Research Article
DOI : 10.33751/jf.v12i2.4736

FITOFARMAKA: Jurnal Ilmiah Farmasi
Vol.12, No.2, December 2022 : 112-121
p-ISSN : 2087-9164 e-ISSN : 2622-755X

Formulation and Characterization of Transfersomal Containing Breadfruit Leaves (*Artocarpus altilis* (Park.) Fsb.) Ethanolic Extract

Elsa Fitria Apriani, Fitrya Fitrya*, Hannan Hanifah
Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, South Sumatra, Indonesia
**Corresponding author : fitrya@unsri.ac.id*

Submit : January 27th, 2022 Revised : October 20th, 2022 Accept : November 30th, 2022

ABSTRACT

Breadfruit (Artocarpus altilis (Park.) Fsb.) leaves have anti-tyrosinase activity due to their flavonoid content. Anti-tyrosinase penetration is deep because the tyrosinase process occurs in the stratum basale. This study aimed to formulate and characterize the transfersomal containing breadfruit (Artocarpus altilis (Park.) Fsb.) leaves ethanolic extract with varying concentrations of Tween 80 and Phospholipon 90G. Breadfruit leaves were extracted using 96% ethanol and quantitative measures of flavonoid content. Breadfruit leaves extract was then formulated into transfersomes using the thin layer hydration method with a concentration ratio of Tween 80 and Phospholipon 90G, respectively, of 5:95; 10:90; 15:85; 20:80; and 25:75. The resulting transfersomes were then characterized by the value of entrapment efficiency (EE), polydispersity index, particle size, and zeta potential to determine the best formula. The %EE result for the 5th formula were 95.13%; 95.42%; 92.78%; 90.48%; and 87.58. The polydispersity result respectively were 0.232; 0.427; 0.236; 0.425; and 0.292. The particle sizes were 276.13 nm, 290.70nm, 222.13nm, 320.80nm, and 280.90 nm. Meanwhile, the zeta potential values respectively were -15.1; -18.2 mV; -20.5 mV; -36.9 mV; and -16.7mV. The concentrations of 20% Tween 80 and 80% Phospholipon 90G were chosen as the best formulas based on the results of their characterization. The best formula also showed a spherical morphology and was most stable with the lowest reduction of entrapment efficiency of 1.7416±0.2033%.

Keywords: Artocarpus altilis; Ethanolic Extract; Transfersome; Tween 80; Phospholipon 90G

INTRODUCTION

Breadfruit or Artocarpus altilis (Parkinson) Fosberg is a plant that grows well in Indonesia. Breadfruit has been widely used empirically by the people of Indonesia, so research on the activity of breadfruit leaves has been widely carried out. Breadfruit leaves have been shown to have anti-tyrosinase activity (Nazliniwaty et al., 2016), anti-inflammatory (Fakhrudin et al., 2015), antidiabetic (Mai et al., 2012; Rante et al., 2019), anthelmintic (Magdeleine et al., 2010), immune suppressant (Palupi et al., 2020), antibacterial (Pradhan et al., 2013), and antihypertensive (Nwokocha et al., 2012).

The activity of breadfruit leaves is related to the secondary metabolites contained therein. Breadfruit leaves contain flavonoids, steroids, mucilage, tannins, phenolics, and anthraquinones (Riasari et al., 2015; Sikarwar et al., 2015). This research will focus on the activity of breadfruit leaves as anti-tyrosinase. Research by Nguyen et al. (2016) proved that the Artocarpus plant contains a flavonoid compound, namely artocarpanone, which has tyrosinase inhibitory activity with an IC50

value of IC50 of 2.0 ± 0.1 M. Tyrosinase plays an essential role in the ageing process because of its ability to produce melanin. Tyrosinase can oxidize L-tyrosine to L-dopaquinone (Pillaiyar et al., 2017). According to research conducted by Sohretoglu et al. (2016), rings A and C of the flavonoid structure, hydroxyl substituents at the seventh position, and hydroxyl substituents at the para or para and meta positions of ring B play critical roles in competitive inhibition of enzymes.

