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Formulation of Natural Mosquito Repellent

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Abstract: Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of diseases and these are the primary source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants made scientists investigate these plants for their uses. The objective of the work was to formulate a mosquito repellent product containing Curcumin and Embelin obtained from *Curcuma longa* and *Embelia ribes* respectively. Isolated Curcumin and Embelin were confirmed by TLC technique. Bands were compared with standards by calculating R_f 0.74 and 0.60 respectively at visual detection and 254 nm. The formulation of cream was evaluated for various parameters like smooth texture and spreadability with a pH 7 which is a non irritant and suitable for the skin. There is no phase separation during thermal stability. From the present work, it was concluded that 1% Curcumin and 1.5% Embelin containing cream is safe, effective, usable for the skin and stable too.

Keywords: Curcumin, Embelin, Natural Mosquito Repellent.

1. INTRODUCTION

Mosquitoes are among the most disturbing blood sucking insects afflicting human beings. Mosquito species belonging to Anopheles, Culex and Aedes genera are vectors for the pathogens of various diseases like Dengue fever, Malaria, Yellow fever, etc.[3] The mosquitoes eject their saliva into the blood of the host that creates an immune response due to the binding of the antibodies IgG and IgE to the antigens. The reactions result in irritations, itching, redness and sometime it develops into the bumps. It is the saliva of the mosquito that often causes an irritating rash that is a serious nuisance. In addition, mosquito bites can cause severe skin irritation through an allergic reaction to the mosquito's saliva by human mosquito contact. Mosquitoes bear set of sensors that have the capability to track their prey's presence, these include:

A. Chemical Sensors: The studies have proved that the mosquitoes have the tendency to sense the lactic acid, carbon dioxide, and the propen-3-ol upto many yards away. On breathing or perspiration, humans and the animals release these compounds. This is the reason that a person who sweats more becomes the target of the species and the one who sweats less don't get as many bites.

B. Heat Sensors: The mosquitoes also have the ability to detect heat and hence can target the warm blooded animals very fast once they get close enough.

C. Visual Sensors: It's been recorded that the mosquitoes are the intelligent insects as they can easily detect you by looking at your clothing if it contrasts with the background. You are easily detected by them as anything that moves is alive and hence full of blood.^[4]

1.1. Control of Mosquito Borne Diseases

Mosquito control and personal protection from mosquito bites are currently the most important measure to control this disease. Prevention of this type of disease involves protecting yourself against mosquito bites.^[3] Mosquito control methods are habitat change, biological control, physical control and chemical control including individual safeguards from the mosquitoes. Among the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission by killing or preventing mosquitoes from biting a human being. This can be achieved by use of repellents.^[5]

1.2. What is a mosquito repellent?

A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages mosquitoes from landing on that surface. It is a substance that is synthesized in such a manner so that it makes the surface unpleasant and unattractive to mosquitoes so as to reduce the human mosquito contact. Mosquito repellents repel insects but do not kill them. Therefore, they are not technically insecticides nor pesticides. They help prevent and control the outbreak of mosquito borne diseases such as Dengue fever, Malaria, Yellow fever, Japanese Encephalitis, etc.^[4] They contain active ingredient which is the only reason to repel mosquitoes by blocking their olfactory senses which detects the carbon dioxide and lactic acid that gets released when the human perspires. These products also contain some more ingredients which aids them with cosmetic finishing.^[3]

1.3. Mosquito repellents of natural origin

Natural ingredients are included in some formulations of insect repellents. In the United States, citronella is a popular botanical ingredient in mosquito repellent formulations. The insecticidal properties of this oil were discovered in 1901, and it was used for a time as a hair dressing for the control of fleas and lice. Despite popular conception, citronella candles or incense were ineffective for reducing the biting pressure of mosquitoes. Neem oil, from *Azadirachta indica*, when formulated as 2% in coconut oil, provided complete protection for 12 hours from *Anopheles* mosquitoes.^[6]

