International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 6, 2024

Original Article

PREPARATION, CHARACTERIZATION, AND *IN VITRO* EVALUATION OF GEL CONTAINING NANOPHYTO-PHOSPHOLIPID COMPLEX OF KOPASANDA LEAF EXTRACT (*CHROMOLAENA ODORATA* (L.) R. M. KING AND H. ROB)

SUCILAWATY RIDWAN[1*], NURUL WAHYUNI[2], GUNAWAN PASARIBU[2], SAFWAN SAFWAN[3], WAHIDA HAJRIN[1], MARIAMA FITRIANA[1], KAMELIA ROIYAN[1]

[1]Departement of Pharmacy, Faculty of Medicine and Health Sciences, Mataram University, Mataram-83125, Indonesia. [2]National Research and Innovation Agency, Indonesia. [3]Department of Pharmacy, University of Muhammadiyah Mataram, Mataram, Indonesia
[*]Corresponding author: Sucilawaty Ridwan; [*]Email: sucilr@unram.ac.id

Received: 17 Dec 2023, Revised and Accepted: 23 Sep 2024

ABSTRACT

Objective: This study aims to prepare, characterize, and *in vitro* evaluation of the gel containing nanophyto-phospholipid complex of kopasanda leaf extract.

Methods: Kopasanda dried leaf was extracted by extraction reflux method, followed by total phenolic content of extract measurement using Spectrophotometry UV-Vis method. The nanophyto-phospholipid complex was prepared using an antisolvent evaporation method with various ratios between extract and phospholipid of 1:1; 1:2; 1:3. The optimum ratio was evaluated by entrapment efficiency (%). The nanophyto-phospholipid complex formation was characterized by polydispersity index, particle size, Fourier Transform Infra-Red (FT-IR), and the Transmission Electron Microscope (TEM) method. The optimum nanophyto-phospholipid complex was formulated into gel preparation. The *in vitro* permeation study was performed to discover the influence of gel containing nanophyto-phospholipid complex compared with gel-containing extract without the nanophyto-phospholipid complex formation.

Results: Thetotal phenolic content of kopasanda leaf extract was 117.214±3.054 mg/GAE. The optimum ratio of kopasanda leaf extract and phospholipid was 1: 2 with entrapment efficiency (%), particle size, and polydispersity index equal to 99.897±0.001%, 130.1 nm and 0.394. The morphology of the nanophyto-phospholipid complex was spherical and the complex formation was confirmed by the FTIR spectrum. The permeation test showed that the gel containing nanophyto-phospholipid complex had better diffusion than the gel without the nanophyto-phospholipid complex formation.

Conclusion: The gel containing nanophyto-phospholipid complex formation exhibited the potential drug delivery system to increase the phenolic content permeation of kopasanda leaf extract.

Keywords: Nanophyto-phospholipid complex, *Chromolaena odorata* (L.) R. M. King and H. Rob), Antisolvent precipitation method, Gel, Permeation studies

DOI: https://dx.doi.org/10.22159/ijap.2024v16i6.50174 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Kopasanda (*Chromolaenaodorata* (L.) R. M. King and H. Rob) has been widely used by Indonesians, particularly in West Nusa Tenggara has been hereditary for treating open wounds and cuts. It is empirically used by squeezing the stem until the liquid comes out and the liquid is dripped onto the injured part. Besides, some people use the leaves for the same treatment by squeezing or mashing them and placing them on the injured part [1]. However, not all people are comfortable with this treatment because of the solid stinging feeling when used. According to the people of West Nusa Tenggara who have used it, the Kopasanda plant can treat wounds using the Kopasanda plant. Heal the wound until the wound dries up [2].

In vitro research on wound healing tests shows that the extract of the kopasanda leaf can increase the proliferation of fibroblasts, endothelial cells, and keratinocytes. Meanwhile, *in vivo* research, ethanol extract of leaves kopasanda of 20% is the most optimal concentration that can increase the amount of collagen, fibroblasts, and thickness of the epidermis in the skin of white mice [2-4]. The stratum corneum of the skin was the main barrier for the transdermal delivery [5].

Kopasanda leaf extract possesses a total phenolic content of 58.34 mgGAE/g-242.3 mgGAE/g [2-4]. On the other hand, the phenolic compound has low bioavailability due to its physicochemical properties. The use of kopasanda plants for treating wounds has the potential to be developed both by increasing effectiveness and availability through formulation. So that it can increase effectiveness and community acceptance. One method to increase effectiveness and community acceptance is a nanophyto-phospholipid complex delivery system created in a gel dosage form. Nanophyto-phospholipid complex is a systems of drug delivery that react to several phospholipids with standardized extracts. Implementing nanophyto-phospholipid complexes as nanocarriers improve compounds' phytochemicals with low absorption and penetration in biological membranes [6]. Nanophyto-phospholipid complex can be made using the antisolvent method [7-9]. The antisolvent methods exhibited higher entrapment efficiency than thin layer formation and solvent evaporation method [10]. This study aims to prepare, characterize, and *in vitro* evaluation of the gel containing nanophyto-phospholipid complex of kopasanda leaf extract.

