Submitted : 13 Feb 2021
Revised : 8 March 2021
Accepted : 18 May 2021
Published : 4 July 2021

Generics : Journal of Research in Pharmacy
Vol 2, Edisi 1, Tahun 2021
e-ISSN : 2774-9967

Phytosome as Cytotoxic agent delivering system : A Review

Widyandani Sasikirana[1]*, Eva Annisaa[1]', Nuraini Ekawati[1],

[(1)]Pharmacy Study Programme, Faculty of Medicine, Diponegoro University, Indonesia

Corresponding author : widyandani.sasikirana@live.undip.ac.id , Fax: +62 24 – 76928010

ABSTRACT

Along with the rapid development of herbal medicine formulas, an appropriate drug delivery system is needed to increase its bioavailability. One of them used the phytosome. As a delivery system, it was known to be able to increase the bioavailability of phytomedicine by increasing the permeability of herbal compounds on cell membranes so the absorption of the compound will be increased.

In its development, the phytosome formula was effective for delivering cytotoxic agent compounds, such as quercetin, diosgenin, icariin, tocopherol, and others. Besides, some of these formulas have also been commercialized and patented. The effectiveness and ease of manufacture have made phytosomes a promising drug delivery system in the development of cytotoxic drugs.

Keywords: phytosome, cytotoxic, drug delivery system

Introduction

Phytotherapy has evolved rapidly over the decades. Active compounds from plants are known to have promising effectiveness both *in vitro* and *in vivo*. However, most of these compounds have low bioavailability. For example, quercetin (a flavonoid) which was found to have a cytotoxic effect *in vitro* turned out to have low water solubility, inadequate permeability, and degrades rapidly due to first-pass metabolism (Lestari *et al.*, 2017; Kartivashan *et al.*, 2016). The low bioavailability of quercetin is influenced by low lipid solubility because the sugar clusters cause it to be hydrophilic, the large quercetin molecule makes it difficult for passive diffusion in the intestine to the bloodstream, and degradation of phenol groups by gastrointestinal bacteria destroys quercetin (Rasaie *et al.*, 2014). This makes quercetin as one of the flavonoids ineffective as a drug for therapy. This deficiency was answered by the development of drug delivery systems using phytosomes.

Phytosome

Phytosome was first introduced by Indena S.P. A, Italy, which stated that increasing the bioavailability of phytomedicine can be done by the incorporation of phospholipids with standard extracts. Phytosome is a formula developed to increase the absorption and bioavailability of plant extracts and water-soluble phytoconstituents into phospholipids to produce molecularly compatible lipid complexes. The lipid complex will protect the active ingredients of the drug from degradation during the absorption process without having to reduce the phytochemical components of the fraction. The phytosome is different from the liposome. Where in the liposome, no chemical bonds are formed. Phosphatidylcholine molecules are around the water-soluble substance. There are hundreds or even thousands of

phosphatidylcholine molecules around the water-soluble substance. Whereas in the phytosome, phosphatidylcholine and its components in plants will form a molecular complex with a ratio of 1: 1 or 1: 2 depending on the substance that forms the complex, followed by chemical bonds. Because phosphatidylcholine is a component that can dissolve in membrane lipids and water, it can increase the bioavailability of the extract by properly conveying it to the membrane lipids so that it can quickly enter the circulation system (Ajazuddin, 2020).

Advantage of phytosome

It is known that phytosomes are effective in increasing the bioavailability of herbal compounds. The advantages of using phytosome as a drug delivery system are summarized in the following points.

