Journal of Young Pharmacists, 2025; 17(2):329-335.
https://www.jyoungpharm.org

Original Article

Development of Microemulsion of Marjoram Oil in the Line of Treatment of Otitis Media

Avinash Balasaheb Gangurde[1,*], Vaibhav Sudam Pandit[2], Kranti Ashok Suryawanshi[2], Bhagyashri Vijay Soundane[2], Sandesh Ragho Nikam[2], Parag Ashok Pathade[2]

[1]Department of Pharmaceutics, KBHSS Trusts Institute of Pharmacy, Malegaon, Nashik, Maharashtra, INDIA.
[2]Department of Pharmaceutics, Institute of Pharmacy, Malegaon, Nashik, Maharashtra, INDIA.

ABSTRACT

Background: Marjoram oil, an antimicrobial agent, has significant potential for industrial applications due to the presence of a high proportion of chemical components such as terpinene-4-ol, sabinene hydrate, thymol, and carvacrol. These components are effective against various broad-spectrum bacteria. Practically, microemulsions of oily drugs improve antibacterial effects by increasing penetration into the skin and bacteria. The topical microemulsion of marjoram oil for otitis media has not yet been explored. Therefore, the present study focuses on the development of a microemulsion of marjoram oil in the context of *in vitro* antibacterial studies against *Streptococcus pneumoniae, Moraxella catarrhalis,* and *Haemophilus influenzae*, the microorganisms responsible for otitis media infections. **Materials and Methods:** Marjoram oil was extracted via hydrodistillation and evaluated for physicochemical properties. It was combined with a co-surfactant and surfactant, emulsified with water to create a microemulsion. A 1:1 ratio of surfactants to co-surfactant was used, with varying concentrations of S_{mix} and oil. Formulations were developed using a triangular diagram and titration method, and evaluated for compatibility, droplet size, polydispersity index, and zeta potential. The stable formulation F7 was tested for antibacterial activity against chloramphenicol ear drops. **Results:** Formulation F7 contained the highest oil quantity (44.99% w/w), with water (35.73% w/w) and S_{mix} (19.28% w/w). Its globule size was 370.3 nm, polydispersity index 0.48, and zeta potential -25.7 mV. FTIR studies confirmed formulation compatibility. The F7 microemulsion (200 µg/mL) showed a greater zone of inhibition compared to chloramphenicol (100 µg/mL) and was effective against *Streptococcus pneumoniae, Moraxella catarrhalis*, and *Haemophilus influenzae*. **Conclusion:** The developed microemulsion of marjoram oil was stable and effective against the microorganisms causing otitis media.

Keywords: Marjoram oil, Microemulsion, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenza*.

Correspondence:
Dr. Avinash Balasaheb Gangurde
Professor, Department of Pharmaceutics, KBHSS Trusts Institute of Pharmacy, Malegaon, Nashik, Maharashtra, INDIA.
Email: avigang2010@gmail.com

Received: 01-07-2024;
Revised: 30-11-2024;
Accepted: 18-03-2025.

INTRODUCTION

Otitis media is caused by bacterial infections entering the middle ear via the Eustachian tube. It can arise from a perforation in the eardrum and can be caused by allergies, respiratory infections, or anatomical issues (Schilder *et al*., 2016; Vanneste & Page, 2019). It can be caused by bacteria, viruses, or co-infections, with *Streptococcus pneumoniae, Moraxella catarrhalis*, and *Haemophilus influenza* (Silva & Sillankorva, 2019; Mittal *et al*., 2018).

Marjoram oil, an essential oil, obtained by extraction from *Origana majorana* plant is used in aromatherapy, cosmetics, skin care, and treating sore muscles and joints. It has therapeutic properties like antibacterial, antimicrobial, antiviral, anticancer, and anti-inflammatory (Dhiman & Bhasin, 2022; Thanh *et al*., 2019). Marjoram oil, a popular antimicrobial agent, has the greatest potential for industrial applications and widely extracted worldwide. It contains mixture of many phytochemicals with high percentage of terpinene-4-ol (29.6%) and sabinene hydrate (3%), and thymol (17.47%) and carvacrol (50%). These phytochemicals are responsible for antimicrobial and antifungal effects (Vera, 1999; Kamari *et al*., 2023; Raina & Negi, 2012). Chemical structure of marjoram oil components shown in Figure 1.

Microemulsions serve as carriers that enhance permeation and penetration through the skin by effectively overcoming the barrier of the stratum corneum. Their unique characteristics enable them to increase the liposolubility of hydrophilic drugs by incorporating a less polar chain into their more polar side, improving the bioavailability of challenging molecules. Similarly,

DOI: 10.5530/jyp.20251347

for lipophilic substances, a less polar side can be combined with a more polar chain to enhance their effectiveness (Souto *et al.*, 2022). These properties of microemulsions are significant in the development of antibacterial microemulsions to penetrate the drug inside bacteria for the effectiveness.

In the view of antimicrobial activity, present work focused to develop microemulsion of marjoram oil in the line of treatment of Otitis media.