The tyrosinase process occurs in the basal layer. The stratum basale is the deepest layer of the epidermis that separates the epidermis from the dermis (Moreiras et al., 2021). The active substance must penetrate deeper to reach the stratum basale even though the skin has the outermost layer in the form of the stratum corneum, which is very tightly arranged, so it is not easy for the active substance to penetrate through the stratum corneum.

Various innovations in drug delivery technology are carried out to increase the penetration of active substances through the skin, one of which is the manufacture of transfersomes. Transfersomes are lipid bilayer vesicles widely used for drug delivery through the skin. Transfersomes are vesicles with high deformability compared to other lipid vesicles, making it easier for active substances to penetrate the skin's stratum corneum (Chaurasiya et al., 2019). Transfersomes are composed of phospholipids and surfactants. Surfactants act as membrane-softening agents that increase the deformability of vesicles (Opatha et al., 2020). The proper concentration of phospholipids and surfactants will produce deformable ones, thereby increasing the permeability ability (Modi & Bharadia, 2012). The number of edge activators is 10-20% (Rai et al., 2017). However, according to Jangdey et al. (2017), using an edge activator of more than 15% will cause the formation of a more significant micelle mixture so that the solubility will be higher, and the impact is vesicle leakage. Research conducted by El Sayyad et al. (2017) proved that using high concentrations of surfactants in the transfer of sildenafil citrate carriers will cause a decrease in entrapment efficiency. In this study, variations of surfactant concentration: lipids were used; namely, 5:95; 10:90; 15:85; 20:80; and 25:75. The surfactant used in this study was Tween 80. Tween 80 can increase transfersome deformability because it can destabilize the lipid bilayer. In addition, Tween 80 can increase absorption and has low toxicity with an LD50 value of 25 g/kgBW (Di Costanzo & Angelico, 2019). Research conducted by Anggraini et al. (2017) and El Sayyed et al. (2017) proved that using Tween 80 resulted in better %EE than Span 80. Phospholipone 80G was chosen as the lipid for forming vesicle transfersomes in this study because it has good compatibility properties, is biodegradable, is a cleaning agent, and can penetrate well (Sachan et al., 2013).

Based on the description above, the ethanol extract of breadfruit leaves was formulated into transfersomes with varying concentrations of Tween 80 and Phospholipon 90G. Transfersomes were then characterized by the percentage of entrapment efficiency (%EE), polydispersity index, particle size, and zeta potential using a particle size analyzer (PSA). The best formula will be tested using a transmission electron microscope (TEM) to see its morphology.

MATERIALS AND METHODS

Equipments

The equipment used in this study included standard laboratory glassware (Pyrex® and Iwaki®), analytical balance (ADAM® Nimbus NBL 254), UV-Vis spectrophotometer (Biobase® BK-UV1900PC), centrifuge (DLAB©: D2012 PLUS), refrigerator (SANYO®), pH-meter (Lutron® pH Electrode PE-03), sonicator (GT SONIC®), particle size analyzer (PSA) (Malvern Zetasizer) and transmission electron microscope (TEM) (JEOLJEM 1400).

Materials

The materials used in this study include breadfruit leaves (Artocarpus altilis (Park.)

Fsb.) (University of Sriwijaya, Indralaya, South Sumatra), 96% ethanol (Bratachem®), distilled water (Bratachem®), Tween 80 (Bratachem®), KH2PO4 (Merck®) Na2CO3 (Merck®), quercetin (Sigma-Aldrich®), AlCl3 (Bratachem®), methanol p.a. (Merck®), sodium acetate (Merck®), NaOH (Bratachem®), Phospholipone 90G (Phospholipid GmbH-Lipoid), FeCl3, anhydrous acetic acid (Merck®), ethyl acetate (Bratachem®) and glass beads (Bratachem®).