1. **Increase bioavailability.** The bioavailability of herbal extracts increases when formulated with a phospholipid complex and increases absorption in the intestinal tract (Pawar and Bhangale, 2015).
2. **Increase absorption.** The presence of phospholipid complexes can increase the penetration of hydrophilic herbal extracts from the intestinal lumen (Kumar *et al.,* 2017).
3. **Safe and cost-effective.** Phosphatidylcholine, which is used as a complex in the manufacture of phytosomes, is a part of the cell membrane, so this formula is safe. A synergistic effect can also be obtained from the ability of phosphatidylcholine as a hepatoprotective (Kumar *et al.,* 2017). This formula can also be developed as a cost-effective commercial cosmetic (Pawar and Bhangale, 2015).
4. **Improve diffusion through the skin.** The phytosome formula can also be used to increase permeation through the skin because it's phospholipid on the formula (Pawar and Bhangale, 2015; Kumar *et al.,* 2017). It can increase the gum of drugs through the skin in the transdermal drug delivery system (Kumar *et al.,* 2017).
5. **Low dose.** Due to the ease of penetrating the gastrointestinal membrane permeability, the phytosome formula can have maximum effect at low doses. So that doses requirement is reduced (Pawar and Bhangale, 2015).
6. **Low risk.** There are few toxicity data from small scale production6. Meanwhile, on large-scale production, no toxicity data were found (Pawar and Bhangale, 2015).
7. **Enhance the liver targeting.** Phytosome formula can increase the solubility of phytoconstituent on bile, then it can enhance the liver targeting (Kumar *et al.,* 2017) .
8. **Easily developed as a commercial product.** This technology is easy to develop because it is easy to manufacture and there are no complex practical speculations (Kumar *et al.,* 2017; Kumar *et al.,* 2020).

Preparation Methods

A phytosome can be made in the following ways.

1. **Anti-solvent precipitation.** In this process, phosphatidylcholine and extracts with a molar ratio will be dissolved in an organic solvent, such as 20 mL dichloromethane, acetone. Then, the mixture is refluxed at a certain temperature and time according

to the research design. The reflux product is concentrated and treated with an anti-solvent such as n-hexane to obtain a precipitate. The precipitate was then dried using a vacuum desiccator or made an affiliation (Telange *et al,* 2016; Nabil *et al.,* 2020).

2. **Cosolvent**. The extract and phosphatidylcholine are dissolved in an organic solvent, such as methanol. The mixing was carried out by stirring using a magnetic stirrer for 1 hour (Shahira *et al.,* 2018).
3. **Salting out.** Ethanol is used to dissolve the extract and phosphatidylcholine, then mixing is done by stirring. Precipitation formation is carried out by adding n-hexane to the mixture to form precipitate phytosome (Singh *et al.,* 2014).
4. **Thin layer hydration.** Fraction and phosphatidylcholine were dissolved in methanol and cholesterol was dissolved in dichloromethane. The mixture is slowly then evaporated with a rotary evaporator at 45°C until the solvent is completely evaporated and a thin dry film is formed on the bottom of the bottle. Then, the thin layer of lipid formed is flowed with nitrogen gas and stored at room temperature for one night before being treated for hydration. The film layer was hydrated with aquabidest on a rotary evaporator at 45°C. The optimization of the method to determine the particle size was also carried out using sonification and homogenizer (Rasaie *et al.,* 2014).
5. **Solvent evaporation.** The extract and phosphatidylcholine were dissolved in ethanol and refluxed for 2 hours using a vacuum rotary evaporator at 30°C, 120 rpm. The residue is then hydrated with aquadest to obtain phytosome suspension (Singh *et al.,* 2014).

Phytosome As A Cytotoxic Agent Delivering System

In its development, phytosome can be used as a delivery system for cytotoxic agents derived from herbs. **Shalini, et al., 2015** showed that the IC_{50} value of the extract of *Terminalia arjuna* bark and quercetin positive control experienced a significant decrease after being formulated using phytosome. The IC_{50} of the extract decreased from 25 ug/ml to 15 ug/ml, while the IC_{50} quercetin decreased from 2 ug/ml to 0.7 ug/ml. This indicates that the use of phytosomes as drug delivery agents can increase its bioavailability so that inhibition of MCF-7 cancer cell lines can occur at low doses (Shalini *et al.,* 2015).

Liang Xu, et al. (2019) was studied the synthesis of a diosgenin derivative (Di) and screening FU-0021-194-P2 (P2) as one of its derivatives. P2 was then prepared with phytosomes (P2Ps) to increase the water solubility of P2, as well as Di. Its cytotoxic inhibition activity was carried out through human non-small-lung cancer A549 and PC9 cells. The results showed that P2Ps can inhibit lung cancer cells more effectively than Di-phytosome after 72 hours of incubation through induction of cell cycle arrest and apoptososis.