MATERIALS AND METHODS

Origanum majorana leaves were purchased from a local nursery in Malegaon. Tween 80 (Loba Chemie), propylene glycol (Loba Chemie), and ethanol (Pure Chem) were purchased from chemical suppliers. The materials used in the study were of pharmaceutical and analytical grade.

Extraction by hydro distillation (Clevenger Apparatus) method

Plant material of *Origanum majorana* L. was collected in Malegaon from June to December 2023 and authenticated with voucher specimen number VSPOM-1 by the Botanical Survey of India, Western Circle, in Pune. The leaves were allowed to dry in the shade. The dried, finely ground sweet marjoram sample was a greyish-brown fine powder with a characteristic essence. This powder was used for the extractions. Dried *Origanum majorana* leaves (100 g) were placed in a 2 L flask containing 1 L of demineralized water. The Clevenger extractor was set up, and the leaves were extracted for 6 hr at 70% heat on a heating mantle. Marjoram oil was obtained using the hydro-distillation method (Ferhat *et al.*, 2006; Dorsaf *et al.*, 2010).

Physicochemical characterization

The obtained marjoram oil was evaluated for various physicochemical properties, including odour, appearance, solubility, pH, boiling point, viscosity (Cps), and density (g/mL). Odour of marjoram oil was determined by nasal perception. Appearance of oil was visually inspected for colour. Solubility of oil was observed in ethanol. pH was determined using previously calibrated digital pH meter. Refractive index was determined using refractometer. Boiling point was determined using capillary method. Viscosity of oil was determined using Brookfield viscometer (DV II+ Pro) using spindle no 3, rpm 100 and temperature 25°C.

Compatibility study

Marjoram oil and the formulation F7 mixture were analyzed by FTIR spectroscopy (JASCO 4600). The samples were screened between 650-4000 cm^{-1}. Liquid samples were directly analyzed for the FTIR spectrum using an ATR attachment.

Formulation and development of microemulsion

Marjoram oil was emulsified with a combination of co-surfactant and surfactant (1:1) i.e. S_{mix} and water. Oil and S_{mix} were mixed by vigorous shaking to obtain oil phase. Water was added to the oil phase in small increments and continuously stirred by a magnetic stirrer at room temperature.

Construction of ternary phase diagram

For the phase diagram, nine formulations (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) of oil and S_{mix} in various proportions were mixed, as shown in Tables 1 and 2. Tween 80 and propylene glycol in a 1:1 ratio were used to produce S_{mix}. Under agitation at sixty rpm, a small amount of water was added to the oil and S_{mix} combination in 0.5% w/w increments, allowing the mixture to equilibrate. The water volume was recorded at the point of the clear-to-turbid change in the microemulsion. The mass percentages of oil, water, and S_{mix} were recorded at the endpoint since the total mass sum was 100% w/w. To obtain appropriate components and their concentration ranges for the microemulsion, a pseudo-ternary phase diagram was constructed. The microemulsion region was identified and formulations were optimized. The shaded area indicated the transparent or clear microemulsion region, while the remaining area showed the turbid microemulsion. The formulations were observed for one week period for clarity of microemulsions (Kumar *et al.*, 2016; Lavanya *et al.*, 2016).

Evaluation parameter of microemulsion

Physical Appearance, viscosity and density of stable microemulsion was determined as per reported methods. Physical appearance was observed for clarity of prepared microemulsion (Yadav *et al.*, 2018). Viscosity of the prepared microemulsion was determined using Brookfield viscometer (DV II pro) at 100 rpm, 25°C and Spindle no 3. Density of the microemulsions were determined using density bottles (Badwi *et al.*, 2009).

Measurement of droplet size, polydispersity index and zeta potential

Formulation F7 was tested for particle size (globule), polydispersibility index and zeta potential by dynamic light scatting detection using Malvern instrument (Zeta sizer). 1 mL of F7 sample was added into 100 mL volumetric flask and diluted with purified water up to the mark. The sample was analysed for particle size (globule), Polydispersibility Index (PDI) and zeta potential (Asmawatia *et al.*, 2014).

In vitro antimicrobial activity by agar diffusion disc method

Antimicrobial activity for *Moraxella catarrhalis, Streptococcus pneumonia* and *Haemophiles influenza* was performed by using agar disc method. Chloramphenicol ear drop was used as a control sample.

Agar plates were inoculated with a standardized inoculum of the test microorganism. Formulation F7 was applied to filter paper discs and placed on the agar surface at concentrations of 50, 100 and 200 µg/mL. Control sample of concentration 100 mg/mL was applied. The appropriate settings were used to incubate the Petri plates. An antimicrobial drug that diffuses into the agar normally inhibits the test microorganism's germination and development, and the diameters of the inhibitory growth zones were calculated. The Petri dishes were examined using a Motic 2.0 microscope (Prabuseenivasan *et al.*, 2006; Borse *et al.*, 2020).

RESULTS

Physicochemical characterization of marjoram oil was performed for various tests and are shown in Table 3. The obtained marjoram oil was found aromatic, pale yellow liquid, soluble in ethanol, pH (6.30-6.95), boiling point (164-172°C), density (0.889±0.012 g/mL), viscosity (166-200 cps) and refractive index at 20°C (1.472±0.054).

