UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF PROCESS PARAMETERS IN PREPARING NANOEMULSION CONTAINING KOJIC MONOOLEATE USING RESPONSE SURFACE METHODOLOGY AND EVALUATION OF TYROSINASE INHIBITION THROUGH IN VITRO AND IN SILICO METHODS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Kojic monooleate (KMO), synthesized from the esterification of kojic acid (KA) and oleic acid, has shown a better depigmenting effect than KA. Previously, our research group had successfully loaded KMO into a nanoemulsion system. The composition of each ingredient was then optimized using mixture experimental design (MED) that resulted in a droplet size of 110.01 nm. Although the nanoemulsion was in nano-sized range, they found that the nanoemulsion only stable at the temperature of 4 and 25°C, and unstable at the temperature of 45°C. Thus, this study aims to further optimize the process parameters in producing the nanoemulsion containing KMO using response surface methodology (RSM). The effects of time of high shear (5-20 min), speed of high shear (4500-6500 rpm), and speed of stirrer (200-300 rpm) were investigated. The optimized process parameters in producing the nanoemulsion containing KMO with nano-sized range were 8.04 min (time of high shear), 4905.41 rpm (speed of high shear) and 271.82 rpm (speed of stirrer). The optimized nanoemulsion containing KMO showed good agreement between predicted droplet size (103.71 nm) and actual droplet size (103.97 ± 0.13 nm) with residual standard error (RSE) value less than 2.0%. An analysis of variance (ANOVA) showed that the fitness of the quadratic polynomial fit the experimental data with large *F*-values (148.79) and small *p*-values ($p<0.0001$) and an insignificant lack-of-fit.

The physicochemical characterization showed that the optimized nano-emulsified KMO was in the nanosize range (103.97 ± 0.13 nm) and had a zeta potential of -45.4 ± 0.05 mV and a polydispersity index (PDI) of 0.312 ± 0.14, indicating that the nanoemulsion produced was stable, and classified as monodispersed. The pH and conductivity of the optimized nanoemulsion (3.98 ± 0.05 mS/cm) signifying the oil-in-water (O/W) nanoemulsion characteristic. A morphology study revealed that the oil droplets in the optimized nanoemulsion containing KMO were spherical in shape, without any aggregation. In addition, the rheological behavior of the optimized nanoemulsion revealed that the nanoemulsion exhibited shear thinning and non-Newtonian behavior. The optimized nanoemulsion containing KMO remained stable under a centrifugation test and storage stability at different storage temperatures of 4, 25 and 45 °C over 90 days.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay performed on the normal 3T3 cell lines showed that the nanoemulsion containing KMO was not cytotoxic with IC_{50} (concentrations of sample required to inhibit the cell viability by 50%) more than 500 µg/mL (IC_{50}>500 µg/mL). The tyrosinase inhibitory assay revealed that the nanoemulsion containing KMO inhibited tyrosinase activity with the IC_{50} value of 68.20 µg/mL, compared to the positive control (KA) with the IC_{50} value of 124.28 µM. The permeation study revealed that 45.94 ± 0.03% of KMO was released from the nanoemulsion and able to permeate the cellulose acetate membrane after 8 h of study time. The total KMO permeated across the membrane per unit area after 8 h of study time was 14355.21 $\mu g.cm^{-2}$, with the flux (J) of 1757.1 $\mu g.cm^{-2}.h^{-1}$ and permeation coefficient (K_p) value of 0.09 $cm.h^{-1}$. The kinetic mechanism analysis revealed that the permeation data was most fitted with the zeroth-order model.

In silico molecular docking revealed that the binding energy for the KMO against mushroom tyrosinase (PDB ID: 2Y9X) is -5.70 kcal/mol, stronger than KA with the binding energy of -4.01 kcal/mol. The interaction of KMO on mushroom tyrosinase is via hydrophobic interaction involving His61, His85, Glu256, His259, Asn260, His263, Phe264, Met280, Gly281, Ser282, Val283, and Ala286 residues. These results predicted that KMO may inhibit mushroom tyrosinase. In conclusion, the nanoemulsion containing KMO with nano-sized range, good stability and physicochemical properties with potent tyrosinase inhibitor properties was successfully optimized in this study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN PARAMETER PROSES DALAM PENYEDIAAN NANOEMULSI MENGANDUNGI KOJIK MONOOLEATE MENGGUNAKAN KAEDAH GERAK BALAS PERMUKAAN DAN PENILAIAN PERENCAT TIROSINA MENGGUNAKAN KAEDAH *IN VITRO* DAN *IN SILICO*

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Kojik monooleate (KMO), yang disintesis melalui proses esterifikasi asid kojik (KA) dan asid oleik telah menunjukkan kesan dipigmentasi yang lebih baik berbanding KA. Terdahulu, kumpulan penyelidikan kami telah berjaya menghasilkan nanoemulsi yang mengandungi KMO. Komposisi optimum bagi setiap bahan nanoemulsi kemudiannya telah disiasat menggunakan reka bentuk eksperimental campuran (MED). Saiz zarah nanoemulsi pada komposisi optimum adalah 110.01 nm. Walaupun nanoemulsi yang dihasilkan adalah bersaiz nano, namun begitu nanoemulsi tersebut hanya stabil pada suhu 4 dan 25°C, dan tidak stabil pada suhu 45°C. Justeru, kajian ini bertujuan untuk mengenal pasti proses parameter yang optimum dalam penghasilan nanoemulsi yang mengandungi KMO menggunakan kaedah gerak balas permukaan (RSM). Kesan masa penyebati berkuasa tinggi (5-20 min), kelajuan penyebati berkuasa tinggi (4500-6500 rpm) dan kelajuan penyebati (200-300 rpm) telah diselidik. Parameter proses optimum dalam penghasilan nanoemulsi yang mengandungi KMO bersaiz nano adalah 8.04 min (masa penyebati berkuasa tinggi), 4905.41 rpm (kelajuan penyebati berkuasa tinggi) dan 271.82 rpm (kelajuan penyebati). Saiz zarah anggaran (103.71 nm) adalah bersesuaian dengan saiz zarah sebenar (103.97 ± 0.13 nm) dengan nilai baki (RSE) kurang daripada 2.0%. Analisis varians (ANOVA) menunjukkan bahawa model kuadratik adalah sesuai dengan data eksperimental dengan nilai-*F* yang besar (148.79) dan nilai-*p* yang kecil ($p<0.0001$).

Pencirian fizikokimia menunjukkan bahawa nanoemulsi yang mengandungi KMO adalah di dalam lingkungan saiz nano (103.97 ± 0.13 nm) dan mempunyai potensi zeta -45.4 ± 0.05 mV dan indeks polidispersiti (PDI) 0.312 ± 0.14. Ini menunjukkan bahawa nanoemulsi yang dihasilkan adalah stabil, dan ia diklasifikasikan sebagai monodispersi. pH nanoemulsi yang dihasilkan adalah 5.75 ± 0.02, menjadikan ia sesuai dengan pH kulit manusia dan nilai konduktivitinya yang tinggi (3.98 ± 0.05 mS/cm) menunjukkan ciri-ciri nanoemulsi minyak-dalam-air. Kajian morfologi menunjukkan bahawa titisan minyak di dalam nanoemulsi yang mengandungi KMO adalah berbentuk sfera, tanpa sebarang pengagregatan di dalam sistem. Ujian reologi telah mempamerkan bahawa nanoemulsi yang mengandungi KMO mempunyai sifat ricih penipisan dan non-Newtonian. Nanoemulsi yang mengandungi KMO kekal stabil apabila diuji dengan daya emparan dan semasa penyimpanan di suhu 4, 25 dan 45 °C selama 90 hari.

Assay MTT (3-(4,5-dimetilthiazol-2-il)-2,5-difenil-tetrazolium bromida) yang dilakukan ke atas sel normal 3T3 menunjukkan bahawa nanoemulsi mengandungi KMO yang dihasilkan adalah tidak sitotoksik, dengan IC_{50} (kepekatan sampel yang diperlukan untuk membunuh sel sebanyak 50%) melebihi 500 µg/mL (IC_{50}>500 µg/mL). Keputusan perencatan tirosina cendawan menunjukkan bahawa nanoemulsi yang mengandungi KMO merencat tirosina dengan nilai IC_{50} 68.20 µg/mL, berbanding kawalan positif (KA) dengan nilai IC_{50} 124.28 µM. Kajian resapan secara *in vitro* menunjukkan bahawa sebanyak 45.94 ± 0.03% KMO berjaya dilepaskan dari sistem nanoemulsi dan meresap merentasi membran asetat selulosa selepas 8 jam. Jumlah KMO yang berjaya meresap merentasi membran per unit keluasan selepas 8 jam adalah 14355.21 $\mu g.cm^{-2}$, dengan nilai flux (J) 1757.1 $\mu g.cm^{-2}.j^{-1}$ dan nilai pemalar resapan (K_p) 0.09 $cm.j^{-1}$. Analisis mekanisma kinetik menunjukkan bahawa data resapan adalah paling bersesuaian dengan model order-sifar.