1.4. Mosquito repellent mode of action

In many cases, it has been found that behavior that can be labeled as repellency may be the result of any number of physiological or biochemical events. Mosquito repellency caused by DEET is thought to be due to the blocking of lactic acid receptors, abolishing upwind flight, resulting in the insect "losing" the host. Further evidence for the role of lactic acid in host seeking comes from studies examining mosquito physiology following a blood meal. Host-seeking behavior in *Aedes aegypti* stops after taking a blood meal. It has been found that following a blood meal, the sensitivity of lactic acid sensitive neurons drops, and this drop is co-incident with the cessation of host-seeking behavior. Lactic acid sensitivity returns to normal after oviposition.^[6]

Turmeric is easily available in the Indian markets. It has been used traditionally in "ayurvedic medicine" as an antiseptic, wound healing, and anti-inflammatory compound. One of the constituents of turmeric, curcumin has stated mosquito repellent activity. Also curcumin can be easily extracted from *Curcuma longa*. Embelin obtained from *Embelia ribes* is reported to have potential insect repellent activity. Synthetic mosquito repellent used for control of vectors are causing irreversible damage to ecosystem and also chemicals are non-degradable in nature. Synthetic repellents are expensive for everyday use and there are concerns about their toxicity and safety. Long term exposure of new born babies and children to pyrethroid based mosquito repellents is associated with clinical, biochemical and neurological effects. *N*-diethyl-3-methylbenzamide (DEET) dissolves synthetic fabrics and plastic on eyeglasses and watches. The other undesirable effects of DEET are undesirable odour, subchronic toxicity, mutagenicity, reproductive and neurological toxicity. To overcome problem, there is need for development of effective non DEET alternatives and prepare repellent by using biodegradable mosquito repellent. Compared to synthetic repellents, plant based repellents are simple, effective, inexpensive, environment friendly and readily available. They are widely accepted by the public even though very few of them have been evaluated for toxicity. The aim of this study was to develop a isolated phytoconstituent based mosquito repellent that is effective and safe to use.^[5]

2. MATERIALS AND METHODS

2.1. Extraction & Isolation of Curcumin

Turmeric (*Curcuma longa*) powder was purchased from Patanjali Ayurved Limited, Mumbai, India. Curcumin, the active ingredient of turmeric was extracted from turmeric powder by using Soxhlet extractor. 50 g of dried powder was placed in a porous bag or "thimble" made of whatmann filter paper, which was placed in chamber of the Soxhlet apparatus. 225 ml ethanol was heated in a round bottom flask, which was attached to the Soxhlet extractor, and its vapors were condensed in condenser. The condensate then dripped into the thimble containing the turmeric powder, and extracted it by contact. When the level of solvent in chamber reached to the top of siphon tube, the liquid contents of chamber were flooded into flask and then the cycle began again. This process was carried out for a total of 21 hours. Yellow, solid crude curcumin was obtained. Embelin, was obtained from our college colleagues. Apparatus used: Soxhlet extractor, condensor, heating mantle, round bottom flask. Reagents used: Ethanol.

2.2. Preparation of Mosquito Repellent Products

2.2.1. Mosquito repellent cream

An aqueous cream (oil in water type) was prepared by emulsifying the essential oils in water with an emulsifying wax. The formula for the mosquito repellent cream is as follows:^[7]

INGREDIENTS	F1	F2	F3
Curcumin/Embelin	0.5%	1%	1.5%
Essential oils	5%	5%	5%
Cetyl alcohol	2%	2%	2%

Lanolin	1%	1%	1%
Mineral oil	2%	2%	2%
Stearic acid	15%	15%	15%
Glycerin	10%	10%	10%
Potassium Hydroxide	1%	1%	1%
Preservatives	Q.S.	Q.S.	Q.S.
Distilled water	Q.S. 100%	Q.S. 100%	Q.S. 100%

The oil in water type cream was prepared by incorporating lanolin, stearic acid, cetyl alcohol, mineral oil, propyl paraben, etc. in the oil phase and Glycerin, potassium hydroxide, methyl paraben etc. in water phase respectively. Both oil and aqueous phases were heated to 75°C. After heating, the oil phase was added into the aqueous phase with continuous stirring until the homogenous cream was formed. After complete emulsification, essential oils were added when the temperature dropped to 55°C± 60°C. Active ingredients- Curcumin & Embelin were then added by levigation method into separate formulations followed by addition of perfume.^[1]