The ternary phase diagram was obtained from developed formulations F1 to F9 shown in Figure 2. When the oil and S_{mix} mixture was titrated against water resulted clear and transparent emulsion after stirring. The oil, S_{mix} (1:1), and water percentages were marked as point in the phase diagram. S_{mix} composed of 1:1 proportion of Tween 80 and Propylene glycol. Total 9 formulations of different proportions of oil, S_{mix} and water was developed. The dark region in the phase diagram represents microemulsion zone. It wss observed that formulation F7, and F8 were stable and were not shown separation of oil and water layers. Formulation F8 was stable but become slightly turbid upon storage after 7 days. The formulation F1, F2, F3, F4, F5, F6 and F9 were not stable and showed phase separation after 1 hour storage.

It was observed that S_{mix} (1:1) at 19.28% concentration was produced stable emulsification of marjoram oil (Formulation F7). It was also observed that at lower S_{mix} concentration, marjoram oil of higher concentration was produced clear microemulsion in water. Formulation F7 ingredient mixture and marjoram oil sample were analysed by JASCO FTIR-4600 for functional group analysis. The chemical compatibility between marjoram oil and S_{mix} was studied. FTIR spectrum shown in Figure 3 revealed characteristic peaks associated with the functional groups present in this essential oil at 3482.81, 2960.2, 2925.48, 1733.69, 1445.39, 1369.21 and 1278.57 cm^{-1}.

Formulation F7 was found stable microemulsion and was further evaluated for physical appearance, viscosity, density, droplet size, polydispersity index and Zeta potential. Prepared microemulsions were found clear and pleasant odor. Formulation F7 was evaluated for viscosity which was found low viscous in the range 102.5 to 124.0 cps at 100 rpm using spindle no 3. Microemulsions were shown density 1.11 to 1.095 g/mL.

The Malvern particle size analyser (Zetasizer Ver. 8.02) was used to measure the mean droplet size, polydispersity index, and zeta potential. The globule size of formulation F7 was found 370.3 nm confirmed the formation of microemulsion which ranges between 100-400 nm. Polydispersibility Index (PDI) is a measure of the heterogeneity of the sample based on size. A polydispersibility index of 0.480 indicated moderate particle size distribution in the microemulsion. A zeta potential of -25.7 mV indicated a strong negative charge at surface of globules in a dispersion. This suggested good stability of micro emulsion and stability against coalescence. Particle size distribution and zeta potential represented graphically in Figure 4.

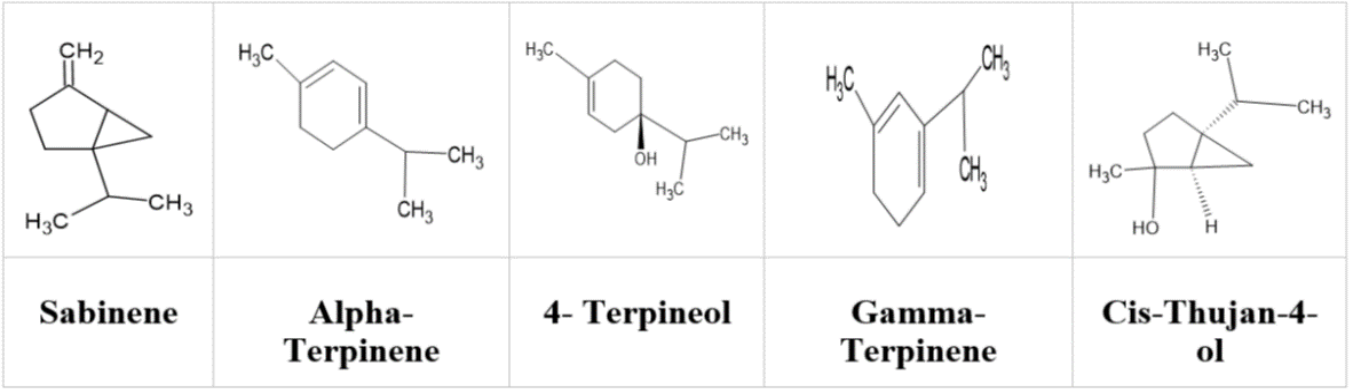

Figure 1: Structure of marjoram oil components.

Table 1: Formulation batches of ternary phase diagram.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Marjoram Oil (mL)	1	2	3	4	5	6	7	8	9
S_{mix} (1:1) (mL)	9	8	7	6	5	4	3	2	1
Water (mL)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