Penyatuan molekul secara *in silico* menunjukkan bahawa tenaga pengikatan antara KMO dan enzim tirosina cendawan (ID PDB: 2Y9X) adalah -5.70 kcal/mol, lebih kuat berbanding KA dengan tenaga pengikatan -4.01 kcal/mol. Interaksi antara KMO dan enzim tirosina cendawan adalah melalui interaksi hidrofobik yang melibatkan residue His61, His85, Glu256, His259, Asn260, His263, Phe264, Met280, Gly281, Ser282, Val283, dan Ala286. Keputusan ini menunjukkan bahawa KMO mungkin merencat enzim tirosina cendawan. Secara kesimpulannya, nanoemulsi mengandungi KMO yang bersaiz nano, mempunyai tahap kestabilan dan ciri fizikokimia yang bagus dengan sifat perencatan tirosina yang cemerlang telah berjaya dioptimumkan.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Central composite design
CCM	Curcumin
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DTX	Docetaxel
IC_{50}	Concentrations of sample that inhibit activity of enzyme or cells by 50%
KA	Kojic acid
KMO	Kojic monooleate
L-DOPA	3,4-dihydroxyphenyl L-alanine
MED	Mixture experimental design
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
OFAT	One-factor-at-a-time
O/W	Oil-in-water
PBS	Phosphate buffer solution
PDI	Polydispersity index
R^2	Coefficient of determination
RSE	Residual standard error
RSM	Response surface methodology
TEM	Transmission electron microscopy
UV	Ultraviolet radiation
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

Skin hyperpigmentation disease occurs when there is an abnormal production of melanin synthesized in the melanocyte. This can be caused by an excess of melanin levels in the skin through several factors such as ultraviolet (UV) radiation, radicals and hormones (Kanlayavattanakul & Lourith, 2018). Melanin is a skin pigment, derived from tyrosine during the melanogenesis process, which determines the color of mammalian skin, eyes and hair (Huang *et al*., 2008). The melanogenesis process is catalyzed by an enzyme known as tyrosinase. Due to its role in melanogenesis, tyrosinase has been considered as an important target for the treatment of hyperpigmentation diseases in the cosmeceuticals applications (Ha *et al*., 2005).

Kojic acid (KA) is commonly used in the cosmetic industry as an antityrosinase, as it can inhibit tyrosinase through copper chelating. However, KA is less stable as it loses its effectiveness as a skin whitening agent when being exposed to sun or air. To overcome this limitation, derivatives of KA such as kojic acid monooleate (KMO) have been synthesized, through the esterification process of KA and oleic acid.

Previously, Syed Azhar *et al*. (2018) had successfully loaded KMO into a nanoemulsion system. The composition of each ingredients was then optimized using mixture experimental design (MED) that resulted in a droplet size of 110.01 nm. Although the nanoemulsion was in nano-sized range, they found that the nanoemulsion only stable at the temperature of 4 and 25°C, and unstable at the temperature of 45°C.

According to Yuan *et al*. (2008), different compositions and processing parameters in the production of the nanoemulsion will result in different physicochemical properties of the nanoemulsion. Thus, in order to obtain a KMO nanoemulsion with nano-sized range and good stability, the process parameters in the production of the nanoemulsion need to be further optimized. Thus, this issue is the main subject in this study to maintain the required droplet size and improved the stability of the nanoemulsion by optimizing the process parameters using a multivariate statistical approach, response surface methodology (RSM).

Furthermore, cytotoxicity and the efficacy of the nanoemulsion will also serve as the main issue to be investigated. The cytotoxic activity of the nanoemulsion was evaluated on the normal mouse fibroblast cell line (3T3). Meanwhile, the efficacy of the nanoemulsion was investigated through antityrosinase assay and permeation study.

Apart from that, the molecular interactions between KMO with its target protein, tyrosinase was predicted using molecular docking simulation. Molecular docking is a computational method that predicts the favored orientation of a ligand (KMO) to its target protein (tyrosinase) and calculates the binding energy, binding mode as well as binding residues of a protein-ligand complex. In other words, molecular docking was performed to understand the interaction between a ligand with its target protein.

The main objective of this research was to optimize nanoemulsion system containing KMO. In order to successfully achieve the main objective, the following specific objectives were carried out:

1. To optimize the process parameters in producing nanoemulsion containing KMO using response surface methodology (RSM).
2. To characterize the physicochemical properties of the optimized nanoemulsion containing KMO.
3. To evaluate the cytotoxicity and efficacy of the optimized nanoemulsion containing KMO; *in vitro* cytotoxicity against normal 3T3 cell line, antityrosinase activity, and permeation release.
4. To predict the binding mechanisms of KMO against mushroom tyrosinase (PDB ID: 2Y9X) through molecular docking simulation.

REFERENCES

Ab Aziz, N. A., Salim, N., Zarei, M., Saari, N., & Yusoff, F. M. (2020). Extraction, anti-tyrosinase, and antioxidant activities of the collagen hydrolysate derived from Rhopilema hispidum. *Preparative Biochemistry and Biotechnology*, 1-10.

Abd Gani, S. S., Basri, M., Abdul Rahman, M. B., Kassim, A., Raja Abd Rahman, R. N. Z., Salleh, A. B., & Ismail, Z. (2011). Engkabang fat as a base in preparing encapsulated titanium dioxide for cosmetics purpose. *Asian Journal of Chemistry, 23*(1), 380–384.

Abdou, E. S., Galhoum, G. F., & Mohamed, E. N. (2018). Curcumin loaded nanoemulsions/pectin coatings for refrigerated chicken fillets. *Food Hydrocolloids*, *83*, 445-453.

Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current Opinion in Colloid and Interface Science*, *14*(1), 3-15.

Akrawi, S. H., Gorain, B., Nair, A. B., Choudhury, H., Pandey, M., Shah, J. N., & Venugopala, K. N. (2020). Development and optimization of naringenin-loaded chitosan-coated nanoemulsion for topical therapy in wound healing. *Pharmaceutics*, *12*(9), 893.

Algahtani, M. S., Ahmad, M. Z., & Ahmad, J. (2020). Nanoemulsion loaded polymeric hydrogel for topical delivery of curcumin in psoriasis. *Journal of Drug Delivery Science and Technology*, 101847.

Ali, A., Ansari, V. A., Ahmad, U., Akhtar, J., & Jahan, A. (2017). Nanoemulsion: An advanced vehicle for efficient drug delivery. *Drug Research*, *67*(11), 617-631.

Alves, P. M., Pohlmann, A. R., & Guterres, S. S. (2005). Semisolid topical formulations containing nimesulide-loaded nanocapsules, nanospheres or nanoemulsion: development and rheological characterization. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *60*(12), 900-904.

Anarjan, N., Mirhosseini, H., Baharin, B. S., & Tan, C. P. (2010). Effect of processing conditions on physicochemical properties of astaxanthin nanodispersions. *Food Chemistry*, *123*(2), 477-483.

Angelova-Fischer, I., Fischer, T. W., Abels, C., & Zillikens, D. (2018). Accelerated barrier recovery and enhancement of the barrier integrity and properties by topical application of a pH 4 vs. a pH 5.8 water-in-oil emulsion in aged skin. *British Journal of Dermatology*, *179*(2), 471-477.

Arbain, N. H., Salim, N., Masoumi, H. R. F., Wong, T. W., Basri, M., & Rahman, M. B. A. (2019). *In vitro* evaluation of the inhalable quercetin loaded nanoemulsion for pulmonary delivery. *Drug Delivery and Translational Research*, *9*(2), 497-507.

Arbain, N. H., Salim, N., Wui, W. T., Basri, M., & Rahman, M. B. A. (2018). Optimization of quercetin loaded palm oil ester based nanoemulsion formulation for pulmonary delivery. *Journal of Oleo Science, 67*(8), 933-940.

Asadzadeh, A., Fassihi, A., Yaghmaei, P., & Pourfarzam, M. (2015). Docking studies of some novel kojic acid derivatives as possible tyrosinase inhibitors. *Biomedical and Pharmacology Journal*, *8*(2), 535-545.

Ashari, S. E., Mohamad, R., Ariff, A., Basri, M., & Salleh, A. B. (2009). Optimization of enzymatic synthesis of palm-based kojic acid ester using response surface methodology. *Journal of Oleo Science, 58*(10), 503-510.

Ashraf, Z., Rafiq, M., Seo, S. Y., & Babar, M. M. (2015). Synthesis, kinetic mechanism and docking studies of vanillin derivatives as inhibitors of mushroom tyrosinase. *Bioorganic and Medicinal Chemistry*, *23*(17), 5870-5880.

Asmawi, A. A., Salim, N., Ngan, C. L., Ahmad, H., Abdulmalek, E., Masarudin, M. J., & Rahman, M. B. A. (2019). Excipient selection and aerodynamic characterization of nebulized lipid-based nanoemulsion loaded with docetaxel for lung cancer treatment. *Drug Delivery and Translational Research*, *9*(2), 543-554.

Asmawi, A. A., Salim, N., Abdulmalek, E., & Abdul Rahman, M. B. (2020). Modeling the effect of composition on formation of aerosolized nanoemulsion system encapsulating docetaxel and curcumin using D-optimal mixture experimental design. *International Journal of Molecular Sciences*, *21*(12), 4357.

