious threat to civilization, affecting about 700 million people annually. *Aedes aegypti* is the primary vector that spreads the RNA virus that causes dengue fever, which is a member of the Flaviviridae family [5]. Lymphatic filariasis is caused by *Wucheria bancrofti*, which is spread by *Culex quinifaciens*. The WHO claims that these illnesses are widespread throughout India, leading to a significant number of cases and fatalities between 2010 and 2016 [6].

The purpose of a mosquito repellent is to keep mosquitoes away so they can't bite people and feed on human blood. Natural and synthetic mosquito repellents are the two varieties available on the market. Certain hazardous chemical compounds found in synthetic repellents are strong carcinogens that are very detrimental to both people and the environment. Taking all of this into account, we created a product containing herbal components that have no known negative effects. Because there are no artificial chemicals, the herbs are readily accessible [7]. For thousands of years, people have been interested in plant latex, and a large portion of the research on its bioactive qualities has been directed toward therapeutic uses. Additionally, the plant's latex may be hazardous to insects and butterflies. The succulent *Euphorbia tirucalli* is a member of the Euphorbiaceae family, which is the largest and most varied family in the kingdom of plants [8]. It is a tiny tree with pencil-like branches that can reach heights of three to six meters. As a result, it is frequently called tirukkali (Tamil) or the pencil tree (English). Because it is not grazed by domestic animals, has few pests, and is resistant to harsher circumstances like drought and salt stress, the tree is commonly employed as a fence plant and for boundary marking [9]. It is a well-known medicinal plant both domestically and abroad, much like many other Euphorbiaceae. It is said to offer therapeutic properties for certain illnesses [10]. Giant milkweed, or *Calotropis gigantea*, is a common weed in arid regions and a member of the Apocynaceae family. The plant features clusters of white or lavender waxy blooms, a milky stem, and oval, light green leaves. In India, *C. gigantea* is widely accessible and utilized in traditional medicine for a number of therapeutic uses. Gigantea has recently been shown to provide a number of therapeutic benefits [2].

MATERIALS AND METHODOLOGY

The plants *Calotropis gigantea* and *Euphorbia tirucalli* were collected and authenticated from Kalaignar Karunanidhi Government Arts College, Tiruvannamalai, by the botanist Dr J Suresh Kumar, M.Sc., M.Phil., PhD, PGDCA, on 27th June 2025. The herbal excipients listed in Table 1 were acquired from Great Scientific Industries in Vengikkal, Tiruvannamalai, Tamil Nadu.

Table 1: Description of Excipients

Name	Biological name	Family	Chemical constituents
1. Orange peel powder 	Fresh and dried outer part of the pericarp of *Citrus aurantium Linn* [11].	Rutaceae	Limonene (90%), Citral (4%), Vitamin C, pectin
2. Turmeric powder 	Dried Turmeric is the dried rhizome of *Curcuma longa Linn* [12].	Zingiberaceae.	Curcuminoids, Curcumin, desmethoxy curcumin.
3. Camphor 	Camphor is a solid ketone, obtained from the volatile oil *Cinnamomum camphora* [13].	Lauraceae	camphor, cineole, pinene, camphene, phellandrene, limonene, diterpenes

4.Gum acacia 	Stems and branches of the Acacia arabica plant [14].	Leguminosae	Arabin, Arabic acid
5. Starch 	Grains of maize *(Zea mays Linn)*, Rice (*Oryza sativa Linn)* [15].	Maize, rice (Gramineae) Tubers of potatoes (solanaceae)	Amylose, Amylopectin.
6. Guar gum 	Powder of endosperm of the seeds *of cyamopsis tetragonolobus linn* [16].	Leguminosae	Galactomannan, Mannose, lipids.
7. Charcoal 	Obtained from the shell of *coconut nucifera linn* [17].	Arecaceae	Vitamin B1, B6 Riboflavin, Lauric acid.

