RESEARCH ARTICLE

Development of Kopasanda Leaves Extract (*Chromolaena odorata* (L.)) Phytosome Using Solvent Evaporation Method

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ABSTRACT

Kopasanda (*Chromolaena odorata* (L.) R.M.King&H.Rob) or siam weed exhibited various pharmacological activities such as anticancer, antidiabetic, and wound healing activities. The phenolic content of kopasanda leaf extract was high in the range of 56.15 mg GAE/g-242.3 mg GAE/g. But, it reported that phenolic compounds exhibited poor bioavailability because of their physicochemical properties. The phytosomes system improves the effectiveness of phytoconstituents. Aims of the research as prepare and evaluate the phytosome from kopasanda leaf extract. Kopasanda dried leaves were extracted by the sonication method. The solvent evaporation method was used in the phytosome preparation with various concentrations between extract and phospholipid (2:1; 1:1; 1:2; and 1:3). Furthermore, phytosome formation was analyzed for its entrapment efficiency, Polydispersity Index (PDI), particle size, spectroscopy infra-red (IR), and Transmission Electron Microscope (TEM) method. The total phenolic content (TPC) of crude was 110.969 ± 0.752 mgGAE/g. The optimum phytosome was gained with a concentration between extract and phospholipid (1:3). It resulted in the entrapment efficiency of 99.930 ± 0.001%, particle size, and polydispersity index equal to 206.5 ± 8.613nm and 0.528 ± 0.038. The morphology of the phytosome was spherical and the phytosome formation was verified by the IR spectrum by comparing the phytosome and the physical mixture of extract and phospholipid. The results obtained showed that phytosome containing kopasanda leaf extract was formed using the solvent evaporation method.

Keywords: Kopasanda leaves extract, Phytosome, a Solvent evaporation method.

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INTRODUCTION

Kopasanda, synonym as Siam weed, is a plant to be one of the 100 most intrusive natural weeds of badlands, roadsides, and other uncovered ranges within the world.[1] The plant grows straight to a height of 2-3 meters, with pointed, smooth stems that the department promptly.[2] Their morphological appearance in terms of blossom color, leaf shape, and the odor of the pulverized clears out. It belongs to *Asteraceae*, the sunflower family.[3]

In West Nusa Tenggara, Indonesia, it is widely used for wound healing. They usually treat their wound by direct application to injured skin. Furthermore, a kopasanda exhibits antipyretic, antiphlogistic, anti-proliferative, antimicrobial, antitumor, antipyretic, antidiabetic, antinociceptive, and wound healing activities.[1,4-9] Some studies showed that kopasanda leaves possess coumarin, phenols, tannins, flavonoids, steroids, isoflavone, flavone, flavanol, and chalcone group compounds. The TPC of kopasanda was in the range of 56.15 mg GAE/g-242.3 mg GAE/g, that significantly higher than gallic acid as the reference standard.[10-12] Phenolic compounds are divided into polyphenols and phenolic acids.[13] The phenolic compound was widely studied and showed various pharmacological activity.[14,15] However, it was reported that phenolic compounds have poor bioavailability due to their physicochemical properties.[16,17] Some studies presented that phytosomes as a drug delivery system exhibited great activity in increasing the bioavailability of phenolic.[18-20]

A stoichiometric amount reaction of phospholipid and standardized extract, such as phenolic compounds formed Phytosomes.[21] Phytosome systems have used some methods, including solvent evaporation, thin layer formation, mechanical dispersion, salting out technique, and lyophilization methods.[22] The solvent evaporation method is widely used for phytosomes preparation because it is convenient for laboratory scale, minimum solvent utilization, and also due to entrapment efficiency.[23]

This study aims to prepare and characterize the phytosome preparation containing kopasanda leaf extract using the solvent evaporation method.

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MATERIALS AND METHODS

The main ingredients used for phytosome preparation were dried leaves of kopasanda (kindly provided by PT. Moringa Organik Lombok) and Phospholipon 90 G (Lipoid GmbH, Germany). The reagent and chemical used were dichloromethane (Merck, Germany), ethanol 70%, ethanol 96% (Merck, Germany), aquadest, gallic acid standard (Sigma Aldrich, Singapore), Na_2CO_3 (Merck, Germany), and Folin-Ciocalteau (Merck, Germany). The instruments used for this study were an Ultrasonication (Elmasonic), vacuum rotary evaporator (Heidolph), UV-Vis spectrophotometer (Analytic Jena Specord 200 plus), Microcentrifugation (Labnet Inc.), Particle Size Analyzer (Nano Particle Analyzer Horiba SZ-100), Fourier Transform InfraRed (Parkin Elmer), and Transmission Electron Microscope (Port Features).

Preparation of Crude Extract

Dried leaves of 300 g were extracted using ethanol 70% as a solvent (1:10) using the sonication extraction method for 2x30 minutes. The solvent of the extract was evaporated using a vacuum rotary evaporator of 50 rpm and 50°C.