Assadpour, E., Jafari, S. M., & Maghsoudlou, Y. (2017). Evaluation of folic acid release from spray dried powder particles of pectin-whey protein nano-capsules. *International Journal of Biological Macromolecules*, *95*, 238-247.

Auwal, S. M., Zarei, M., Tan, C. P., & Saari, N. (2018). Comparative physicochemical stability and efficacy study of lipoid S75-biopeptides nanoliposome composite produced by conventional and direct heating methods. *International Journal of Food Properties*, *21*(1), 1646-1660.

Azam, S. S., & Abbasi, S. W. (2013). Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. *Theoretical Biology and Medical Modelling*, *10*(1), 63.

Azami, F., Tazikeh-Lemeski, E., & Mahmood-Janlou, M. A. (2017). Kojic acid effect on the inhibitory potency of tyrosinase. *Journal of Chemical Health Risks*, *7*(2).

Aziz, Z. A. A., Mohd-Nasir, H., Mohd Setapar, S. H., Peng, W. L., Chuo, S. C., Khatoon, A., Mohamad Ibrahim, M. N., Umar, K., & Ahmad, A. (2019). Role of nanotechnology for design and development of cosmeceutical: application in makeup and skin care. *Frontiers in Chemistry*, *7*, 739.

Bahari, A. N., Saari, N., Salim, N., & Ashari, S. E. (2020). Response factorial design analysis on papain-generated hydrolysates from *Actinopyga lecanora* for determination of antioxidant and antityrosinase activities. *Molecules*, *25*(11), 2663.

Bali, V., Ali, M., & Ali, J. (2011). Nanocarrier for the enhanced bioavailability of a cardiovascular agent: *in vitro*, pharmacodynamic, pharmacokinetic and stability assessment. *International Journal of Pharmaceutics*, *403*(1-2), 46-56.

Bandgar, B. P., Adsul, L. K., Chavan, H. V., Shringare, S. N., Korbad, B. L., Jalde, S. S., Lonikar, S. V., Nile, S. H., & Shirfule, A. L. (2012). Synthesis, biological evaluation, and molecular docking of N-{3-[3-(9-methyl-9H-carbazol-3-yl)-acryloyl]-phenyl}-benzamide/amide derivatives as xanthine oxidase and tyrosinase inhibitors. *Bioorganic and Medicinal Chemistry*, *20*(18), 5649-5657.

Barradas, T. N., Senna, J. P., Cardoso, S. A., e Silva, K. G. D. H., & Mansur, C. R. E. (2018). Formulation characterization and *in vitro* drug release of hydrogel-thickened nanoemulsions for topical delivery of 8-methoxypsoralen. *Materials Science and Engineering: C*, *92*, 245-253.

Benson. H.A.E., & Watkinson, A.C. (2012). *Transdermal and topical drug delivery: principles and practice*. John Wiley & Sons.

Bernardi, D. S., Pereira, T. A., Maciel, N. R., Bortoloto, J., Viera, G. S., Oliveira, G. C., & Rocha-Filho, P. A. (2011). Formation and stability of oil-in-water nanoemulsions containing rice bran oil: *In vitro* and *in vivo* assessments. *Journal of Nanobiotechnology, 9*, 1–9.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965-977.

Boonme, P., Junyaprasert, V. B., Suksawad, N., & Songkro, S. (2009). Microemulsions and nanoemulsions: novel vehicles for whitening cosmeceuticals. *Journal of Biomedical Nanotechnology*, *5*(4), 373-383.

Bossart, S., Cazzaniga, S., Willenberg, T., Ramelet, A. A., Baumgartner, M., Hunger, R. E., & Seyed Jafari, S. M. (2020). Skin hyperpigmentation index: a new practical method for unbiased automated quantification of skin hyperpigmentation. *Journal of the European Academy of Dermatology and Venereology*.

Briganti, S., Camera, E., & Picardo, M. (2003). Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Research*, *16*(2), 101-110.

Burdock, G. A., Soni, M. G., & Carabin, I. G. (2001). Evaluation of health aspects of kojic acid in food. *Regulatory Toxicology and Pharmacology, 33*(1), 80-101.

Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*(3), 143.

Cafaggi, S., Leardi, R., Parodi, B., Caviglioli, G., & Bignardi, G. (2003). An example of application of a mixture design with constraints to a pharmaceutical formulation. *Chemometrics and Intelligent Laboratory Systems*, *65*(1), 139-147.

Campos, F. F., Campmany, A. C. C., Delgado, G. R., Serrano, O. L., & Naveros, B. C. (2012). Development and characterization of a novel nystatin-loaded nanoemulsion for the buccal treatment of candidosis: Ultrastructural effects and release studies. *Journal of Pharmaceutical Sciences*, *101*(10), 3739-3752.

Cardoso, S. A., & Barradas, T. N. (2020). Developing formulations for drug follicular targeting: Nanoemulsions loaded with minoxidil and clove oil. *Journal of Drug Delivery Science and Technology*, 101908.

Castro-Alvarez, A., Costa, A. M., & Vilarrasa, J. (2017). The performance of several docking programs at reproducing protein-macrolide-like crystal structures. *Molecules*, *22*(1), 136.

Çelik, B., Özdemir, S., Barla Demirkoz, A., & Üner, M. (2017). Optimization of piribedil mucoadhesive tablets for efficient therapy of Parkinson's disease: physical characterization and ex vivo drug permeation through buccal mucosa. *Drug Development and Industrial Pharmacy*, *43*(11), 1836-1845.

Cenobio-Galindo, A. d. J., Campos-Montiel, R. G., Jiménez-Alvarado, R., Almaraz-Buendía, I., Medina-Pérez, G., & Fernández-Luqueño, F. (2019). Development and incorporation of nanoemulsions in food. *International Journal of Food Studies*, *8*(2).

Cestelli, A., Catania, C., D'Agostino, S., Di Liegro, I., Licata, L., Schiera, G., Pitarresi, G. L., Savettieri, G., De Caro, V., Giandalia, G., & Giannola, L. I. (2001). Functional feature of a novel model of blood brain barrier: studies on permeation of test compounds. *Journal of Controlled Release*, *76*(1-2), 139-147.

Channar, P. A., Saeed, A., Larik, F. A., Batool, B., Kalsoom, S., Hasan, M. M., Erben, M. F., El-Seedi, H. R., Ali, M., & Ashraf, Z. (2018). Synthesis of aryl pyrazole via Suzuki coupling reaction, *in vitro* mushroom tyrosinase enzyme inhibition assay and *in silico* comparative molecular docking analysis with kojic acid. *Bioorganic Chemistry, 79*, 293–300.

Che Sulaiman, I. S., Basri, M., Masoumi, H. R. F., Ashari, S. E., & Ismail, M. (2016). Design and development of a nanoemulsion system containing extract of *Clinacanthus nutans* (L.) leaves for transdermal delivery system by D-optimal mixture design and evaluation of its physicochemical properties. *RSC Advances, 6*, 67378-67388.

Chen, C. S., Liu, K. J., Lou, Y. H., & Shieh, C. J. (2002). Optimisation of kojic acid monolaurate synthesis with lipase PS from *Pseudomonas cepacia*. *Journal of the Science of Food and Agriculture*, *82*(6), 601-605.

Chen, J., Li, Q., Ye, Y., Huang, Z., Ruan, Z., & Jin, N. (2020a). Phloretin as both a substrate and inhibitor of tyrosinase: inhibitory activity and mechanism. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *226*, 117642.

Chen, J., Liu, S., Huang, Z., Huang, W., Li, Q., & Ye, Y. (2018). Molecular inhibitory mechanism of dihydromyricetin on mushroom tyrosinase. *Journal of Biomolecular Structure and Dynamics*, *36*(14), 3740-3752.

Chen, J., Ye, Y., Ran, M., Li, Q., Ruan, Z., & Jin, N. (2020b). Inhibition of Tyrosinase by Mercury Chloride: Spectroscopic and Docking Studies. *Frontiers in Pharmacology*, *11*, 81.

Chen, W. C., Tseng, T. S., Hsiao, N. W., Lin, Y. L., Wen, Z. H., Tsai, C. C., Lee, Y. C., Lin, H. H., & Tsai, K. C. (2015). Discovery of highly potent tyrosinase inhibitor, T1, with significant anti-melanogenesis ability by zebrafish *in vivo* assay and computational molecular modeling. *Scientific Reports, 5*, 1-8.

Cheong, J. N., Tan, C. P., Man, Y. B. C., & Misran, M. (2008). α-Tocopherol nanodispersions: Preparation, characterization and stability evaluation. *Journal of Food Engineering*, *89*(2), 204-209.

Costa, P., & Lobo, J. M. S. (2001). Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences*, *13*(2), 123-133.

Costin, G. E., & Hearing, V. J. (2007). Human skin pigmentation: melanocytes modulate skin color in response to stress. *The FASEB journal*, *21*(4), 976-994.

da Silva Marques, T. Z., Santos-Oliveira, R., de Siqueira, L. B. D. O., da Silva Cardoso, V., de Freitas, Z. M. F., & da Silva Ascenção, R. D. C., Vazquez Villa, A. L., de Bustamante Monteiro, M. S. D. S., dos Santos, E. P., Ricci-Junior, E. (2018). Development and characterization of a nanoemulsion containing propranolol for topical delivery. *International Journal of Nanomedicine, 13*, 2827-2837.