2.2.2. Mosquito repellent candle

The candle was made up of a mixture of hard paraffin and stearic acid as the hydrocarbon bases. The formula for the insect repellent candle is as follows:^[8]

INGREDIENTS	F1	F2	F3
Curcumin/Embelin	0.5%	1%	1.5%
Lemon oil	10%	10%	10%
Eucalyptus oil	10%	10%	10%
Stearic acid	19%	19%	19%
Hard paraffin	Q.S. 100%	Q.S. 100%	Q.S. 100%

The candle was prepared by heating (70°C) hard paraffin and stearic acid until they were melted. The essential oils were added when temperature dropped to 55°C± 60°C. Active ingredients- Curcumin & Embelin were then added to separate formulations. The wick was plunged repeatedly in liquid wax, in order to obtain the required diameter. It was introduced into a cup shaped mould, which was then filled with liquid wax. After cooling, the solidified candle was removed from it.

2.3. TLC of isolated drug and formulation. (Cream only)

Identification of curcumin and embelin in formulated cream was determined using TLC. The cream sample was dissolved in methanol so that cream was broken and filtered. Then, resulting solution was applied to TLC silica gel plate using capillary against that of standard and isolated samples of curcumin and embelin. It showed the presence of curcumin and embelin in the cream formulation at Rf 0.74 and 0.60 respectively at visual detection and 254 nm. ^[1]

Mobile phase for Curcumin

n-PROPANOL	WATER	GLACIAL ACETIC ACID
8 ml	1 ml	1 ml

Mobile phase for Embelin

n-PROPANOL	n-BUTANOL	AMMONIA
7 ml	1 ml	2 ml

2.4. Evaluation of Cream

The cream was evaluated for appearance, spread ability, irritancy test, viscosity, pH, thermal stability tests.

2.4.1. Appearance

The appearance of the cream was judged by its color, texture, roughness and its odour.

2.4.2. Spreadability

The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under a certain load. Lesser the time is taken for separation of the two slides, better the Spread ability.

Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the formulation, such that the cream was sandwiched between the two slides weight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. The upper slide allowed slipping off freely by the force of weight tied to it. The time taken for the upper slide was noted.

2.4.3. Irritancy Test

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

2.4.4. PH

The pH meter was calibrated using standard buffer solution. About 5 ±0.01 g of the cream was weighed in a 100 ml beaker and dissolved in 45.0 ml of distilled water and dispersed the cream in it. pH of cream was measured at room temperature using the pH meter.

2.4.5. Thermal Stability

The formulated cream was kept in petriplates inside the incubator at 45° ± 1° for 48 hrs. The sample passed the test if on removal from the incubator shows no oil separation or any other phase separation. ^[1]

3. RESULTS AND DISCUSSION

The present study was conducted to determine the mosquito repellent activities of curcumin and embelin in order to obtain safe and efficient herbal mosquito repellent formulations. This was evaluated on the grounds of feedback obtained from volunteers. In the investigation, the creams formulated with curcumin and showed a significant and embelin showed moderate repellent activity.

The formulated cream was evaluated for the parameters like visual appearance, spreadability, irritancy test, pH, thermal stability. The appearance of cream was good having a smooth texture and easily spreadable property. Irritancy test was performed and there was no sign of redness and itching which indicated that the cream is safe for topical application. pH of cream was found to be 7 which is nearer to the required pH of the skin. The cream was thermally stable at 45°C ± 1°C and no phase separation was seen after 48 hours.

To confirm isolated phytoconstituent TLC was performed using n-propanol: water: glacial acetic acid (8: 1: 1) and n-propanol: n-butanol: ammonia (7: 1: 2) as mobile phase Along with that TLC showed the presence of Curcumin and Embelin respectively at Rf 0.74 and 0.60 respectively at visual detection and 254 nm in the formulated cream.

