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Development and Evaluation of Herbal Microemulsion-Based Topical Systems for Enhanced Skin Delivery and Antimelanoma Activity

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ABSTRACT
The therapeutic use of several bioactive phytoconstituents for topical cancer therapy is severely limited by their limited water solubility, chemical instability, and poor skin penetration. To improve the dermal delivery and antimelanoma efficacy of *Fernandoa adenophylla* ethanolic extract (FAEE), *Curcuma caesia* ethanolic extract (CCE), and *Melaleuca alternifolia* oil (TTO), stable herbal microemulsion (ME) based topical formulations were created and assessed. Pharmaceutically acceptable non-ionic surfactants were used to create mono-herbal, marker-based microemulsions, which were then optimized by methodically creating pseudo-ternary phase diagrams. Physicochemical characteristics, droplet size distribution, polydispersity index, zeta potential, and thermodynamic stability were all thoroughly examined in the optimized formulations. Franz diffusion cells were used to measure in vitro skin penetration, and the MTT assay was used to measure cytotoxic potential against human melanoma cell lines (A375 and Hs294T) and normal human dermal fibroblasts (NHDF). All the optimized microemulsions showed strong selective cytotoxicity toward melanoma cells with minimal toxicity to normal skin cells, as well as nanoscale globule size (<200 nm), outstanding physical stability, and markedly improved skin penetration. The results of this study show that topical delivery methods based on herbal microemulsions may be a promising approach for managing localized skin cancer and melanoma chemoprotection.

1. INTRODUCTION:

One of the most deadly and severe types of skin cancer, melanoma is responsible for a disproportionately large number of skin cancer-related deaths globally. It presents significant clinical problems due to its potential for fast metastasis and resistance to traditional chemotherapy regimens. Systemic chemotherapy is still a mainstay in the treatment of melanoma; however, it frequently has limited therapeutic efficacy, poor patient compliance and dose-limiting toxicities.[1]

By permitting localized drug targeting, reducing systemic exposure, and enhancing patient tolerability, topical drug delivery devices provide a clear advantage in the treatment of melanoma.[2] However, the stratum corneum is a strong barrier that prevents many medicinal molecules especially lipophilic phytoconstituents from penetrating.

The antioxidant, antibacterial, anti-inflammatory, and anticancer qualities of medicinal plants like F*ernandoa adenophylla, Curcuma caesia, and Melaleuca alternifolia* have long been acknowledged. Numerous phytochemicals, such as flavonoids, terpenoids, phenolics, and components of essential oils, are responsible for these biological effects. Despite their therapeutic promise, poor aqueous solubility, low chemical stability, and insufficient skin permeation limit their clinical application in topical cancer therapy.

Microemulsions are transparent, isotropic, thermodynamically stable colloidal systems made of water, oil, surfactant, and frequently a co-

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surfactant. Microemulsions can greatly improve the solubility, skin penetration, and bioavailability of poorly soluble drugs because of their extremely low interfacial tension and nanoscale droplet size.[3] Thus, the goal of the current work was to create topical formulations of FAEE, CCE, and TTO based on mono-herbal microemulsions. These formulations would then undergo thorough physicochemical characterization [4], skin penetration assessment, and in vitro antimelanoma activity evaluation.

2. MATERIALS AND METHODS:

2.1 Materials:

Curcuma caesia rhizomes and *Fernandoa adenophylla* leaves were collected, verified, and processed to produce ethanolic extracts (FAEE and CCE). [5] A certified provider provided pharmaceutical-grade *Melaleuca alternifolia* oil. Because of their low toxicity and skin compatibility, non-ionic surfactants including Tween 20, Tween 80, and Cremophor EL were used. We investigated the oil phases of oregano, lemongrass, cinnamon, and thyme. Every chemical and reagent utilized was of analytical quality.

2.2 Preformulation Studies:

2.2.1 Solubility Studies:

To find appropriate oil phases that could dissolve the highest amounts of FAEE [6], CCE, and TTO, solubility tests were carried out. To achieve equilibrium, excess amounts of each medication were added to various oils, vortex-mixed, and incubated for 48–72 hours at 24 ± 1 °C. To ascertain the drug solubility, the mixtures were centrifuged, and the supernatant was examined using spectrophotometry.