MATERIALS AND METHODS

Materials

Dried leaves kopasanda (kindly provided by PT. Moringa Organik Lombok). It was determined in the Biology Laboratory, the Faculty of Mathematics and Natural Science, University of Mataram, with an identification number of 18/UN18.7/lBL/2003. Phospholipon 90 G, containing 97% phosphatidylcholine (LipoidGmbH, Germany), gallic acid (Sigma Aldrich, Singapore), dichloromethane, ethanol 96%, ethanol 70%, Folin-Ciocalteu, Na_2CO_3, aquadestillata, and other chemical reagents a grade of analytic. The formulation material was pharmaceutical grade consisting of NaCMC, Carbopol 940, Propylene glycol, DMDM hydantoin, citric acid, and triethanolamine.

Methods

Preparation of crude extract

Kopasanda dried leaf was extracted by reflux extraction method.30 g of kopasanda dried leaf extract was put into a round bottom flask

with the addition of 300 ml 70% ethanol as a solvent. The temperature during extraction was 60 °C for 2x2 h. The liquid extract was evaporated using vacuum rotary evaporator at temperature 40 °C, speed of 50 rpm, and a pressure bar of 200 mBar.

Total phenolic content

The total phenolic content was measured using Follin-Ciocalteu method with gallic acid as the standard. Standard gallic acid was dissolved in aqua distillate with various concentration 5µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml, and 50 µg/ml. Briefly, 0.3 ml of the extract was mixed with 1.5 of Folin-Ciocalteu reagent. The solution was incubated for 3 min at ambient temperature. The 1.2 ml of sodium bicarbonate was added to the mixed solution then incubated for 25 min. The mixture solution was read at the absorbance of 753 nm [11, 12].

Phyto-phospholipid complex preparation

The phyto-phospholipid complex preparation of kopasanda leaf extract was utilized by antisolvent precipitation method. The known quantities of kopasanda leaves extract was dissolved in 96% of ethanol, while the known phospholipid was dissolved in dichloromethane. The solution mixture was refluxed at a temperature of 60 °C for 1 h. Furthermore, the solution was concentrated using a vacuum rotary evaporator of 50 °C to almost $1/4^{th}$ amount. 20 ml of n-hexane was added to the concentrated mixture to gain the precipitate. The precipitate was filtered and placed in a desiccator overnight. The precipitate was added with the water as a hydration process for 30 min in a round bottom flask and the speed of 210 rpm resulting in the suspension of the phyto-phospholipid complex. The phyto-phospholipid complex preparation was optimized into various concentrations between kopasanda leaf extract and phospholipid (1:1;1:2;1:3). The optimum variation concentration of the phyto-phospholipid complex was evaluated by entrapment efficiency (%) [10, 12, 13].

The entrapment efficiency (%)

The entrapment efficiency (%) of variation concentration between kopasanda leaf extract and phospholipid (1:1:1:2:1:3) were measured using a Spectrophotometry UV-Vis method. 1 ml of phyto-phospholipid complex suspension was taken into the centrifuge tube. The water was added to the solution up to 10 ml. The mixture solution was centrifuged at 14000 rpm for 1 hour. 1 ml of supernatant was taken and the total phenolic content was measured using spectrophotometer UV-Vis. The absorbance was determined at a wavelength of 753 nm. The measurement was done triplicate of each sample. The calculation of entrapment efficiency (%) followed the equation [10]:

$$\text{Entrapment efficiency (\%)} = \frac{T-S}{T} \times 100$$

Where,

T = Total phenolic content present in a phyto-phospholipid complex taken

S = Total phenolic content present in the supernatant

T – S = Total phenolic content entrapped in phyto-phospholipid complex

Preparation of nanophyto-phospholipid complex

The optimum ratio between kopasanda leaf extract and phospholipid was selected to prepare for nanophyto-phospholipid complex using antisolvent precipitation method. Furthermore, the optimum phyto-phospholipid complex was reduced in particle size using ultrasonic homogenizer with the various sonication time (0 min, 1 min, 2 min, and 3 min).

Characterization of nanophyto-phospholipid complex

Particle size and polydispersity index

The measurement of particle size and polydispersity index was determined using particle size analyzer. The solution of nanophyto-phospholipid complex was scattered the light at a temperature 25 °C [12].

Fourier transform infrared spectroscopy (FT-IR)

The spectrum analysis of nanophyto-phospholipid complex was gained using Fourier Transform Infrared Spectroscopy. The spectrum characterized was consisted of kopasanda leaves extract, phospholipid, physical mixture of kopasanda leaves extract and phospholipid, and nanophyto-phospholipid complex. Each sample was mixed with Kalium Bromide at a ratio 1:100. The mixture then pressed deeply into a mold and analysed at a wavelength of 4000-400 cm-1 [12].

