MedCrave
Step into the World of Research
Journal of Analytical & Pharmaceutical research

Research Article

Open Access
CrossMark

The cytotoxicity assessment of beackea frutescens emulsion stabilizes with mixed surfactant system (sodium dodecyl sulphate: twenty 20) using HIG-82 cell line

Abstract

The toxicity assessment shows the potential of beackea frustescence emulsion samples with IC50 value more than 30µg/mL were considered as non-toxic on HIG-82 cell lines. Thus, the four formulation samples were safe to be used on human skin. Beackea frutescens comes in the family of Mrytaceae and is well known aromatic and medicinal herb. In view of this it is more economical to grow and commercialize this product instead of importing other source of similar chemical properties such tea tree. In this study, six formulation of emulsion determined from phase diagram with mixed surfactant ratio 70:30 as it showed the largest liquid crystal region. This emulsion formulations consisting of different composition's percentage of mixed surfactant (SDS: Tween 20): benzyl alcohol: water, 2.5g beeswax and 10 drops of Beackea frutescens essential oil. The stability of an emulsion also depends on the combination of surfactant and the surfactant mixing ratio.[1]

Keywords: cytotoxicity, hig-82 cell lines, beackea frutescens, cosmetic emulsion, sodium dodecyl sulphate, tween 20

Volume 10 Issue 6 - 2021

Mohd Zul Helmi Rozaini,[1] Anis Nadilla Ahmad,[1,2] Mohd Fairuz Affandi Aziz,[1] Mohd Safuan Azhar[1]

[1]Faculty of Fisheries and Food Sciences, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Contaminant Management for Oil & Gas, Research, Innovation & Commercialisation, Universiti Teknologi PETRONAS, 32610 Seri Iskandar Perak, Malaysia

Correspondence: Mohd Zul Helmi Rozaini, Faculty of Fisheries and Food Sciences, University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia, Email zulhelmi@umt.edu.my

Received: November 29, 2021 | **Published:** December 13, 2021

Introduction

Cosmetics formulation is more than just simple mixing of all ingredients. There must be chemistry behind putting together all ingredients.[2,3] Each cosmetic formulation produced needs to be assessed for toxicity. Without chemistry it would be called a mixture rather than a formulation. There is increasing attention on the use of natural components in human cosmetics as well as in other domestic uses lately. For instance the use of natural components in cosmetic product can give the better effects to our body due to their good properties.[4,5] Thus, this study was conducted to examine the aspect of stability, texture and also the viscosity of the cosmetic's emulsion of Beackea frutescens (Cucur Atap) as it posses antioxidant property and display antibacterial activity.[4] This plant can be found grown in the wild on bris soil along the border road of Terengganu and Kelantan, Malaysia.[6,7]

Methodology

Emsulsification process by construction of ternary phase diagram

In constructing phase diagram for a system, titration method is employed.[8,9] Ten samples of 0.5g of mixture containing the mix surfactant (SDS and Twenty 20) and benzyl alcohol are prepared at the ration of 1:9, 2:8, 3:7, 4:6, 7:3, 8:2, 9:1 and 10:0 in respective test tubes. A small amount of water is titrated into each test tube. Upon each addition of water, the mixtures are mixed till homogeneity is achieved with vortex and followed by centrifuge for 5minutes at 4500rpm. After that the samples are left in the water bath at 25°C to allow the temperature of the samples to reach equilibrium. The resulting mixtures are evaluated under cross polarized light in order to observe the phase changes.[10,11]

Cytotoxicity test

Cytotoxicity test was carried out for the evaluation of cell compatibility in direct contact with brushite crystals. HIG-82 cell line (synoviocytes) was purchased from American Type Culture Collection (ATCC, Manassas, USA) and cultured in the Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 4.5g/L glucose, 1.0mM sodium pyruvate, 2,0mM L-glutamine, 50µg/ml streptomycin and 50µg/ml penicillin at 37°C in a humidified atmosphere of 5% CO_2 in the air. Cells at a confluency of 80-90% were detached by typsin-EDTA (0.25%) and centrifuged at 110 x g at 4°C for 5minutes. The concentration was then adjust to 8 x 105 cells/ml and cell viability was> 95% as determined by trypan blue dye exclusion.[9]

Prior to cell seeding, the beackea frustescence emulsion were dissolved and sterilized by washing three times with ethanol and gamma irradiated at 40kGy. The dissolve samples were examined with stability chamber test to ensure that there was no change in the emulsification phases. Cell suspension (100µl) was dispersed into wells of 96-well culture plates (8 x 104 cells/well) containing the crystals, respectively and incubated for 5days at 37°C and 5% CO2. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dephenyl tetrasodium bromide) assay and SEM examination were conducted at days 1,3 and 5. The culture medium was replaced every alternate day with care to ensure minimum disruption of cells.[17]

Result and discussions

Figure 1 showed the comparison between the regions in every phase diagrams of the three systems. Each every phase diagrams has similar type of phases which is one phase, two phase, three phase and liquid crystal phase region. For the one phase region, System B showed the widest area in the region compared to the others two systems. Each of every phase diagrams formed one phase region in the center of the diagrams.