Preparation of Breadfruit Leaves Extract

Breadfruit leaves (Artocarpus altilis (Park.) Fsb.) were obtained at Sriwijaya University, Palembang and plant identification was carried out at the Plant Conservation Center of the Purwodadi Botanical Gardens, LIPI. Fresh leaves are washed with running water, chopped, and then dried in the sun. Then the simplicia is mashed using a blender. Some simplicia were macerated with 96% ethanol for 48 hours and then remecerated for 24 hours. The obtained macerate was concentrated with a rotary evaporator at a temperature of 50ºC to obtain a concentrated extract (Fitrya et al., 2020).

Determination of Total Flavonoid Content

Determination of total flavonoid content in breadfruit leaves ethanolic extract using UV-Vis Spectrophotometry. Quercetin was used as a standard to make calibration curves with various concentrations of 1;5;10;15;20;25;30 and 35 µg/mL. A total of 1.5 mL of the standard solution was reacted with 1 M Sodium acetate and 0.1 mL of 10% AlCl3 each, then diluted with distilled water to a total volume of 5 mL (Indarti et al., 2019). Let stand for 30 minutes and measure the absorbance at a maximum wavelength of 434 nm. Then, the total flavonoid content of the breadfruit leaves ethanolic extract was determined with a concentration of 1 mg/mL. Total flavonoid content was calculated using Equation 1.

Formulation of Transferosomes Containing Breadfruit Leaves Ethanolic Extract

The design of the transfersome formula can be seen in Table 1. The independent variables in this study were the concentration ratio of Tween 80 and Phospholipon 90G, namely 5:95; 10:90; 15:85; 20:80; and 25:75. The ratio of ethanol extract and vesicle used is 1:2 (Ahmed et al., 2014).

Production of Transferosomes Containing Breadfruit Leaves Ethanolic Extract

The manufacture of transfersome ethanol extract from breadfruit leaves was manufactured using a thin-layer hydration method using glass beads. First, a thin layer was made by mixing the ethanol extract of breadfruit leaves, Phospholipon 90G and Tween 80, which was then dissolved in 96% ethanol in a round bottom flask. The solution was evaporated using a rotary evaporator at a temperature of 54 ºC (Apriani et al., 2019). The initial rotation speed of the round bottom flask was 50 rpm, then it was increased by 25 rpm until it reached 150 rpm. The thin layer formed is then stored for 24 hours in the refrigerator.

After the thin layer was formed, then the hydration process was carried out by adding a solution of phosphate buffer pH 7.4 until all the thin layers were peeled off using glass beads. The hydration process was carried out with an initial rotation speed of 50 rpm and then increased by 25 rpm to reach 250 rpm. Furthermore, after the thin layer was utterly hydrated, the transfersome suspension was put into a glass bottle then the particle size was reduced using a sonicator for 10 minutes for 3 cycles (Apriani et al., 2019). The resulting transfersome suspension was observed organoleptically and at pH.

$$F = \frac{X\,(ppm)\ x\ V\,(L)}{m}\ \text{x Dilution Factor} \qquad (1)$$

Where F: Total flavonoid content; V: Total volume of extract (L); M: Sample weight (g); X: Concentration (ppm)

Table 1. Formula Design of Transferosomes

Ingredients	Concentration				
	TF1	**TF2**	**TF3**	**TF4**	**TF5**
Extract (%)	3,5	3,5	3,5	3,5	3,5
Tween 80 : Phospholipon 90G (%)	5:95	10:90	15:85	20:80	25:75
Etanol 96% (%)	10	10	10	10	10
Dapar fosfat pH 7,4 ad (mL)	100	100	100	100	100

Characterization of Transferosomes Containing Breadfruit Leaves Ethanolic Extract

Entrapment Efficiency (%EE)

The percentage of entrapment efficiency was carried out by the indirect method. The transfersome suspension was centrifuged at 9500 rpm for 90 minutes to obtain two phases: supernatant and precipitate (Apriani et al., 2019). The supernatant was used to determine total flavonoids following the procedure above. After obtaining the total flavonoid content in the supernatant, the %EE was calculated using Equation 2.

Polydispersity Index, Particle Size, and Zeta Potential

The polydispersity index (PDI), particle size, and zeta potential were measured using a particle size analyzer (PSA). Measurements were made with transfersome suspension, which had been separated from free flavonoids and then resuspended in transfersome solvent. The suspension was diluted in 10 mL of distilled water, and then 1 mL was taken and put into a cuvette.