Surface morphology

The surface morphology of nanophyto-phospholipid complexes was studied using Transmission Electron Microscope (TEM) method. The sample was put in a copper grid covered by carbon for 30 min until dried. Then, the excess solution was filtered. The sample was read using Transmission Electron Microscope apparatus [14].

Formulation of gel

The gel formula was explained in table 1.

Table 1: Gel formulation of nano-phytophospholipidcomplex and extract

Ingredients	Percentage of amount (%)		Function
	F1	F2	
Kopasanda leaf extract in nanophyto-phospholipid complex system	5	-	Active pharmaceutical ingredients
Kopasanda leaf extract without nanophyto-phospholipid complex system	-	5	Active pharmaceutical ingredients
NaCMC	1.5	1.5	Gelling agent
Carbopol 940	1	1	Gelling agent
Propylene glycol	15	15	Humectant
DMDM hydantoin	0.6	0.6	Preservative agent
Citric acid	0.1	0.1	Antioxidant
Triethanolamine (TEA)	q. s	q. s	Alkalizing agent
Aquadest	Ad to 100	Ad to 100	Solvent

NaCMC was soaked in the heated water for 30 min. Carbopol 940 was also soaked in the heated water for 30 min. Citric acid was dissolved in the aquadest, then mixed with propylene glycol and DMDM hydantoin resulting in a clear mixture. The clear mixture was added to the gelling agent containing NaCMC and Carbopol 940. The suspension of active pharmaceutical ingredients was added to the gel [15].

Gel evaluation of nanophyto-phospholipid complex

Stability studies

The gel stability was analysed by organoleptic, pH measurement, and viscosity measurement. The stability study was observed during 9 days. Each test was done in triplicate [10].

***In vitro* diffusion studies**

The permeation studies were performed using Franz diffusion cells on gel-containing extract and gel-containing neophyte-phospholipid. The Spangler's membrane was used to evaluate the permeation of the effect of preparation of nano-phytophospholipid. Initially, the Spangler's membrane was prepared following the formula in table 2. All ingredients of Spangler's solution were melted in the water bath at a temperature of 80 °C and mixed until homogenous. The paper Whatman no.1 was soaked in the Spangler's solution for 15 min and dried at room temperature [16].

A phosphate buffer of pH 7.4 was filled in a receptor compartment. The temperature of the system was kept at 37 °C. The gel

preparation of 1 g was put on the membrane cell. The receptor compartment was stirred to homogenize the solution. 2 ml of solution in the compartment receptor was taken for total phenolic content measurement using Spectrophotometry UV Vis at 0, 5, 10, 15, 30, 60, 90, and 120 min. Each solution taken was refilled with a buffer solution of pH 7.4 [17].

Table 2: Composition of spangler's membrane

Ingredients	Weight (g)
Palmitic acid	10
Oleic acid	15
Stearic acid	5
Squalene	5
Paraffin	10
Cholesterol	5
White wax	15
Coconut oil	15

Statistic study

Each sample was analyzed by statistical analysis using ANOVA (*P≤0.05). All results were presented as the means (±SD) of at least three times experiments.

RESULTS AND DISCUSSION

Preparation of crude extract

The extraction used the reflux extraction method 2 x 2 h. The ethanol 70% was used as a solvent for phenolic content due to its polarity. The solvent was reported to influence the amount of total phenolic content. Ethanol was more efficient in extracting solvent for TPC than ethyl acetate and acetone [18].

Total phenolic content

The total phenolic content used was Folin-Ciocalteu reagent. The phenolic content in plant extract would react with the Folin-Ciocalteu reagent as a particular redox reagent. It forms a blue colour complex. The colour is read by Spectrophotometry UV Vis at the optimum wavelength [19]. The optimum wavelength of this study was 753 nm. Gained total phenolic content of kopasanda leaf extract was 117.214±3.054 mg/GAE. Depending on some previous studies, the total phenolic content of kopasanda leaf extract was around 58.34 mgGAE/g-242.3 mgGAE/g [2-4].

Preparation and evaluation of nano-phytophospholipidcomplex

Nanophyto-phospholipid complex, well known as phytosome is a novel drug delivery system (NDDS) [20]. Phytosomes have a role in converted water soluble phytoconstituents into a lipid-compatible molecular complex by reacting the phytoconstituent with phosphatidylcholine to increase the bioavailability of phytoconstituent [21]. The consideration of solvent addition depends on the solubility of both drug and phospholipid. Most aprotic solvents were recently replaced with ethanol for safety consideration [22].

The antisolvent preparation method was used to utilize the phyto-phospholipid complex. N-hexane was the required solvent to add to the precipitation process [23]. The preparation involved various ratios of kopasanda leaf extract and phospholipid. The optimum ratio was assessed by the entrapment efficiency (%). The entrapment efficiency (%) was explained in table 3.

