
EVALUATION OF REPELLENCY ACTIVITY OF EMULGEL FORMULATIONS AND THEIR CHARACTERIZATION BY GC-MS

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Abstract

The present study based on the preparation of emulgel formulations by incorporating the combinations of essential oils with aloe-vera gel and flaxseed oil. Total four formulations were prepared during the study and they further characterize by GC-MS. All the formulations evaluated for their repellency percentage by two different methods i.e., Arm-cage method and diagonal method. F2 formulation showed the best result in terms of its repellency activity in both the methods as it contains the combination of ylang-ylang and thyme oil. The GC-MS results indicated the presence of linalool and d-limonene as their active constituents along with others. The combination of essential oil was effective in their repellency power and suitable for longer duration protection also.

Keywords: Essential oil, Emulgel, Arm-cage, Diagonal method, Linalool, Repellency activity

1. Introduction

Mosquitoes are at the top of the list when it comes to the most disturbing bloodsucking creatures that humans have to deal with. Mosquitoes, which are members of the genera Anopheles, Culex, and Aedes, are responsible for the spreading of a different diseases, including Dengue fever, malaria, yellow fever, Japanese encephalitis, and plenty of others. In the span of only one year, mosquitoes are accountable for about 700 million incidences of disease transmission and more

than one million deaths around the globe. Malaria is transmitted from one person to another through the bites of female *Anopheles* mosquitoes. In areas where the disease is common, neonates and young children continue to bear a disproportionate share of the amount of illness caused by malaria.^{4, 5} Malaria was responsible for the deaths of approximately 627,000 persons over the world in the year 2012. There were around 207 million people who were impacted by the sickness (Ranasinghe, 2016). The majority of mosquito repellent lotions and devices that are available for purchase on the market are thought to have adverse effects on individuals; therefore, the purpose of this research is to find effective alternatives that are derived from plants. The control of mosquitoes is a significant challenge for public health on a global scale.

Although essential oils work wonders when used topically. Due to their vapour-phase nature, essential oils usually only have a fleeting impact on mosquitoes (Zhu et al., 2001; Barnard, 2000). This is probably because of how volatile they are; formulas that cause the active ingredients to penetrate the skin for longer might improve this feature. The product's unique characteristics determine the extent to which an essential oil's repelling effect is enhanced. According to Oyedele et al. (2002), the effectiveness of hydrophilic, emulsion, and oleaginous bases decreased over time. To make the essential oil repellent work better, researchers have tried utilizing coconut oil and liquid paraffin as fixatives (Das et al., 1999; Zhu et al., 2001; Barnard, 2000; Tawatsin et al., 2001). The present study is conducted to develop the emulgel formulation containing the essential oils and further evaluated for its repellency activity by different methods.

2. Material and Methods

2.1 Material

Essential oil used in the preparation extracted from the plant material and used in the formulation i.e., Lavender oil, thyme oil, nagarmotha oil, ylang-ylang oil. The aloe vera gel was collected from the local garden plant. Flax seed oil and Xanthine was procured from the local dealers.

2.2 Methods

2.2.1 Preparation of emulgel formulations

Essential oil extracted from the plant samples by the hydro-steam distillation process and used in the emulgel preparation. The gel of aloe vera leaves removed and further stabilized it by heating for 4-5 minutes (Navale S et al., 2022). The emulgel was prepared by the method described by Kumar D et al., 2022 with doing slight modification in it. Stabilized aloe vera gel (30 gm) taken and mixed with 100 gm of xanthine. Another phase was prepared by adding the 2m of essential oil with 18 ml of flaxseed oil. Both the phases mixed together by continuous stirring at 3000 rpm for about 15 minutes drop by drop until the clear smooth emulgel was obtained (Kumar D et al., 2022). Different combinations of essential oil were used in formulations.

