

Design and Development of Mosquito Repellent Formulation Containing Carom Seed

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ABSTRACT

Article Info

Volume 7, Issue 5

Page Number: 88-94

Publication Issue :

September-October-2020

Mosquito bites can give an unpleasant feeling and may transmit the vector disease such as dengue or malaria to humankind. Mosquitoes are attracted to human blood containing protein to develop their eggs, biting human to another human, thereby transmitting the vector disease to human. Topical mosquito repellent gel was prepared with different concentration of extracted oil from carom seed all the formulation showed satisfied result with regular evaluation parameters like spreadability, washability, mosquito repellent activity, irritancy test. The formulation containing 7.5% of Carom seed oil extract showed repellent activity up to 80% these results are encouraging and promising. At last it was concluded that Carom seed oil extract has potential of mosquito repellent and can be converted into a marketed formulation like essence stick, repellent Gel etc.^[1]

Keywords : Mosquito Repellents, Carom Seed Oil, Skin Sensitivity, *Trachyspermum Ammi*, Screen Cage Method.

Article History

Accepted : 16 Sep 2020

Published : 23 Sep 2020

I. INTRODUCTION

A mosquito repellent doesn't actually kill mosquitoes. Repellents work by making people less attractive to mosquitoes, so they're less likely to bite you. The Environmental Protection Agency (EPA) says mosquito repellents that contain natural oils are safe for adults and children over the age of 2 months, when used correctly. Many repellents are nowadays available which can easily fend off the mosquitoes but are not good for the health as it contains a harmful chemical called DEET. It is fine to use a natural

repellent which can make you unattractive in the eyes of mosquitoes. The use of natural oils as an insect repellent is one of the wisest choices you can make. The biggest advantages of using only natural oils is that they are non-toxic and safe with no side effect.

Trachyspermum ammi is a native of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. In India, it is cultivated in Madhyapardesh, Uttarapardesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal. *Trachyspermum ammi* L. belonging

to family Apiaceae is a highly valued medicinally important seed spice. The roots are diuretic in nature and the seeds possess excellent aphrodisiac properties. The seeds contain 2–4.4% brown colored oil known as Ajwain oil. The main component of this oil is thymol, which is used in insecticide. the carom seeds are also a popular addition to borders and vegetable gardens. According to NYBG, not only can they keep away mosquitoes, but they also dissuade aphids, thrips, whiteflies, Mexican bean beetles, squash bugs, and tomato hornworms. Thus, a research will be conduct to study on formulation of natural mosquito repellent solution using extraction process for Carom seeds. Thymol has been extracted from Carom seed using at various extraction conditions such as different temperature, extraction time and volume of solvent. The summary of this attempt on claiming and efficacy of plant-based repellents are suggested for further drug development program. These plant seeds may represent an alternative in formulating potent and affordable products in the control of mosquitoes.^[2]

after a heating time of about 40 minutes and was continued till no more essential oil was obtained after 5-6 hours. After the distillation process was complete, the volatile essential oils were removed from the top of the hydrosol and dried over anhydrous sodium sulphate (Na₂SO₄) and stored in small sealed tubes at low temperature for preliminary screening of phytometabolites, qualitative screening by FT-IR and quantitative GC-MS analysis.^[2]

II. MATERIAL & METHOD:

Table No.1. List of instruments

Sr. No.	Instrument Name	Make
1	GC-MS	QP2010 Plus, Shimadzu, Japan
2	FTIR	Bruker
3	pH meter	Labtronics LT - 11
4	Clevenger apparatus	Borosil lab

I. Extraction of Carom seed oil:

Hydro-distillation using Clevenger’s method:

The plant parts of Carom seed oil i.e. flower (fresh and dry) were subjected to hydro-distillation using a Clevenger’s apparatus. The collection of oil started.

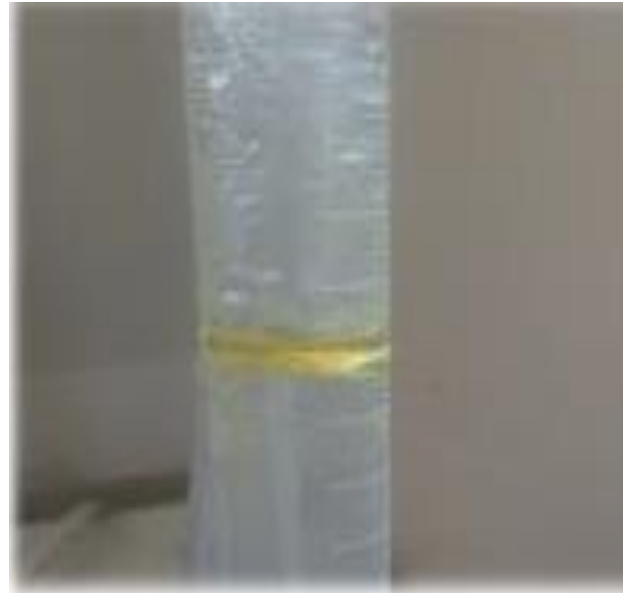


Fig No.1 Oil extraction from fresh carom seed by Clevenger method

Preparation of Mosquito repellent product:**Formulation of herbal mosquito repellent Cream.**

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 insect repellent cream is as follows:

Table no.2 Formulation concentration

Ingredient	F1	F2	F3
Carom seed oil	2.5 %	5 %	7.5 %
Cetyl alcohol	2 %	2 %	2 %
Lanolin	1%	1%	1%
Mineral oil	2 %	2 %	2 %
Stearic acid	15%	15%	15%
Glycerine	10%	10%	10%
Pot. Hydroxide	1%	1%	1%
Water	Q.S.	Q.S.	Q.S.