Table 3: Entrapment efficiency (%)

Ratio Extract: phospholipid	Mean entrapment efficiency (%)
1: 1	99.842±0.003
1: 2	99.897±0.001
1: 3	99.815±0.001

*Data given in mean±SD of three experiments

The entrapment efficiency (%) was determined by the total phenolic compound incorporated in the neophyte-phospholipid complex system. The differences in the phospholipid and extract molarity ratio affect the value of entrapment efficiency (%). A higher ratio of phospholipid and extract molarity in the system results in more extract incorporated into the nanophyte-phospholipid complex [24, 25].

The optimum ratio of extract and phospholipid was elected to continue in reducing particle size process and gel formulation. The ratio between extract and phospholipid optimum was 1:2. The ultrasonic homogenizer was used to reduce the particle size with various sonication times (0 min, 1 min, 2 min, and 3 min). The effect of sonication time was displayed in table 4 and fig. 1 to 4.

Table 4: Particle size and polydispersity index of nanophyto-phospholipid complex

Sonication time (min)	Z-Average (nm)	Polydispersity index (PI)
0	514.7	0.629
1	173.5	0.393
2	216.1	0.410
3	130.1	0.394

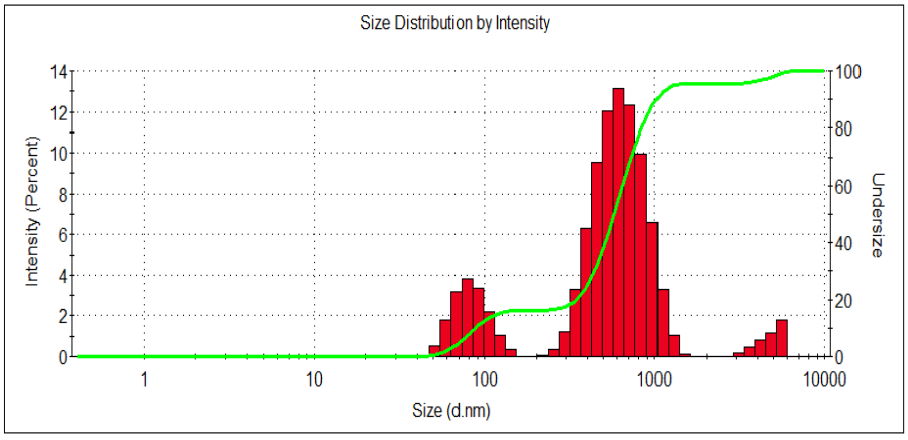

Fig. 1: Size distribution of sonication time 0 min

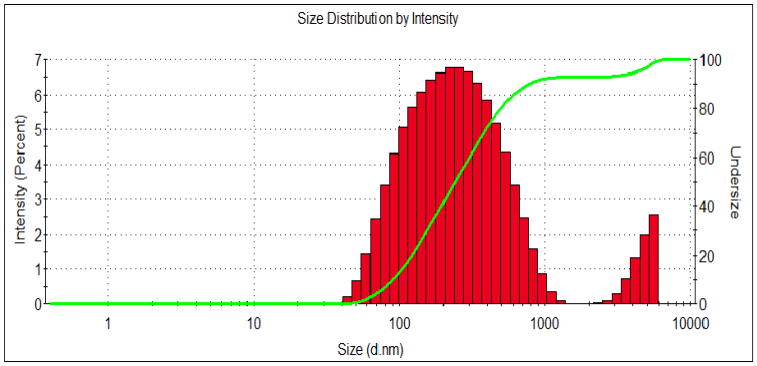

Fig. 3: Size distribution of sonication time 2 min

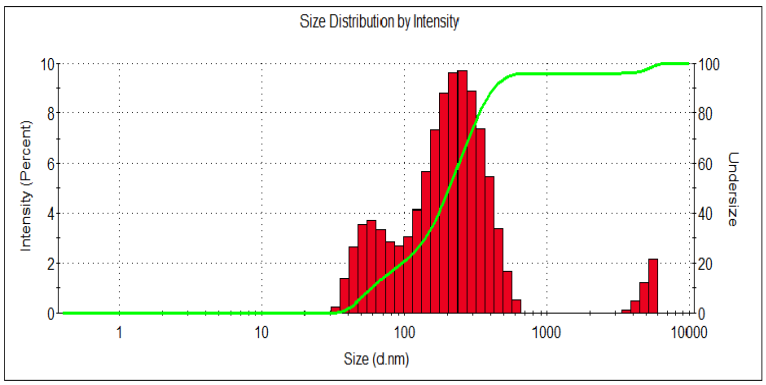

Fig. 2: Size distribution of sonication time 1 min

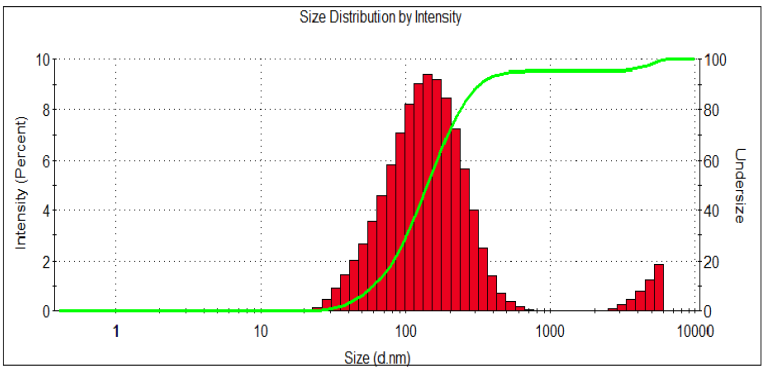

Fig. 4: Size distribution of sonication time 3 min

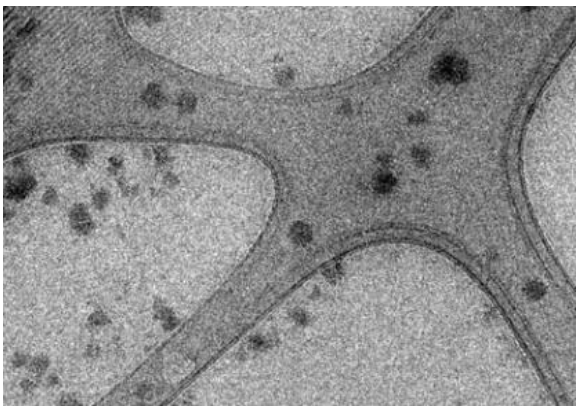

Fig. 5: Surface morphology using transmission electron microscope

The particle size of nanophyto-phospholipid complex containing kopasanda leaf extract showed that the 3-minute sonication time has a lower particle size than the other sonication time (p value=0.025). The longer sonication time resulted in stronger disintegration of particles due to a reduction in the particle size [26]. High