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, *67*(3), 217-223.

Date, A. A., Hanes, J., & Ensign, L. M. (2016). Nanoparticles for oral delivery: Design, evaluation and state-of-the-art. *Journal of Controlled Release*, *240*, 504-526.

de Almeida Borges, V. R., Simon, A., Sena, A. R. C., Cabral, L. M., & de Sousa, V. P. (2013). Nanoemulsion containing dapsone for topical administration: a study of *in vitro* release and epidermal permeation. *International Journal of Nanomedicine*, *8*, 535.

De Azevedo Ribeiro, R. C., Barreto, S. M. A. G., Ostrosky, E. A., Da Rocha-Filho, P. A., Veríssimo, L. M., & Ferrari, M. (2015). Production and characterization of cosmetic nanoemulsions containing *Opuntia ficus-indica* (L.) Mill extract as moisturizing agent. *Molecules, 20*(2), 2492–2509.

de Oca-Ávalos, J. M. M., Candal, R. J., & Herrera, M. L. (2017). Nanoemulsions: Stability and physical properties. *Current Opinion in Food Science*, *16*, 1-6.

Dehghani, M. H., Yetilmezsoy, K., Salari, M., Heidarinejad, Z., Yousefi, M., & Sillanpää, M. (2020). Adsorptive removal of cobalt (II) from aqueous solutions using multi-walled carbon nanotubes and γ-alumina as novel adsorbents: Modelling and optimization based on response surface methodology and artificial neural network. *Journal of Molecular Liquids*, *299*, 112154.

dos Santos Fonseca, A. B., Beringhs, A. O. R., Ferraz, H. G., Stulzer, H. K., Sonaglio, D., & Pezzini, B. R. (2020). Liquisolid pellets: Mixture experimental design assessment of critical quality attributes influencing the manufacturing performance via extrusion-spheronization. *Journal of Drug Delivery Science and Technology*, 101630.

dos Santos Matos, A. P., Lopes, D. C. D. X. P., Peixoto, M. L. H., da Silva Cardoso, V., Vermelho, A. B., Santos-Oliveira, R., Lifsitch Viçosa, A., Holandino, C., & Ricci-Júnior, E. (2020). Development, characterization, and anti-leishmanial activity of topical amphotericin B nanoemulsions. *Drug Delivery and Translational Research*, 1-19.

Du, Z., Wang, C., Tai, X., Wang, G., & Liu, X. (2016). Optimization and characterization of biocompatible oil-in-water nanoemulsion for pesticide delivery. *ACS Sustainable Chemistry & Engineering*, *4*(3), 983-991.

Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., & Liang, J. (2006). CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Research*, *34*(suppl_2), W116-W118.

Einhorn-Stoll, U., Weiss, M., & Kunzek, H. (2002). Influence of the emulsion components and preparation method on the laboratory-scale preparation of o/w emulsions containing different types of dispersed phases and/or emulsifiers. *Food/Nahrung, 46*(4), 294-301.

El-Leithy, E. S., Makky, A. M., Khattab, A. M., & Hussein, D. G. (2018). Optimization of nutraceutical coenzyme Q10 nanoemulsion with improved skin permeability and anti-wrinkle efficiency. *Drug Development and Industrial Pharmacy*, *44*(2), 316-328.

Espinosa-Andrews, H., & Páez-Hernández, G. (2020). Optimization of ultrasonication curcumin-hydroxylated lecithin nanoemulsions using response surface methodology. *Journal of Food Science and Technology*, *57*(2), 549-556.

Fang, J. Y., Hung, C. F., Hua, S. C., & Hwang, T. L. (2009). Acoustically active perfluorocarbon nanoemulsions as drug delivery carriers for camptothecin: drug release and cytotoxicity against cancer cells. *Ultrasonics*, *49*(1), 39-46.

Farahin, A. W., Yusoff, F. M., Basri, M., Nagao, N., & Shariff, M. (2019). Use of microalgae: Tetraselmis tetrathele extract in formulation of nanoemulsions for cosmeceutical application. *Journal of Applied Phycology*, *31*(3), 1743-1752.

Fard Masoumi, H. R., Basri, M., Kassim, A., Kuang Abdullah, D., Abdollahi, Y., Abd Gani, S. S., & Rezaee, M. (2013). Statistical optimization of process parameters for lipase-catalyzed synthesis of triethanolamine-based esterquats using response surface methodology in 2-liter bioreactor. *The Scientific World Journal, 2013.*

Fong, P., & Tong, H. H. (2012). *In silico* prediction of the cosmetic whitening effects of naturally occurring lead compounds. *Natural Product Communications*, *7*(10), 1934578X1200701010.

Gallarate, M., Carlotti, M. E., Trotta, M., Grande, A. E., & Talarico, C. (2004). Photostability of naturally occurring whitening agents in cosmetic microemulsions. *Journal of Cosmetic Science, 55*(2), 139-148.

Gou, L., Lee, J., Hao, H., Park, Y. D., Zhan, Y., & Lü, Z. R. (2017a). The effect of oxaloacetic acid on tyrosinase activity and structure: Integration of inhibition kinetics with docking simulation. *International Journal of Biological Macromolecules*, *101*, 59-66.

Gou, L., Lee, J., Yang, J. M., Park, Y. D., Zhou, H. M., Zhan, Y., & Lü, Z. R. (2017b). The effect of alpha-ketoglutaric acid on tyrosinase activity and conformation: Kinetics and molecular dynamics simulation study. *International Journal of Biological Macromolecules*, *105*, 1654-1662.

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., Bouffard, G.G., Blakesley, R.W., Murray, P.R., Green, E.D., & Turner, M. L. (2009). Topographical and temporal diversity of the human skin microbiome. *Science*, *324*(5931), 1190-1192.

Grice, E. A., Kong, H. H., Renaud, G., Young, A. C., Bouffard, G. G., Blakesley, R. W., Wolfsberg, T. G., Turner, M. L., & Segre, J. A. (2008). A diversity profile of the human skin microbiota. *Genome Research*, *18*(7), 1043-1050.

Gutiérrez, J. M., González, C., Maestro, A., Solè, I. M. P. C., Pey, C. M., & Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid and Interface Science*, *13*(4), 245-251.

Guttoff, M., Saberi, A. H., & McClements, D. J. (2015). Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. *Food Chemistry*, *171*, 117-122.

Ha, S. K., Koketsu, M., Lee, K., Choi, S. Y., Park, J. H., Ishihara, H., & Kim, S. Y. (2005). Inhibition of tyrosinase activity by N, N-unsubstituted selenourea derivatives. *Biological and Pharmaceutical Bulletin*, *28*(5), 838-840.

Hadizadeh, E., Pazokifard, S., Mirabedini, S. M., & Ashrafian, H. (2020). Optimizing practical properties of MMA-based cold plastic road marking paints using mixture experimental design. *Progress in Organic Coatings*, *147*, 105784.

Heo, S. J., Ko, S. C., Kang, S. M., Cha, S. H., Lee, S. H., Kang, D. H., Jung, W. K., Affan, A., Oh, C., & Jeon, Y. J. (2010). Inhibitory effect of diphlorethohydroxycarmalol on melanogenesis and its protective effect against UV-B radiation-induced cell damage. *Food and Chemical Toxicology*, *48*(5), 1355-1361.

Hidajat, M. J., Jo, W., Kim, H., & Noh, J. (2020). Effective droplet size reduction and excellent stability of limonene nanoemulsion formed by high-pressure homogenizer. *Colloids and Interfaces*, *4*(1), 5.

Homayoonfal, M., Khodaiyan, F., & Mousavi, S. M. (2014). Optimization of walnut oil nanoemulsions prepared using ultrasonic emulsification: A response surface method. *Journal of Dispersion Science and Technology*, *35*(5), 685-694.

Hu, S., & Li, W. X. (2018). Influence of particle size distribution on lifetime and thermal stability of Ostwald ripening of supported particles. *ChemCatChem*, *10*(13), 2900-2907.

Hu, W. J., Yan, L., Park, D., Jeong, H. O., Chung, H. Y., Yang, J. M., Ye, Z. M., & Qian, G. Y. (2012). Kinetic, structural and molecular docking studies on the inhibition of tyrosinase induced by arabinose. *International Journal of Biological Macromolecules*, *50*(3), 694-700.

Huang, S. Y., & Zou, X. (2010). Advances and challenges in protein-ligand docking. *International Journal of Molecular Sciences*, *11*(8), 3016-3034.

Huang, H. P., Shih, Y. W., Chang, Y. C., Hung, C. N., & Wang, C. J. (2008). Chemoinhibitory effect of mulberry anthocyanins on melanoma metastasis involved in the Ras/PI3K pathway. *Journal of Agricultural and Food Chemistry*, *56*(19), 9286-9293.

Huey, R., Morris, G. M., Olson, A. J., & Goodsell, D. S. (2007). A semiempirical free energy force field with charge-based desolvation. *Journal of Computational Chemistry*, *28*(6), 1145-1152.