Table 2: Plant profile

Plant profile	Description
	Common name: Giant milkweed, Crown flower, Swallow-wort. Scientific name: *Calotropis gigantea* Family: Apocynaceae Chemical constituents: Calotropin, calotoxin, Uscharin & syriogenin.
	Common name: Pencil cactus, Milk bush, Indian tree spruce, Fire Stick plants. Scientific name: Euphorbia tirucalli Family: Euphorbiaceae Chemical Constituents: Phorbol esters, triterpenoids & ingenol esters.

Collection of latex

Fresh latex was acquired locally from *Calotropis gigantea* and *Euphorbia tirucalli*, by cutting an incision on the tree's adult branches, from which latex seeped into little sample bottles. To prevent latex solarization, which would cause the sample bottles to deteriorate, they were corked and covered with aluminium foil.

Figure 1: Collection of latex.

METHODOLOGY

Latex collection: Both plants' fresh latex was gathered from the medicinal garden. Mixing: To create a dough-like consistency, latex was combined with water, camphor, turmeric powder, wood powder, orange peel powder, and acacia. Rolling: The 3g chunks of dough were manually rolled onto unadorned bamboo sticks. Drying: For at least seven days, the sticks were left to dry in the shade. Sandalwood oil was used to perfume the dried sticks before they were packaged [(7)]

EVALUATION PARAMETER PROCEDURE FOR HERBAL DHOOP STICKS

Physical evaluation: The formulated herbal Dhoop stick was visually evaluated for colour and odour.

Flammability: The Dhoop stick was burned to test its flammability and burning time. The Dhoop stick was seen to be fully burned, producing little smoke, and the burning time was recorded.

Moisture Content Test: The prepared Dhoop stick's initial weight is burned, and the dried Dhoop stick's ultimate weight is recorded.

Calculated by using the formula Moisture Content= Wet weight – Dry weight x 100.

Ash Value Test: The Dhoop stick was burnt completely, and the ash was collected & weighed using the formula, Ash value= final weight -initial weight [(18)].

Repellence Test: A rectangle repellent test is conducted with an aperture to allow mosquitoes to flee. Inside the net, the Dhoop stick caught fire. It is important to record how long it takes the mosquitoes to attempt to flee, obtain a number, or die [(19)].

Irritation Test: to assess Dhoop sticks' potential for irritation. Burn the Dhoop sticks, look for irritation symptoms like sneezing, coughing, or eye irritation, and note any flaws we see [(20)].

Larvicidal bioassay:

i. Larvae culture: Using a standard technique from the World Health Organization, latex bioassays were conducted on mosquito larvae [(21)]. The instar larvae from Arunai College of Pharmacy, Tiruvannamalai, were gathered from an open, exposed tank in a field of standing water. Larvae were initially housed in a plastic container filled with tap water to establish the colony. They were kept at the standard room temperature. Yeast, dog biscuits, and algae gathered from ponds were the larvae's food sources.

ii Bioassay: The larvae recovered during the laboratory trail screening process were placed in plastic glasses. First, 100 millilitres of distilled water (stock solution) were used to dissolve 1 millilitre of new latex. In a volumetric flask, 1 mcg/ml of the stock solution was made using distilled water. With a few minor adjustments, the WHO technique was used to evaluate the larvicidal activity. The larvae were separated into six batches of ten different concentrations (100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, and 350 ppm) for the bioassay test. After 24, 48, 72 hours of exposure, the number of dead larvae was tallied, and the average of three days was used to calculate the % mortality.

iii Statistical analysis: Abbott's (1925) formula was used to adjust the mortality data, and Finney's method of probit analysis was applied to determine the concentration that killed 50% of the tested larvae (LC50) and 90% of the tested

larvae (LC90). To determine the significance of the relationship between time and concentration, the data were additionally subjected to a one-way ANOVA.