Procedure:

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 Glycerine, potassium hydroxide, etc. in water phase respectively. Both oil and aqueous phases were heated to 75^oc. 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.^[5]

Evaluation:**Evaluation of extracted oil;****1. Gas Chromatography- Mass Spectroscopy (GC-MS)**

Carom seed oil was analyzed using GC-MS (QP2010 Plus, Shimadzu, Japan), equipped with an Rtx-5 MS capillary column (30.0 m x 0.20.25 mm i.d., 0.25 µm film thickness) for the separation of its components. The injector was maintained at 260°C and operated in split injection mode (split ratio 50) with the split valve closed for one min and injection volume was 6 µL. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50°C for two min, raised to 210°C at 3°C/min, then to 280°C at 10°C/min. The interface temperature was 270°C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 650 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionization Voltage (EI) 70 eV, peak width 2s, mass.

2. Fourier Transform Infra-red Spectroscopy (F.T.I.R):

IR spectroscopy of Carom seed oil was done by using FT-IR spectrophotometer (JASCO FTIR-410). The spectra were scanned over wavelength region of 4000 to 400 cm⁻¹ at resolution of 4 cm⁻¹ the procedure consisted of dispersing sample of KBr and compressing into disc by applying of pressure consisted of 5 tons for 5 minutes in hydraulic press. The oil was placed in light path and the spectrum was obtained.

Evaluation of formulation:**Evaluation of herbal mosquito repellent Cream:****1. Primary test:**

- a. **Colour:** - Colour of cream was observed visually.
- b. **Odour:** - The odour of herbal cream was observed.

2. pH: The pH of various formulations was determined by using digital pH meter. About 1 g of the cream was weighed and dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3. 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 certain load. Lesser the time taken for separation of the two slides, better the Spreadability. 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.

$$\text{Spreadability} = m \times l / t$$

Were;

m = weight tied to the upper slide (30g)

l = length of glass slide (5cm)

t = time taken in seconds.

1. Washability: Wash ability A small amount of cream applied on hand & washed under running tap water.

2. Extrudability: The extrudability of the prepared formulation was determined by first filling the cream (100 g) into a capped collapsible aluminum tube and sealed by using manual ointment sealing machine. The tubes (containing different formulations) were placed in between two slides and properly clamped. It was followed by placing 500 g weight over the slides and ultimately

opening the cap where the extruded ribbon length was noted after 10 min.

3. Determination of viscosity: Brookfield Viscometer (Model RVT) with helipath stand was used for rheological studies. The sample (50 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-D spindle at 10, 20,30,50,60,100 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted. The measurements were carried in triplicate at ambient temperature. Direct multiplication of the dial readings with factors given in the Brookfield viscometer catalogue gave the viscosity in centipoises. Average of three triplicates was computed.

4. Stability Study: Stability study was performed as per ICH guideline. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Therefore, stability studies provide data to justify the storage condition and shelf-life of the drug product. For drug substance, such studies establish the retest date in addition to the storage condition of raw material. Stability studies were performed for selected formulation with $25 \pm 2^\circ \text{C}$ and $60 \pm 5\% \text{RH}$ and $40 \pm 2^\circ \text{C}$ and $75 \pm 5\% \text{RH}$ conditions for 6 months. The samples were analyzed at 0,3- and 6-months interval for colour, physical appearance and pH.

5. Irritancy test: 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.^[7]

Evaluation of mosquito repellent activity:

1.Screened Cage Method

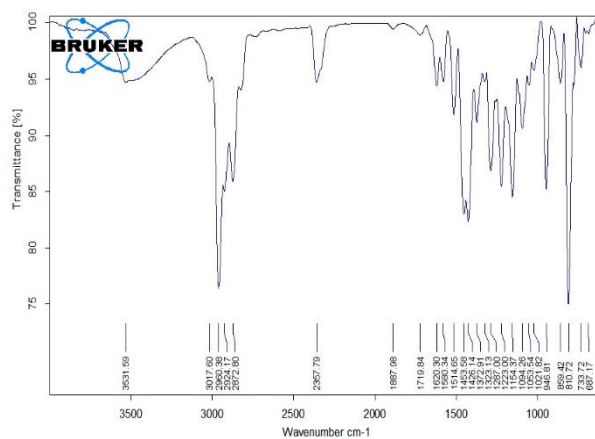
The screened cage bioassay method employs a 40 cm glass-frame cage with a wooden bottom, screened top and back, clear acrylic sides (for viewing), and a front stockinette sleeve for access. 7-8-day-old female mosquitoes are placed in the cage 1 h before the test. The treated forearm is inserted into the cage (a glove is used to protect the hand from mosquito bites.) and the number of mosquitoes that land and probe the skin in 3 min is observed and recorded. observations are repeated every 30 or 60 min.