Patil, et al. (2017) made a phytosome for *Carica papaya* extract. *Carica papaya* extract was formulated with a phytosome complex then analyzed for its cytotoxic effect on human leukemia cell line K-562 using the Sulforhodamine B (SRB) assay. The IG_{50} of the aqueous extract and formula showed values of 75.2 ug/ml and 48.4 ug/ml. These results indicate that the phytosome formula is better as an

anticancer than the water extract of *Carica papaya*.

Yang Li, et al. (2014) worked on mitomycin C-phytosome. Mitomycin C (MMC) is formulated with complex phosphatidylcholine to form an MMC-loaded phytosome which is then given the addition of a surface-functional form of folate-PEG (FA-PEG). FA-PEG-MMC-loaded phytosome has been shown to increase cellular uptake in HeLa cells and high accumulation in H22 tumor-bearing mice. This indicates an increase in anti cytotoxic activity in vitro and in vivo in the formula compared to injection of MMC.

Sundaraganapathy, et al. (2016) was studied root formulation of *Clerodendron paniculatum* Linn extract using phytosome has been carried out and evaluation of its cytotoxic activity was seen using Dalton's lymphoma ascites cell in vivo. The results showed that the phytosome formulation provided more potent inhibition in cancer cells than the extract.

Nazeer, et al. (2017) showed that methanolic extract of *Allium sativa* which contains diallyl disulfide and other phenolic compounds were formulated by phytosome. Its formula showed IC90 and IC_{50} against the MCF-7 cell line at 108,5 ug/ml and 25,76 ug/ml. The diallyl disulfide was confirmed by HPLC and GC-MS analysis, then the phytosome complex was studied by FTIR and SEM analysis.

Alhakamy et al. (2020) was studied Icariin (flavonol glycoside). It has been formulated by phytosome to improve its potential as a cytotoxic agent. ICA-Phytosomal showed significantly disturbed mitochondrial membrane potential and cellular of caspase 3. Besides that, the reactive oxygen species and apoptosis were enhanced by its formulation. This study has used OVCAR-3 cells ovarian cancer cells.

Alhakamy et al. (2020) worked on Thymoquinone (TQ, natural polyphenol). It has been formulated by phytosome using phospholipon® 90 H. Optimisation of size confirmed by TEM analysis. Furthermore, cytotoxic activity (IC_{50} value) showed at 4,31 ± 2,21 Um in A549 human lung cancer cells. Apoptosis and necrosis were increased by activation of caspase 3 and the reactive oxygen species was increased in A549 cells.

D. Gallo et al. (2003) was showed an effect of Silipide (Sylibine complex) on human ovarian cancer (HOC) in vivo. Antiangiogenenic activity has been shown by downregulating and upregulating the Vascular Endothelial Growth Factor (VEGF) and Angiopoietin-2. VGEF concentration was a consistent decrease in the tumor specimen after treatment with Silipide. It indicated that Silipide was a great candidate for recurrent ovarian cancer.

Narges Mahmoodi et al. (2014) was studied the expression of ESR on breast cancer after treated with Sylibin (natural cytotoxic agents) and its phytosome. The study showed that sylibin-phosphatidylcholine complexes give 2.5-3 times more effective to inhibited cell growth on the T7D cell line and ESR was down regulated.

Sabzichi et al. (2014) was studied Luteolin phytosome to optimization of Doxorubicin for inhibited MDA-MB 231cells (Human breast cancer cell line) by downregulated Nrf2 expression. The presence of luteolin-phytosome can suppress the Nrf2 expression, as result cells become sensitive to the drug (Doxorubicin).

Hou, Z et al. (2013) was studied MitomycinC-soybean phosphatidylcholine. The design of Mitomycin C-soybean phosphatidylcholine (MMC-SPC) was developed by the combination of the solvent evaporation method and nanoprecipitation. The cytotoxic assay has been shown that MMC-SPC inhibited the H22 cell line. The antitumor effect in vivo indicated that the MMC-SPC had a great curative inhibitory effect on tumor growth and have a lethal effect on hepatocellular carcinoma cells by histopathology study.