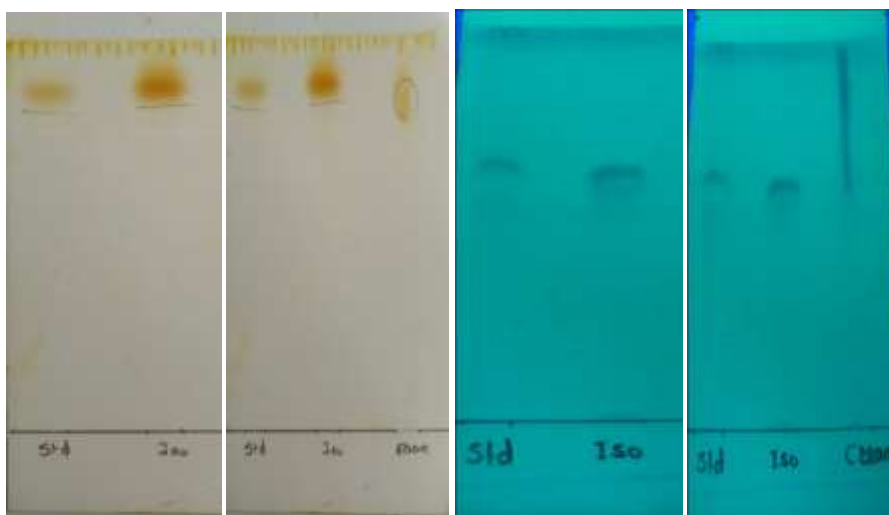


Image 1: TLC of Curcumin

Image 1: TLC of Embelin

Mobile phase for Curcumin -			Mobile phase for Embelin-		
n-propanol :	Water :	Glacial acetic acid	n-propanol :	n-butanol :	Ammonia
(8 ml :	1 ml :	1 ml)	(7ml :	1 ml :	2 ml)

All the Results are Tabulated Below:

Table 1: Results of Evaluation of Mosquito Repellant Cream. ^[1]

SR. No.	PARAMETERS	CURCUMIN CREAM	EMBELIN CREAM
1	DESCRIPTION	Pale yellow colored, smooth cream having a characteristic odour.	Pink colored, smooth cream having a characteristic odour.
2	SPREADABILITY	6.1 g cm/sec	6.2 g cm/sec
3	IRRITANCY TEST	Non irritant	
4	pH	7	
5	THERMAL STABILITY	Stable at 45°C ± 1°C for 48 hours.	

Feedback from 60 volunteers was taken for evaluating the properties of formulations of three concentrations (0.5%, 1% & 1.5%) tabulated in the following tables.

Table 2: Feedback of volunteers: For Curcumin cream

FORMULATION	MOSQUITO REPELLENCY	SPREAD ABILITY	AESTHETIC APPEAL	PLEASANT ODOUR	WASHABLE
0.5% Curcumin	1.8	2	2.4	1.8	2.2
1% Curcumin	3	2.2	3	3	2.8
1.5% Curcumin	2.2	1.8	2.2	1.8	2.2

Table 3: Feedback of volunteers: For Embelin cream

FORMULATION	MOSQUITO REPELLENCY	SPREAD ABILITY	AESTHETIC APPEAL	PLEASANT ODOUR	WASHABLE
0.5% Embelin	1.8	1.8	2	1.8	2.4
1% Embelin	1.8	2	2.4	1.8	2.2
1.5% Embelin	1.6	2	2.2	2.2	2