iv Data Analysis: The formula of was used to compute mortality. This accounts for changes in an ecosystem's larval population. Mortality as a percentage = Number of deceased larvae divided by the number of imported larvae (X 100). Two latex treatments were shown to be highly effective based on calculated larval mortality. This formula was used to determine the LC50 [22]:

LC_{50}=C1+ (50-M1)/ (M2-M1) x (C2-C1)

C1- low conc 1(below 50% mortality)
C2- higher conc (above 50% mortality)
M1- % mortality at C1
M2- % mortality at C2
LC90 were the calculated by using this formula:

LC_{90}=C1+ (90-M1)/ (M2-M1) x (C2-C1)

C1- low conc (below 90% mortality)
C2- higher conc (above 90% mortality)
M1- % mortality at C1
M2- % mortality at C2

RESULT AND DISCUSSION:

Herbal mosquito repellent Dhoop sticks should burn steadily, slowly, and completely, emit little smoke, and keep mosquitoes away for an extended period of time. The mosquito repellent stick is lighter, burns for longer, and has less ash [23]. In the current study, five different formulations of a mosquito repellent stick were made using plant latex and additional excipients. The F5 met the aforementioned ideal parameters and was very effective. **Table 3** represents the ingredients used in the preparation of Herbal mosquito-repellent Dhoop sticks along with their respective functions.

Table 3: Uses of Ingredients in Herbal Dhoop Sticks

S.NO	INGREDIENTS	USES
1.	Latex *(Euphorbia tirucalli, Calotropis gigantea*)	Larvicidal activity
2.	Orange peel powder	Aromatic
3.	Turmeric Powder	Coloring agent
4.	Camphor	Enhance combustion
6.	Acacia	Binding agent
7.	Guar gum	Binding agent
8.	Starch	Binding agent
9.	Charcoal	Enhance combustion Process
10.	Sandalwood oil Rose oil	Flavoring agent
11.	Water	Vehicle

latex from *Euphorbia tirucalli* and *Calotropis gigantea* has larvicidal properties against mosquito larvae, it was utilized as the active ingredient. Turmeric powder is utilized as a natural coloring agent, and orange peel powder is added as an aromatic to provide a pleasing scent. In order to improve combustion and guarantee even, smooth burning of the Dhoop sticks, camphor and charcoal were added. Starch, guar gum, and acacia were used as binding agents. To add a scent while burning, sandalwood and rose oils were added as flavorings. In order to achieve an appropriate mass for moulding Dhoop sticks and to enable homogeneous mixing of all elements, water was utilized as a transport. **Table 4** shows the formulation composition of herbal Dhoop sticks prepared in five different batches (F1-F5) with varying concentrations of ingredients.

Table 4: Formulation of Herbal Dhoop Sticks

S. No	INGREDIENTS	F1	F2	F3	F4	F5
1	Latex-*Euphorbia tirucalli*	6ml	6.5ml	7.5ml	7.5ml	**7.5ml**
2	Latex-*Calotropis gigantea*	6ml	6.5ml	7.5ml	7.5ml	**7.5ml**
3	Orange peel powder	4.7g	6.25g	-	6g	**5g**

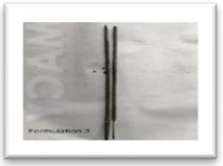
4	Wood powder	-	6.9g	8.5g	10.5g	**4g**
5	Charcoal powder	4.7g	6.25g	9g	-	**-**
6	Turmeric powder	4.7g	5g	4g	4g	**5g**
7	Camphor	7g	6.9g	8.5g	10.5g	**10g**
8	Acacia	-	3.1g	-	5g	**5.5g**
9	Guar gum	-	-	5g	-	**-**
10	Starch	5.8g	3.1g	-	-	**-**
11	Rose oil	q.s	q.s	q.s	-	**-**
12	Jasmine oil	-	-	-	q.s	**-**
13	Lemon oil	-	-	-	-	**-**
14	Sandalwood oil	-	-	q.s	-	**q.s**
15	Water	q.s	q.s	q.s	q.s	**q.s**