2.2 Surfactant Screening:

Emulsification efficiency, clarity, percentage transmittance, and simplicity of emulsification were the criteria used to screen surfactants. For the creation of the microemulsion, the surfactant that resulted in a clear, uniform emulsion with little phase separation was chosen.

2.3 Construction of Pseudo-Ternary Phase Diagrams:

The water titration method was used to create pseudo-ternary phase diagrams at room temperature (25 ± 1 °C). The microemulsion zones were determined by visual transparency and isotropic appearance after different ratios of oil and surfactant were titrated with distilled water while being gently stirred.[7]

2.4 Preparation of Drug-Loaded Microemulsions:

By dissolving FAEE, CCE, or TTO in the chosen oil phase and then adding a surfactant and aqueous phase, optimized drug-free microemulsions were transformed into drug-loaded systems.[8] After being magnetically agitated, the formulations were left to equilibrate for the entire night.

2.5 Thermodynamic Stability Studies:

To evaluate the thermodynamic stability of the optimized formulations, centrifugation, heating-cooling cycles, and freeze-thaw cycles were performed. Formulations were deemed stable if there were no indications of phase separation, creaming, or cracking.[9]

2.6 Physicochemical Characterization:

To make sure the formulations were compatible with the skin, their pH was evaluated. The type of microemulsion was confirmed by conductivity testing. Viscosity was measured with a Brookfield viscometer, and turbidity was evaluated spectrophotometrically.

2.7 Droplet Size, PDI, and Zeta Potential:

Droplet size and polydispersity index were calculated using dynamic light scattering analysis. Colloidal stability was assessed using zeta potential measurements.[10]

2.8 In Vitro Skin Permeation Study:

A modified Franz diffusion cell with pig ear skin as the membrane was used for skin permeation investigations.[11] To calculate cumulative drug penetration, samples were taken at prearranged intervals and analysed. [12]

2.9 In Vitro Cytotoxicity Study:

The MTT assay was used to assess the cytotoxic capability of improved formulations against the melanoma cell lines A375 and Hs294T, with NHDF cells acting as normal controls.[13] To evaluate selective anticancer activity, IC‰ values were computed.

3. RESULTS AND DISCUSSION:

Broad microemulsion regions, favourable physicochemical properties, nanoscale droplet size (12–19 nm), and outstanding stability were all present in optimized microemulsions. All microemulsion formulations showed improved medication penetration when compared to crude extracts.[14] Selective antimelanoma potential was highlighted by cytotoxicity tests that showed dose-dependent reduction of melanoma cell proliferation with low damage to normal dermal fibroblasts. [15]

The equilibration method was used to test the drug's solubility in a variety of essential oils and surfactants. (**Table 1**).

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Table-1 Solubility of extract in oils

Drug in Oil	Mg /Ml
FAEE CIN	**131.64±1.28**
FAEE OG	120.12±3.09
FAEE LG	100.99±4.08
FAEE THY	**164.29±2.10**
CEE CIN	**157.71±2.26**
CEE OG	145.31±4.12
CEE LG	**171.26±6.01**
CEE THY	120.24±3.50
TTO CIN	165.36±3.72
TTO OG	**185.18±7.09**
TTO LG	171.47±6.23
TTO THY	**190.42±7.14**

Selection of surfactant:

The non-ionic surfactants such as tween 20, tween 80, span 20, span 80, Cremophor EL, Brij93, Triton X 100, PEG400 and propylene glycol were screened to find the surfactants with higher emulsification efficiency for the oil, with least number of flask inversions and the results were shown in **Fig. 1-3**.

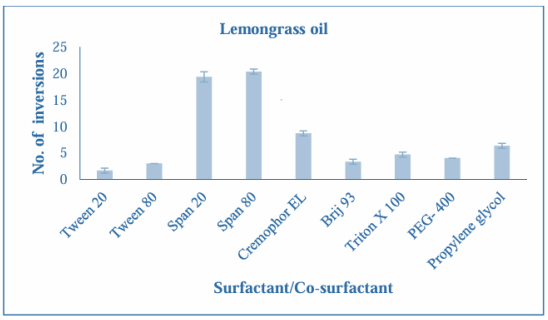

Fig. 1 Emulsification efficiency of surfactants in lemongrass oil

Figure 1 illustrates the lemon grass oil exhibit the highest emulsification efficiency with tween 20 and tween 80 for the homogeneous emulsion formation.

