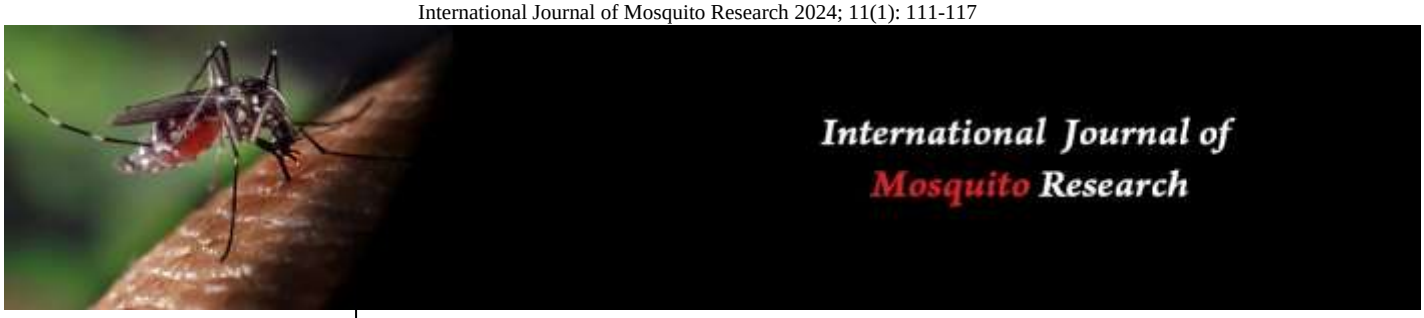

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Evaluation of mosquito repellency activity using mosquito cage method

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Abstract
Essential oils are the well-known ingredients for their repellency activity. But the essential oils have the volatile constituents which vaporize due to course of time. The approach for encapsulation of essential oils in the micro-range provides the controlled release for the oils and gave the activity for longer duration of time. In the present study, Lavender and ylang-ylang oil encapsulated by the polymer ethyl-cellulose in the micron range and further incorporated in the carbopol gel. The FTIR data of lavender and ylang-ylang oils showed the peak at 1735.5 cm^{-1} and 1749 cm^{-1} respectively for the presence of C=O group. The prepared gel evaluated for its mosquito repellency activity, the gel showed the repellency of about 85% and was safe for the application. The MTT assay on the cell line indicates the IC50 value was 724.9±0.162 µg/ml. GC-MS confirms the linalool as its main constituent.

Keywords: Mosquito repellency, essential oil, lavender oil, ylang-ylang oil, GC-MS

Introduction
The most significant insects for public health are mosquitoes, as they can spread a variety of diseases as Japanese encephalitis, dengue, and Chikungunya, malaria, filariasis, and encephalitis, which together account for millions of fatalities annually. Mosquitoes are members of the Culicidae family of (Verma *et al*. 2023) [10] *Aedes aegypti* (Ae. aegypti) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) are the primary urban vectors of Japanese encephalitis, chikungunya, dengue fever, and dengue hemorrhagic fever. Additionally, allergic reactions from mosquito bites can result in systemic reactions including urticarial and localized cutaneous reactions. One way to avoid mosquito bites is to wear protective gear or using the mosquito repellent formulations. The formulations based on essential oils are also showing effective results in terms of repellency (Sritabutra *et al*., 2013) [14]. Mosquitoes are mainly invertebrates. About 3000 of mosquito's species transmit disease when compared to other creatures in the world. Both female and male mosquitoes feed on the same kind of food. However, the female mosquitoes are only responsible for the transmission of the pathogens (Mishra *et al*., 2023) [7]. Many cultures have been using essential oils for medical and therapeutic purposes for thousands of years. Concentrated hydrophobic liquid with volatile substances that readily evaporate at room temperatures are the plant-derived chemical compounds. Their antidepressant, stimulating, detoxifying, antiviral, anti-bacterial, repellency power (Mishra P *et al*., 2023) [8] and relaxing qualities have led to their recent surge in popularity as a safe, natural, and affordable therapy for various health issues. Essential oils (EOs) are fragrant substances that are abundant in oil sacs or oil glands located at varying depths within the fruit peel, primarily in the flavedo portion and cuticles (Harman *et al*., 2019) [6].