The active larvicidal ingredient in all formulations was the latex of *Euphorbia tirucalli* and *Calotropis gigantea*, with amounts progressively increasing from (F1-F5). To enhance aroma and combustion qualities, varying amounts of orange peel powder, wood powder, and charcoal powder were added. All formulations used turmeric powder as a natural coloring agent, and different amounts of camphor were added to improve burning efficiency and ignition. In certain formulations, starch, guar gum, and acacia were employed as binding agents to improve cohesiveness. To add scent, essential oils like sandalwood, rose, and jasmine were added in the necessary amounts (q.s). All formulations employed water as a carrier in the necessary amount.

Figure No 2: Formulation of Herbal Dhoop Sticks

Table 5 describes the defects observed in different formulations of herbal Dhoop sticks along with their probable causes.

Table 5: Defects of Herbal Dhoop Sticks

FORMULATIONS	DEFECTS	CAUSES
FORMULATION 1: 	Appear cracks	May be much amount of coconut fibre and starch.
FORMULATION 2: 	A breaking effect is produced.	Maybe More Amount Of charcoal and starch.
FORMULATION 3: 	Breaking effect produced.	Addition of guar gum.
FORMULATION 4: 	The stick is too hard.	Usage of a combination of acacia and guar gum.
FORMULATION 5: 	The above formulation defects do not arise.	Removal of charcoal, guar gum, starch, coconut fibre, Addition of acacia.

Formulation 1 contained an excessive amount of coconut fiber and starch, cracks appeared in this formulation. Because Formulation 2 contained more starch and charcoal, it had a breaking effect. Formulation 3 showed the Dhoop sticks' breaking effect, which might have been brought on by the guar gum addition upsetting the equilibrium of the binding ingredients. Because acacia and guar gum were used together, Formulation 4 was discovered to be too hard. The elimination of charcoal, guar gum, starch, and coconut fiber, coupled with the addition of acacia as an appropriate binding agent, resulted in Formulation 5, which did not exhibit any of the aforementioned flaws.

The evaluation parameters of Herbal Dhoop sticks made in five distinct formulations (F1–F5) are shown in **Table 6**, along with a comparison to standard values.

Table 6: Evaluation test for Herbal Dhoop Stick

EVALUATION PARAMETERS	F1	F2	F3	F4	F5
1. Physical test: A. Herbal Dhoop stick: Colour Odour Texture	 Black Smoky Rough	 Black Smoky Rough	 Black Smoky Rough	 Yellow Aromatic Rough	 Yellow Aromatic Rough
B. Standard (Moon relax): Colour Odour Texture	Green Smoky Smooth				
2. Flammability test: A. Herbal Dhoop stick: Burning time (mins)	5 mins	12 mins	15 mins	35 mins	47 mins
B. Standard: Burning time (mins)	1hrs ±05 mins				
3. Moisture content: (wet weight-dry weight) x100	80%	85%	90%	90%	90%
4. Irritation test: A. Herbal Dhoop stick:	Normal	Mild	Mild	Fair	Fair
B. Standard	Mild				
5. Ash test: A. Herbal Dhoop stick:	-	-	-	-	0.08g
B. Standard	0.38g				
6. Smoke test: A. Herbal Dhoop stick	visible	visible	visible	visible	visible
B. Standard	visible				
7. Repellence test: A. Herbal Dhoop Stick	0	0	2	2	5
B. Standard	9				

Physical analysis revealed that the normal Dhoop stick was green in color, with a smoky odor and a smooth texture; formulations F1, F2, and F3 were black in color, with a smoky odor and rough texture; formulations F4 and F5 were yellow in color, with an aromatic odor and rough texture. In comparison to the conventional burning duration of one hour and five minutes, the flammability test revealed a progressive increase in burning times F1–F5, with burning times of five, twelve, fifteen, thirty-five, and forty-seven minutes, respectively. in contrast to the typical burning time of one hour and five minutes. An appropriate moisture level for consistent burning was indicated by moisture content values ranging from 80% to 90%. Testing for irritation showed that F1 was normal, F2 and F3 were mildly irritated, and F4 and F5 were fair, showing mild irritation that was comparable to the benchmark. The herbal Dhoop sticks should have very little ash residue after an ash content study; F5 produced 0.08g of ash as opposed to the typical 0.38g. The results of the smoke test showed that all formulations had visible smoke, which was comparable to the norm. Mosquito repellent activity increased from F1 to F5, exhibiting the most repellency effects of all the formulations, according to repellence testing.