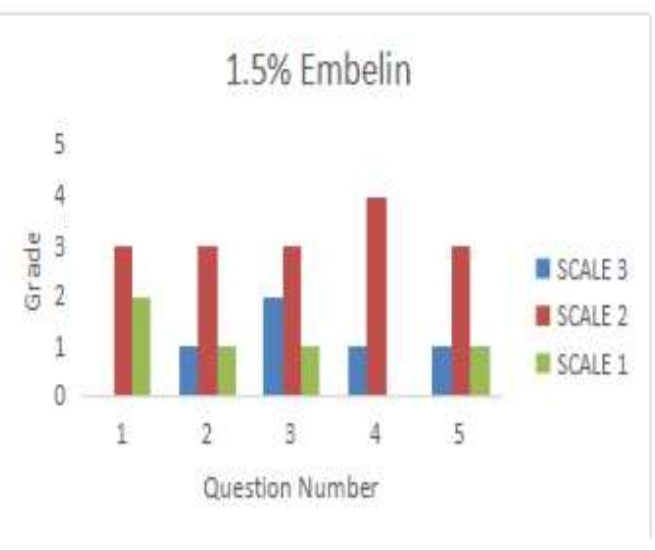
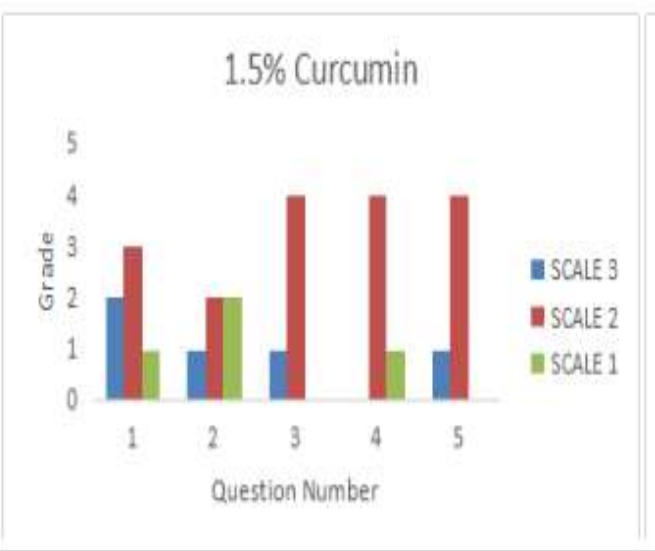
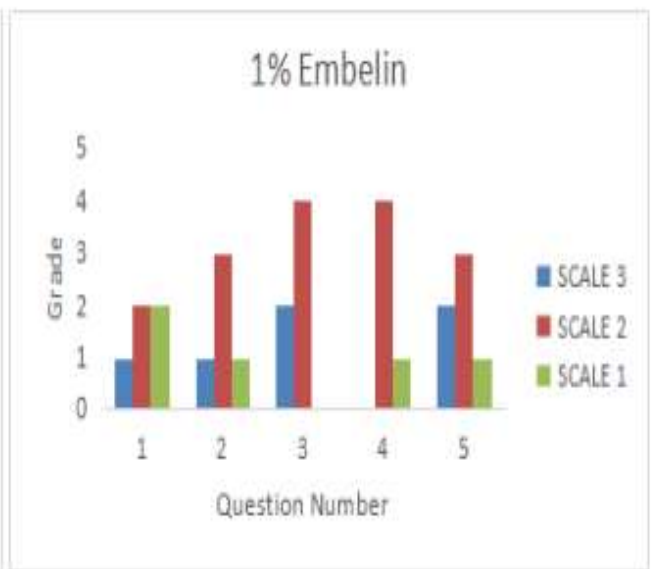
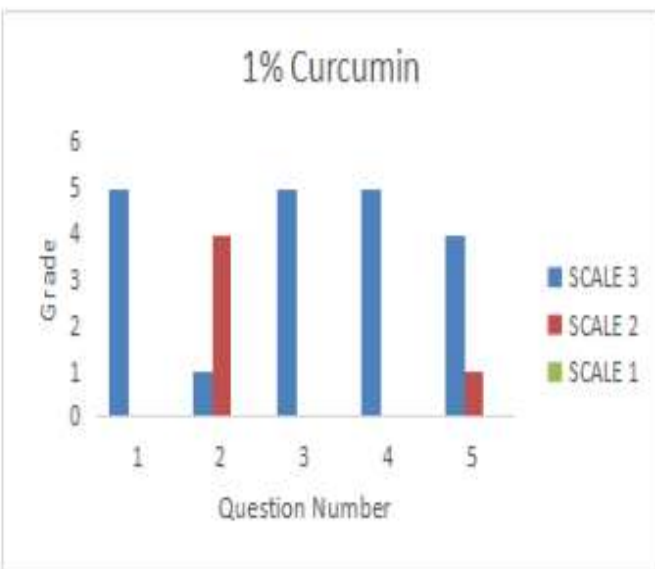