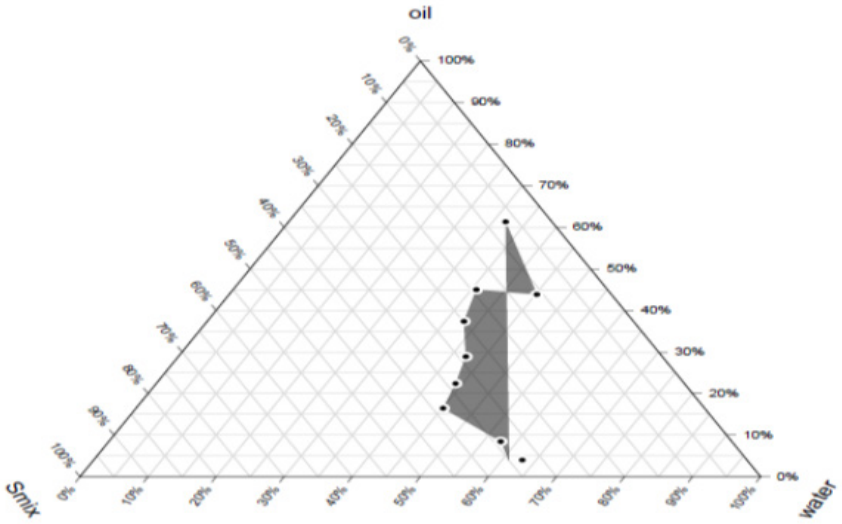

Figure 2: Ternary phase diagram of marjoram oil microemulsion (The dark area represents the microemulsions existence field where stable, clear and transparent formulations are produced).

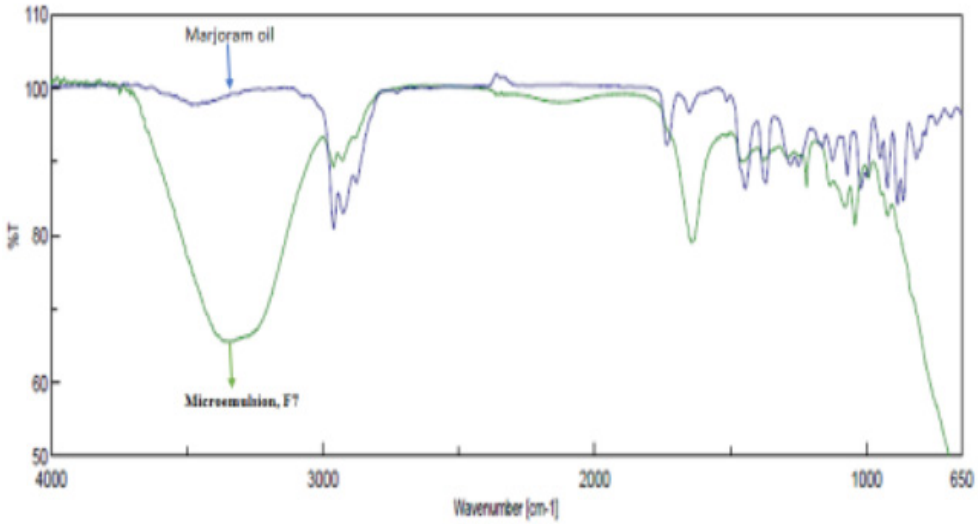

Figure 3: FTIR marjoram oil and Micro emulsion F1.

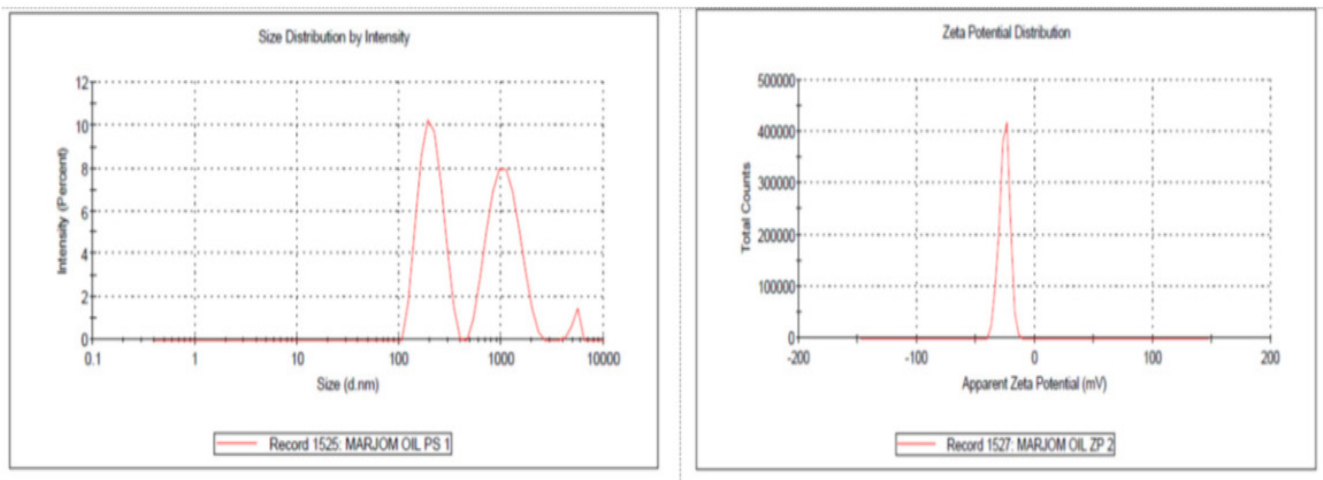

Figure 4: Particle size distribution and Zeta Potential of formulation F7.