Here, Table **7** represents the larvicidal bioassay results of *Euphorbia tirucalli* & *Calotropis gigantea* latex against mosquito larvae, evaluating the dose and time-dependent mortality effect.

Table 7: Larvicidal Bioassay

S. No.	Test sample	No. of larvae tested	Mosquito species	Larval instar (i–iv)	Concentration (ppm or mg/l)	Control	No of dead mosquitoes on day 1	No of dead mosquitoes on day 2 (cumulative)	No of dead mosquitoes on day 3 (cumulative)	Mortality at 24 hrs (%)	Mortality at 48 hrs (%)	Mortality at 72 hrs (%)
1	***(E.tirucalli,C.giganteaa*** **) Latex**	10	Varieties of mosquitoes species	Stage (III and IV)	100	10(alive)	0	3	4	0%	30%	70%
2		10			150	10(alive)	1	4	4	10%	50%	90%
3		10			200	10(alive)	0	6	4	0%	60%	100 %
4		10			250	10(alive)	0	8	2	0%	80%	100 %
5		10			300	10(alive)	0	9	1	0%	90%	100 %
6		10			350	10(alive)	2	8	0	20%	100 %	100 %

Larvae of different mosquito species at late larval instars (3 and 4) were exposed to varying concentrations of the test sample ranging from 100 to 350 ppm (mg/L), as the above table explains. The control group demonstrated 100% survival, confirming the absence of natural mortality. The cumulative mortality for days two and three was recorded, along with the number of dead larvae at 24, 48, and 72 hours. At 100 ppm, there was no death at 24 hours, but at 48 and 72 hours, mortality rose to 30% and 70%, respectively. As concentration rose, 150 ppm showed 10% mortality at 24 hours and 90% mortality at 72 hours, whereas 200–300 ppm produced 100% mortality by 72 hours despite little to no death at 24 hours. With 20% mortality at 24 hours and 100% mortality within 48 hours, the maximum concentration, 350 ppm, demonstrated a quick impact.

Table No 8: Probit Regression Analysis

Concentration (Ppm)	Log Concentration	Percentage Mortality	Probit Value
100	2.00	70%	5.52
150	2.18	90%	6.28
200	2.30	100%	7.33
250	2.40	100%	7.33
300	2.48	100%	7.33
350	2.54	100%	7.33

Table No 9: Determination of Lc_{50} and Lc_{90} Value

Exposure time	Lc_{50}	95 % LCL-UCL	Lc_{90}	95% LCL-UCL	χ(Chi-Square) df=4	Probit Regression	Slope(±SE)	Significant value <0.05
24h	390	310-510	620	510-820	1.12	Y=2.31+1.48 $Log_{10}C$	1.48±0.32	0.05
48h	165	145-185	285	255-330	0.86	Y=1.87+2.94 $Log_{10}C$	2.94±0.41	0.05
72h	112	95-128	175	150-205	0.42	Y=1.42+3.76 $Log_{10}C$	3.76±0.38	0.05