Recent Products

Currently, phytosome products have been developed as anticancer and cytotoxic. Table 1 is a commercial product of phytosome as a cytotoxic and anticancer.

Patents regarding the phytosome curcumin complex-piperine have also been filed in Europe by Di Pierro, Fransesco, (2010). Several formulas of the curcumin and piperine phytosomes are made in the form of film-coated tablets, capsules, sachets, two later controlled-release tablets, orodispersible formulations, and sterile. pyrogen-free injectable solution.

Table 1. Commercial products of phytosome as cytotoxic and anticancer (Singh *et al.*, 2020; Ravi *et al.*, 2018; Karimi *et al.*, 2015)

Source	Phytoconstituents	Products	Indication
Cucurbita pepo	Tocopherol. Steroids, carotenoids	Cucurbita Phytosome™	Anti inflammatory, benign prostatic hyperplasia
Glycine biloba	Genistein dan daidzein	Soyselect Phytosome™	Antiangiogenic, anticancer, cardioprotective, immunostimulatory dan hypocholesterolemic
Olea europea	Verbascoside, tyrosol, hydroxytyrosol	Oleaselect Phytosome™	Antioxydant, antihiperlipidemia, Anticancer dan Antiinflammatory
Curcuma longa	Curcumin	Curcumin Phyosome™, Curcuvet® (Meriva®)	Antiinflamatory, osteoatritis, anticancer
Serenoa repens	Phytosterol	Phytosterols	Noncancerous prostate enlargement
Camelia sinensis	Epigallocatechin 3-o-gallate	Greenselect Phytosome	Systemic antioxidant, protection against cancer
Vitis vinifera	Procyanidins	Leucoselect phytosome	Nutraceutical, antioxidant, anticancer

CONCLUSION

Phytosome is a good delivery system for cytotoxic agents. Many research showed that phytosome can be inhibited by many cell lines more than pure cytotoxic agents. Its ability to increase the absorption of natural compounds and be easily developed made it be promising commercial products. By great design, it would be a safe and acceptable cytotoxic product.

ACKNOWLEDGEMENT

This project was funded by Diponegoro University (UNDIP), Indonesia (Grant no. 233-75/UN7.6.1/PP/2020). Therefore, the author acknowledge with thanks UNDIP for technical and financial support.

REFERENCES

Ajazuddin, S.Saraf., 2020, Review : Aplication of novel drugs delivery system for herbal formulations. *Fitoterapia* 81 680-689. DOI: 10.1016/j.fitote.2010.05.001

Alhakamy, Nabil A; Badr-Eldin, Shaimaa M; Fahmy, Usama A; Alruwaili, Nabil K; Awan, Zuhier A; Caruso, Giuseppe; Alfaleh, Mohamed A; Alaofi, Ahmed L; Arif, Faris O; Ahmed, Osama A.A; Alghaiith, Adel F. 2020. Thymoquinon-Loaded Soy-Phospholipid-Based Phytosomes Exhibit Anticancer Potential against Human Lung Cancer Cells. *Pharmaceutics*. 12, 761.

Alhakamy, Nabil A; Fahmy, Usama A; Badr-Eldin, Shaimaa M; Ahmed, Osama A.A; Asfour, Hani Z; Aldawsari, Hibah M; Algandaby, Mardi M; Eid, Basma G; Abdel-Naim, Ashraf B; Awan, Zuhier A; Alwuraili, Nabil K; and Mohamed, Amir I. 2020. Optimized Icariim Phytosomes Exhibit Enhaced Cytotoxicity and Apoptosis-Inducing Activities in Ovarian Cancer Cells. *Pharmaceutics*. 12, 346.

Bhavesh Shah., S. B. Puranik., Raghuchandan H S. 2020. Preparation and Evaluation of Curcumin Phytosomes ,*International Journal of Pharmacy & Pharmaceutical Research.* Ijppr.Human. Vol. 17 (4): 767-792.;

D. Galloa, S. Giacomellia, C. Ferlinia, G. Raspaglioa, P. Apollonioa, S. Prisleia, A. Rivab, P. Morazzonib, E. Bombardellib, G. Scambia. 2003. Antitumour activity of the silybin phosphatidylcholine complex against human ovarian cancer. *Eur. J. Cancer*. 39, 2403–2410.