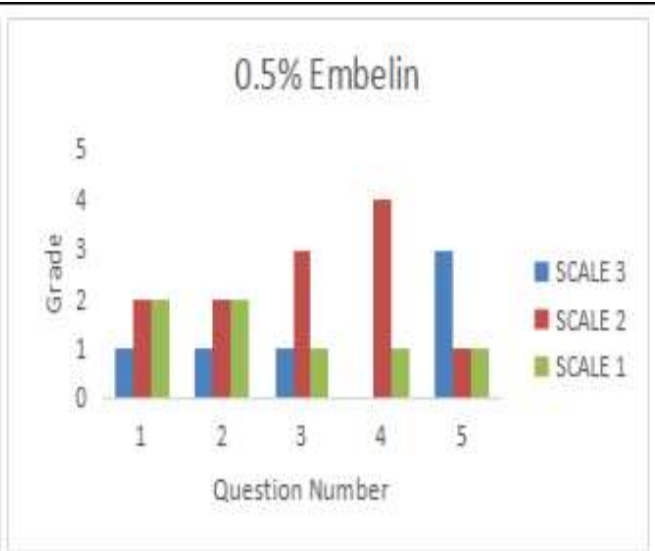
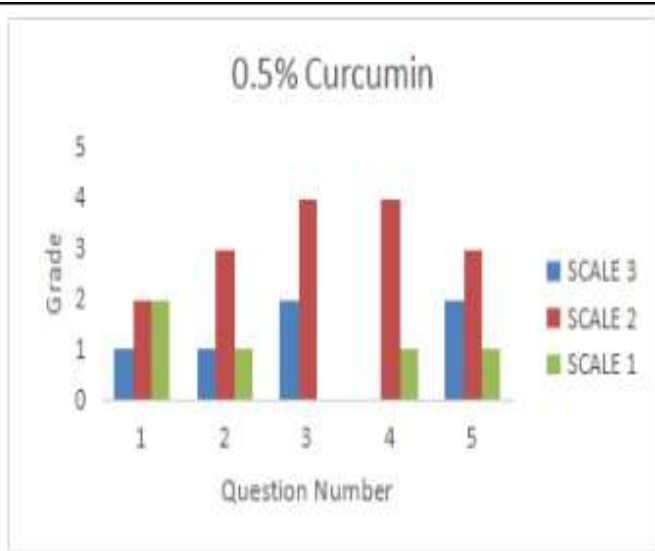
Table 4: Feedback of volunteers: For Curcumin candle