lipid composition in the formula could increase the tendency to form agglomerates, which tend to the bigger size of vesicles. The results of the polydispersity index show the polydisperse system of nanophyto-phospholipid complex. A lower polydispersity index indicates a better homogeneity of particles [25]. As shown in table 4, the nanophyto-phospholipid complex with 1 min sonication time was the most homogeneous particle (p value=0.025). This result is close to nanophyto-phospholipid complexwith a 3 min sonication time.

Fourier transform infrared spectroscopy (FT-IR)

The purpose of characterization using FTIR was to discover the interaction between extract of kopasanda and phospholipid. The spectrum of kopasanda extract indicated the absorption at around

2100-2250 (C-H), 3700-3584 cm^{-1} (O-H), and 1650-1580 cm^{-1} (N-H). The spectrum of physical mixture between kopasanda extract and phospholipid were superimposable with the kopasanda extract and phospholipid spectrum. Besidesthat, the nanophyto-phospholipidspectrum involves only weak physical bonding [27]. The spectrum was explained in fig. 6.

Gel evaluation of nanophyto-phospholipid complex

Stability studies

The stability studies of gel formulation was illustrated in fig. 7 and fig. 8. The pH measurement for 9 d exhibited there was not significantly changed. Statistic data showed that the significance of pH during 9 d was more than 0.01. The pH of gel formulation was influenced by the composition of the formula. These results concluded that the pH of the gel was stable and had no chemical reaction or interaction during storage. The pH of gel formulation was suitable for skin application 4.5 and 7.5 [28]. The viscosity measurement of gel decreased during 9 d. Statistical data showed that there was a significant change during the storage. The significance value was less than 0.01. The viscosity during storage decrease of Carbopol and NaCMC base was influenced by the temperature during the storage [28, 29].