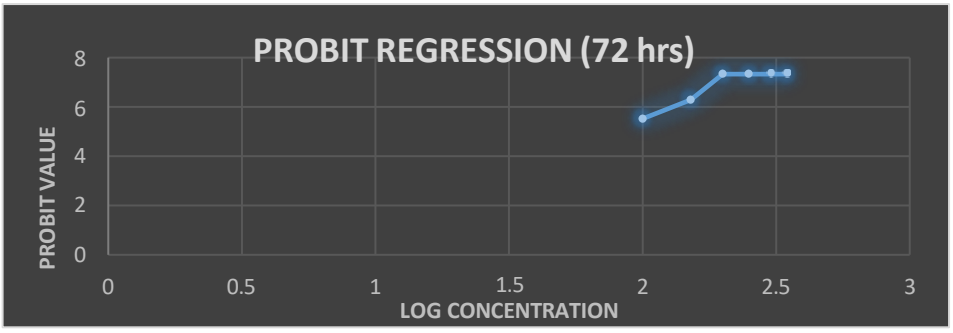


Figure 3: Graphical Representation of Probit Regression Analysis(72hrs)

This table 8 and 9 represents the probit analysis of larvicidal activity of the test sample at different exposure periods (24, 48 and 72 h), showing the lethal concentration values required to cause 50% (LC_{50}) and 90% (LC_{90}) mortality along with their 95% confidence limits (LCL–UCL) following the Finney method. As control mortality was zero, Abbott's correction was not applied. At 24 h exposure, the LC_{50} was 390 ppm (310–510 ppm), and the LC_{90} was 620 ppm (510–820 ppm), indicating comparatively lower toxicity at shorter exposure duration. With an increase in exposure time to 48 h, the LC_{50} and LC_{90} values decreased to 165 ppm (145–185 ppm) and 285 ppm (255–330 ppm), respectively, demonstrating enhanced larvicidal effectiveness. The highest toxicity was observed at 72 h, where the LC_{50} and LC_{90} values further reduced to 112 ppm (95–128 ppm) and 175 ppm (150–205 ppm), confirming a clear time-dependent increase in larval mortality. The chi-square (χ^2) values for all exposure periods were low (1.12, 0.86 and 0.42; df = 4), indicating a good fit of the probit regression model. The probit regression equations and slope values (1.48 ± 0.32 at 24 h, 2.94 ± 0.41 at 48 h and 3.76 ± 0.38 at 72 h) suggest a steep dose–response relationship with increasing exposure time. The goodness of fit of the probit model was assessed using the chi-square test. All statistical analyses were carried out
assuming a significant level of $p \leq 0.05$

Figure 4: Larvicidal Bioassay

In figure 3 represents the regression analysis was performed on the treatment means of the larvae that were discovered alive following treatment. Probit analysis results ($P \leq 0.05\%$) were deemed statistically significant. The latex extract's Lc50 values were 390 ppm at 24 hours, 165 ppm at 48 hours, and 112 ppm at 72 hours, according to the probit analysis, suggesting a time-dependent rise in larvicidal activity. The reliability of the bioassay data was confirmed by the probit regression analysis, which revealed a good fit ($p<0.05$). For the maximum dilution (1:350), LC50 and LC90 were reached within 24 hours of application, respectively, achieving overall mortality in 3 days. The number of mosquito larvae alive decreased between days two and three following treatment, but it began to increase again after the third day in all treatments, suggesting that latex was more effective during this time.

CONCLUSION

The Herbal Dhoop sticks made with the aforementioned ingredients demonstrated mosquito-repellent activity and had no negative side effects. The prepared Dhoop stick was economical, safe and environmentally beneficial. It is easily transportable and suitable for use by people of all ages. The above formulation was shown to be highly effective at eliminating mosquitoes; formulation 5 was more so compared to the standard (moon relax-citronella incense sticks). This research led to the creation of a natural mosquito repellent that is risk-free for people. The research findings also suggested that a mosquito repellent made from latex would be effective in reducing mosquito populations.

ACKNOWLEDGEMENT
The authors are thankful to the Dr J Geetha (Department of Pharmacognosy) for providing the innovative ideas, infrastructure and lab facilities to carry out the Research work.

CONFLICT OF INTEREST:
The authors declare no conflict of interest.

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