FORMULATION	MOSQUITO REPELLENCY	AESTHETIC APPEAL	PLEASANT ODOUR	CHARRIN G	SMOKE CREATION
0.5% Curcumin	1.8	2	2.4	1.8	2.2
1% Curcumin	3	2.2	3	3	2.8
1.5% Curcumin	2.2	1.8	2.2	1.8	2.2

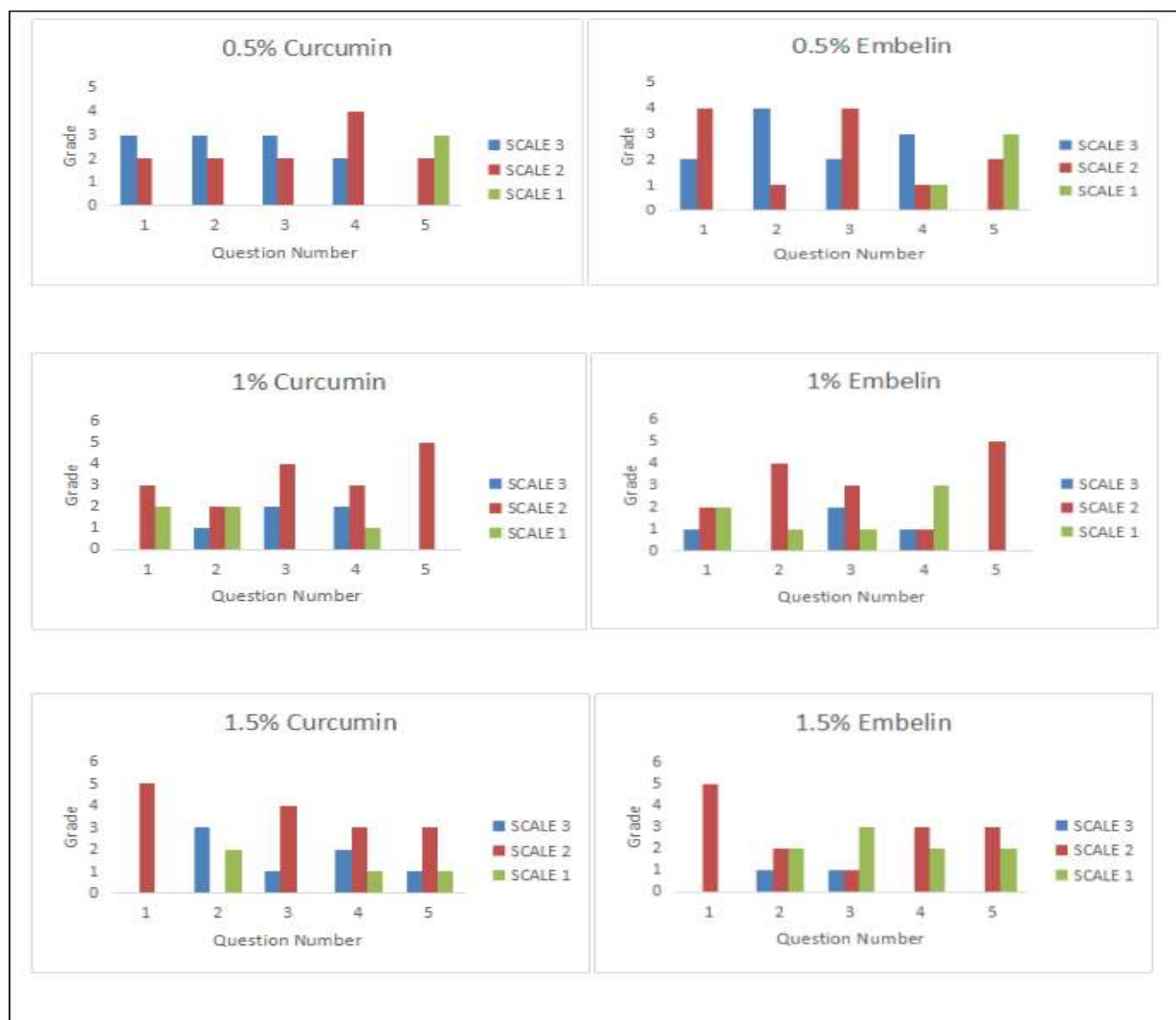
Table 5: Feedback of volunteers: For Embelin candle

FORMULATION	MOSQUITO REPELLENCY	AESTHETIC APPEAL	PLEASANT ODOUR	CHARRIN G	SMOKE CREATION
0.5% Embelin	2.4	2.8	2.4	2.4	1.4
1% Embelin	1.8	1.8	2.2	1.6	2
1.5% Embelin	2	1.8	1.6	1.6	1.6

Graph 1: The graphs below illustrate the grades for various properties of Curcumin and Embelin Cream having concentrations 0.5%, 1% and 1.5% on the scale of 3, 2, 1.



Graph 2: The graphs below illustrate the grades for various properties of Curcumin and Embelin Candles having concentrations 0.5%, 1% and 1.5% on the scale of 3, 2, 1.



5. CONCLUSION

Thus in the present study both the formulations i.e. 1% Curcumin and 1.5% Embelin containing creams offer a remarkable mosquito repellency. Based on these results, both phytoconstituents find potential applications in the pharmaceutical use. Further studies are needed to explore the other allied activities of Curcumin and Embelin.

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