Combinations of essential oil were:

- F1 = Lavender oil + Ylang-Ylang oil
- F2 = Thyme oil + Ylang-Ylang oil

- F3 = Thyme oil + Lavender oil
- F4 = Nagarmotha oil + Ylang-Ylang oil

2.2.2 Characterization of formulations by GC-MS

GC-MS of the prepared emulgel was done by the instrument Alginat. 1 μ l of the sample injection in the injection port for the identification of major constituents present in the emulgel.

2.2.3 Evaluation for Mosquito Repellency

2.2.3.1 Testing of the Mosquito Repellency Activity by Arm Cage method

The mosquitoes used to catch with the help of net during the evening time like between the 5 pm to 10 pm for the further study. They were kept in starved conditions for 24 hours and then twenty mosquitoes placed in the cage. As mosquitoes generally bite during the night so the test timing chosen for the study was 6 pm and 10 pm. Before the start of experiment, the host seeking nature of mosquitoes determined. This was done by placing the dummy hand (pre-cleaned) in cage and observing the mosquitoes aligned within 10 minutes.

Hand rubbed with the formulation about 1.5 ml and then placed in the cage (Fig 1, Fig 2). The number of mosquitoes aligned and used to sit on the hand observed visually and recorded for each minute for 5 minutes. Mosquitoes further given for 1 hour rest period then the same procedure repeated for each four formulation one by one. Test repeated thrice and mean was calculated (Ranasinghe, MSN et al., 2016).



Fig. 1: Arm-Cage Model (Front view)



Fig. 2: Arm-Cage (Upper View)

2.2.3.2 Testing of the mosquito repellency activity by Diagonal method

The repellency activity for the prepared formulations evaluated by the methods described by Reis, et al., 2016 and Mossi, et al., 2011 after some modifications. The 5 chambers with specific shape and size get interlinked with the central one. All chambers contain the formulations. The central chamber remain to be empty, two diagonal chamber contain the prepared formulation while two chamber in diagonal referred as control i.e., contain only emulgel base preparation. The mosquitoes remain to be free to move through the attached pipe and the observation will be done for specific time interval. The repellency percentage will be calculated. All the observations and data collection will be carried out in triplicate form (Mossi et al., 2011).

3. Result and Discussion

The emulgel containing the combinations of essential oil when characterize by the GC-MS it shows the presence of active constituents. All the four formulations were stable throughout the study period as separation of phases was not observed visually.

3.1 Results for Characterization of Emulgel by GC-MS

• Formulation F1

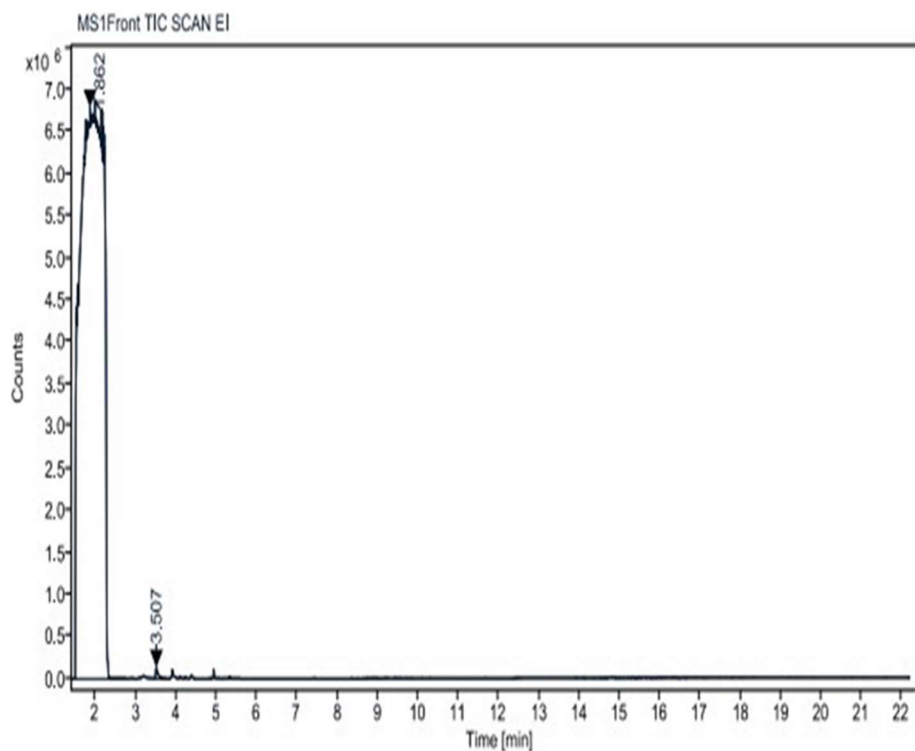
The major constituent identified by the GC-MS in the formulation 1 was Linalool and 2-ethyl oxetane (Fig. 3, Table 1).