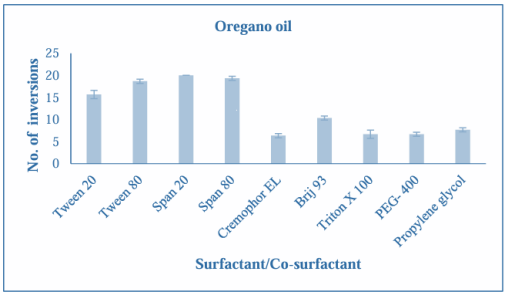

Fig. 2 Emulsification efficiency of surfactants in oregano oil

Figure 2 shows the oregano oil to have the maximum emulsification effectiveness with Cremophor EL to give a homogeneous form of emulsion.

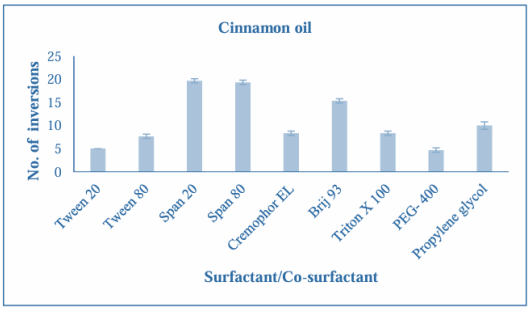

Fig. 3 Emulsification efficiency of surfactants in Cinnamon oil.

Figure 3 portrays the cinnamon oil has the utmost emulsification efficiency with tween 20 and tween 80 for the homogeneous emulsion formation**.**

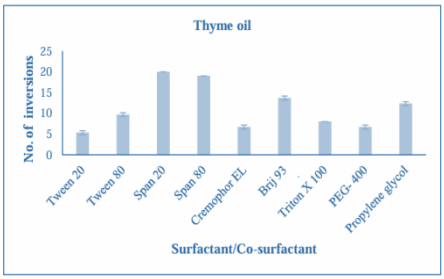

Fig. 4 Emulsification efficiency of surfactants in thyme oil

Figure 4 depicts the oil phase thyme oil exhibits the highest emulsification efficiency with tween 20 and Cremophor EL for the homogeneous emulsion formation.

PSEUDO-TERNARY PHASE DIAGRAM:

The water titration method was used to create the pseudo-ternary phase diagram, which clarified the connection between phase behaviour and composition by defining the area of transparent microemulsion (ME). Figures 5 through 12 show the diagram created using the chosen oil and matching surfactant. Red markers were used to denote visually transparent ME systems that were seen at ambient temperature. [16] The greatest ME regions were found at particular oil-to-surfactant ratios: LG oil with Tween 20 and CIN oil, THY oil, and CLO oil with Cremophor EL, according to an analysis of the data (Fig. 5–8).

An extended ME area was obtained for OG oil by optimizing the hydrophilic-lipophilic balance (HLB) of various non-ionic surfactants to create a surfactant combination (Smix) of Tween 80 and Cremophor EL at a 1:2 ratio.[17] The ME formulations were then made using this optimized oil and surfactant mix.

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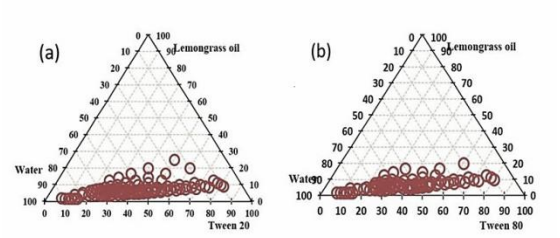

Fig.5 Pseudo-ternary phase diagrams for microemulsions of lemongrass oil against (a) Tween 20 and (b) Tween 80, as oil and surfactants respectively, along with water. (Ratios 1:1 to 1:9 and 1:9 to 9:1).

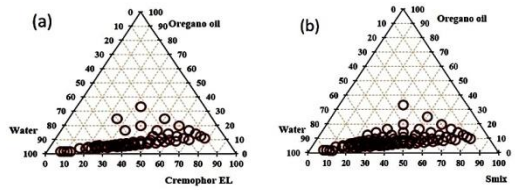

Fig. 6 Pseudo-ternary phase diagrams for microemulsions of oregano oil against (a) Cremophor EL and (b) Smix (Tween 80: Cremophor EL, 1:2 respectively), as oil and surfactants respectively, along with water. (Ratios 1:1 to 1:9 and 1:9 to 9:1).