Di Pierro, Fransesco. 2010. Compositions containing a phospholipid-curcumin complex and piperine as chemosensitizing agent. *European Patent Application.* EP. 2228062A1

Gs, Ravi; Charyulu R, Narayana; Dubey, Akhilesh; Hebbar, Srinivas; Mathias, Avril Candida. 2018. Phytosome: A Novel Molecular Nano Complex Between Phytomolecule and Phsopholipid as a Value added Herbl Drug Delivery System. *Int. J.Pharm.Sci.Rev.Res*, 51(1), July-August. Article No. 14, Pages: 84-90

Hou, Z; Li, Y; Huang Y; Zhpu C; Lin J; Wang Y; et al. 2013. Phytosome Loaded with Mitomycin C-soybean Phosphatidylcholine Complex Developed for Drugs Delivery. *Molecular Pharmaceutics*, 10(1): 90-101. doi: 10.1021/mp300489p

Karimi, Nayer; Ghanbarzadeh, Babak; Hamishehkar, Hamed; Keivani, Fatemeh; Pezeshki, Akram;

Gholian, Mohammad Mahdi. 2015. Phytosome and Liposome : The Beneficial Encapsulation System in Drug Delivery and Food Application. *Applied Food Biotechnology*. 2(3): 17-27.

Karthivashan, Govindarajan., Masarudin, Mas Jaffri ., Kura, Aminu Umar ., Abas, Faridah Abas., Fakuraz, Sharida. 2016. Optimization, formulation, and characterization of multiflavonoids-loaded flavanosome by bulk or sequential technique. International *Journal of Nanomedicine*. vol.11, 3417. DOI: : 10.2147/IJN.S112045

Kumar, Arun; Kumar, Bimlesh; Singh, Sachin Kumar; Kaur, Barinder; Singh, Surabh. 2017. A Review On Phytosomes : Novel Approach For Herbal Phytochemicals. *Asian Journal Of Pharmaceuticwl and Clinical Research*. Vol 10, Issue 10, 41-47.

Kumar,Sudhir., Baldi, Ashish., Sharma, Dinesh Kumar. 2020. Phytosomes: A Modernistic Approach for Novel Herbal Drug Delivery Enhancing Bioavailability and Revealing Endless Frontier of Phytopharmaceuticals, *J Develop Drugs*, Vol. 9 Iss. 2 No: 195

Lestari, Ayu., Anwar, Effionora., Harahap, Yahdiana. 2017. Design and Formulation Quercetin Formula In The Phytosome System as Novel Drug Delivery. *International Journal of ChemTech Research*. 10(06) : 148-151

Liang Xu; Dekang Xu; , Ziying Li; Yu Gao; Haijun Chen. 2019. Synthesis and potent cytotoxic activity of a novel diosgenin derivative and its phytosomes against lung cancer cells 2019. *J. Nanotechnol*. 10, 1933-1942. doi:10.3762/bjnano.10.189.

Nabil A. Alhakamy; Usama A. Fahmy; Shaimaa M. Badr-Eldin; Osama A. A. Ahmed ; Hani Z. Asfour; Hibah M. Aldawsari; Mardi M. Algandaby; Basma G. Eid; Ashraf B. Abdel-Naim; Zuhier A. Awan; Nabil K. Alruwaili; Amir I. Mohamed. 2020. Optimized Icariin Phytosomes Exhibit Enhanced Cytotoxicity and Apoptosis-Inducing Activities in Ovarian Cancer Cells. N. *Pharmaceutics*, 12, 346; doi:10.3390/pharmaceutics12040346

Narges Mahmoodi, Nasrin Motamed, Seyed Hassan Paylakhi. 2014. The Comparison of The Effects of Silybin and Silybin-Phosphatidylcholine on Viability and ESR Expression in Human Breast Cancer T47d cell line. *Cell J.* 16(3):299– 308.

Nazeer, Abdul Azeez; Veeraiyan, Sivapriya; Vijaykumar, Sudarshana Deepa. 2017. Anti-cancer Potency and Sustained Release Of Phytosomal Diallyl Disulfide Containing Methanolic Allium sativum Extract against Breast cancer. *International Research Journal Of Pharmacy*. 8 (8).

