Encapsulation of cinnamic acid and galangal extracts in coconut (Cocos nucifera L.) liposomes

D Hudiyanti, M F Al-Khafiz and K Anam
Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Semarang 50275, Indonesia

Corresponding author’s e-mail: dwi.hudiyanti@live.undip.ac.id

Abstract. Coconut liposomes are used to encapsulate cinnamic acid, red and white galangal extracts. Their encapsulation efficiencies and leakage rates are explored. The encapsulation efficiencies of cinnamic acid, red galangal and white galangal are 62 %, 42 % and 52 % respectively. Addition of 7 % cholesterol increases the encapsulation efficiency of cinnamic acid red galangal and white galangal to 70 %, 50 % and 68 % correspondingly. The red galangal extract has as much leakage rate as the cinnamic acid compare to white galangal extract at all liposomal systems.

Keywords: cinnamic acid, encapsulation, galangal extract, leakage rate, liposome.

1. Introduction
Cinnamic acids are semi polar unsaturated carboxylic acid compounds. They are used as precursor for the synthesis of valuable cinnamic esters, i.e. cinnamic acid derivatives (CAD). Cinnamic acid and cinnamic acid derivatives (CAD) have pharmacological profile range from anti TB, antidiabetic, antioxidant, antimicrobial, UV rays absorbent, and anticancer agents [1–3]. Cinnamic acid are commonly found in herbs such as galangal, either red or white galangal. They were obtained in methanol extract of galangal rhizome [1]. In red galangal rhizome there are 9–11% cinnamic acid and its derivatives while white galangal contains 2–5% [2]. Cinnamic acid and CAD are easily degraded by oxygen, change of pH, media, and enzymatic activity [1]. The degradation can be prevented by encapsulating them into a carrier system, namely liposomes.

Liposomes are spherical structures made out from self-assembly process of phospholipid molecules. Due to the nature of phospholipid molecules liposomes have polar and nonpolar compartments thus they are able to carry polar and nonpolar materials. This ability to carry different type of molecules or active substances makes them important in pharmaceutical industries since encapsulation in liposomes structures shields the molecules from early inactivation, degradation and dilution in the blood circulation [4]. So far there are more than a few liposomal-based drugs in the markets and in clinical trials [5]. They are used for assisting delivery vehicle in numerous drugs for various diseases. Some of them are Daunorubicin for leukemia and solid tumors [6,7], Cytarabine or cytosine arabinoside for neoplastic meningitis and lymphomatous meningitis [6,8]. Morphine sulfate for pain management [6,7], Vertepeorfin for molecular degeneration [6,7,9], Vincristine for non-hodgkin lymphoma [7,10] and Doxorubicin and bortezomib for Relapsed or refractory multiple myeloma [11].

As the building blocks of liposomes phospholipid molecules are lipids containing phosphorous, fatty acid residues and a polar group in their structures. They have an excellent biocompatibility and a especial amphiphilicity. These unique characters make phospholipids most suitable to be utilized as pharmaceutical excipients and have successfully employed in many drug delivery system (DDS) designs [12]. Some of carriers based on phospholipids including drug-phospholipids complexes [13,14], cochleates [15], micelles [16], intravenous lipid emulsions [17], and liposomes [18].
Phospholipid molecules used to manufacture liposomes are either synthetic or natural origin. Natural phospholipids obtained from plant or animal sources are preferred for selection of phospholipid in pharmaceutical excipients compared to synthetic phospholipids [19]. Various sources for natural phospholipids have been cultivated e.g., egg yolk, soybean [20], rapeseed, sunflower seed [21] and coconut [22].

Coconut phospholipids in the form of liposomes have been utilized as carrier for carboxyfluorescein [23] and vitamin C [24]. These data demonstrate that coconut liposomes can be used to encapsulate polar compounds. Moreover adding cholesterol to the liposome’s membrane increases the encapsulation efficiency (EE). Problem with coconut liposomes to encapsulate these compounds was their fast leakage during storage. However the addition of cholesterol to the membrane displayed improvement in their storability profiles. These information lead to further quest for coconut liposomes behaviour in encapsulating other materials with different properties such as cinnamic acid as mentioned above.

Therefore, the scope of this work is to investigate the ability of coconut liposomes to encapsulate cinnamic acid and galangal extracts. We determine EE and leakage rate of cinnamic acid and galangal extracts in coconut liposomes with and without cholesterol in the liposome’s membrane. In this work we used soybean liposomes from commercial soybean phospholipids as comparison. This finding could advance our understanding of coconut liposomes behaviors in relation to further application in pharmaceutical and food industries.