The primary issue that has been resolved by the development of numerous encapsulation techniques is the management of excessive volatility of EOs. By use of a physical or chemical contact with a matrix, encapsulation can hold essential oils longer. Nearly all applications of EOs necessitate longer retention periods and various release schedules (Maes *et al*., 2019) [2]. Essential oils are volatile substances that quickly evaporate and lose their effectiveness in the presence of even a small amount of heat.

By trapping the essential oil in the matrix and encapsulating it with polymer, these volatile oils are protected from harsh environmental conditions and their volatility is reduced. When included in the formulation, the trapping of oils allows for the gradual release of the volatile chemical. Microspheres shield the oil from heat deterioration and enable the regulated release of the core substance. Better handling of the essential oil and simple, uniform dispersion of the micro particles into the formulation are made possible by the encapsulation (Radünz *et al*. 2018) [5].

The main aim of the present study is to prepare the encapsulated micro-particles of essential oils (Lavender oil and ylang-ylang oil) and their incorporation into the gel for the evaluation of repellency activity of encapsulated oils against the mosquitoes.

Materials and Methods

Materials

The essential oil used in the study purchased from the authentic sources. The carbopol 940 was used for the preparation of gel and ethyl cellulose for the matrix formation. Tween 80 and Dichloromethane purchased from Merck Life Sciences Private Limited.

Preparation of micro-encapsulated particles of essential oil

Lavandula angustifolia and *Cananga odorata* used for their encapsulation in the form of micro-particles by the method described by Nagavarma BV *et al*., 2012 [11] with slight modifications. 2 ml of Lavender oil mixed in the dichloromethane (15 ml) with continuous stirring. Ethyl cellulose added to the mixture in quantity of 2-3 gm and shake on magnetic stirrer for homogenous mixing. This referred to the organic phase. The aqueous phase was prepared by the incorporation of Tween 80 into the 50 ml of distilled water in the concentration of 1%. The above prepared organic phase was added slowly into the Tween 80 solution with continuous stirring. The particles were formed then filter it, dry in atmospheric condition. The same procedure repeated for the Ylang-ylang oil.

Evaluation of encapsulated particles

FTIR

FTIR spectra of the encapsulated oils of Lavender and ylang-ylang oil in ethyl cellulose were studied using an FTIR spectrometer (Shimadzu, Kyoto, Japan). Spectra were obtained using KBr pellets in the spectral range of 4000-600 cm (Yilmaztekin *et al*. 2019) [16].

SEM

For the analysis of prepared microcapsules for Lavender oil and ylang-ylang oil scanning electron microscopy was done. The image was captured by scanning electron microscope through the instrument JSM-6490 and micro-particles were observed at different resolution.

Preparation of gel containing encapsulated micro-particles

Cold mechanical method employed for the preparation of gel with Carbopol gel. Weigh quantity of Carbopol added to the required quantity of water and mixed homogenously for about 2- 3 hours to avoid any lumps in the prepared formulation. Following then, a mechanical stirrer was used continuously to agitate it until the polymer entirely dissolved in the water. After optimizing microencapsulated Lavender oil and Ylang-ylang oil was added to it. Lastly, the mixture was mixed with the appropriate amount of glycerin at the suitable interval (Pattnaik S *et al*., 2016) [12].

GCMS of gel formulation

Characterization by GC-MS for the gel containing encapsulated lavender and ylang-ylang oil was done by the Instrument of Agilent Technology by injecting the volume of 1 µl of sample for the detection of major constituents.

Determination of mosquito repellency activity of gel by arm-cage method

Mosquitoes captured with the help of net for the study in evening period. Starvation period was for 24 hours and total 25 mosquitoes were utilized in the study. The study conducted at the evening period due to the tendency of mosquitoes to bite the humans usually in evening. The host-seeking behavior determined before starting the experiment. After that the dummy hand rubbed with the gel was exposed to the cage and alignment of mosquitoes on the dummy-hand was recorded for 5 minutes. The above describe method repeated in an interval of one hour. The experiment was repeated in triplicate to get the mean value (Ranasinghe, *et al*., 2016) [13].