Table 1: Major compounds of Formulation 1

| S. No. | Compound name | Retention time |
|--------|---------------|----------------|
| 1. | Linalool | 3.507 |

| | | |
|----|-----------------|-------|
| 2. | 2-Ethyl oxetane | 1.862 |
|----|-----------------|-------|

Figure 3: GC-MS of formulation F1



• **Formulation F2**

The major constituent identified by the GC-MS (Fig. 4) in the formulation 2 mentioned in table 2

Table 2: Major compounds in Formulation 2

| S. No. | Compound name | Retention time |
|--------|--|----------------|
| 1. | Acetic acid | 3.99 |
| 2. | D-Limonene | 3.168 |
| 3. | Linalool | 3.58 |
| 4. | 2-Ethyl oxetane | 1.847 |
| 5. | Benzoic acid, 2-hydroxy-phenylmethyl ester | 7.394 |
| 6. | 9,12-Octadecadienoic acid (Z,Z)- | 8.447 |

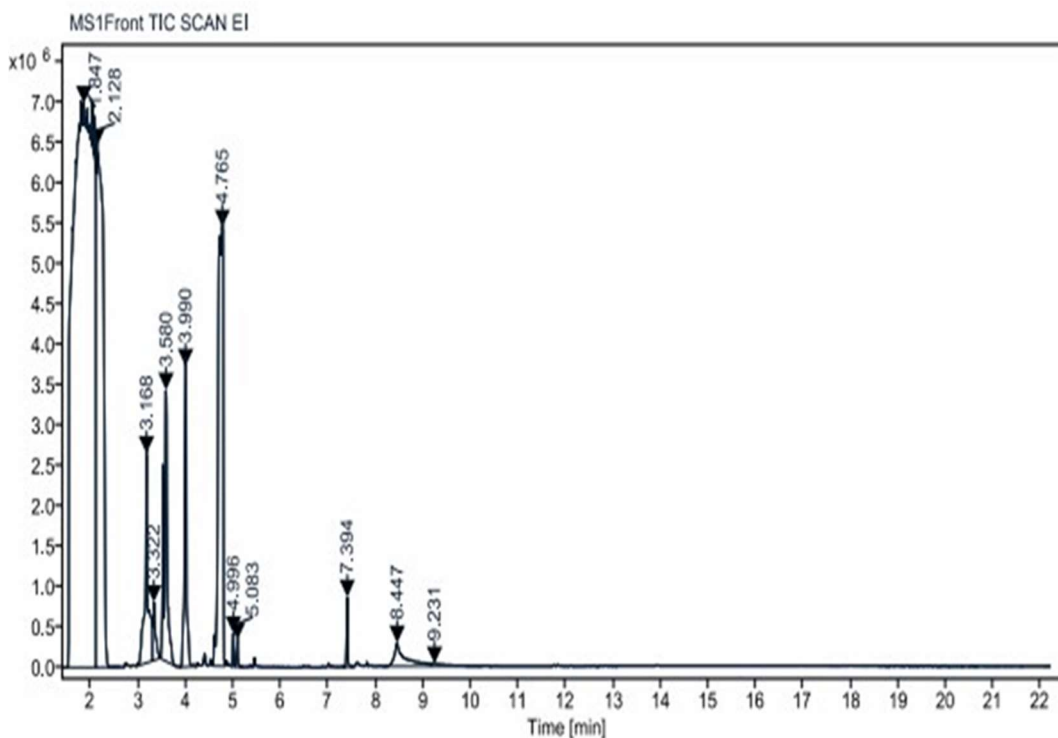


Figure 4: GC-MS of formulation F2

- **Formulation F3**

The major constituent identified by the GC-MS (Fig. 5) in the formulation 3 was benzyl alcohol, n- Hexane, 3-methyl pentane mentioned in table 3.