Ibrahim, Z., Tejo, B. A., Latif, M. A. M., Karjiban, R. A., Salleh, A. B., & Rahman, M. B. A. (2016). *In-silico* identification of potential Protein Arginine Deiminase IV (PAD4) inhibitors. *Malaysian Journal of Analytical Sciences*, *20*(6), 1269-1277.

Ismaya, W. T., Rozeboom, H. J., Weijn, A., Mes, J. J., Fusetti, F., Wichers, H. J., & Dijkstra, B. W. (2011). Crystal structure of *Agaricus bisporus* mushroom tyrosinase: identity of the tetramer subunits and interaction with tropolone. *Biochemistry*, *50*(24), 5477-5486.

Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H., & Raman, A. (2004). In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *Journal of Ethnopharmacology*, *90*(1), 33-38.

Izadiyan, Z., Basri, M., Masoumi, H. R. F., Karjiban, R. A., Salim, N., & Shameli, K. (2017). Modeling and optimization of nanoemulsion containing Sorafenib for cancer treatment by response surface methodology. *Chemistry Central Journal*, *11*(1), 21.

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids*, *22*(7), 1191-1202.

Jafari, S. M., He, Y., & Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering*, *82*(4), 478-488.

Jaslina, N. F., Faujan, N. H., Mohamad, R., & Ashari, S. E. (2020). Effect of addition of PVA/PG to oil-in-water nanoemulsion kojic monooleate formulation on droplet size: three-factors response surface optimization and characterization. *Cosmetics*, *7*(4), 73.

Jaworska, M., Sikora, E., Zielina, M., & Ogonowski, J. (2013). Studies on the formation of O/W nano-emulsions, by low-energy emulsification method, suitable for cosmeceutical applications. *Acta Biochimica Polonica, 60*(4), 779–782.

Jiang, J., Mei, Z., Xu, J., & Sun, D. (2013). Effect of inorganic electrolytes on the formation and the stability of water-in-oil (W/O) emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *429*, 82-90.

Jumbri, K., Al-Haniff Rozy, M. F., Ashari, S. E., Mohamad, R., Basri, M., & Fard Masoumi, H. R. (2015). Optimisation and characterisation of lipase catalysed synthesis of a kojic monooleate ester in a solvent-free system by response surface methodology. *PLoS One, 10*(12), 1-13.

Jusril, N. A., Muhamad Juhari, A. N. N., Abu Bakar, S. I., Md Saad, W. M., & Adenan, M. I. (2020). Combining *in silico* and *in vitro* studies to evaluate the acetylcholinesterase inhibitory profile of different accessions and the biomarker triterpenes of *Centella asiatica*. *Molecules*, *25*(15), 3353.

Kale, S. N., & Deore, S. L. (2017). Emulsion micro emulsion and nano emulsion: a review. *Systematic Reviews in Pharmacy*, *8*(1), 39.

Kanlayavattanakul, M., & Lourith, N. (2018). Skin hyperpigmentation treatment using herbs: A review of clinical evidences. *Journal of Cosmetic and Laser Therapy*, *20*(2), 123-131.

Karimian, S., Ranjbar, S., Dadfar, M., Khoshneviszadeh, M., Gholampour, M., Sakhteman, A., & Khoshneviszadeh, M. (2020). 4 H-benzochromene derivatives as novel tyrosinase inhibitors and radical scavengers: synthesis, biological evaluation, and molecular docking analysis. *Molecular Diversity*, 1-11.

Kaur, K., Kumar, R., Goel, S., Uppal, S., Bhatia, A., & Mehta, S. K. (2017). Physiochemical and cytotoxicity study of TPGS stabilized nanoemulsion designed by ultrasonication method. *Ultrasonics Sonochemistry*, *34*, 173-182.

Khatib, S., Nerya, O., Musa, R., Tamir, S., Peter, T., & Vaya, J. (2007). Enhanced substituted resorcinol hydrophobicity augments tyrosinase inhibition potency. *Journal of Medicinal Chemistry*, *50*(11), 2676-2681.

Kim, C. K., Cho, Y. J., & Gao, Z. G. (2001). Preparation and evaluation of biphenyl dimethyl dicarboxylate microemulsions for oral delivery. *Journal of Controlled Release*, *70*(1-2), 149-155.

Kim, C. S., Noh, S. G., Park, Y., Kang, D., Chun, P., Chung, H. Y., Jung, H. J., & Moon, H. R. (2018). A potent tyrosinase inhibitor,(E)-3-(2, 4-Dihydroxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one, with anti-melanogenesis properties in α-MSH and IBMX-induced B16F10 melanoma cells. *Molecules*, *23*(10), 2725.

Kim, H., Choi, J., Cho, J. K., Kim, S. Y., & Lee, Y.-S. (2004). Solid-phase synthesis of kojic acid-tripeptides and their tyrosinase inhibitory activity, storage stability, and toxicity. *Bioorganic and Medicinal Chemistry Letters, 14*(11), 2843-2846.

Koirala, P., Seong, S. H., Zhou, Y., Shrestha, S., Jung, H. A., & Choi, J. S. (2018). Structure-activity relationship of the tyrosinase inhibitors Kuwanon G, Mulberrofuran G, and Albanol B from morus species: a kinetics and molecular docking study. *Molecules*, *23*(6), 1413.

Komaiko, J. S., & McClements, D. J. (2016). Formation of food-grade nanoemulsions using low-energy preparation methods: A review of available methods. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 331-352.

Koroleva, M. Y., & Yurtov, E. V. (2012). Nanoemulsions: the properties, methods of preparation and promising applications. *Russian Chemical Reviews*, *81*(1), 21.

Kotta, S., Khan, A. W., Ansari, S. H., Sharma, R. K., & Ali, J. (2015). Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method. *Drug Delivery*, *22*(4), 455-466.

Kwong, H. C., Chidan Kumar, C. S., Mah, S. H., Chia, T. S., Quah, C. K., Loh, Z. H., Chandraju, S., & Lim, G. K. (2017). Novel biphenyl ester derivatives as tyrosinase inhibitors: Synthesis, crystallographic, spectral analysis and molecular docking studies. *PLoS one*, *12*(2), e0170117.

Lajis, A. F. B., Hamid, M., & Ariff, A. B. (2012). Depigmenting effect of kojic acid esters in hyperpigmented B16F1 melanoma cells. *Journal of Biomedicine and Biotechnology, 2012*.

Lajis, A. F. B., Hamid, M., Ahmad, S., & Ariff, A. B. (2017). Lipase-catalyzed synthesis of kojic acid derivative in bioreactors and the analysis of its depigmenting and antioxidant activities. *Cosmetics*, *4*(3), 22.

Lee, A., Chaibakhsh, N., Rahman, M. B. A., Basri, M., & Tejo, B. A. (2010). Optimized enzymatic synthesis of levulinate ester in solvent-free system. *Industrial Crops and Products, 32*(3), 246-251.

Lee, E. S., Kim, J. H., Im, S., Lee, K. B., Sohn, S., & Kang, W. H. (2001). Application of computerized image analysis in pigmentary skin diseases. *International Journal of Dermatology*, *40*(1), 45-49.

Leelananda, S. P., & Lindert, S. (2016). Computational methods in drug discovery. *Beilstein Journal of Organic Chemistry*, *12*(1), 2694-2718.

Li, H., Leung, K. S., Wong, M. H., & Ballester, P. J. (2015). Improving AutoDock Vina using random forest: the growing accuracy of binding affinity prediction by the effective exploitation of larger data sets. *Molecular Informatics*, *34*(2-3), 115-126.

Li, J., Shi, Y., Ren, Y., Cong, Z., Wu, G., Chen, N., Zhao, X., Li, L. (2017a). Development and evaluation of self-nanoemulsifying drug delivery system of rhubarb anthraquinones. *Journal of Drug Delivery Science and Technology, 39*, 283–295.

Li, X., Wang, L., & Wang, B. (2017b). Optimization of encapsulation efficiency and average particle size of Hohenbuehelia serotina polysaccharides nanoemulsions using response surface methodology. *Food Chemistry, 229*, 479-486.

Liu, R., Sun, L., Fang, S., Wang, S., Chen, J., Xiao, X., & Liu, C. (2016). Thermosensitive in situ nanogel as ophthalmic delivery system of curcumin: development, characterization, in vitro permeation and in vivo pharmacokinetic studies. *Pharmaceutical Development and Technology*, *21*(5), 576-582.

Lima, C. R., Silva, J. R. A., De Tássia Carvalho Cardoso, É., Silva, E. O., Lameira, J., Do Nascimento, J. L. M., Brasil, D. D. S. B., & Alves, C. N. (2014). Combined kinetic studies and computational analysis on kojic acid analogs as tyrosinase inhibitors. *Molecules*, *19*(7), 9591-9605.

MacNeil, S. (2007). Progress and opportunities for tissue-engineered skin. *Nature*, *445*(7130), 874-880.

Makeneni, S., Thieker, D. F., & Woods, R. J. (2018). Applying pose clustering and MD simulations to eliminate false positives in molecular docking. *Journal of Chemical Information and Modeling*, *58*(3), 605-614.