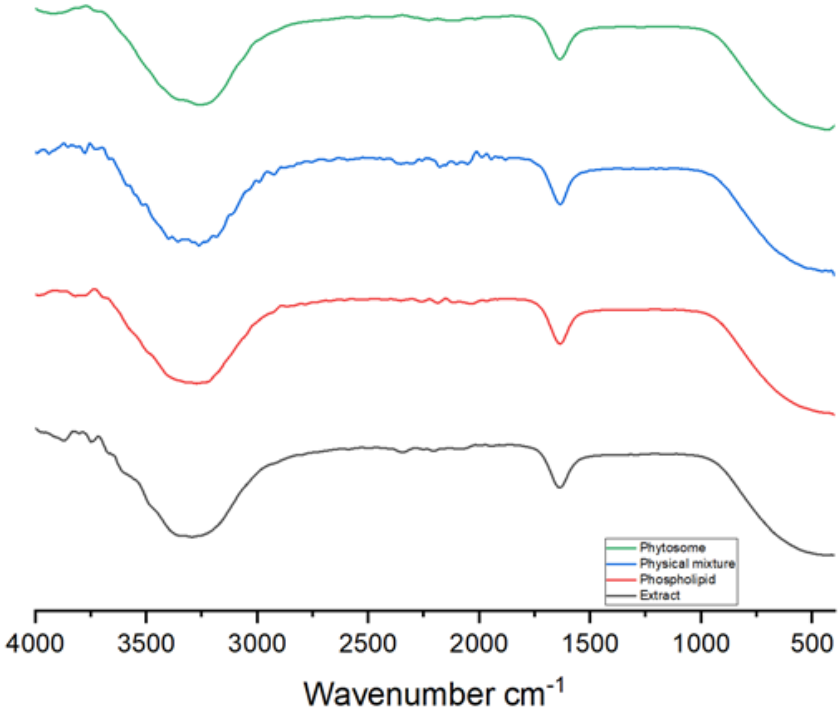

Fig. 6: Infrared spectrum

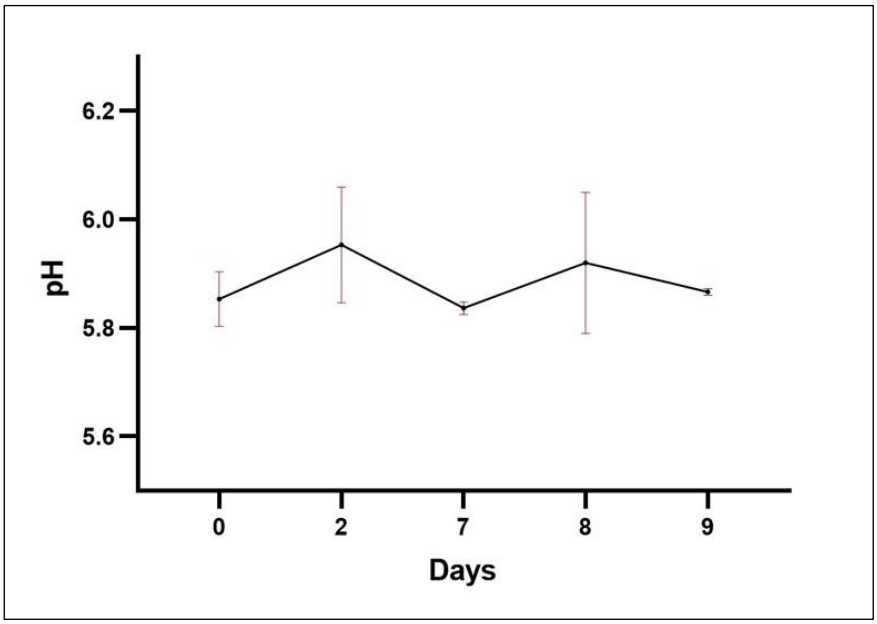

Fig. 7: pH stability of gel formulation during 9 days, *Data given in mean±SD of three experiments

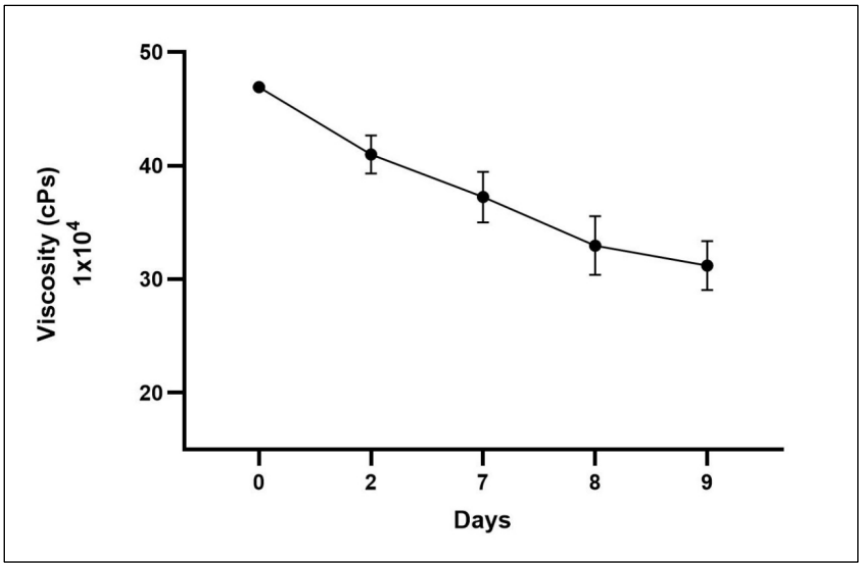

Fig. 8: Viscosity stability of gel formulation during 9 d, *Data given in mean±SD of three experiments