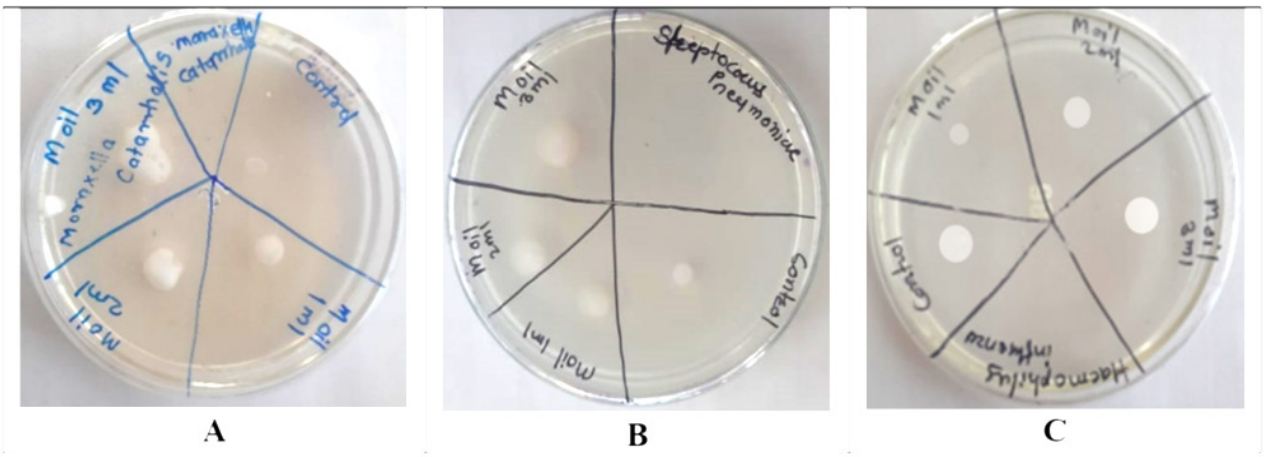

Figure 5: Zone of inhibition for formulation F7 against A. *Moraxella catarrhalis* B. *Streptococcus pneumonia* and C. *Haemophiles influenza.*

Table 2: Microemulsion formulations for Ternary phase diagram.

Ingredients	F1 (1:9)	F2 (2:8)	F3 (3:7)	F4 (4:6)	F5 (5:5)	F6 (6:4)	F7 (7:3)	F8 (8:2)	F9 (9:1)
Marjoram oil %	3.89	8.47	16.47	22.41	28.87	37.36	44.99	43.84	61.31
S_{mix} (1:1) %	35.02	33.90	38.42	33.61	28.87	24.91	19.28	10.96	6.81
Distilled water %	61.09	57.63	45.12	43.98	42.26	37.73	35.73	45.21	31.88

It was observed that as the concentration of Marjoram oil was increased in the disc method, zone of inhibition was found increased as compared to chloramphenicol control sample. The results of zone of inhibition are shown in Figure 5 and Table 4. Microemulsion formulation (F7) was used at 50 µg/mL, 100 µg/mL, 200 µg/mL concentration for the respective sample code 1 mL-M Oil, 2 mL-M oil and 3 mL-M Oil. The control sample chloramphenicol ear drop used at 100 µg/mL concentration for antibacterial test. Microemulsion F7 was shown 17 to 29 mm, 09 to 20 mm and 20 to 27 mm concentration dependent zone of inhibition for *Moraxella catarrhalis, Streptococcus pneumonia* and *Haemophiles influenza* bacterial species respectively. The zone of inhibition was found increased with increase in concentration of microemulsion against all three bacterial species. The chloramphenicol ear drop (control sample) was shown lower zone of inhibition against *Moraxella catarrhalis* and *Streptococcus pneumonia* and better zone of inhibition against *Haemophiles influenza.*

DISCUSSION

The most of the volatile oils are transparent or pale yellow due to presence of linalool (Sadgrove *et al.*, 2022). Volatile oils generally showed less density compared to water. Volatile oils are immiscible in water but soluble in organic solvents (Dhifi *et al.*, 2016). Naturally, skin pH values lies between 4.0 to 7.0 and neutral pH value of marjoram oil suggested the no irritation of skin surface (Lambers *et al.*, 2006). The higher boiling point of marjoram oil suggested long lasting aroma, extended release from formulations and more heat stability. The higher boiling point of marjoram oil is due to presence of 4- terpeneol (Warsito *et al.*, 2017). Low viscosity of oil are easily pourable liquids. Generally, the refractive index value of volatile oils falls between 1.45 to 1.55 (Siejak *et al.*, 2021). The results of physicochemical studies confirmed the purity of marjoram oil and its properties.

Table 3: Marjoram oil physicochemical properties.