Percentage mosquito repellency was calculated using the formula (Ranasinghe, *et al*., 2016) [13].

$$\% \text{ repellency for mosquitoes} = \frac{\text{aligned mosquitoes in control- aligned mosquitoes in gel}}{\text{aligned mosquitoes in control}}$$

Fig 1: Arm-Cage model front view

Fig 2: Arm cage model upper view

In-vitro cytotoxicity evaluation of Gel by MTT assay (On

HaCaT cell line)

Cytotoxicity of the gel determined on HaCaT cell lines. The live cells was cultured for 24 hours in Dulbecco's Modified Eagle Medium in 96 well plate supplemented with Fetal Bovine Serum (10%) and antibiotic solution (1%) with 5% carbon-dioxide at 37° C. After suitable incubation period cell lines treated from different concentrations into MTT solution into the cell culture. Further incubation required for 2 hours. Culture supernatant was removed at the end of experiment and dissolved in dimethyl sulfoxide in 100 µL and read in ELIA plate reader at 540nm and 660nm. Then IC-50 value was calculated for gel formulation (Morgan DML, 1998, Tihauan, *et al*., 2020) [4, 15].

Results

FTIR and SEM analysis of micro-encapsulated oils

FTIR analysis

FTIR of Lavender oil

Lavender oil FTIR data shows the 1735.5 cm^{-1} peak related to the vibrations of C=O group (Fig. 1), peak at 2956 cm^{-1} associated with the bending vibrations of C-H bonds. Peak at 833 cm^{-1} assigned for C-H molecular bond vibrations and at 923 cm^{-1} C-H deformation vibrations from the structure of Linalool.