TPC Measurement

The TPC measurement of kopasanda leaf extracts using a reagent of Folin-Ciocalteu's by UV visible spectrophotometry. The standard of this measurement was Gallic acid with several concentrations (5, 10, 20, 30, 40, and 50 µg/mL). 300 µL of kopasanda extract with a concentration of 25 µg/mL in dH_2O was added with Folin-Ciocalteu's reagent in aqua dest (1:10). The mixture was incubated for 3 minutes, added Na_2CO_3 7.5%, incubated for 25 minutes, then was measured at wavelength 765 nm.[24]

Phytosomes Preparation

Optimization of extract: Phospholipid

The solvent evaporation method was used to phytosome of Kopasanda leaves extract preparation. Weight variations of extract and phospholipid were used (1:1; 1:2; 1:3; 2:1). Firstly, extracts and phospholipids were dissolved in 96% ethanol solvent and dichloromethane (3:2). The solution was refluxed at 50°C for 1-hour in a bottom flask 100 mL. Ethanol was then evaporated under vacuum for 1 hour at 50°C at a speed of 150 rpm. The resulting compounds were stored in a desiccator overnight at room temperature. Then, hydrate with water for 30 minutes at a speed of 210 rpm.[25]

Phytosome Evaluation

Percentage of entrapment efficiency

UV-vis Spectrophotometer method was used in entrapment efficiency measurements. As 1-mL of phytosomes in 5 mL of dH_2O was prepared and then centrifuged at 14,000 rpm for 1-hour at 27°C, then the supernatant was used for TPC by measurement with a UV-Vis Spectrophotometer at 765 nm. The entrapment efficiency (EE%) was analyzed following our previous research.[20]

Particle size and PDI analysis

Particle size and PDI values of phytosome solutions were analyzed by dynamic light scattering, which uses connected photon beams on a spectrometer that can detect light scattering fluctuations caused by Brownian motion. The scattering of light is monitored at 25°C with a scattering angle of 90°C.

IR spectroscopy analysis

Spectrum determination was obtained using a spectroscopy infrared. The analysis determination was carried out on extracts, phospholipids, a physical mixture, and phytosome. The sample was mixed with KBr at a ratio of 1:100. The mixture was stirred in the mortar until homogeneous, then pressed deeply into a mold and analyzed at a wavelength of 4000 to 400 cm^{-1}.

TEM analysis

TEM was used for the surface morphology of the sample. About 10 µL of phytosomes were dropped on top. The specimen was then covered with a 300 mesh grid and left for 1-minute to be adsorbed as a thin layer, with the excess sample removed with filter paper. Then, samples were treated with uranyl acetate solution (2% w/v). Samples were inserted into a TEM tool to take pictures.

Statistic study

The data were presented as the means ± Deviation Standard (SD). Analysis of Variance (ANOVA) was used for comparing the groups ($*p \leq 0.05$).

RESULT AND DISCUSSION

Crude extract

The sonication method was used as an extraction method for this research. This method is a green and advanced technology for compound extraction due to its fast processing speed, low cost, and environmental friendliness.[26] The yield extract in this study was 13.711%. The yield was significantly higher than the percolation extraction method which gained a yield of 5.7%.[27] The sonication can generate shock waves and shear forces generated by mechanical and cavitation effects to promote cell disruption, and to improve the dissolution rate and the amount of active ingredients during the extraction process.[28,29]

TPC result

The Folin Ciocalteu test is the method for determining the TPC of crude extracts using F-C reagent.[30] The standard of this measurement was gallic acid. In this study, the optimum wavelength for the complex solution between gallic acid and F-C reagent was 765 nm. The phenolic content of kopasanda leaves extract was 110.969 ± 0.752 mgGAE/g. Based on some research, the kopasanda leaves extract contained a TPC in the range of 56.15 mg GAE/g-242.3 mg/gGAE.[10-12]

Phytosome Preparation

Optimization of extract: Phospholipid

Phytosome is a drug delivery system by forms the complex between phytoconstituents and phospholipids.[31] Phospholipid

was a primer substance in phytosome preparation. It was an amphiphilic molecule that consisted of a positive head group and two neutral tail clusters.[32] In this research, the solvent evaporation method was used to form the phytosome. This method includes the mixture of phytoconstituents and organic solvent in the process at a temperature optimum. The temperature used for this study was 50°C, depending on the stability of the phenolic compound. The optimum time for this process was 1-hour, based on the optimum time for the reaction.[31] To discover the optimum variation of the mixture of phospholipid and extract, the weight of mixture variations was used (1:1; 1:2; 1:3; 2:1). The phytosome suspension resulted after the hydration process. The entrapment efficiency percentage of TPC was determined to find out the optimum mixture between phospholipid and extract.

Phytosome Evaluation

Entrapment efficiency (%)

The entrapment efficiency was a critical physicochemical characteristic of phytosome preparation. The microcentrifuge was performed to segregate the phytosome in phytosome suspension. The measurement method was used the indirect method.[32] The TPC was read in supernatant equal to the unentrapped phenolic content. The percentage of entrapment efficiency is shown in Table 1. The optimum variation was 1:3, with mean entrapment efficiency of 99.930 ± 0.001. The entrapment efficiency exhibited in the range of 80 to 100%, showed that the system has a good entrapment.[33] Depending on this study, higher phospholipids resulted in higher entrapment efficiency. It's due to the ability of phospholipid to entrap the phytoconstituent in forming a vesicle.