The two phase region in all of the diagrams located at the same position that is lower surfactants composition and below than 70% of benzyl alcohol. Next, we can see the pattern of the formation of

the three phase region. Overall, the three phase region were started to be formed when the amount of the water was titrated less than 14 %. The largest and widest area for three phase region was formed on System A.

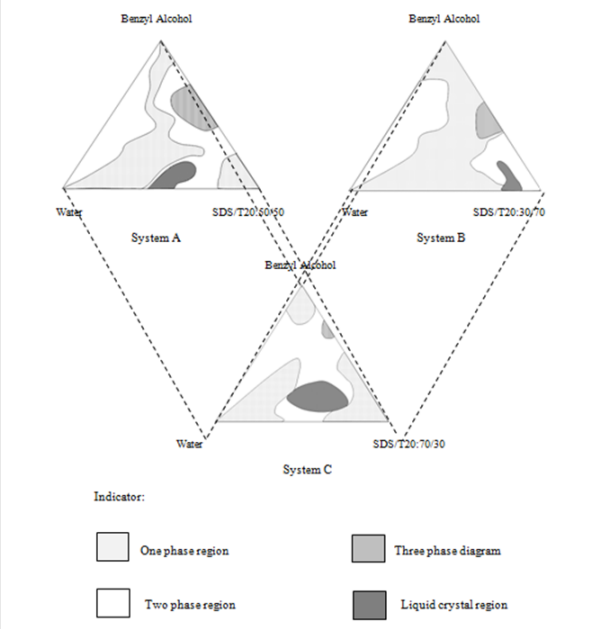

Figure 1 Comparison of Phase Diagram.

The most stable phase in the diagrams is liquid crystal phase. Formation of liquid crystal phase is very important for the stability of emulsions.[12-14] Between the three systems, phase diagram of System C showed the largest region for the liquid crystal phase. Thus, System C was chosen to obtain six emulsion formulations as System C is the most stable phase diagram among the other two diagrams.[15,16]

As much as six samples emulsion formulation prepared by using different material percentage. The formulation can be seen at Table 1. The stability test have been carried out in the stability chamber at 40degree celcius for 3month and the pH is recorded at 6.5. The composition of surfactant formulation is different because it is taken from different phase diagram points. These different surfactant content didn't effected the bioactivity of the essential oil.[9] Therefore, we need a stable emulsion to be a stable medium of beakcea furtescence essential oil against cell line only. The surfactant composition is considered as a catalyst because it does not affect the end result of the reaction.[17] It is just a catalyst to make the emulsion state more stable.

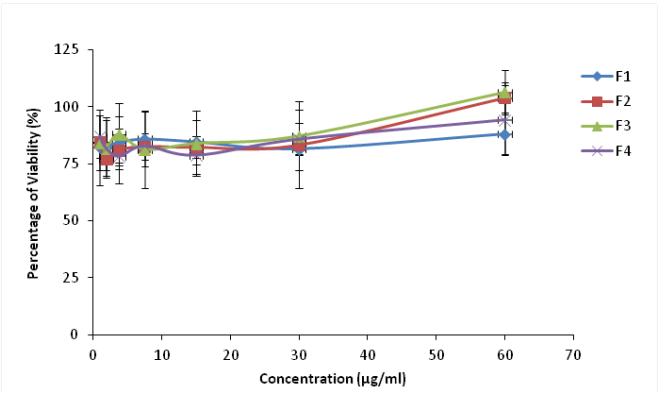

Figure 2 Cytotoxicity property of microemulsion formulations against HIG-82 cell line.

Table 1 Beackea frustecence Emulsion Formulation taken from System C phase diagram

	Sample					
Material	**Formulation 1**	**Formulation 2**	**Formulation 3**	**Formulation 4**	**Formulation 5**	**Formulation 6**
SDS:Tween20 (70:30)	67%	56%	47%	50%	46%	44%
Benzyl Alcohol	7%	14%	12%	22%	8%	16%
Water	26%	30%	41%	28%	46%	40%
Beeswax	2.5g	2.5 g	2.5 g	2.5 g	2.5g	2.5 g
Essential oil of BF	10 drops	10 drops	10 drops	10 drops	10 drops	10 drops

According to the result in Figure 2, all of the microemulsion formulations were defines as non-toxic against HIG-82 cell line. The HIG-82 have been selected in this study because it was extensively used as the test system for the prediction of toxicity, inflammatory and this cell line is also suitable for transfection host.[17] Regarding to the obtained results, it was found that the IC_{50} of the samples is not less than 30µg/ml and even we were used the highest concentration, there are still live cells as well.

Further in depth discussing, the cytotoxicity property of the samples was according to the range of their inhibition value. It can be interpreted as follows; non-toxic IC_{50} value is defines as more than 30.0µg/mL, highly toxic recorded with less than 1.0µg/mL is, ranges between 1.0 to 10.0 µg/mL is toxic, and ranges from 10.0 to 30.0µg/mL is moderately toxic.[16] As a result, all microemulsion samples with value of IC_{50} higher than 30 µg/mL were not toxic to HIG-82 cell lines. Thus, the four formulation samples were safe to be further used in the next examination on human skin analysis.