Sl. No.	Properties	Observation
1.	Odour	Aromatic
2.	Appearance	Pale Yellow
3	Solubility	Water insoluble, soluble in ethanol
4	pH	6.30-6.95
5	Boiling point	164-172°C
6	Viscosity cps	166-200
7	Density g/mL	0.889±0.012
8	Refractive index	1.472±0.054

Formulation F7 ingredient mixture and marjoram oil sample were analysed by JASCO FTIR -4600 for functional group analysis. The chemical compatibility between marjoram oil and S_{mix} was studied. FTIR spectrum shown in Figure 3 revealed characteristic peaks associated with the functional groups present in this essential oil at 3482.81, 2960.2, 2925.48, 1733.69, 1445.39, 1369.21 and 1278.57 cm^{-1}. Peaks around 2900-3000 cm^{-1} indicated the stretching vibrations of C-H bonds in aliphatic hydrocarbons, which commonly found in essential oils (Agatonovic-Kustrin *et al.*, 2020). A peak around 1700-1750 cm^{-1} indicated the presence

Table 4: Zone of inhibition.

Sl. No.	Bacteria species	Zone of inhibition in mm for sample code (Quantity applied)			
		1 mL-M Oil (50 µg/mL)	2 mL-M Oil 100 µg/mL	3 mL-M Oil 200 µg/mL	Control (100 µg/mL)
1	*Moraxella catarrhalis*	17 mm	21mm	29 mm	08 mm
2	*Streptococcus pneumonia*	09 mm	12 mm	20 mm	08 mm
3	*Haemophiles influenzae*	20 mm	23 mm	27 mm	26 mm

of carbonyl groups, which could be from ketones, aldehydes, or esters present in the oil. A broad peak in the region of 3200-3600 cm^{-1} indicated the presence of hydroxyl groups typically found in alcohols and phenols. Peaks in the range of 1500-1600 cm^{-1} and 800-900 cm^{-1} might represent vibrations associated with aromatic ring structures present in the oil (Nandiyanto *et al.*, 2019). All the peaks of marjoram oil were observed in microemulsion F7 with small shift in frequency indicated compatibility between oil and the excipients used.

The marjoram oil was emulsified with the Tween 80 surfactant and propylene glycol co-surfactant. The emulsification was produced due to solubilization of oil in the water at critical micelle concentration of surfactant and co-surfactant effect (Fernández-Peña *et al.*, 2019). Non-ionic surfactant, Tween 80, stabilizes the emulsions based on dynamic interfacial theory which produces greater resistance to phase disruption and coalescence of oil droplets. Stearic repulsion slows down the droplet flocculation (Roldan-Cruz *et al.*, 2016).

The prepared microemulsion of marjoram oil F7, was shown the globule size 370.3 nm. Generally, an emulsion with average droplet size ≥6000 nm is classified as having poor stability (Adejokun & Dodou, 2020). Formulation F7 produced Zeta potential of -25.7 mV. The zeta potential of the emulsion system is an important parameter for particle – particle aggregation, and stability. The ideal zeta potential between ±10 to 30 mV is considered as stable formulation. The negative potential prevents coalescence of droplets (Kotakadi *et al.*, 2013). The negative charge of microemulsion could be due to presence of anionic groups of fatty acids, and glycols present in oil, surfactant and co-surfactant (Shinde UA *et al.*, 2018). Negatively charged carriers are considered more effective than positive charged for drug accumulation in skin and diffusion (Gillet *et al.*, 2011).

It is investigated that marjoram oil shows antimicrobial activity against *S. aureus, S. epidermitis, E. Coli, P. aeruginosa, H. influenza, S. pneumonia* due to presence of alcoholic and phenolic monoterpinoids (Karen *et al.*, 2023; Leigh-de *et al.*, 2021; Inouye *et al.*, 2001). Hydrophobic essential oils components are present in the microemulsion which partition with the lipids present in the cell membrane of bacteria and mitochondria. The penetrated components disturb the bacterial cell structures and lead to death (Devi *et al.*, 2010; Malik & Upadhyay, 2023). The results of antimicrobial activity on marjoram oil microemulsion confirms the effectiveness against Otitis media causing microorganisms.

CONCLUSION

The developed microemulsion formulation of marjoram oil was shown better antimicrobial effect against otitis media causing micro-organisms than the chloramphenicol control sample (marketed ear drop). This research study reveals that marjoram oil used in microemulsion system showed antibacterial effect and have a potential to use as a drug delivery system. In future, *in vivo* preclinical and clinical test should be conducted to evaluate toxicity and clinical effectiveness against Otitis media infection.

ACKNOWLEDGEMENT

The authors are thankful to Sandip Institute of Pharmacy, Nashik and R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur for providing facility to study microbiological testing and Malvern particle size analyser instrument facility respectively.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Mv: Millivolt; **mL:** Millilitre; **PDI:** Polydispersibility Index; **g/mL:** Gram per millilitre; **cps:** Centipoise; **rpm:** Revolutions per minute; **FTIR:** Fourier-Transform Infrared Spectroscopy; **nm:** Nanometer; **cm^{-1}:** per centimetre; **% w/w:** Percent weight by weight.