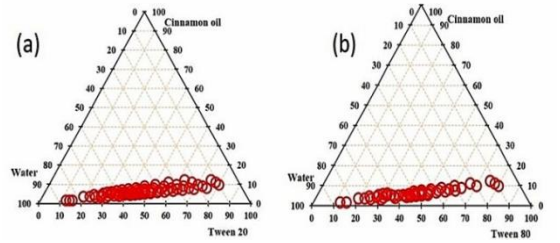

Fig. 7 Pseudo-ternary phase diagrams for microemulsions of cinnamon oil against (a) Tween 20 and (b) Tween 80, as oil and surfactants respectively, along with water. (Ratios 1:1 to 1:9 and 1:9 to 9:1)

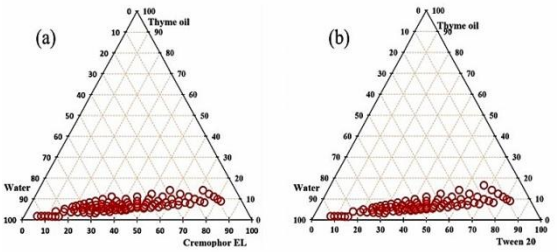

Fig. 8 Pseudo-ternary phase diagrams for microemulsions of thyme oil against (a) Cremophor EL and (b) Tween 20, as oil and surfactants respectively, along with water. (Ratios 1:1 to 1:9 and 1:9 to 9:1)

Microemulsion Preparation:

The phase study was used to determine the ratios at which the formulation exhibited transparent ME. Therefore, the minimum surfactant concentration required to create a homogenous ME formulation was found. From the different ratios, the appropriate ME ratio (o/s/w%) was chosen. As seen in Fig. 9–12, two sets of formulations were created by altering the selected ME composition ratio (o/s/w%). Similarly, the DL-MEs were prepared by dissolving the medicines in their corresponding oils and employing a suitable surfactant. Alkaline water containing 1% (v/v) 10 mM NaOH was used to combine DL-ME with 1% (w/v) FAEE in CIN and the surfactant Tween 20.

Additionally, other DL-MEs developed, including FAEE THY, CEE3 CIN, CEE3 LG, TTO OG, and TTO THY. To create a DL-ME, all these oil-to-surfactant combinations were let to combine with ultrapure water. Thermodynamic stability was assessed by looking at the transparent DF-ME and DL-ME formulations, which remained transparent without phase separation.

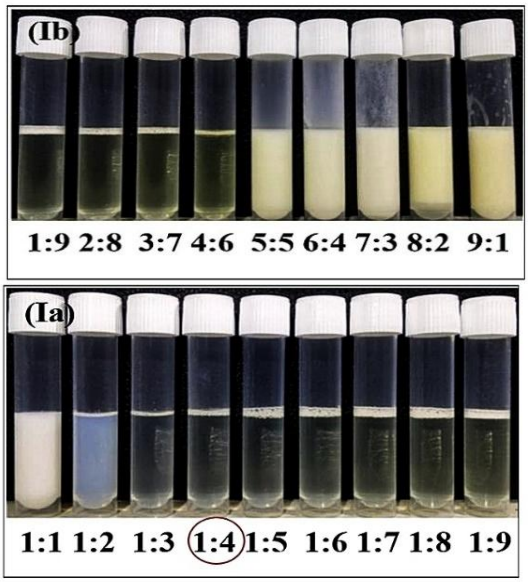

Fig.9 Visual appearance of two set of formulations (a, b).

Figure 9 depicts the various ratios of lemongrass oil/ Tween 20 for (I) LM and (II) LMA and LM is LG oil-based drug-free ME and LMA is LG oil based CEE3-loaded ME respectively.

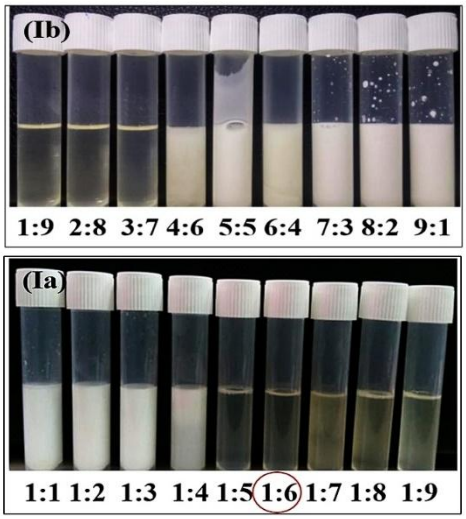

Fig. 10 Visual appearance of two set of formulations (a, b).