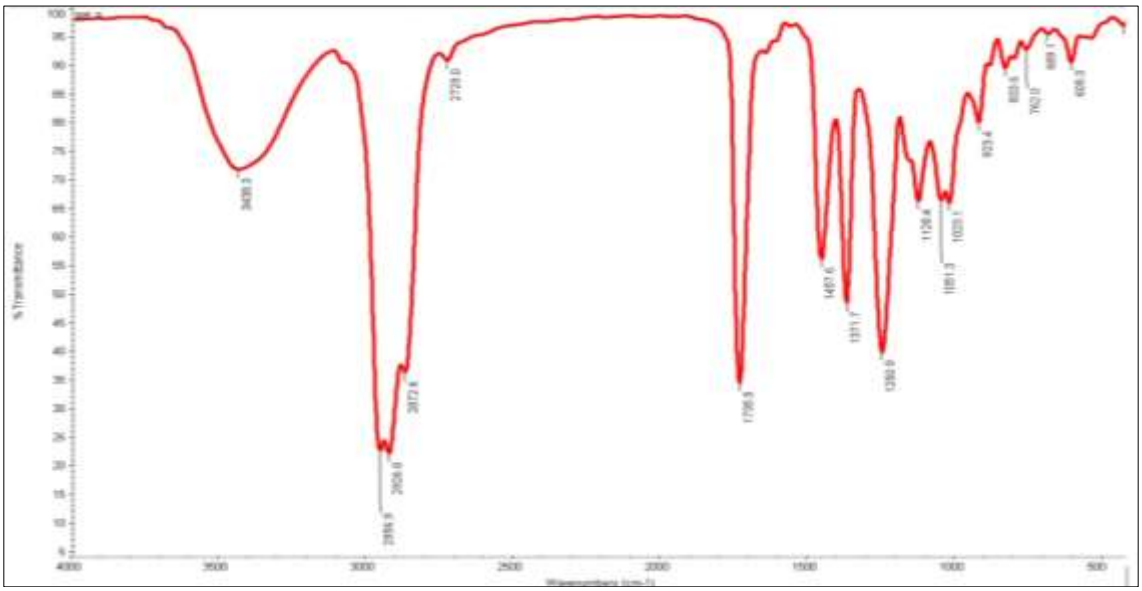

Fig 3: FTIR of Lavender oil

FTIR of ylang-ylang oil

The existence of the -OH group was suggested by the emergence of a thin band at 3753.9, 3437.4 cm^{-1} (Fig 2). These three distinct wavenumbers could indicate the presence of -OH in one of its three forms. The four bands, 2924.0 and 2862.4 cm^{-1}, that are slightly below 3000 cm^{-1} indicate the presence of methyl groups (sp3 hybridized carbon atoms). The presence of aromatic chemicals in the sample can be determined by the combination band or faint overtones in the 2000-1667 cm^{-1} range. Furthermore, the ortho substitution of the benzene ring is demonstrated by a single strong absorption band below 928 cm^{-1}. C=O stretching causes a prominent absorption band at 1749 cm^{-1}, indicating that the molecule in the sample may be an amide, aldehyde, ketone, or carboxylic acid.

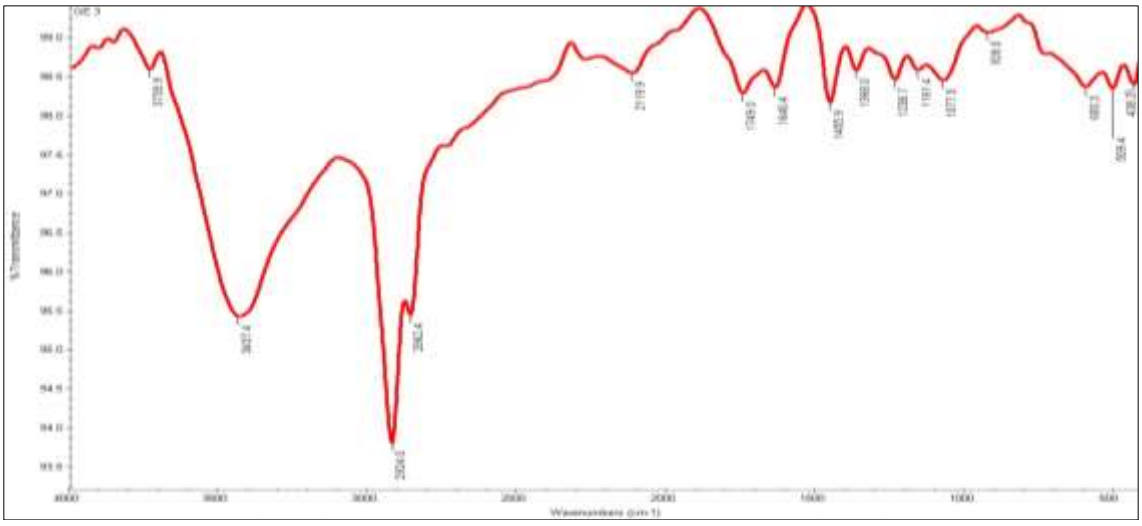

Fig 4: FTIR of Ylang-ylang oil

SEM analysis for Lavender oil and ylang-ylang oil microcapsules

The microcapsules prepared by the method describe above was analyze by the scanning electron microscopy for the determination of its size in micron range. In was found that the microcapsules of lavender and ylang-ylang oil was in the range or below of 100 µm and 50 µm. The resolution was performed on 100 X (Fig 3), 200 X (Fig 5, Fig 6) and 500 X (Fig 4). The particles were found in optimum range for both the oils.