Phytosomes particle size and PDI

Optimum weight variation of phospholipid and extract was continued in the measurement of particle size and PDI. Dynamic light scattering (DLS) may obtain particle sizes and PDI, it is a useful method for a full characterization of nanoparticles.[34] DLS is used to evaluate the dispersion of nanoparticles in nanofluids as dangling drops and levitated droplets. This method can be used to determine particle diffusivity in pendant droplets when combined with a heterodyne detection scheme. Using an ultrasonic acoustic levitator for levitated droplets, dynamic light scattering studies are conducted, allowing the dynamics of the particle number density fluctuations inside the droplet to be evaluated.[35]

The size range index of particles is shown by the PI. A very broad particle size range is indicated by a PI score >0.7 (a value near 1.0 indicates a highly polydisperse sample), whereas a *p-value* <0.05 suggests a very monodisperse standard (a value near zero demonstrates a uniform sample).[36] In contrast, the Z average means the particle size of the vesicle. The particle size in the range of 100 to 1000 nm was classified as a large unilamellar vesicle (LUV).[37] In this study, the mean of particle size and polydispersity index of optimum phytosome was 206.5 ± 8.614 and 0.528 ± 0.038, respectively. It concluded that phytosomes were classified as LUV and monodispersed samples. The particle size and PDI of phytosome were described in Table 2.

Spectroscopy infrared

Characterization using IR Spectroscopy was aimed to find out the interaction between extract and phospholipid. The spectrum of kopasanda extract indicated absorption at around 3700 to 3584 cm^{-1} (O-H), 2100 to 2250 (C-H stretching), and 1650 to 1580 cm^{-1} (N-H bending).

The spectrum of FTIR of the physical mixture is superimposable with the kopasanda extract and Phospholipon 90G (phospholipid) spectrum (Figure 1). Furthermore, the phytosome spectrum involves only weak physical bonding and exhibited a decreasing of %transmittance to physical mixture due to the entrapment of kopasanda in phytosome.[38]

TEM result

TEM was a critical analysis to provide information about the morphology of the vesicle. It is broadly used for phytosome visualization.[39] Depending on TEM visualization, the phytosome of Kopasanda showed a spherical morphology and had a particle size of around 200 nm (Figure 2). It was confirmed with particle size data using a particle size analyzer that the phytosome of Kopasanda had a particle size average of

Table 1: Entrapment efficiency results with variation extract and phospholipid

Extract: Phospholipid ratio	*Entrapment efficiency average (%)*
2:1	99.813 ± 0.003
1:1	99.908 ± 0.004
1:2	99.919 ± 0.003
1:3	99.930 ± 0.001

Table 2: Particle size and polydispersity index of phytosome

Replicate	*Z-Average (nm)*	*PDI*
1	199.2	0.565
2	204.3	0.529
3	216.0	0.489
Mean of particle size	206.5 ± 8.614	0.528 ± 0.038

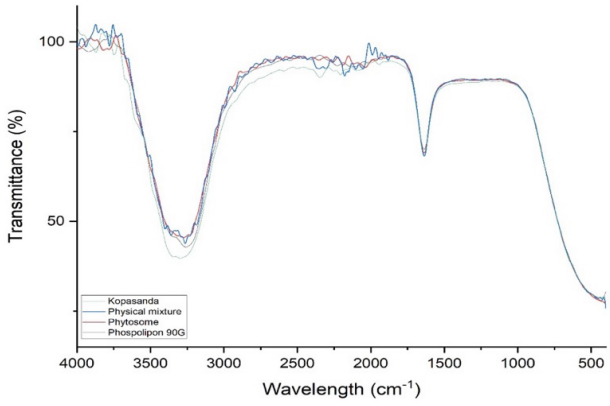

Figure 1: Spectrum FTIR

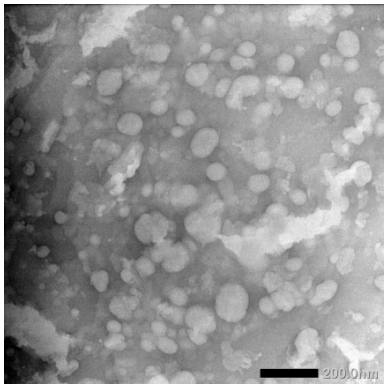

Figure 2: TEM visualization of phytosome

206.5 ± 8.614. The particle size average was calculated from the average of particle size analyzer data which was done in three replications (Table 2).

CONCLUSION

The optimum phytosome was gained with a concentration between extract and phospholipid (1:3). It resulted in the entrapment efficiency of 99.930 ± 0.001%, particle size, and polydispersity index equal to 206.5 ± 8.613 nm and 0.528 ± 0.038. The morphology of the phytosome was spherical and the phytosome formation was confirmed by the FTIR spectrum by comparing the phytosome and the physical mixture between the extract and phospholipid.

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