Stability Test

Transfersome stability test was carried out using the cycling test method. The test was carried out by storing the preparations at 4±2ºC in the refrigerator for 24 hours and then transferring them to a high temperature of 40±2ºC for 24 hours (1 cycle) alternately. The test was carried out for 6 cycles and observed at the beginning and end of the cycle. At the beginning and end of each cycle, the percentage of entrapment efficiency is measured, and then the percentage decrease is calculated (Apriani et al., 2018).

Determination of the Best Formula

Based on the transfersome characterization that has been done, the best transfersome formulation will be selected. The formulation that will be selected is a formula with a high percentage of entrapment efficiency of more than 50%, particle size less than 1000 nm, zeta potential less than -30 mV or more than +30mV, polydispersity index less than 0.5, pH range 4-7 and the minor decrease in %EE (Apriani et al., 2019).

Determination of Vesicle Morphology

Determination of the morphology of the transfersome vesicles was analyzed using a TEM. One sample drop was dropped on a carbon-coated copper grid, dried, and the TEM voltage was adjusted to 20-25 kV (El Zaafarani et al., 2010).

Data Analysis

Data analysis was carried out using the SPSS program using Shapiro-Wilk to see the normality of the data. If the data is normally distributed, it is continued with the one-way Anova test.

$$\%\text{Entrapment Efficiency (EE)} = \frac{Qt-Qs}{Qt} x100\% \quad (2)$$

Information:
Qt: Total flavonoid content in transfersome suspension (µg/mL)
Qs: Flavonoid content in supernatant (µg/mL)

RESULT AND DISCUSSION

Breadfruit leaves were extracted using 96% ethanol as solvent. Breadfruit leaves contain quercetin, a flavonol compound in the form of an aglycone, so it is suitable to be extracted with 96% ethanol solvent, which can dissolve semipolar and polar compounds (Soifoini et al., 2021). The breadfruit leaves ethanolic extract produced is greenish-brown, thick and has a distinctive smell. The per cent yield value obtained is 10.33%. The breadfruit leaves ethanolic extract was also proven to contain 98.349 mg QE/g extract flavonoids. The presence of flavonoids in the extract of has the potential to provide anti-tyrosinase activity. However, because the tyrosinase process occurs in the basal layer, the deepest layer of the epidermis, it is necessary to deliver the breadfruit leaves ethanolic extract to penetrate the basal layer. One of them is by making ethanol extract from breadfruit leaves into transfersome preparations.

Transfersomes were prepared by the thin-layer hydration method. The manufacture of transfersomes using the thin-layer hydration method is divided into two processes: the thin-layer formation process and the thin-layer hydration process. The thin layer was formed at the transition temperature of Phospholipon 90G of 54°C. In the transition state, the lipid molecule will be more permeable because it can move freely to absorb the drug optimally (Kraft et al., 2013). Phospholipids are amphiphilic compounds consisting of a hydrophilic head and a lipophilic tail (Drescher & Hoogevest, 2020). Phospholipids consisting of a hydrophilic head and a hydrophobic tail will automatically join and direct the hydrophilic head to the surface in contact with the phosphate buffer, while the tail will be between the lamellar vesicles and form a hydrophobic layer so that it can encapsulate drug compounds (Choudhury et al., 2020). Furthermore, Tween 80 will increase vesicle flexibility and drug permeability because amphiphilic surfactants can form micelles (Pandey et al., 2014). The transfersome suspension obtained had the same appearance, namely the distinctive aroma of breadfruit leaves, homogeneous and dark green, as shown in Figure 1. Furthermore, the 5 transferosome formulas, such as pH test, sorption efficiency, particle size, PDI and zeta potential, were characterized. The results of these measurements are listed in Table 2.

The pH results of the 5 transfersome formulas showed that the highest pH value was in the TF5 formula because it contained the highest Tween 80. The pH of Tween 80 is in the range of 6-8 (Rowe et al., 2009). Based on its structure, Tween 80 contains ethylene oxide compounds which will increase the pH of tween 80. So the higher the concentration of Tween 80, the higher the pH value.