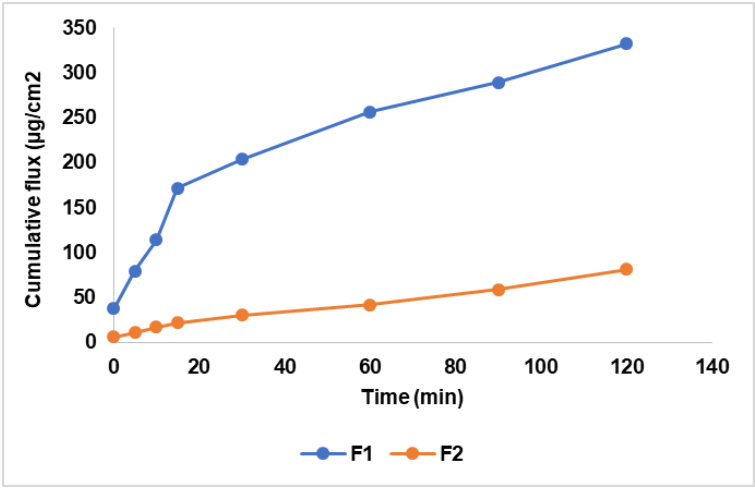

Fig. 9: Permeation profile of total phenolic content from nanophyto-phospholipid complex gel (F1) and extract gel (F2)

In vitro diffusion studies

The permeation profile was described in fig. 9. It indicated that nanophyto-phospholipid complex enhancing the phenolic compound. Nanophyto-phospholipid complex have a role in converted phenolic content as water-soluble phytoconstituents into a lipid-compatible molecular complex by reacting the phytoconstituent with phosphatidylcholine to increase the bioavailability of phytoconstituent [20].

CONCLUSION

The gel containing nanophyto-phospholipid complex formation resulted in the potential drug delivery system to increase the phenolic content permeation of kopasanda leaf extract. This study found that the optimum nanophyto-phospholipid complex has entrapment efficiency (%), particle size, and polydispersity index equal to 99.897±0.001%, 130.1 nm and 0.394, respectively. The morphology of the nanophyto-phospholipid complex was spherical and the complex formation was confirmed by the FTIR spectrum.

FUNDING

This research was funding by National Research and Innovation Agency of Indonesia.

AUTHORS CONTRIBUTIONS

Ridwan, S., Wahyuni, N. and Safwan, S. Conceived and design the experiment. Pasaribu, G. and Hajrin, W. Collected data and contributed tools analysis. Ridwan, S., Fitriana, M. and Roiyan, K. Performed the experiment. Ridwan, S. Wahyuni, N., Safwan, S., and Hajrin, W. Wrote the paper.

CONFLICT OF INTERESTS

All the authors report none to declare

REFERENCES

1. Gaurav V, Paliwal S, Singh A, Pandey S, Aqil M. Phytosomes: preparation evaluation and application. Int J Res Eng Sci. 2021;9(21):35-9.
2. Alara OR, Nour AH, Binti Abdul Mudalip SK. Screening of microwave-assisted batch extraction parameters for recovering total phenolic and flavonoid contents from *Chromolaena odorata* leaves through two-level factorial design. Indones J Chem. 2019;19(2):511-21. doi: 10.22146/ijc.40863.
3. Srinivasa Rao KS, Chaudhury PK, Pradhan A. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. Food Chem Toxicol. 2010;48(2):729-32. doi: 10.1016/j.fct.2009.12.005, PMID 20026159.