Mallick, A., Gupta, A., Hussain, A., Aparajay, P., Singh, S., Singh, S. K., & Dev, A. (2020). Intranasal delivery of gabapentin loaded optimized nanoemulsion for augmented permeation. *Journal of Drug Delivery Science and Technology*, *56*, 101606.

Marrakchi, S., & Maibach, H. I. (2007). Biophysical parameters of skin: map of human face, regional, and age-related differences. *Contact Dermatitis*, *57*(1), 28-34.

Martínez-Pla, J. J., Martín-Biosca, Y., Sagrado, S., Villanueva-Camañas, R. M., & Medina-Hernández, M. J. (2004). Evaluation of the pH effect of formulations on the skin permeability of drugs by biopartitioning micellar chromatography. *Journal of Chromatography A, 1047*(2), 255–262.

Maruno, M. & da Rocha-Filho, P. A. (2010). O/W nanoemulsion after 15 years of preparation: A suitable vehicle for pharmaceutical and cosmetic applications. *Journal of Dispersion Science and Technology, 31*(1), 17–22.

Masoumi, H. R. F., Basri, M., Samiun, W. S., Izadiyan, Z., & Lim, C. J. (2015). Enhancement of encapsulation efficiency of nanoemulsion-containing aripiprazole for the treatment of schizophrenia using mixture experimental design. *International Journal of Nanomedicine, 10*, 6469.

Masum, M. N., Yamauchi, K., & Mitsunaga, T. (2019). Tyrosinase inhibitors from natural and synthetic sources as skin-lightening agents. *Reviews in Agricultural Science*, *7*, 41-58.

Mat Hadzir, N., Basri, M., Abdul Rahman, M. B., Salleh, A. B., Raja Abdul Rahman, R. N. Z., & Basri, H. (2013). Phase behaviour and formation of fatty acid esters nanoemulsions containing piroxicam. *AAPS PharmSciTech, 14*(1), 456–463.

Maupas, C., Moulari, B., Béduneau, A., Lamprecht, A., Pellequer, Y. (2011). Surfactant dependent toxicity of lipid nanocapsules in HaCaT cells. *International Journal of Pharmaceutics*, *411*, 136-141.

McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter, 7*(6), 2297–2316.

Mehmood, T. (2015). Optimization of the canola oil based vitamin E nanoemulsions stabilized by food grade mixed surfactants using response surface methodology. *Food Chemistry*, *183*, 1-7.

Mehmood, T., Ahmad, A., Ahmed, A., & Ahmed, Z. (2017). Optimization of olive oil based O/W nanoemulsions prepared through ultrasonic homogenization: A response surface methodology approach. *Food Chemistry*, *229*, 790-796.

Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, *7*(2), 146-157.

Miastkowska, M., Sikora, E., Ogonowski, J., Zielina, M., & Łudzik, A. (2016). The kinetic study of isotretinoin release from nanoemulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *510*, 63-68.

Miastkowska, M., & Śliwa, P. (2020). Influence of Terpene Type on the Release from an O/W Nanoemulsion: Experimental and Theoretical Studies. *Molecules*, *25*(12), 2747.

Mohd Faudzi, S. M., Leong, S. W., Auwal, F. A., Abas, F., Wai, L. K., Ahmad, S., Tham, C. L., Shaari, K., Lajis, N. H., & Yamin, B. M. (2020). *In silico* studies, nitric oxide, and cholinesterases inhibition activities of pyrazole and pyrazoline analogs of diarylpentanoids. *Archiv der Pharmazie*, e2000161.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785-2791.

Mousazadeh, M., Mousavi, M., Emam-Djomeh, Z., Hadinezhad, M., & Gharibzahedi, S. M. T. (2014). Formulation optimization of pistachio oil spreads by characterization of the instrumental textural attributes. *International Journal of Food Properties, 17*(6), 1355–1368.

Murugesu, S., Ibrahim, Z., Ahmed, Q. U., Nik Yusoff, N. I., Uzir, B. F., Perumal, V., Abas, F., Saari, K., El-Seedi, H., & Khatib, A. (2018). Characterization of α-glucosidase inhibitors from *Clinacanthus nutans* lindau leaves by gas chromatography-mass spectrometry-based metabolomics and molecular docking simulation. *Molecules*, *23*(9), 2402.

Musa, S. H., Basri, M., Masoumi, H. R. F., Karjiban, R. A., Malek, E. A., Basri, H., & Shamsuddin, A. F. (2013). Formulation optimization of palm kernel oil esters nanoemulsion-loaded with chloramphenicol suitable for meningitis treatment. *Colloids and Surfaces B: Biointerfaces, 112*(7), 113-119.

Musa, S. H., Basri, M., Masoumi, H. R. F., Shamsudin, N., & Salim, N. (2017). Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: *in vitro* diffusion and *in vivo* hydrating action. *International Journal of Nanomedicine*, *12*, 2427.

Nakazawa, H., Ohta, N., & Hatta, I. (2012). A possible regulation mechanism of water content in human stratum corneum via intercellular lipid matrix. *Chemistry and Physics of Lipids*, *165*(2), 238-243.

Nasti, T. H., & Timares, L. (2015). MC 1R, Eumelanin and Pheomelanin: Their role in determining the susceptibility to skin cancer. *Photochemistry and Photobiology*, *91*(1), 188-200.

Nemitz, M.C., von Poser, G.L., Teixeira, H.F. (2019). *In vitro* skin permeation/retention of daidzein, genistein and glycitein from a soybean isoflavone rich fraction-loaded nanoemulsions and derived hydrogels. *Journal of Drug Delivery Science and Technology*, *51*, 63–69.

Ng, S. P., Lai, O. M., Abas, F., Lim, H. K., & Tan, C. P. (2014). Stability of a concentrated oil-in-water emulsion model prepared using palm olein-based diacylglycerol/virgin coconut oil blends: Effects of the rheological properties, droplet size distribution and microstructure. *Food Research International*, *64*, 919-930.

Ngan, C. L., Basri, M., Lye, F. F., Masoumi, H. R. F., Tripathy, M., Karjiban, R. A., & Abdul-Malek, E. (2014). Comparison of Box–Behnken and central composite designs in optimization of fullerene loaded palm-based nano-emulsions for cosmeceutical application. *Industrial Crops and Products*, *59*, 309-317.

Nigro, F., Cerqueira, C., Rossi, A., Cardoso, V., Vermelho, A. B., Ricci-Júnior, E., dos Santos, E. P., & Mansur, C. R. E. (2020). Development, characterization and in vitro toxicity evaluation of nanoemulsion-loaded hydrogel based on copaiba oil and coenzyme Q10. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *586*, 124132.

Nishad, J., Dutta, A., Saha, S., Rudra, S. G., Varghese, E., Sharma, R. R., Tomar, M., Kumar, M., & Kaur, C. (2020). Ultrasound-assisted development of stable grapefruit peel polyphenolic nano-emulsion: Optimization and application in improving oxidative stability of mustard oil. *Food Chemistry*, *334*, 127561.

Noh, J. M., Kwak, S. Y., Seo, H. S., Seo, J. H., Kim, B. G., & Lee, Y. S. (2009). Kojic acid-amino acid conjugates as tyrosinase inhibitors. *Bioorganic and Medicinal Chemistry Letters, 19*(19), 5586–5589.

Norddin, F. A. A., Azhar, S. N. A. S., & Ashari, S. E. (2017). Evaluation of direct esterification of fatty acid derivative of kojic acid in co-solvent system: A statistical approach. *Journal of Chemical Engineering Process and Technology*, *8*, 331.

Panich, U., Kongtaphan, K., Onkoksoong, T., Jaemsak, K., Phadungrakwittaya, R., Thaworn, A., Akarasereenont, P., & Wongkajornsilp, A. (2010). Modulation of antioxidant defense by *Alpinia galanga* and *Curcuma aromatica* extracts correlates with their inhibition of UVA-induced melanogenesis. *Cell Biology and Toxicology*, *26*(2), 103-116.

Pereira, T. A., Guerreiro, C. M., Maruno, M., Ferrari, M., & Rocha-Filho, P. A. (2016). Exotic vegetable oils for cosmetic O/W nanoemulsions: *In vivo* evaluation. *Molecules, 21*(3), 248.

Pey, C. M., Maestro, A., Solé, I., González, C., Solans, C., & Gutiérrez, J. M. (2006). Optimization of nano-emulsions prepared by low-energy emulsification methods at constant temperature using a factorial design study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 288*(1-3), 144-150.

Pongsumpun, P., Iwamoto, S., & Siripatrawan, U. (2020). Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochemistry*, *60*, 104604.

Poomanee, W., Khunkitti, W., Chaiyana, W., & Leelapornpisid, P. (2020). Optimization of *Mangifera indica* L. kernel extract-loaded nanoemulsions via response surface methodology, characterization, stability, and skin permeation for anti-acne cosmeceutical application. *Pharmaceutics*, *12*(5), 454.

Pratama, M. R. F., Mulyani, E., & Suratno, S. (2019). Molecular docking study of akar kuning (*Arcangelisia flava*) secondary metabolites as Src Inhibitor. *Indonesian Journal of Cancer Chemoprevention*, *10*(3), 122-130.