Conclusion

Based on this study found that the phase diagram of mixed surfactant SDS and Tween 20 on 70:30 ratio showed the largest region for the liquid crystal phase. Formation of liquid crystal phase is very important for the stability of emulsions. Thus, six emulsion formulations were obtain from this phase diagram because it is the most stable phase diagram among the other two diagrams. The emulsion formulations contain different composition's percentage of mixed surfactant (SDS:Tween 20): benzyl alcohol: water, 2.5g beeswax and 10 drops of Beackea Frutescens essential oil. The toxicity assessment conclude that all beackea frustescence emulsion samples with IC50 value more than 30µg/mL were considered as non-toxic on HIG-82 cell lines. Thus, the four formulation samples were safe to be used on human skin.

Acknowledgments

None.

Citation: Rozaini MZH, Ahmad AN, Aziz MFA, et al. The cytotoxicity assessment of beackea frutescens emulsion stabilizes with mixed surfactant system (sodium dodecyl sulphate: twenty 20) using HIG-82 cell line. *J Anal Pharm Res.* 2021;10(6):246–248. DOI: 10.15406/japlr.2021.10.00391

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

References

1. Hasham R, Choi HK, Sarmidi MR, et al. Protective effects of a Ficus deltoidea (Mas cotek) extract against UVB-induced photoageing in skin cells. *Biotechnol Bioprocess Eng*. 2013;(18):185–193.
2. Huang HC, Chiu SH, Chang TM. Inhibitory effect of [6]-gingerol on melanogenesis in B16F10 melanoma cells and a possible mechanism of action. *Biosci Biotechnol Biochem.* 2011;75(6):1067–1072.
3. Kanlayavattanakul M, Lourith N. Therapeutic agents and herbs in topical application for acne treatment. *Int J Cosmet Sci*. 2011;33(4):2:89–297.
4. Karim AA, Azlan A. Fruit pod extracts as a source of nutraceuticals and pharmaceuticals. *Molecules.* 2012;17(10):1931–11946.
5. Kim JK, Kim M, Cho SG. Biotransformation of mulberroside A from Morus alba results in enhancement of tyrosinase inhibition. *J Ind Microbiol Biotechnol.* 2010;37(6):631–637.
6. Kim J, Lee CW, Kim EK. Inhibition effect of Gynura procumbens extract on UVB induced matrix metalloproteinase expression in human dermal fibroblast. *J Ethnopharmacol*. 2011;137(1):427–433.
7. Ko HJ, Kim HJ, Kim SY. Hypopigmentary effects of ethyl P-methoxycinnamate isolated from Kaempferia galanga. *Phytother Res.* 2014;28(2):274–279.
8. Lin CH, Ding HY, Kuo SY. Evaluation of in vitro and in vivo depigmenting activity of raspberry ketone from Rheum officinale. *Internatioanl Journal of Molecular Sciences*. 2011;12(8):4819–4835.
9. Mohd Zul Helmi Rozaini, Habibah Hamzah, Nor Fazliyana Mahtar. Calcium Hydroxiapatite-Based Marine Origin: Novel Sunscreen Materials for Cosmeceutical Treatments. *Oriental Journal of Chemistry*. 2018;34(8):2207–2210.
10. Oh MJ, Hamid MA, Ngadiran S. Ficus deltoidea (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity in vitro and by suppressing tyrosinase gene expression in B16F1 melanoma cells. *Arch Dermatol Res.* 2011;303(3):161–170.
11. Ong HC, Ruzalila BN, Milow P. Traditional knowledge of medicinal plants among the Malay villagers in Kampung Tanjung Sabtu, Terengganu, Malaysia. *Indian J Tradit Knowl*. 2011;10(3):460–465.
12. Ong HC, Zuki RM, Milow P. Traditional knowledge of medicinal plants among the Malay villagers in Kampung Mak Kemas, Terengganu, Malaysia. *Ethnomedicine.* 2011;5(3):175–185.
13. Panich U, Kongtaphan K, Onkoksoong T. Modulation of antioxidant defense by Alpinia galanga and Curcuma aromatica extracts correlates with their inhibition of UVA-induced melanogenesis. *Cell Biol Toxicol.* 2010;26(2):103–116.
14. Park HM, Moon E, Kim AJ. Extract of Punica granatum inhibits skin photoaging induced by UVB irradiation. *Int J Dermatol.* 2010;49(3):276–282.
15. Phetdee K, Rattanamanee K, Teaktong T, et al. Tamarind seed coat extract reduces melanin production via tyrosinase in melanocyte. *J Biol Sci.* 2012;12(4):239–245.
16. Takasao N, Tsuji-Naito K, Ishikura S. Cinnamon extract promotes type I collagen biosynthesis via activation of IGF-I signaling in human dermal fibroblasts. *J Agric Food Chem.* 2012;60(5):1193–1200.
17. Vedastus, W M Edmund, J. P. The assessment of inflammatory activity and toxicity of treated sewage using RAW264.7 cells. *Water and Environment Journal*. 2015;29(3):353–359.