2. Materials and methods

The apparatus used in this research were standard laboratory glassware, a set of thin layer hydration tools equipped with N, gas, vortex, hot plate magnetic stirrer (Thermolyne Cimarec #1), magnetic bar, thermometer, centrifuge (Hettich EBA20), ultrasonic cleaner (EUMAX) and UV-Vis spectrophotometer T60. The materials used in the present study were coconut phospholipid from coconut endosperm obtained in house, chloroform (p.a.), methanol (p.a.), cholesterol, phosphate buffer solution 0.1 M pH 7.4 prepared from NaHPO4·2H2O (Merck) and NaHPO4·2H2O (Merck).

Galangal extracts were prepared by macerating 250 g powder of red and white galangal rhizomes in methanol for 2 days. The filtrate of each sample then was collected and evaporated to produce galangal extracts. Phytochemical screening was performed for both red and white galangal extracts in order to identify the contents of alkaloids, flavonoids, tannins, quinones, and saponins in both extracts.

Encapsulation of cinnamic acid was performed using thin layer hydration method. A 0.6 mg of cinnamic acid, 113 mg of coconut phospholipid and 7.9 mg of cholesterol were dissolved in 100 mL of chloroform/methanol with ration of 9:1. A 25 mL of solution was placed in a tube to prepare a thin layer by syphoning N gas into the tube. A 25 mL of buffer phosphate solution (pH 7.4) was added to the thin layer and followed by freeze thawing process then ultrasonication for 2 hours. The dispersion was centrifuged for 1 hour in 6000 rpm. The absorbance at λ = 265 nm of the filtrate was measured by a UV-Vis spectrophotometer to obtain the concentration of the unencapsulated substance. Encapsulation of red and white galangal extracts were conducted as above by replacing the cinnamic acid with 31.14 mg of red galangal and 43.75 mg of white galangal respectively. The absorbance of red galangal filtrate was at λ = 260 nm while for white galangal extract was at λ = 285 nm.

The storability profile of the encapsulated substance was investigated by monitoring the concentration of the unencapsulated substance every day for 6 days. The encapsulation efficiency was calculated using equation (1):

\[
Encapsulation\ efficiency\ (EE) = \frac{[\text{encapsulated\ material}]}{[\text{total\ material\ before\ encapsulation}]} \times 100\%
\]

3. Results and discussion

The encapsulation efficiency of cinnamic acid in coconut liposomes as well as in soybean liposomes were presented in figure 1. The results showed that the encapsulation efficiency of coconut liposome was 10 % lower than soybean liposomes. The encapsulation efficiency of cinnamic acid was significantly influenced by addition of cholesterol in the liposome’s membrane both coconut and soybean liposomes. In the presence of 7 % cholesterol, coconut liposomes showed a 7 % increase in the efficiency of encapsulation for cinnamic acid compared to 15 % of soybean liposomes. These results indicated the presence of cholesterol optimized the encapsulation efficiency of both liposomes. Cholesterol regulates the arrangement of phospholipids composing liposome’s membrane by occupying empty spaces among the phospholipids. The different increment of the encapsulation efficiency of coconut and soybean liposomes was allegedly due to the dissimilar composition of
phospholipid species that build the membranes which has previously been reported for liposomal carboxyfluorescence [23]. Furthermore cholesterol has different influence to the membrane width depending on the chain length of the phospholipids [25] as already known coconut phospholipids mostly consisted of C12 and C8 acyl chains [22] while soybean phospholipids were C18:2 and C18:1 [26].

In encapsulation of red and white galangal extracts, the efficiency of encapsulation is shown in figure 2 and figure 3. Both results were similar in essence that the encapsulation efficiency of red and white galangal extracts in coconut was lower than in soybean liposomes. The cholesterol increased the encapsulation efficiency of coconut and soybean liposomes for both galangal extracts. The encapsulation efficiency of cholesterol added coconut liposomes surpassed the soybean liposomes. However the figures also displayed that encapsulation efficiency of red galangal was smaller than white galangal extract in all accounts. This circumstances most likely as a result of the chemical content of both extracts. The phytochemical screening test of both red and white galangal extracts disclosed that both the red and white galangal extracts were positive containing flavanoid, quinone, tannin, and saponin compounds. Meanwhile, alkaloid compounds was only present in the red galangan extract. Alkaloid compounds have been found to interact with membranes to change their fluidity [27].
Figure 3. Encapsulation efficiency of white galangal extract in coconut and soybean liposomes.