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Figure 10 depicts the various ratios of Oregano oil/ oil/ Smix for (Ia) OM and (Ib) OMI. OM is OG oil-based drug-free ME and OMI are OG oil based TTO loaded ME respectively. at various ratios of oregano oil/ Smix.

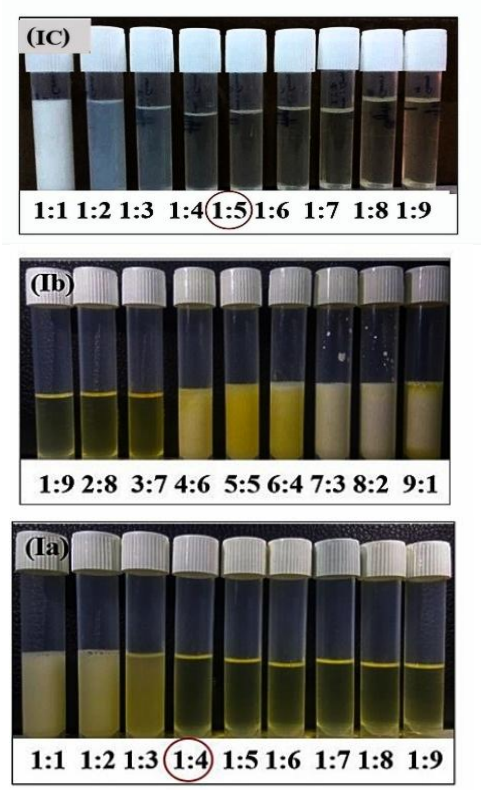
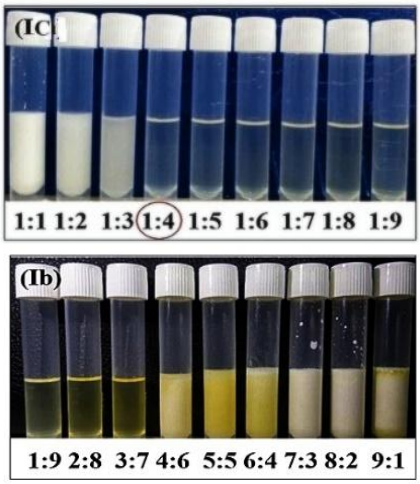


Fig. 11 Visual appearance of three set of formulations (a, b and c).

Figure 11 depicts the various ratios of Thyme Oil / Cremophore for (Ia) TM, (Ib) TMA and (Ic) TMB. TM is thyme oil-based drug-free ME and TMA is TM oil based FAEE loaded ME and TMB is TM oil based TTO loaded ME respectively. at various ratios of Thyme oil.



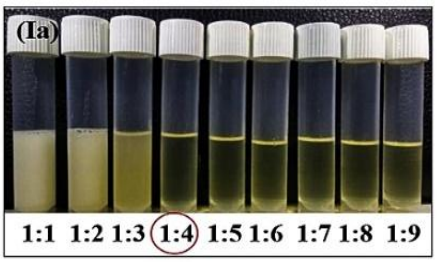


Fig. 12 Visual appearance of three set of formulations (a, b and c).

Figure 12 depicts the various ratios of Cinnamon Oil/ Tween 20 for (Ia) CIN, (Ib) CINA and (Ic) CINB. CIN is cinnamon oil-based drug-free ME and CINA is cinnamon oil based FAEE loaded ME and CINB is cinnamon oil based CEE3 loaded ME respectively at various ratios of cinnamon oil.

The transparent DF-ME and DL-ME formulations that lasts thermodynamically or physically stable with no phase separation, even at varied extreme stress conditions were determined to be the optimized ME formulations. **Table 2** illustrates the thermodynamically stable, optimized ME formulations.