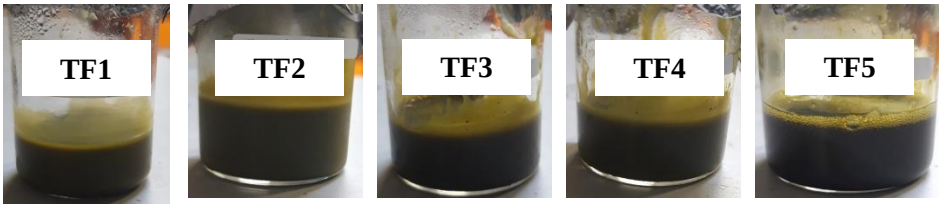

Figure 1. Transfersomal of breadfruit leaves ethanolic extract TF1 (5:95), TF2 (10:90), TF3 (15:85), TF4 (20:80) dan TF5 (25:75)

Table 2. Characterization Result

Formula	pH	EE (%)	Particle Size (nm)	PDI	Zeta Potential (mV)
TF1	6.03	95,1277	276,13	0,232	-15,1
TF2	6.08	95,4189	290,70	0,427	-36,9
TF3	6.10	92,7770	222,13	0,236	-18,2
TF4	6.11	90,4799	320,80	0,425	-36,9
TF5	6.13	87,5830	280,90	0,292	-16,7

Based on Table 2, the EE values of the 5 formulas show an inverse relationship with the concentration of Tween 80 used. The higher the concentration of Tween 80 used, the smaller the %EE obtained. Based on statistical analysis, there were significant differences in each formula ($p<0.05$). This is due to the difference in the concentration of Tween 80 used so that it occurs. Mixed micelles formed from the use of Tween 80 will increase the solubility of vesicles; as a result, the vesicles formed will leak more quickly so that the %EE will decrease (Seo et al., 2020). The value of particle size and PDI of the 5 formulas meet the requirements where the particle size must be less than 1000 nm and PDI <0.5. The results showed a particle size of about 200-350 nm and a PDI of 0.2-0.5. Particle size is an essential parameter in determining the penetration ability of the preparation, safety, effectiveness, stability, and solubility (Chauhan et al., 2017). The PDI value indicates the uniformity of the particles. PDI value less than 0.5 indicates that the particles obtained are uniform. In addition, based on the zeta potential results, transfersome suspensions have a negative surface charge due to the presence of phosphate and carboxyl groups of phospholipids (Chuacharoen et al., 2019). Based on the zeta potential value results, F2 gives a zeta potential result that meets the requirements, namely -36.9 mV. The zeta potential value of a nanoparticle is said to be stable if it is at a value less than -30 mV or more than +30 mV.

The zeta potential value of TF3, TF2 and TF1 decreased with increasing concentration of Phospholipone 90G. At this value, the particles are at great distances so that they will repel each other and prevent flocculation (Lowry et al., 2016). The higher concentration of Phospholipone 90G will cause the transfersome vesicles to become more flexible and permeable so that they leak more easily (Hosny, 2016; Ullmann et al., 2021). Vesicle leakage can also quickly occur at high surfactant concentrations due to mixed micellar events (Ahad et al., 2017). When the vesicle leaks, the flavonoids trapped in it will be released and then become free flavonoids and release H+ ions which will cause the zeta potential value to decrease in negativity. The zeta potential results are also in line with the stability tests carried out. TF4 had a minor decrease in %EE, namely 1.7416±0.2033 compared to the others, because there was vesicle leakage in TF1, TF2, F3, and F5 (Table 3). Therefore, the TF4 formula was chosen as the optimum formula in this study.

Table 3. Decreasing of EE Percentage (%)

Formula	Percentage of EE decrease (%)
TF1	5,7063±0,0040
TF2	4,5568±0,0116
TF3	3,7734±0,0399
TF4	1,7416±0,2033
TF5	5,4907±0,0178

The best formula was tested for morphology using a transmission electron microscope (TEM) magnification 150,000 times. The results of the morphology test can be seen in Figure 2. Based on Figure 2, the vesicle morphology of TF2 produced is spherical. The spherical shape of the particles will make it easier for vesicles to penetrate the cell, thereby increasing drug penetration (Dasgupta et al., 2014).