Proksch, E. (2018). pH in nature, humans and skin. *The Journal of Dermatology*, *45*(9), 1044-1052.

Pund, S., Borade, G., & Rasve, G. (2014). Improvement of anti-inflammatory and anti-angiogenic activity of berberine by novel rapid dissolving nanoemulsifying technique. *Phytomedicine*, *21*(3), 307-314.

Qamar, R., Saeed, A., Larik, F. A., Abbas, Q., Hassan, M., Raza, H., & Seo, S. Y. (2019). Novel 1, 3-oxazine-tetrazole hybrids as mushroom tyrosinase inhibitors and free radical scavengers: Synthesis, kinetic mechanism, and molecular docking studies. *Chemical Biology and Drug Design*, *93*(2), 123-131.

Ramli, F., Hamid, M. A., Wahab, R. A., Ismail, I. S., & Karunakaran, T. (2020). Ultrasonic-assisted extraction of phalerin from *Phaleria macrocarpa*: response surface methodology and artificial neural network modelling. *Arabian Journal for Science and Engineering*, *45*(9), 7635-7644.

Raviadaran, R., Chandran, D., Shin, L. H., & Manickam, S. (2018). Optimization of palm oil in water nano-emulsion with curcumin using microfluidizer and response surface methodology. *LWT*, *96*, 58-65.

Rebolleda, S., Sanz, M. T., Benito, J. M., Beltrán, S., Escudero, I., & González San-José, M. L. (2015). Formulation and characterisation of wheat bran oil-in-water nanoemulsions. *Food Chemistry, 167*, 16–23.

Ribeiro, R., Barreto, S., Ostrosky, E., Rocha-Filho, P., Veríssimo, L., & Ferrari, M. (2015). Production and characterization of cosmetic nanoemulsions containing *Opuntia ficus-indica* (L.) mill extract as moisturizing agent. *Molecules, 20*(2), 2492-2509.

Rocha-Filho, P., Ferrari, M., Maruno, M., Souza, O., & Gumiero, V. (2017). *In vitro* and *in vivo* evaluation of nanoemulsion containing vegetable extracts. *Cosmetics, 4*(3), 32.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science*, *391*, 95-102.

Sajjadi, S. E., Ghanadian, M., Haghighi, M., & Mouhebat, L. (2015). Cytotoxic effect of Cousinia verbascifolia Bunge against OVCAR-3 and HT-29 cancer cells. *Journal of HerbMed pharmacology*, *4*(1), 15-19.

Salamanca, C. H., Barrera-Ocampo, A., Lasso, J. C., Camacho, N., & Yarce, C. J. (2018). Franz diffusion cell approach for pre-formulation characterisation of ketoprofen semi-solid dosage forms. *Pharmaceutics*, *10*(3), 148.

Samiun, W. S., Ashari, S. E., Salim, N., & Ahmad, S. (2020). Optimization of processing parameters of nanoemulsion containing Aripiprazole using response surface methodology. *International Journal of Nanomedicine*, *15*, 1585.

Samson, S., Basri, M., Masoumi, H. R. F., Malek, E. A., & Karjiban, R. A. (2016). An artificial neural network based analysis of factors controlling particle size in a virgin coconut oil-based nanoemulsion system containing copper peptide. *PloS One*, *11*(7).

Saravana, P. S., Shanmugapriya, K., Gereniu, C. R. N., Chae, S. J., Kang, H. W., Woo, H. C., & Chun, B. S. (2019). Ultrasound-mediated fucoxanthin rich oil nanoemulsions stabilized by κ-carrageenan: process optimization, bio-accessibility and cytotoxicity. *Ultrasonics Sonochemistry*, *55*, 105-116.

Scholz, P., & Keck, C. M. (2015). Nanoemulsions produced by rotor–stator high speed stirring. *International Journal of Pharmaceutics*, *482*(1-2), 110-117.

Secer, A., & Hasanoğlu, A. (2020). Evaluation of the effects of process parameters on co–gasification of Çan lignite and sorghum biomass with response surface methodology: An optimization study for high yield hydrogen production. *Fuel*, *259*, 116230.

Segger, D., Aßmus, U., Brock, M., Erasmy, J., Finkel, P., Fitzner, A., Heuss, H., Kortemeier, U., Munke, S., Rheinländer, T. & Schmidt-Lewerkühne, H. (2008). Multicenter study on measurement of the natural pH of the skin surface. *International Journal of Cosmetic Science*, *30*(1), 75-75.

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., & Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. *European Journal of Pharmaceutics and Biopharmaceutics*, *66*(2), 227-243.

Shahidan, N. S., Salim, N., & Ashari, S. E. (2019). Preparation and optimization of ibuprofen-loaded nanoemulsion formulation. *Journal of Multidisciplinary Engineering Science and Technology*, *6*(12), 89-96.

Shaker, D. S., Ishak, R. A., Ghoneim, A., & Elhuoni, M. A. (2019). Nanoemulsion: A review on mechanisms for the transdermal delivery of hydrophobic and hydrophilic drugs. *Scientia Pharmaceutica*, *87*(3), 17.

Shang, C., Zhang, Y., You, X., Guo, N., Wang, Y., Fan, Y., & Liu, W. (2018). The effect of 7, 8, 4´-trihydroxyflavone on tyrosinase activity and conformation: Spectroscopy and docking studies. *Luminescence*, *33*(4), 681-691.

Sharif, H. R., Abbas, S., Majeed, H., Safdar, W., Shamoon, M., Khan, M. A., Shoaib, M., Raza, H., & Haider, J. (2017). Formulation, characterization and antimicrobial properties of black cumin essential oil nanoemulsions stabilized by OSA starch. *Journal of Food Science and Technology*, *54*(10), 3358-3365.

Sharma, N., Bansal, M., Visht, S., Sharma, P., & Kulkarni, G. (2010). Nanoemulsion: A new concept of delivery system. *Chronicles of Young Scientists, 1*(2), 2-6.

Shi, Y., Li, H., Li, J., Zhi, D., Zhang, X., Liu, H., Wang, H., Li, H. (2015). Development, optimization and evaluation of emodin loaded nanoemulsion prepared by ultrasonic emulsification. *Journal of Drug Delivery Science and Technology, 27*, 46-55.

Shoaib, M. H., Tazeen, J., Merchant, H. A., & Yousuf, R. I. (2006). Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. *Pakistan Journal of Pharmaceutical Sciences*, *19*(2), 119-124.

Shtay, R., Tan, C. P., & Schwarz, K. (2018). Development and characterization of solid lipid nanoparticles (SLNs) made of cocoa butter: A factorial design study. *Journal of Food Engineering*, *231*, 30-41.

Si, Y. X., Wang, Z. J., Park, D., Chung, H. Y., Wang, S. F., Yan, L., Yang, J.M., Qian, G. Y., Yin, S. J., & Park, Y. D. (2012). Effect of hesperetin on tyrosinase: Inhibition kinetics integrated computational simulation study. *International Journal of Biological Macromolecules*, *50*(1), 257-262.

Silva, E. K., Rosa, M. T. M., & Meireles, M. A. A. (2015). Ultrasound-assisted formation of emulsions stabilized by biopolymers. *Current Opinion in Food Science*, *5*, 50-59.

Simon, A., Amaro, M. I., Healy, A. M., Cabral, L. M., & de Sousa, V. P. (2016). Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with *in vivo-in vitro* correlation. *International Journal of Pharmaceutics*, *512*(1), 234-241.

Singhvi, G., & Singh, M. (2011). *In-vitro* drug release characterization models. *International Journal of Pharmaceutical Studies and Research*, *2*(1), 77-84.

Sinha, P., Srivastava, N., Rai, V. K., Mishra, R., Ajayakumar, P. V., & Yadav, N. P. (2019). A novel approach for dermal controlled release of salicylic acid for improved anti-inflammatory action: combination of hydrophilic-lipophilic balance and response surface methodology. *Journal of Drug Delivery Science and Technology*, *52*, 870-884.

Sobhani, H., Tarighi, P., Ostad, S.N., Shafaati, A., Nafissi-Varcheh, N., Aboofazeli, R. (2015). Formulation development and toxicity assessment of triacetin mediated nanoemulsions as novel delivery systems for rapamycin. *Iranian Journal of Pharmaceutical Research*, *14*, 3–21.

Şöhretoğlu, D., Sari, S., Barut, B., & Özel, A. (2018). Tyrosinase inhibition by a rare neolignan: Inhibition kinetics and mechanistic insights through *in vitro* and *in silico* studies. *Computational Biology and Chemistry*, *76*, 61-66.

Son, S. M., Moon, K. D., & Lee, C. Y. (2001). Inhibitory effects of various antibrowning agents on apple slices. *Food Chemistry*, *73*(1), 23-30.

Song, M. M., Branford-White, C., Nie, H. L., & Zhu, L. M. (2011). Optimization of adsorption conditions of BSA on thermosensitive magnetic composite particles using response surface methodology. *Colloids and Surfaces B: Biointerfaces*, *84*(2), 477-483.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N., & Garcia-Celma, M. J. (2005). Nano-emulsions. *Current Opinion in Colloid and Interface Science, 10*(3), 102–110.