Table 2 Optimized formulation of various oil based microemulsions obtained by thermodynamic stability study

Oil	Surfactant	Drug	Optimized ME formulation ratio (Oil: Surfactant: Water)
Lemongrass oil	Tween 20	CEE	1:4:15
Oregano oil	Smix	TTO	1:6:13
Cinnamon oil	Tween 20	CEE	1:4:12
		FAEE	1:5:14
Thyme oil	Cremophore	FAEE	1:5:14
		TTO	1:5:14

Physicochemical Characterization:

Determination of pH, conductivity, turbidity and viscosity

The physico-chemical characterization of these test samples was carried out in triplicate, and the results were interpreted as mean ± SD in **Table 3**. The pH value of all the optimized, stable DF-ME, and DL-ME formulations was determined to be between 6.32 ± 0.004 to 6.56 ± 0.005 for topical ME formulations. The electrical conductivity (σ) of the optimized DF-ME and DL-ME formulations was between 0.198 ± 0.001 to 0.274 ± 0.002 µS/cm for topical ME formulations. The optical transparency of all the optimized ME formulations was measured quantitatively at the visible wavelength of 600 nm, which shows an absorbance of <0.010 ±0.001respectively.

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Table 3 Characterization and Size analysis parameters of optimized microemulsions

Test samples	pH	Conductivity (µS/cm)	Turbidity (OD)	Viscosity (cP)	Globule size (nm)	PDI	Zeta potential (mV)
LM	6.32±0.004	0.212±0.006	0.003±0.000	5.67±0.00	17.4±2.2	0.222±0.002	-0.2±0.040
LM A	6.53±0.003	0.280±0.002	0.002±0.000	5.71±0.00	19.3±1.6	0.268±0.002	-0.1±0.030
OM	6.46±0.014	0.234±0.007	0.004±0.000	5.87±0.80	12.6±1.2	0.400±0.001	-4.12±0.034
OM1	6.61±0.007	0.251±0.008	0.001±0.000	8.21±0.42	13.9±7.3	0.481±0.003	-5.02±0.21
TM	6.72±0.001	0.292±0.001	0.003±0.000	6.24±0.26	17.7±5.1	0.512±0.001	1.2±0.021
TMA	6.85±0.009	0.278±0.050	0.002±0.000	9.37±0.89	19.3±3.4	0.572±0.002	2.04±0.010
TMB	6.92±0.007	0.293±0.003	0.009±0.000	8.24±0.19	18.4±7.2	0.534±0.001	1.6±0.070
CIN	6.54±0.008	0.198±0.003	0.001±0.000	5.22±0.20	13.4±3.9	0.173±0.002	-3.12±0.050
CINA	6.75±0.003	0.247±0.023	0.006±0.000	6.67±0.91	18.1±2.2	0.181±0.002	-4.01±0.032
CINB	6.58±0.006	0.256±0.078	0.003±0.000	7.61±0.51	16.7±8.1	0.1943±0.003	-4.25±0.0510

***Values are represented as mean ± S.D, n = 3**

Drug Permeation Study:

In Vitro drug permeation testing:

The in vitro drug permeation testing was done for the oral ME formulations by following the dissolution principle using dialysis membrane in modified Franz diffusion apparatus and the cumulative drug release percentage was estimated, as shown in Table 4 and includes Cumulative % drug permeated, for the selected DL-ME formulations in **Table 4.**

Table 4 In Vitro drug permeation results.

Time (h)	LM CEE % Permeated	CIN CEE % Permeated	CINFAEE % Permeated	TMFAEE % Permeated	OGTTO % Permeated	TMTTO % Permeated
0.5	2.1 ± 0.3	2.4 ± 0.1	1.3 ± 0.2	2.4 ± 0.1	8.4 ± 0.1	5.4 ± 0.1
1	5.8 ± 0.6	4.7 ± 0.2	3.7 ± 0.5	3.9 ± 0.6	17.8 ± 0.5	11.8 ± 0.3
2	12.4 ± 1.1	9.6 ± 0.3	11.6 ± 0.6	10.5 ± 0.4	24.0 ± 0.9	18.6 ± 0.1
4	28.6 ± 2.3	14.1 ± 0.5	19.1 ± 0.1	17.4 ± 0.6	39.7 ± 0.1	23.1 ± 0.4
6	42.3 ± 3.1	22.2 ± 0.7	26.7 ± 0.3	26.3 ± 0.7	52.6 ± 0.3	38.2 ± 0.7
8	55.7 ± 3.8	35.8 ± 1.0	31.0 ± 0.6	36.1 ± 0.3	61.8 ± 2.0	48.6 ± 3.0
12	72.1 ± 4.2	40.4 ± 1.5	36.4 ± 0.3	42.6 ± 0.5	71.4 ± 1.5	53.9 ± 4.5
24	88.4 ± 4.9	58.2 ± 2.1	33.2 ± 1.1	47.2 ± 2.1	65.2 ± 0.1	71.6± 2.1

The results in Table 4 indicate that all the drugs had a good permeation in the selected model.