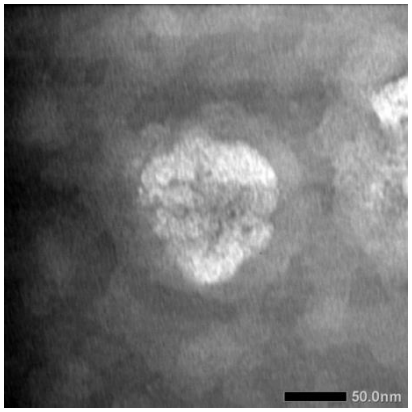

Figure 2. Vesicle morphology TF4 magnification 150,000 times

CONCLUSIONS

In this study, the transfersome characterization of breadfruit leaf extract was strongly influenced by the concentrations of Tween 80 and Phospholipon 90G. The TF4 formula containing 20%:80% Tween 80 and Phospholipon 90G was chosen as the best formula with an entrapment efficiency percentage of 90.4799%; polydispersity index of 0.425, a particle size of 320.8 nm, and zeta potential of -36.9 mV. TF4 also showed a minor decrease in %EE, namely 1.7416±0.2033%, which indicates that TF4 is stable. The resulting morphology is also spherical. Based on this conclusion, TF4 has the potential to be further developed into anti-tyrosinase preparations.

ACKNOWLEDGEMENT

The author would like to thank PT. DKSH Indonesia for its assistance in testing the Particle Size Analyzer for free.

REFERENCES

Ahad A, Al-Saleh AA, Al-Mohizea AM, Al-Jenoobi FI, Raish M, Yassin AEB, Alam MA (2017) Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of eprosartan mesylate. *Saudi Pharmaceutical Journal.* 25:1040-1046.

Ahmed TA (2014) Preparation of transfersomes encapsulating sildenafil aimed for transdermal drug delivery: Plackett-Burman design and characterization. *J. Liposome Res.* 25 (1): 1-10.

Anggraini W, Sagita E, and Iskandarsyah I (2017) Effect Of Hydrophilicity Surfactants Toward Characterization and In Vitro Transfersomes Penetration In Gels Using Franz Diffusion Test. *Int J App Pharm.* 9 (1): 112-115.

Apriani EF, Nurleni N, Nugrahani HN, Iskandarsyah I (2018) Stability Testing of Azelaic Acid Cream Based Ethosome. Asian Journal of Pharmaceutical and Clinical Research. 11 (5): 270-273.

Apriani EF, Rosana Y, Iskandarsyah I (2019) Formulation, characterization, and in vitro testing of azelaic acid ethosome-based cream against Propionibacterium acnes for the treatment of acne. *J Adv Pharm Technol Res.* 10 (2): 75-80.

Chauhan, Neelam, Kapil K, and Navin CP (2017) An updated review on transfersomes: a novel vesicular system for transdermal drug delivery. *Universal Journal of Pharmaceutical Researc*. 2 (4): 49-52.

Chaurasiya P, Ganju E, Upmanyu N, Ray S, and Jain P (2019) Transfersomes: a novel technique for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics.* 9 (1): 279-285.

Choudhury A, Sonowal K, Laskar RE, Deka D, Dey BK (2020) Liposome: A Carrier for Effective Drug Delivery. *Journal of*

Applied Pharmaceutical Research. 8 (1): 22 – 28.

Chuacharoen T, Prasongsuk S, and Sabliov SM (2019) Effect of Surfactant Concentrations on Physicochemical Properties and Functionality of Curcumin Nanoemulsions Under Conditions Relevant to Commercial Utilization. *Molecules* 24 (2744): 1-12

Dasgupta S, Auth T, Gompper G (2014) Shape and orientation matter for the cellular uptake of nanospherical particles. *Nano Lett* 14: 687 – 693.