Sood, S., Jain, K., & Gowthamarajan, K. (2014). Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment. *Colloids and Surfaces B: Biointerfaces*, *113*, 330-337.

Sosa, L., Clares, B., Alvarado, H. L., Bozal, N., Domenech, O., & Calpena, A. C. (2017). Amphotericin B releasing topical nanoemulsion for the treatment of candidiasis and aspergillosis. *Nanomedicine: Nanotechnology, Biology and Medicine*, *13*(7), 2303-2312.

Spagnol, C. M., Zaera, A. M., Isaac, V. L. B., Corrêa, M. A., & Salgado, H. R. N. (2018). Release and permeation profiles of spray-dried chitosan microparticles containing caffeic acid. *Saudi Pharmaceutical Journal*, *26*(3), 410-415.

Strecker-McGraw, M. K., Jones, T. R., & Baer, D. G. (2007). Soft tissue wounds and principles of healing. *Emergency Medicine Clinics of North America*, *25*(1), 1-22.

Syed Azhar, S. N. A., Ashari, S. E., & Salim, N. (2018). Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. *International Journal of Nanomedicine*, *13*, 6465-6479.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science, 108-109*, 303-318.

Tan, S. F., Masoumi, H. R. F., Karjiban, R. A., Stanslas, J., Kirby, B. P., Basri, M., & Basri, H. Bin. (2016). Ultrasonic emulsification of parenteral valproic acid-loaded nanoemulsion with response surface methodology and evaluation of its stability. *Ultrasonics Sonochemistry, 29*, 299-308.

Tang, S. Y., Manickam, S., Wei, T. K., & Nashiru, B. (2012). Formulation development and optimization of a novel Cremophore EL-based nanoemulsion using ultrasound cavitation. *Ultrasonics Sonochemistry*, *19*(2), 330-345.

Tangyuenyongwatana, P., & Jongkon, N. (2016). Molecular docking study of tyrosinase inhibitors using ArgusLab 4.0. 1: A comparative study. *Thai Journal of Pharmaceutical Sciences*, *40*(1), 21-25.

Teo, B. S. X., Basri, M., Zakaria, M. R. S., Salleh, A. B., Rahman, R. N. Z. R. A., & Rahman, M. B. A. (2010). A potential tocopherol acetate loaded palm oil esters-in-water nanoemulsions for nanocosmeceuticals. *Journal of Nanobiotechnology, 8*, 4.

Thakur, R. K., Vial, C., Djelveh, G., & Labbafi, M. (2004). Mixing of complex fluids with flat-bladed impellers: effect of impeller geometry and highly shear-thinning behavior. *Chemical Engineering and Processing: Process Intensification*, *43*(10), 1211-1222.

Verma, S., Kumar, S., Gokhale, R., & Burgess, D. J. (2011). Physical stability of nanosuspensions: investigation of the role of stabilizers on Ostwald ripening. *International Journal of Pharmaceutics*, *406*(1-2), 145-152.

Wahgiman, N. A., Salim, N., Rahman, M. B. A., & Ashari, S. E. (2019). Optimization of nanoemulsion containing gemcitabine and evaluation of its cytotoxicity towards human fetal lung fibroblast (MRC5) and human lung carcinoma (A549) cells. *International Journal of Nanomedicine, 14*, 7323.

Wang, Y., Zhang, G., Yan, J., & Gong, D. (2014). Inhibitory effect of morin on tyrosinase: Insights from spectroscopic and molecular docking studies. *Food Chemistry, 163*, 226-233.

Wójcik-Pastuszka, D., Krzak, J., Macikowski, B., Berkowski, R., Osiński, B., & Musiał, W. (2019). Evaluation of the release kinetics of a pharmacologically active substance from model intra-articular implants replacing the cruciate ligaments of the knee. *Materials*, *12*(8), 1202.

Wooster, T. J., Golding, M., & Sanguansri, P. (2008). Impact of oil type on nanoemulsion formation and Ostwald ripening stability. *Langmuir*, *24*(22), 12758-12765.

Xie, J., Dong, H., Yu, Y., & Cao, S. (2016). Inhibitory effect of synthetic aromatic heterocycle thiosemicarbazone derivatives on mushroom tyrosinase: Insights from fluorescence, 1H NMR titration and molecular docking studies. *Food Chemistry*, *190*, 709-716.

Yap, W. N., Zaiden, N., Xu, C. H., Chen, A., Ong, S., Teo, V., & Yap, Y. L. (2010). Gamma-and delta-tocotrienols inhibit skin melanin synthesis by suppressing constitutive and UV-induced tyrosinase activation. *Pigment Cell and Melanoma Research*, *23*(5), 688-692.

Yilmaz, E., & Borchert, H. H. (2006). Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema-an *in vivo* study. *International Journal of Pharmaceutics*, *307*(2), 232-238.

Yin, S. J., Si, Y. X., Chen, Y. F., Qian, G. Y., Lü, Z. R., Oh, S., Lee, J., Lee, S., Yang, J. M., Lee, D. Y., & Park, Y. D. (2011). Mixed-type inhibition of tyrosinase from *Agaricus bisporus* by terephthalic acid: Computational simulations and kinetics. *The Protein Journal*, *30*(4), 273-280.

Yuan, Y., Gao, Y., Mao, L., & Zhao, J. (2008). Optimisation of conditions for the preparation of β-carotene nanoemulsions using response surface methodology. *Food Chemistry*, *107*(3), 1300-1306.

Yusuf, D., Davis, A. M., Kleywegt, G. J., & Schmitt, S. (2008). An alternative method for the evaluation of docking performance: RSR vs RMSD. *Journal of Chemical Information and Modeling*, *48*(7), 1411-1422.

Zanatta, C. F., Ugartondo, V., Mitjans, M., Rocha-Filho, P. A., & Vinardell, M. P. (2008). Low cytotoxicity of creams and lotions formulated with Buriti oil (*Mauritia flexuosa*) assessed by the neutral red release test. *Food and Chemical Toxicology*, *46*(8), 2776-2781.

Zeng, H. J., Sun, D. Q., Chu, S. H., Zhang, J. J., Hu, G. Z., & Yang, R. (2020). Inhibitory effects of four anthraquinones on tyrosinase activity: Insight from spectroscopic analysis and molecular docking. *International Journal of Biological Macromolecules*, *160*, 153-163.

Zhang, Z., & McClements, D. J. (2018). Overview of Nanoemulsion Properties: Stability, Rheology, and Appearance. In *Nanoemulsions* (pp. 21-49). Academic Press.

Zirak, M., & Eftekhari-Sis, B. (2015). Kojic acid in organic synthesis. *Turkish Journal of Chemistry*, *39*(3), 439-496.

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LIST OF PUBLICATIONS

Research Papers:

Muhammad Azimuddin Roselan, Siti Efliza Ashari, Nur Hana Faujan, Siti Munirah Mohd Faudzi & Rosfarizan Mohamad (2020). An Improved Nanoemulsion Formulation Containing Kojic Monooleate: Optimization, Characterization and In Vitro Studies. *Molecules*, *25*(11), 2616. (Published, Q2, IF 3.060)

Muhammad Azimuddin Roselan, Norzalina Zakaria, Sharifah Nurfadhlin Afifah Syed Azhar, Nur Hana Faujan & Siti Efliza Ashari (2020). A Preliminary Study: Molecular Docking, Optimization and Characterization of Kojic Monooleate Nanoemulsion for Cosmeceuticals Application. *Journal of Sustainability Science and Management*. (Accepted, Q4, IF 0.630)

Muhammad Azimuddin Roselan, Norzalina Zakaria, Nur Hana Faujan, Muhammad Alif Mohammad Latif, Siti Munirah Mohd Faudzi, Hazrina Ab Hadi & Siti Efliza Ashari (2020). In Vitro Cytotoxicity Assay, Mushroom Tyrosinase Inhibitory Activity and Franz Diffusion Cell Analysis of Nanodelivery System and In Silico Molecular Docking Study Against 2Y9X Target Enzyme with Kojic Monooleate. *Applied Biochemistry and Biotechnology*. (Submitted, Q3, IF 2.140)

Conferences and Exhibitions:

Siti Efliza Ashari, Rosfarizan Mohamad, Nur Hana Faujan, Nur Farzana Izzati Mohd Jaslina, Muhammad Azimuddin Roselan, Sharifah Nurfadhlin Afifah Syed Azhar & Mohamad Ridzuan Yahya. Advanced Skin Technology: Upgrading into Whitemask Nanocosmeceutical Formula Incorporated with Palm-Based Kojic Monooleate in Thin Film System. International Conference and Exposition on Inventions by Institutions of Higher Learning 2019 (PECIPTA'19). 22-23 September 2019. (Silver medal)

Muhammad Azimuddin Roselan, Norzalina Zakaria, Sharifah Nurfadhlin Afifah Syed Azhar, Nur Hana Faujan & Siti Efliza Ashari. Three-Factors-Five-Levels Central Composite Design of Kojic Monooleate Nanoemulsion and Its Molecular Docking. 2nd Symposium on Multidisciplinary Science 2020 (V-SMS2020). 12 August 2020.

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