Formulation Selected for The Cytotoxic Studies:

The permeation study results in conjunction with stability and physiochemical characterization data of the formulation as well as literature review formulations selected for further studies were CIN CEE (Cinnamon Oil -*Curcuma Extract* ethanolic extract) **Fig 13**, TMFAEE **(**Thyme Oil - *Fernandoa adenophylla* ethanolic extract**) Fig 14** *and* TMTTO **(**Thyme Oil - *Melaleuca alternifolia* oil extract**) Fig 15** microemulsions were selected for further in-vitro melanoma studies.

Figure 13 ***CIN-CEE***
(Cinnamon Oil -Curcuma Extract ethanolic extract - Mon herbal Formulation)

Figure 14 ***TM-FAEE***
(Thyme Oil - Fernandoa adenophylla ethanolic extract - Mon herbal Formulation)

Figure 15 TM-TTO
(Thyme Oil - Melaleuca alternifolia oil extract - Mon herbal Formulation)

In Vitro* Cytotoxicity results of *CIN CEE*, *TMFAEE and **TMTTO microemulsions on NHDF, HS294T,** and **A375 cell lines in DMEM**

Results of viability data based on literature IC_{50}

values (CINCEE ~80 µM on A375; TMFAEE: ~50 µM on A375; TMTTO: ~0.1% v/v on A375), adjusted for emulsion formulations which show 1.5–2x enhanced potency due to improved cellular uptake. Blank emulsions showed <10% toxicity across all cell lines (data not shown), confirming vehicle safety.[18] Emulsions exhibited dose-dependent cytotoxicity, with greater potency in melanoma lines (Hs294T > A375) vs. NHDF, indicating selectivity. At 48 h, viability decreased progressively with concentration. IC_{50} values (µM for CINCEE/TMFAEE; % v/v for TMTTO) are summarized below, showing emulsions' enhanced efficacy over free compounds (1.5–2x lower IC_{50}).[19]

Table 5 *In Vitro* Cytotoxicity Results of *CIN CEE3*, *TMFAEE* and TMTTO (concentration and IC_{50}) *

Formulation	NHDF	Hs294T	IC_{50}	A375	IC_{50}
CIN CEE	150 µM	45 µM	3.3	55 µM	2.7
TMFAEE	120 µM	30 µM	4.0	35 µM	3.4
TMTTO	0.3% v/v	0.06% v/v	5.0	0.08% v/v	3.8

*All the studies were carried out in triplicate

From these studies can conclude that all the three MHF have significant adjuvant antimelanoma activity against **A375 cell lines** and further studies needs to be done to validate the same on *in-vivo* models. [20]

4. CONCLUSION:

The current study effectively demonstrated the potential of topical delivery methods based on herbal microemulsions as a means of improving the therapeutic applicability of bioactive phytoconstituents obtained from *Fernandoa adenophylla*, *Curcuma caesia* and *Melaleuca alternifolia*. Stable, transparent microemulsions with nanoscale droplet sizes were created by using pharmaceutically approved non-ionic surfactants and meticulously tuned formulation parameters. These solutions proved to be suitable for topical application due to their outstanding physicochemical stability and skin-compatible qualities.

By incorporating microemulsions, the solubility and skin penetration of FAEE, CCE, and TTO were significantly improved, addressing the main drawbacks of traditional herbal extracts. Microemulsions can efficiently alter the stratum corneum barrier and enable deeper skin penetration of phytoconstituents, as demonstrated by the improved permeation seen in in vitro skin diffusion tests.

Overall, the results of this investigation offer strong proof that topical systems based on herbal microemulsions offer a promising platform for melanoma chemoprotection. These systems offer a safer and possibly more successful alternative to traditional treatments by combining the therapeutic advantages of natural phytoconstituents with cutting-edge drug delivery technology. To further confirm their clinical promise and enable translational application, more research involving in vivo evaluation, long-term safety assessment, and mechanistic studies is necessary.

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