Fig no.2 Evaluation by cage method

III. Result : (Evaluation result of extract oil)

1. F.T.I.R: IR spectrum of carom seed oil shows characteristic peaks at 3551.59,2960.38,1453.58,810.72



FT-IR spectrum of carrom oil

Table No.3: FT-IR results of Carrom oil

Sr.no	Bond	functional group	frequency
1	O-H stretch	Phenol	3551.59
2	C-H stretch	Aromatic ring	2960.38
3	C-C stretch	Aromatic ring	1453.58
4	C-H substituted	Meta disubstituted aromatic	810.72

Gas Chromatography- Mass Spectroscopy (GC-MS):

Gas chromatography- Mass spectroscopy of carom seed oil shows characteristic peaks at 4.93,5.33,5.69,8.65.

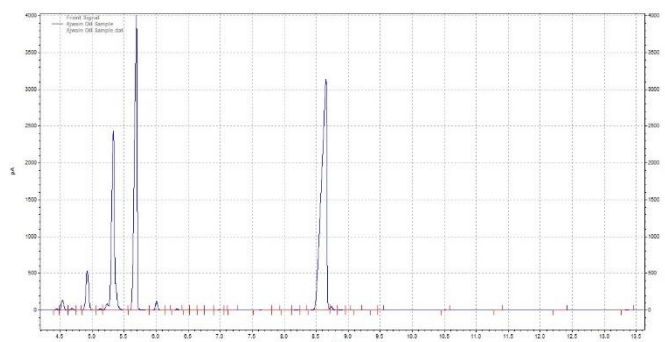


Fig. no.14: Gas chromatography- Mass spectroscopy of carom seed oil.

Table No. 4 : GC results of carrom oil

Sr.No.	RT (min)	Constituent
1.	4.93	β -pinene
2.	5.33	p-cymene
3.	5.69	γ -terpinene
4.	8.65	thymol

Result : (Evaluation result of formulation)**pH:**

pH of all the formulations were found to be between 6.20 ± 0.185 to 6.24 ± 0.158 that is within the range, which are presented in the table. Although alkaline, this pH is typical of creams acceptable by the all standards.

Table No.5 : Result of pH for all formulation

Formulation no.	pH (mean \pm SD)
F 1	6.22 ± 0.152
F 2	6.20 ± 0.189
F 3	6.23 ± 0.196

Washability:

The washability of all formulations was determined and it was observed that all formulation shows good washability.

Table No. 6 : Result of Washability for all formulation

Formulation no.	Washability
F 1	Good
F 2	Good
F 3	Good

Extrudability:

The extrudability of all formulations was determined and it was observed that all formulation shows good extrudability. The extrudability of all formulation was carried out in percentage (%) and formulation F3 has greater extrudability as compared to other formulations.

Table No. 7: Result of Extrudability for all formulation

Formulation no.	Extrudability (%)
F 1	70.42
F 2	74.33
F 3	79.45

Determination of viscosity:

Viscosities of all the formulations were noted and found in the range of 2545 ± 7.83 to 3597 ± 7.25 CPS at 10 rpm as shown in table. All the formulations were showed pseudo plastic flow.

Table No.8: Result of viscosity for all formulation

Formulation no.	Viscosity at 10 rpm (CPS) (mean \pm SD)
F 1	2545 ± 7.83
F 2	3225 ± 7.22
F 3	3597 ± 7.25

Irritancy test:

The irritancy test of all formulations was determined and it was observed for Irritancy, erythema, edema. All the formulation does not show any Irritancy, erythema, edema.

IV. Conclusion

The formulation containing 7.5% of Carom seed oil extract showed repellent activity up to 80% these results are encouraging and promising. At last it was concluded that carom seed oil extract has potential of

mosquito repellent and can be converted into a marketed formulation like essence stick, repellent cream etc.

V. ACKNOWLEDGMENTS

Authors are thankful to Prof. Dr. B. P. Ronge Secretary, SVERI's College of Pharmacy, Gopalpur, Pandharpur for providing necessary facilities to carry out this work. his valuable moral support.

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Cite this article as :

Nikam Mangesh Maruti, More V.V., "Design and Development of Mosquito Repellent Formulation Containing Carom Seed", *International Journal of Scientific Research in Science and Technology (IJSRST)*, Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 7 Issue 5, pp. 88-94, September-October 2020. Available at doi : <https://doi.org/10.32628/IJSRST207520>
Journal URL : <http://ijsrst.com/IJSRST207520>