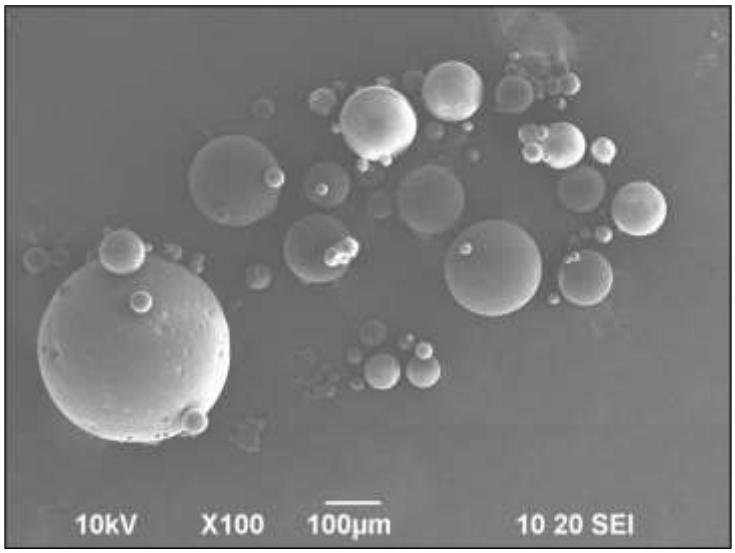

Fig 5: Lavender oil microcapsules at 100X

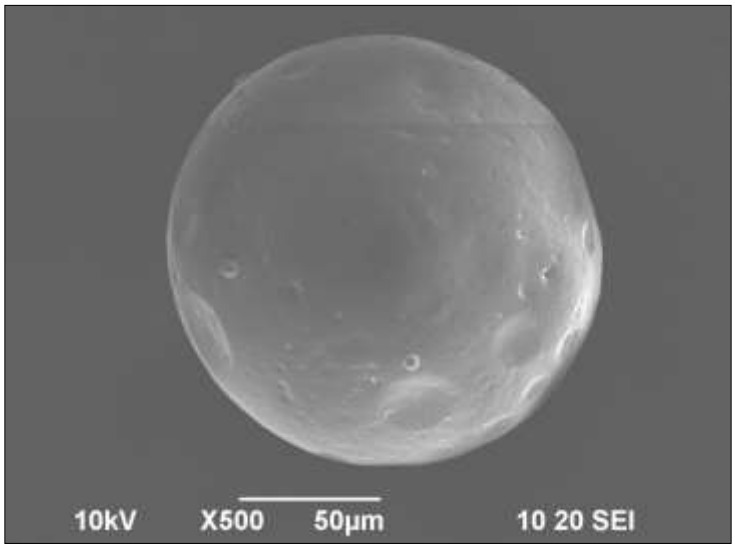

Fig 6: Lavender oil microcapsules at 500X

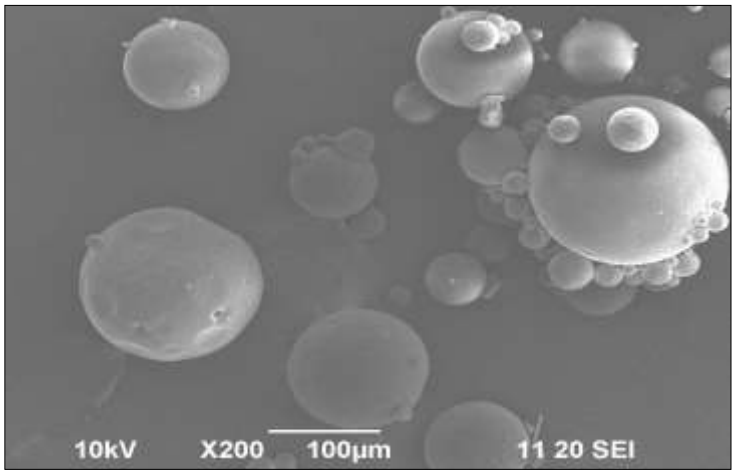

Fig 7: Lavender oil microcapsules at 200X

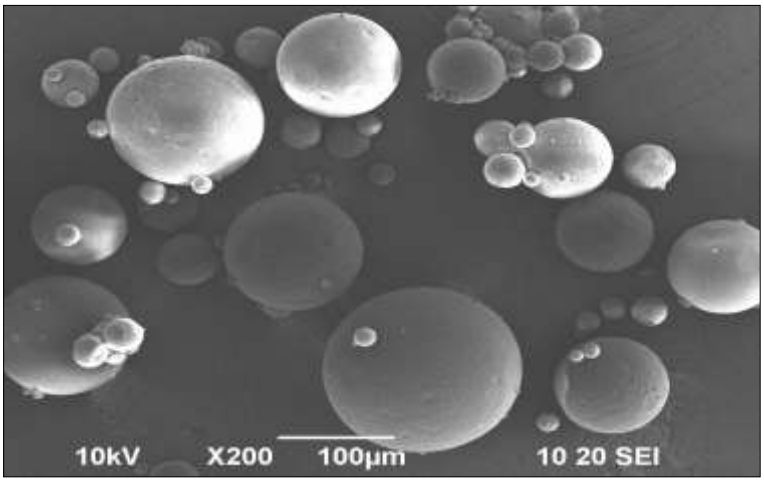

Fig 8: Ylang-ylang oil microcapsules at 200X

GC-MS analysis of gel formulation

GC-MS analysis indicate the presence of major constituents in the gel formulations as lavender oil and ylang-ylang oil both contain the linalool as their main constituents (Ciocarlan *et al*., 2021; Brokl *et al*., 2018) [1, 5] and the peak of linalool in the graph confirm (Fig 7) its presence at the retention time of 3.513 minute (Table 1).

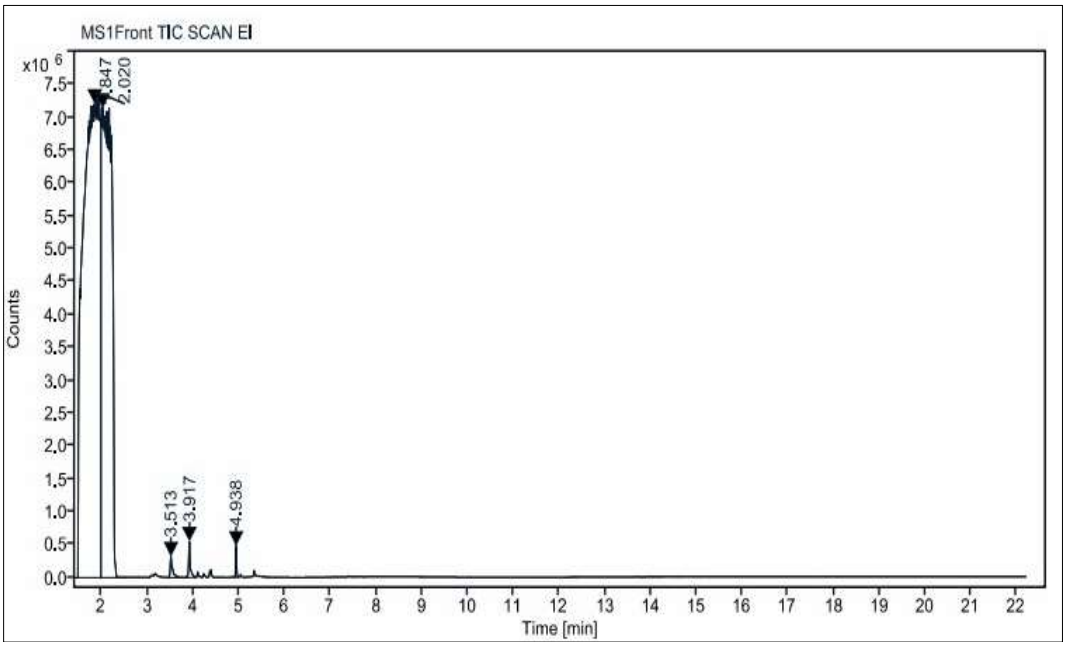

Fig 9: Major peak in GC-MS graph

Table 1: Major compounds in the gel

S. No.	Compound name	Retention time	Prob. %
1.	α-Terpinyl acetate	4.938	48.61
2.	Linalool	3.513	66.59
3.	3-methyl pentane	2.02	36.23
4.	Acetic acid	3.917	73.45

3.3 Results for repellency activity of gel formulation

The alignment of mosquitoes observed visually in the arm-cage on the hand (Table 2). In the case of control the mosquitoes aligned on the hand for the time period more than 5 minutes. But in the case of gel containing micro-encapsulated oils the mosquitoes not aligned on it or if aligned the time period was very short i.e., 10-15 sec only. The gel was found to be effective for the period of 7-8 hours. The longer activity period is due to the presence of oil in the encapsulated form.