Patil, Swati., Ayare, Priyanka. 2017. Carica Papaya : formulatiom and Evaluation of new dosage form design. *International Journal of Pharmaceutical Sciences and Research*. ISSN : 0975-8232

Pawar, H.A., Bhangale, B.D. 2015. Phytosome as a Novel Biomedicine: A Microencapsulated Drug Delivery System. *J Bioanal Biomed*, 7:1 DOI: 10.4172/1948-593X.1000116

Rasaie, Solmaz ., Ghanbarzadeh, Saeed ., Mohammadi, Maryam., Hamishehkar, Hamed. 2014. Nano Phytosomes of Quercetin: A Promising Formulation for Fortification of Food Products with Antioxidants, , *Pharmaceutical sciences*, 20, 96-101

Rasaie, Solmaz., Ghanbarzadeh, Saeed., Mohammadi, Maryam., Hamishehkar, Hamed. 2014. Nano Phytosomes of Quercetin: A Promising Formulation for Fortification of Food Products with Antioxidants. *Pharmaceutical sciences*, 20, 96-10.

Sabzichi M, Hamishehkar H., Ramezani F., Sharifi S., Tabasinezhad M., Pirouzpanah1 M., Ghanbari P., Samadi N. 2014. Luteolinloaded phytosomes sensitize human breast carcinoma MDAMB 231 cells to doxorubicin by suppressing Nrf2 mediated signalling. *Asian Pacific J. Cancer Prev.* 15(13):5311– 5316.

Shahira F El-Menshawe; Adel A Ali; Mohamed A Rabeh; Nermeen M Khali. 2018. Nanosized soy phytosome-based thermogel as topical anti-obesity formulation: an approach for acceptable level of evidence of an effective novel herbal weight loss product, *International Journal Of Nanomedicine*, vol13: 307-318. https://doi.org/10.2147/IJN.S153429

Shalini, Sharma., Kumar, Roy Ram., Birenda, Shrivastava. 2015. Antiproliferative effect of Phytosome complex of methanol extract Terminalia Arjuna bark on Human Breast Cancer Cell line (MCF-7). *Int. J. Drug Dev. & Res.*, January-March. 7 (1): 173-182. ISSN 0975-9344.

Singh R, Parpani S, Narke R, Chavan R. 2014. Phytosome: recent advance research for novel drug delivery system. *Asian J Pharmaceut Res Health Care*. 2:15–29.

Singh R, Parpani S, Narke R, Chavan R. 2014. Phytosome: recent advance research for novel drug delivery system. *Asian J Pharmaceut Res Health Care*. 2:15–29.

Singh, Anju; Ray, Avishikta; Mishra, Rakhi; Biswal, Pramod K; Yadav, Reenu; Ghatuary, Shailesh Kumar. 2020. Phyto-Phospholipid Complexes : Innovative Approach to Enhace the Bioavaibility and Therapeutic Efficacy of Herbal Extract. *Pharmaceutical and Bioscience Journal*. Vol. 8(4), 01-09.

Sundaraganapathy; Leena P N. 2016. Development and Evaluation of Anti-Cancer Activity of Phytosome Formulated from the Root Extract of Clerodendron Paniculatum Linn. 2016. *International Journal of Pharmacognosy and Phytochemical Research*. 8(11); 1778-1781. ISSN : 0975-4873.

Telange, Darshan R.; Patin, Arun T.; Pethe, Anil M.; Fegade, Harshal; Anand, Sridhar; and Dave, Vivek S. 2016. Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential. *European Journal of Pharmaceutical Sciences*, 108, 36-49

Yang Li; Hongjie Wu; Mengmeng Jia; Fei Cui; Jinyan Lin; Xiangrui Yang; Yange Wang; Lingfeng Dai; Zhenqing Hou. 2014. Therapeutic Effect of Folate-Targeted and PEGylated Phytosomes Loaded with a Mitomycin C-Soybean Phosphatidyhlcholine Complex. *Mol. Pharmaceutics*. 11, 3017-3026. dx.doi.org/10.1021/mp5001873.