4. Akib NI, Hendra NS, Putri AE, Armadhani I, Adjeng AN. Preparation of phytosome of kersen leaves (*Muntingia calabura* L.) ethanol extract as antioxidant. J Farmasi Sains Praktis. 2021;7(3):393-404. doi: 10.31603/pharmacy.v7i3.6206.
5. Chandrakala V, Chandrakala V, Srinivasan S. An overview: recent development in transdermal drug delivery. Int J Pharm Pharm Sci. 2022;14(10):1-9. doi: 10.22159/ijpps.2022v14i10.45471.
6. Ittadwar PA, Puranik PK. Novel umbelliferone phytosomes: development and optimization using experimental design approach and evaluation of photoprotective and antioxidant activity. Int J Pharm Pharm Sci. 2017;9(1):218-28. doi: 10.22159/ijpps.2017v9i1.14635.
7. Alharbi WS, Almughem FA, Almehmady AM, Jarallah SJ, Alsharif WK, Alzahrani NM. Phytosomes as an emerging nanotechnology platform for the topical delivery of bioactive phytochemicals. Pharmaceutics. 2021;13(9):1475. doi: 10.3390/pharmaceutics13091475, PMID 34575551.
8. Barani M, Sangiovanni E, Angarano M, Rajizadeh MA, Mehrabani M, Piazza S. Phytosomes as innovative delivery systems for phytochemicals: a comprehensive review of literature. Int J Nanomedicine. 2021 Oct 15;16:6983-7022. doi: 10.2147/IJN.S318416, PMID 34703224.
9. Varadkar M, Gadgoli C. Preparation and evaluation of wound healing activity of phytosomes of crocetin from Nyctanthes arbour tristis in rats. J Tradit Complement Med. 2022;12(4):354-60. doi: 10.1016/j.jtcme.2021.10.002, PMID 35747356.
10. Ridwan S, Hartati R, Pamudji JS. Development and evaluation of cream preparation containing phytosome from amla fruit extract (Phyllanthus emblica L.). Int J App Pharm. 2023;15(4):91-8. doi: 10.22159/ijap.2023v15i4.48116.
11. Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Motevalizadeh Ardekani AM, Mostafazadeh A. Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on hela and BT-474 cell lines. Int J Mol Cell Med. 2014;3(2):102-7. PMID 25035860.
12. Rani A, Kumar S, Khar RK. Murraya koenigii extract loaded phytosomes prepared using antisolvent precipitation technique for improved antidiabetic and hypolidemic activity. Indian J Pharm Educ Res. 2022;56(2s):s326-38. doi: 10.5530/ijper.56.2s.103.
13. Damle M, Mallya R. Development and evaluation of a novel delivery system containing phytophospholipid complex for skin aging. AAPS Pharm Sci Tech. 2016;17(3):607-17. doi: 10.1208/s12249-015-0386-x, PMID 26285673.
14. Deleanu M, Toma L, Sanda GM, Barbalata T, Niculescu LS, Sima AV. Formulation of phytosomes with extracts of ginger rhizomes and rosehips with improved bioavailability antioxidant and anti-inflammatory effects *in vivo*. Pharmaceutics. 2023;15(4):1066. doi: 10.3390/pharmaceutics15041066, PMID 37111552.
15. Roy S, Bose S, Sarkar D, Mandal S, Sarkar S, Mandal SK. Formulation and evaluation of anti-acne gel containing *Murraya koeinigii* extract. Int J Curr Pharm Sci. 2020;12(4):108-13. doi: 10.22159/ijcpr.2020v12i4.39095.
16. Chaerunisaa AY, Abdassah M, Levita J, Febrina E, Hafni U. Piroxicam percutaneous permeation from gels through membrane models of shed snakeskin and cellulose. Indonesian J Pharm Sci Technol. 2021;8(2):66-75. doi: 10.24198/ijpst.v8i2.29017.
17. Maru AD, Lahoti SR. Formulation and evaluation of ointment containing sunflower wax. Asian J Pharm Clin Res. 2019;12(8):115-20.
18. Ezez D, Tefera M. Effects of solvents on total phenolic content and antioxidant activity of ginger extracts. J Chem. 2021;2021:1-5. doi: 10.1155/2021/6635199.
19. Blainski A, Lopes GC, DE Mello JC. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules. 2013;18(6):6852-65. doi: 10.3390/molecules18066852, PMID 23752469.
20. Vadnere GP, Phytosomes URM. A mini-review. Food Biotechnol. 2021;2(3):17-27.
21. Semalty A, Semalty M, Rawat MS, Franceschi F. Supramolecular phospholipids-polyphenolics interactions: the phytosome strategy to improve the bioavailability of phytochemicals. Fitoterapia. 2010;81(5):306-14. doi: 10.1016/j.fitote.2009.11.001, PMID 19919847.
22. Gnananath K, Sri Nataraj K, Ganga Rao B. Phospholipid complex technique for superior bioavailability of phytoconstituents. Adv Pharm Bull. 2017;7(1):35-42. doi: 10.15171/apb.2017.005, PMID 28507935.
23. Nanavati B. Phytosome: a novel approach to enhance the bioavailability of phytoconstituent. Asian J Pharm. 2017;11(03). doi: 10.22377/ajp.v11i03.1445.
24. Maryana W, Rachmawati H, Mudhakir D. Formation of phytosome containing silymarin using thin layer hydration technique aimed for oral delivery. Mater Today Proc. 2016;3(3):855-66. doi: 10.1016/j.matpr.2016.02.019.
25. Anwar E, Farhana N. Formulation and evaluation of phytosome loaded maltodextrin gum arabic microsphere system for delivery of Camellia sinensis extract. J Young Pharm. 2018;10 Suppl 56:S56-62. doi: 10.5530/jyp.2018.2s.11.
26. Putri NR, Firdausi SI, Najmina M, Amelia S, Timotius D, Kusumastuti Y. Effect of sonication time and particle size for synthesis of magnetic nanoparticle from local iron sand. J Eng Sci Technol. 2020;15(2):894-904.
27. Sarkar P, Bhagavatula S, VS. A brief research study on novel antibiotic-producing isolate from VIT Lake Vellore Tamil Nadu. J Appl Pharm Sci. 2014;4(1):61-5. doi: 10.7324/JAPS.2014.40110.
28. Safitri FI, Nawangsari D, Febrina D. editors. Overview: application of Carbopol 940 in gel. International Conference on Health and Medical Sciences (AHMS 2020). Atlantis Press; 2021. doi: 10.2991/ahsr.k.210127.018.
29. Wang F, Sun Z, LI X, Wang K, Chen D, LI Z. Study on factors influencing the viscosity of sodium carboxymethyl cellulose used in capsule mucous sealers. Geofluids. 2022;2022(1):1-9. doi: 10.1155/2022/4198311.