Table 3: Major compound in formulation 3

| S. No | Compound name | Retention time (min) |
|-------|-------------------------------------|----------------------|
| 1. | Phenol, 2-methyl-5-(1-methylethyl)- | 4.641 |
| 2. | n-Hexane | 2.192 |
| 3. | n-Hexane | 2.125 |
| 4. | Pentane, 3-methyl | 1.902 |
| 5. | Benzyl alcohol | 3.185 |

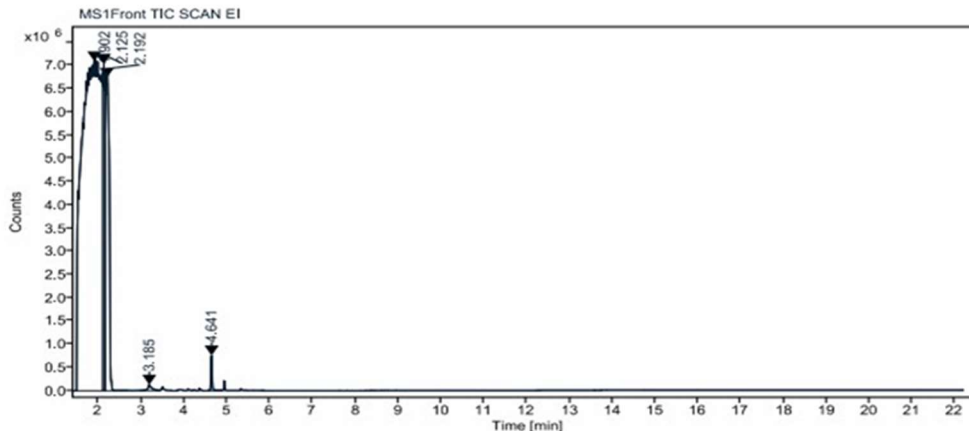


Figure 5: GC-MS of formulation F3

- Formulation F4**

GCMS graph (Fig 6) shows the compound in the retention time of 4.236 min, 4.432 min, 3.585 min and 1.829 min. The major compound in formulation 4 mentioned in table 4.

Table 4: Major compounds in formulation 4

| S. No. | Compound name | Retention time |
|--------|----------------------------------|----------------|
| 1. | Acetic acid, phenyl methyl ester | 3.11 |
| 2. | Citronellol | 4.263 |
| 3. | Geraniol | 4.432 |
| 4. | Caryophyllene oxide | 6.243 |
| 5. | 2-ethyl oxetane | 1.829 |
| 6. | Linalool | 3.585 |
| 7. | n-Hexane | 1.99 |