REFERENCES

Adejokun, D. A., & Dodou, K. (2020). A Novel Method for the evaluation of the long-term stability of cream formulations containing natural oils. *Cosmetics*, *7*(4), 86. https://doi.org/10.3390/cosmetics7040086

Agatonovic-Kustrin, S., Ristivojevic, P., Gegechkori, V., Litvinova, T. M., & Morton, W. (2020). Essential oil quality and purity evaluation via FT-IR spectroscopy and pattern recognition techniques. *Applied Sciences*, *10*(20), Article 7294. https://doi.org/10.3390/app10207294

Badawi, A. A., Nour, S. A., Sakran, W. S., & El-Mancy, S. M. S. (2009). Preparation and evaluation of microemulsion systems containing salicylic acid. *AAPS PharmSciTech*, *10*(4), 1081–1084. https://doi.org/10.1208/s12249-009-9301-7

Borse, V. A., Gangude, A. B., & Deore, A. B. (2020). Formulation and evaluation of antibacterial topical gel of doxycycline hyclate, neem oil, and tea tree oil. *Indian Journal of Pharmaceutical Education and Research*, *54*(1), 206–212. https://doi.org/10.5530/ijper.54.1.24

Devi, K. P., Nisha, S. A., Sakthivel, R., & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of Ethnopharmacology*, *130*(1), 107–115. https://doi.org/10.1016/j.jep.2010.04.025

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, *3*(4), Article 25. https://doi.org/10.3390/medicines3040025

Dhiman, N., & Bhasin, A. (2022). *Origanum majorana*: An essential oil with potential pharmacological properties and health benefits. *The pharma innovation journal*, SP-11, *7* (pp. 4454–4460).

Dorsaf, B. H., Hanen, B. I., Chokri, J., Larbi, K. M., & Manef, A. (2010). Chemical composition of some Tunisian Eucalyptus essential oils as obtained by hydrodistillation using Clevenger type apparatus. *Biosciences Biotechnology Research Asia*, *7*(2), 647–656.

Ferhat, M. A., Meklati, B. Y., Smadja, J., & Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *Journal of Chromatography. A*, *1112*(1–2), 121–126. https://doi.org/10.1016/j.chroma.2005.12.030

Fernández-Peña, L., Gutiérrez-Muro, S., Guzmán, E., Lucia, A., Ortega, F., & Rubio, G. (2019). Oil-In-water microemulsions for thymol solubilization. *Colloids and Interfaces*, *3*(4), Article 64. https://doi.org/10.3390/colloids3040064

Gillet, A., Compère, P., Lecomte, F., Hubert, P., Ducat, E., Evrard, B., & Piel, G. (2011). Liposome surface charge influence on skin penetration behavior. *International Journal of Pharmaceutics*, *411*(1–2), 223–231. https://doi.org/10.1016/j.ijpharm.2011.03.049

Inouye, S., Takizawa, T., & Yamaguchi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *The Journal of Antimicrobial Chemotherapy*, *47*(5), 565–573. https://doi.org/10.1093/jac/47.5.565

Kamari, F. E., Chlouchi, A., El Hamzaoui, N. E., Harmouzi, A., Lhilali, I., Amrani, J., & E., ElMouhdi, K., Omari, H. E., & Abdellaoui, A. (2023). Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of Origanum majorana Growing in Middle Atlas of Morocco. *Tropical Journal of Natural Product Research*, *7*(10), 4232–4237. https://doi.org/10.26538/tjnpr/v7i10.16

Kotakadi, V. S., Rao, Y. S., Gaddam, S. A., Prasad, T. N. V. K. V., Reddy, A. V., & Gopal, D. V. R. S. (2013). Simple and rapid biosynthesis of stable silver nanoparticles using dried leaves of *Catharanthus roseus*. Linn. G. Donn and its anti microbial activity. *Colloids and Surfaces. B, Biointerfaces*, *105*, 194–198. https://doi.org/10.1016/j.colsurfb.2013.01.003

Kumar, R., Kumar, S., & Sinha, V. R. (2016). Evaluation and optimization of water-in-oil microemulsion using ternary phase diagram and central composite design. *Journal of Dispersion Science and Technology*, *37*(2), 166–172. https://doi.org/10.1080/01932691.2015.1038351

Lambers, H., Piessens, S., Bloem, A., Pronk, H., & Finkel, P. (2006). Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science*, *28*(5), 359–370. https://doi.org/10.1111/j.1467-2494.2006.00344.x

Lavanya, N., Aparna, C., & Umamahesh, B. (2016). Formulation and evaluation of glipizide microemulsion. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(8), 171–176.

Leigh-de Rapper, S., Viljoen, A., & van Vuuren, S. (2021). Essential oil blends: The potential of combined use for respiratory tract infections. *Antibiotics*, *10*(12), Article 1517. https://doi.org/10.3390/antibiotics10121517

Malik, P., & Upadhyay, P. (2023). Formulation and evaluation of tea tree/rosemary essential oil-based microemulsion for antimicrobial activity. *Biosciences Biotechnology Research Asia*, *20*(1), 229–239. https://doi.org/10.13005/bbra/3084

Mazza, K. E. L., Costa, A. M. M., da Silva, J. P. L., Alviano, D. S., Bizzo, H. R., & Tonon, R. V. (2023). Microencapsulation of marjoram essential oil as a food additive using sodium alginate and whey protein isolate. *International Journal of Biological Macromolecules*, *233*, Article 123478. https://doi.org/10.1016/j.ijbiomac.2023.123478