Di Costanzo A, Angelico R (2019) Formulation Strategies for Enhancing the Bioavailability of Silymarin: The State of the Art. *Molecules*. 24 (11): 2155.

Drescher S, and Hoogevest PV (2020) The Phospholipid Research Center: Current Research in Phospholipids and Their Use in Drug Delivery. *Pharmaceutics* 12 (1235): 1-36.

El Sayyad MK, Zaky AA, and Samy AM (2017) Fabrication and characterization of sildenafil citrate loaded transfersomes as a carrier for transdermal drug delivery. *Pharm Pharmacol Int J*. 5 (2): 37–46.

El Zaafarany GM, Awad GAS, Holayel SM, and Mortada ND (2010) Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery. *International Journal of Pharmaceutics* 397: 164-172.

Fakhrudin N, Hastuti S, Andriani A, Widyarini S, and Nurrochmad A (2015) Study on the antiinflammatory activity of Artocarpus altilis leaves extract in mice. *International Journal of Pharmacognosy and Phytochemical Research*. 7 (6): 1080-1085.

Fitrya F, Amriani A, Novita R, Elfita, Setiorini D, Garrido G (2020) Immunomodulatory effect of Parkia speciosa Hassk. pods extract on rat induced by Salmonella typhimurium. *Journal of Pharmacy & Pharmacognosy Research*. 8: 457-465.

Hosny KM (2016) Alendronate Sodium as Enteric Coated Solid Lipid Nanoparticles; Preparation, Optimization, and In Vivo Evaluation to Enhance Its Oral Bioavailability. *PLoS One*. 11 (5): 1-14.

Indarti K, Apriani EF, Wibowo AE, Simanjuntak P (2019) Antioxidant Activity of Ethanolic Extract and Various Fractions from Green Tea (Camellia sinensis L.) Leaves. *Pharmacognosy Journal*. 11 (4): 771-776.

Jangdey MS, Gupta A, Saraf S, and Saraf S (2017) Development and optimization of apigenin-loaded transfersomal system for skin cancer delivery: in vitro evaluation. *Artif Cells Nanomed Biotechnol*. 4: 1452-1462

Kraft JC, Freeling JP, Wang Z, and Ho RJ (2014) Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. *J. Pharm. Sci*. 103: 29–52.

Lowry GV, Hill RJ, Harper S, Rawle AF, Hendren CO, Klaessig F, Nobbmann U, Sayre P, and Rumble J (2016) Guidance to improve the scientific value of zeta potential measurements in nanoEHS. *Environ. Sci. Nano*. 3: 953-965.

Magdeleine CM, Maurice M, Marie-Laure L, and Harry A (2010) In vitro evaluation of the nematicidal value of *Artocarpus altilis* (Parkinson) var. seminifera and non seminifera and Terminalia cattapa L. against Haemonchus contortus. *Advances in Animal Biosciences* 1 (02): 440-441.

Mai NTT, Hai NX, Phu DH, Huu PN, Nguyen T, and Nhan T (2012) Three new geranyl aurones from the leaves of *Artocarpus altilis*. *Phytochemistry Letters*. 5 (3): 647-650.

Modi CD, and Bharadia PD (2012) Transfersomes: New Dominants for

Transdermal Drug Delivery. *Am. J. PharmTech Res*. 2 (3): 71-91.

Moreiras H, Seabra MC, and Barral DC Melanin Transfer in the Epidermis: The Pursuit of Skin Pigmentation Control Mechanisms. *Int. J. Mol. Sci*. 22 (4466): 1-11.

Nazliniwaty, Arianto A, and Nasution KRA (2016) Formulation and anti-aging effect of cream containing breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract. *International Journal of PharmTech Research*. 9 (12): 524-530.

Nguyen HX, Nguyen NT, Nguyen MH, Le TH, Van Do TN, Hung TM, Nguyen MT (2016) Tyrosinase inhibitory activity of flavonoids from Artocarpus heterophyllous. *Chem Cent J*. 10 (2): 1-6.