Table 2: Result for Mosquito repellency Test by Arm-Cage method

Formulations	Total no. of mosquitoes aligned (Replicate 1)	Total no. of mosquitoes aligned (Replicate 2)	Total no. of mosquitoes aligned (Replicate 3)	Calculated Mean Value of Mosquitoes Aligned	Percentage Mosquito Repellency (%)
Gel formulation	1	1	0	0.666	85
Control	5	4	5	4.666	00

***In-vitro* cytotoxicity evaluation of Gel by MTT assay (HaCaT)**

The MTT assay is a colorimetric viability test that relies on the conversion of the MTT molecule to formazan by enzymatic reduction in the presence of live cells. The MTT molecule changes color as a result of the reduction. The percentage of live human cells that survive after being treated with different concentrations of a tested drug is determined by absorbance measurements in comparison to a control. This information is translated to its IC50 values. The MTT assay's precision, speed, and relative ease of use make it a popular choice for cytotoxicity investigations (Ganot *et al*., 2013) [3]. MTT assay determined for the cytotoxicity evaluation of gel on the HaCaT cell line at different concentrations of the gel. The IC50 value was found to be 724.9 ± 0.162 µg/ml (Fig 8).

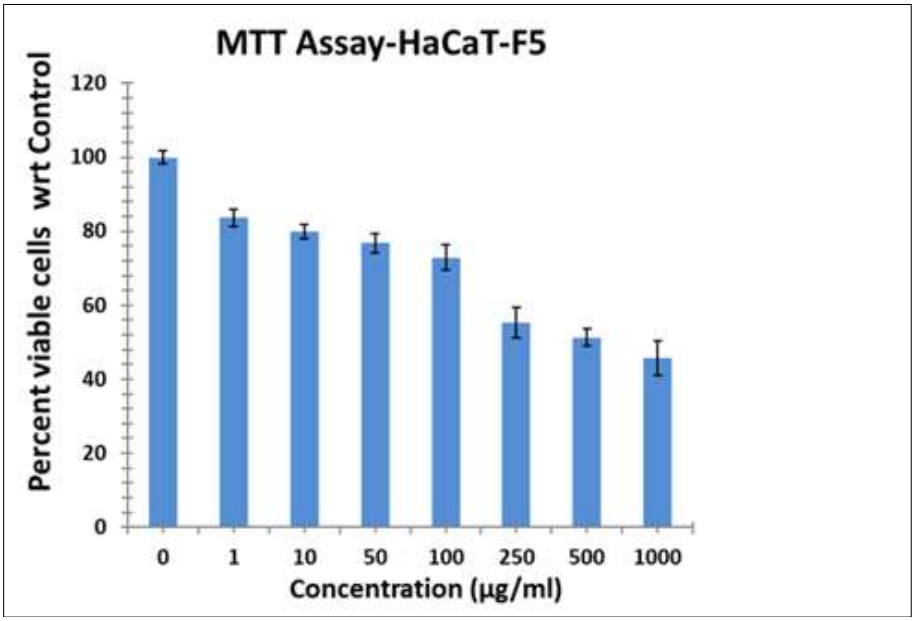

Fig 10: Percent viable cells with respect to control at different concentrations

4. Conclusion

Ylang-ylang and lavender was effective against the mosquitoes in the encapsulated form also. The encapsulation no-doubt slow down the release and also lighten the fragrance associated with the oils but it releases the components in controlled manner which works for longer duration of time. The combination of essential oils provides the synergistic effect in the respective activity. There is a great need for the development of more effective mosquito repellent products on the commercial purpose which can also replace the synthetic repellents.

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