Mittal, R., Parrish, J. M., Soni, M., Mittal, J., & Mathee, K. (2018). Microbial otitis media: Recent advancements in treatment, current challenges and opportunities. *Journal of Medical Microbiology*, *67*(10), 1417–1425. https://doi.org/10.1099/jmm.0.000810

Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscopy of organic material. *Indonesian Journal of Science and Technology*, *4*(1), 97–118. https://doi.org/10.17509/ijost.v4i1.15806

Prabuseenivasan, S., Jayakumar, M., & Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine*, *6*, Article 39. https://doi.org/10.1186/1472-6882-6-39

Raina, A. P., & Negi, K. S. (2012). Essential oil composition of Origanum majorana and Origanum vulgare ssp. hirtum growing in India. *Chemistry of Natural Compounds*, *47*(6), 1015–1017. https://doi.org/10.1007/s10600-012-0133-4

Roldan-Cruz, C., Vernon-Carter, E. J., & Alvarez-Ramirez, J. (2016). Assessing the stability of Tween 80-based o/w emulsions with cyclic voltammetry and electrical impedance spectroscopy. *Colloids and Surfaces. Part A, Physicochemical and Engineering Aspects*, *511*, 145–152. https://doi.org/10.1016/j.colsurfa.2016.09.074

Sadgrove, N. J., Padilla-González, G. F., & Phumthum, M. (2022). Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentication. *Plants*, *11*(6), Article 789. https://doi.org/10.3390/plants11060789

Asmawatia, Wan, Salma, A. W. M., M. Y., Mohamad, Y. M., & Ahmad, F. S. (2014). Characteristics of cinnamaldehyde nanoemulsion prepared using APV-high pressure homogenizer and ultra turrax. *AIP Conference Proceedings*, *1614*, 244–250. https://doi.org/10.1063/1.4895203

Schilder, A. G. M., Chonmaitree, T., Cripps, A. W., Rosenfeld, R. M., Casselbrant, M. L., Haggard, M. P., & Venekamp, R. P. (2016). Otitis media. *Nature Reviews. Disease Primers*, *2*(1), Article 16063. https://doi.org/10.1038/nrdp.2016.63

Shinde, U. A., Modani, S. H., & Singh, K. H. (2018). Design and development of repaglinide microemulsion gel for transdermal delivery. *AAPS PharmSciTech*, *19*(1), 315–325. https://doi.org/10.1208/s12249-017-0811-4

Siejak, P., Smułek, W., Fathordobady, F., Grygier, A., Baranowska, H. M., Rudzińska, M., Masewicz, Ł., Jarzębska, M., Nowakowski, P. T., Makiej, A., Kazemian, P., Drobnik, P., Stachowiak, B., Jarzębski, M., & Pratap-Singh, A. (2021). Multidisciplinary studies of folk medicine "five thieves' oil" (Olejek Pięciu Złodziei) Components. *Molecules, 26*(10), Article 2931. https://doi.org/10.3390/molecules26102931

Silva, M. D., & Sillankorva, S. (2019). Otitis media pathogens – A life entrapped in biofilm communities. *Critical Reviews in Microbiology*, *45*(5–6), 595–612. https://doi.org/10.1080/1040841X.2019.1660616

Souto, E. B., Cano, A., Martins-Gomes, C., Coutinho, T. E., Zielińska, A., & Silva, A. M. (2022). Microemulsions and nanoemulsions in skin drug delivery. *Bioengineering*, *9*(4), Article 158. https://doi.org/10.3390/bioengineering9040158

Thanh, V. M., Bui, L. M., Bach, L. G., Nguyen, N. T., Thi, H. L., Hoang Thi, T. T., & TT. (2019). *Origanum majorana* L. essential oil-associated polymeric nano dendrimer for antifungal activity against Phytophthora infestans. *Materials*, *12*(9), Article 1446. https://doi.org/10.3390/ma12091446

Vanneste, P., & Page, C. (2019). Otitis media with effusion in children: Pathophysiology, diagnosis, and treatment. A review. *Journal of Otology*, *30*(4), 1–7. https://doi.org/10.1016/j.joto.2019.01.005

Vera, R. R. (1999). Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island. *Food Chemistry*, *66*(2), 143–145. https://doi.org/10.1016/S0308-8146(98)00018-1

Warsito, W., Palungan, M. H., & Utomo, E. P. (2017). Profiling study of the major and minor components of kaffir lime oil (*Citrus hystrix* DC.) in the fractional distillation process. *The Pan African Medical Journal*, *27*, Article 282. https://doi.org/10.11604/pamj.2017.27.282.9679

Yadav, V., Jadhav, P., Kanase, K., Bodhe, A., & Dombe, S. (2018). Preparation and evaluation of microemulsion containing antihypertensive drug. *International Journal of Applied Pharmaceutics*, *10*(5), 138–146. https://doi.org/10.22159/ijap.2018v10i5.27415

Cite this article: Gangurde AB, Pandit VS, Suryawanshi KA, Soundane BV, Nikam SR, Pathade PA. Development of Microemulsion of Marjoram Oil in the Line of Treatment of Otitis Media. J Young Pharm. 2025;17(2):329-35.