Nwokocha CR, Owu DU, McLaren M, Murray J, Delgoda R, Thaxter K, McCalla G, and Young L (2012) Possible mechanisms of action of the aqueous extract of *Artocarpus altilis* (breadfruit) leaves in producing hypotension in normotensive Sprague-Dawley rats. *Pharmaceutical Biology*. 50 (9): 1096-1102.

Opatha SAT, Titapiwatanakun V, and Chutoprapa R (2020) Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery. *Pharmaceutics*. 12 (855): 1-23.

Palupi DHS, Retnoningrum DS, Iwo MI, and Soemardji AA (2020) Leaf extract of *Artocarpus altilis* (Park.) Fosberg has potency as anti-inflammatory, antioxidant and immunosuppressant. *Rasayan Journal of Chemistry*. 13 (1): 636-646.

Pandey A, Mittal A, Chauhan C, and Alam S (2014) Role of Surfactants as Penetration Enhancer in Transdermal Drug Delivery System. *J Mol Pharm Org Process Res*. 2 (113): 1-10.

Pillaiyar T, Manickam M, and Namasivayam V (2017) Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *J Enzyme Inhib Med Chem*. 32 (1): 403-425.

Pradhan C, Mohanty M, and Rout A (2013) Phytochemical screening and comparative bioefficacy assessment of *Artocarpus altilis* leaf extracts for antimicrobial activity. *Frontiers in Life Science*. 6 (3–4): 71–76.

Rai S, Pandey V, and Rai G (2017) Transfersomes as versatile and flexible nanovesicular carriers in skin cancer therapy: the state of the art. *Nano Reviews & Experiments*. 8: 1- 15.

Rante H, Alam G, and Irwan M (2019) α – Glucosidase inhibitory activity of breadfruit leaf extract (Artocarpus altilis (parkinson) fosberg). *Journal of Physics: Conference Series*. 1341: 1-9.

Riasari H, Sukrasno, and Ruslan K (2015) Metabolite Profile of Various Development Bread Fruit Leaves (Artocarpus Altilis. Parkinson. Fosberg) and the Identification of Their Major Componens. *International Journal of Pharmaceutical Sciences and Research*. 6 (5): 2170-2177.

Rowe RC, Sheskey PJ, and Quinn ME (2009) *Handbook of Pharmaceutical Excipients Sixth Edition*. London: Pharmaceutical Press.

Sachan R, Tarun P, Soniya, Vishal S, Gaurav S, and Satyanand T (2013) Drug carrier transfersomes: a novel tool for transdermal drug delivery system. *International Journal of Research and Development in Pharmacy and Life Sciences* 2(2): 309 – 316.

Seo SH, Kim E, Joo Y, Lee J, Oh KT, Hwang SJ, and Choi KY (2020) A Mixed Micellar Formulation for the Transdermal Delivery of an Indirubin Analog. *Pharmaceutics*. 12 (175): 1-10.

Sikarwar MS, Hui B, Subramaniam K, Valeisamy BD, KarYean L, and Balaji K (2015) Pharmacognostical, Phytochemical and Total Phenolic Content of Artocarpus altilis (Parkinson)

Fosberg Leaves. *Journal of Applied Pharmaceutical Science*. 5 (5): 94-100.

Sohretoglu D, Sari S, Barut B, and Özel A (2018) Tyrosinase inhibition by some flavonoids: Inhibitory activity, mechanism by in vitro and in silico studies. *Bioorganic Chemistry*. 81: 168-174.

Soifoini T, Donno D, Jeannoda V, Rakoto DD, Msahazi A, Farhat SMM, Oulam MZ, Beccaro GL (2021) Phytochemical Composition, Antibacterial Activity, and Antioxidant Properties of the Artocarpus altilis Fruits to Promote Their Consumption in the Comoros Islands as Potential Health-Promoting Food or a Source of Bioactive Molecules for the Food Industry. Foods. 10, 2136.

Ullmann K, Leneweit G, and Nirschl H (2021) How to Achieve High Encapsulation Efficiencies for Macromolecular and Sensitive APIs in Liposomes. *Pharmaceutics*. 13 (691): 1-13.