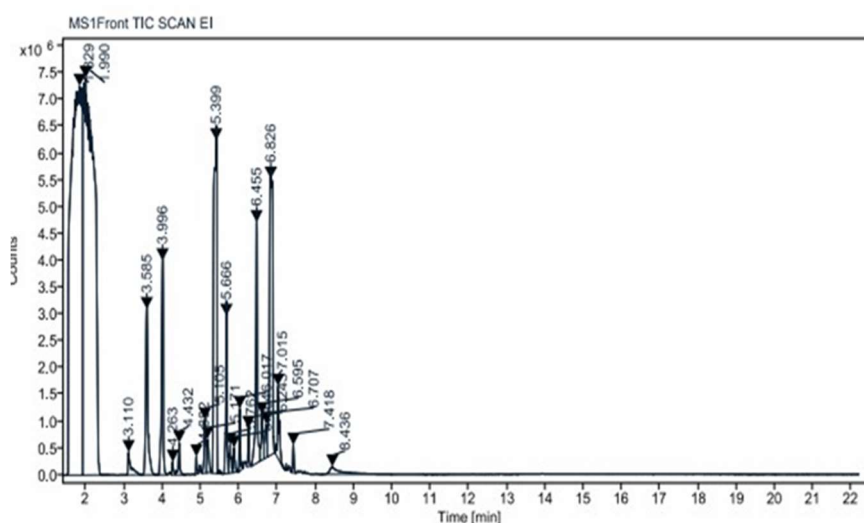


Figure 5: GC-MS of formulation F4

3.2 Results for Mosquito Repellency

3.2.1 Mosquito repellency Test by Arm-Cage method

All the four formulations evaluated for their mosquito repellency (Table 5). The alignment of mosquitoes was observed visually inside the cage and the number of aligned mosquito was noted for further calculation. The observation was done in the triplicate form for the calculation of mean and it was found that the formulation F2 showed the maximum repellency percentage against the mosquitoes which was about 97% followed by formulation F1 i.e., 89.5%. In all the formulations F2 gave the best results in terms of repellency.

Table 5: Result for Mosquito repellency Test by Arm-Cage method

| Formulations | Total no. of mosquitoes aligned (Replicate 1) | Total no. of mosquitoes aligned | Total no. of mosquitoes aligned | Calculated Mean Value of Mosquitoes Aligned | Percentage Mosquito Repellency (%) |
|--------------|---|---------------------------------|---------------------------------|---|------------------------------------|
| F1 | 2 | 0 | 3 | 1.666 | 89.5 |
| F2 | 1 | 0 | 0 | 0.33 | 97.94 |
| F3 | 4 | 1 | 4 | 3 | 81.25 |
| F4 | 3 | 4 | 2 | 3 | 81.25 |

3.2.2 Result for Mosquito repellency test by Diagonal method

When the mosquito repellency evaluated by another method it was found that the mosquitoes resist to enter in the chambers which contain the formulations due to their smell. As presence of volatile constituents in the chamber impart the mosquito repellency activity. Total number of mosquitoes (N = 10) trapped with the help of net and settled into the chamber and then covered with transparent lid and observation done visually. The repellency percentage was calculated (Table 6).

Table 6: Repellency % by Diagonal method

| Formulations | Total no. of mosquitoes in chamber with formulations | Total no. of mosquitoes in chamber with formulations | Total no. of mosquitoes in chamber with formulations | Calculated Mean Value | Percentage Mosquito Repellency (%) |
|--------------|--|--|--|-----------------------|------------------------------------|
| F1 | 0 | 1 | 2 | 1 | 69.96 |
| F2 | 2 | 0 | 0 | 0.66 | 80.18 |
| F3 | 2 | 1 | 1 | 1.33 | 60.06 |
| F4 | 1 | 0 | 2 | 1 | 69.96 |
| Base emulgel | 4 | 4 | 2 | 3.33 | 0.00 |

Conclusion

The prepared emulgel formulation analyzed by the GC-MS (Gas Chromatography-Mass Spectroscopy) for the identification of main components present in the formulations. F1 showed the presence of linalool, F2 showed the presence of linalool, and D-limonene, F3 indicate the presence of n-hexane and methyl alcohol and F4 contain the citronellol, geraniol, carophyllene oxide and many others. All the four formulations evaluated for their mosquito repellency. Two methods were used in the determination of repellency activity one was arm-cage method and other was the modified diagonal chamber method. In the arm cage method F2 showed the best repellency activity in comparison to other and in the diagonal method the same result obtained means F2 was found to be effective by this method also followed by F1 and F4 as they showed same repellency

percentage. Many of the researches conducted to evaluate the repellency activity of formulation based on essential oil against the mosquitoes. As linalool has good repellency power against mosquitoes and the combination of the essential oils provide the synergistic effects in terms of its repellent activity.

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