Figure 4. Storability profile of the encapsulated cinnamic acid, red and white galangal extracts in 6 days storage.

Such interaction may affect the encapsulation process of red galangal extract in liposomes leading to lessening of encapsulation efficiency of red galangal extract than of white galangal. In all cases above we found out that encapsulation of cinnamic acid, red and white galangal extracts in coconut liposomes were optimized by addition of cholesterol although they were still slighter compared to soybean liposomes.

The storability profile of the encapsulated cinnamic acid, red and white galangal extracts was presented in figure 4. Data were obtained during 6 days of storage. In general cinnamic acid leak faster then the red and white galangal extracts in similar carrier systems except for red galangal in cholesterol added coconut liposomes. It seemed that even though coconut liposomes had high encapsulation efficiency for cinnamic acid, they also leaked fast. We assume that this fact caused by the high partition coefficient of cinnamic acid \[28\]. This effect was then deterred by the addition of cholesterol in the liposome’s membrane as can be seen from the leakage rate after cholesterol addition. The bulky structure of cholesterol with tetracyclic ring made the movement of cinnamic acid across the membrane impeded which then eventually lowered the leakage rate. The red galangal extract had as much leakage rate as the cinnamic acid compare to white galangal extract at all liposomal systems. The alkaloid components in red galangal were not only influence the encapsulation efficiency but also affect the leakage rate as well.
4. Conclusions
The encapsulation efficiencies of cinnamic acid, red galangal and white galangal are 62%, 42% and 52% respectively. Addition of 7% cholesterol increases the encapsulation efficiency of cinnamic acid red galangal and white galangal to 70%, 50% and 68% correspondingly. The red galangal extract has as much leakage rate as the cinnamic acid compared to white galangal extract at all liposomal systems.

5. Acknowledgements
DH would like to acknowledge the financial support from DIPA FSM UNDIP 2016.

References
[1] Sharma P 2011 J. Chem. Pharm. Res. 3 403–23
[2] De P, Baltas M and Bedos-Belval F 2011 Curr. Med. Chem. 18 1672–703
[3] Guzman J D 2014 Molecules 19 19292–349
[4] Ulrich A S 2002 Biosci. Rep. 22 129–50
[5] Sercombe L, Veevati T, Moheimani S, Wu S Y, Sood A K and Hua S 2015 Front. Pharmacol. 6 286
[6] Chang T C, Shiah H S, Yang C H, Yeh K H, Cheng A L, Shen B N, Wang Y W, Yeh C G, Chiang N J, Chang J Y and Chen L T 2015 Cancer Chemother. Pharmacol. 75 579–86
[7] Allen T M and Cullis P R 2004 Science 303 1818–22
[8] Jahn F, Jordan K, Behlendorf T, Globig C, Schmoll H J, Müller-Tidow C and Jordan B 2015 Oncology 89 137–42
[9] Gross N, Ranjbar M, Evers C, Hua J, Martin G, Schulze B, Michaelis U, Hansen L L and Agostini HT 2015 Mol. Vis. 19 54–61
[10] Wang X, Song Y, Su Y, Tian Q, Li B, Quan J, and Deng Y 2015 Drug Deliv. 29 1–9
[11] Ning Y M, He K, Dagher R, Sridhara R, Farrell A T, Justice R and Pazdur R 2007 Oncology (Williston Park) 21 1503–8
[12] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X and Deng Y 2015 Asian J. Pharm. Sci. 10 81–98
[13] Bombardelli E 1991 Boll. Chim. Farm. 130 431–8
[14] Pathan R A and Bhandari U 2011 J. Incl. Phenom. Macrocycl. Chem. 69 139–47
[15] Rao R, Squillante E 3rd and Kim K H 2007 Crit. Rev. Ther. Drug Carrier Syst. 24 41–61
[16] Trubetskoy V S and Torchilin V P 1996 S T P Pharm. Sci 6 79–86
[17] Hippalgaonkar K, Majumdar S and Kansara V 2010 AAPS PharmSciTech 11 1526–40
[18] Gregoriadis G, Leathwood P D and Ryman B E 1971 FEBS Lett. 14 95–99
[19] van Hoogeveest P and Wendel A 2014 Eur. J. Lipid Sci. Technol. 116 1088–107
[20] Wendel A 1995 Lecithin Kirk & Othmer Encyclopedia of Chemical Technology vol 15, ed M Grayson (New York: John Wiley & Sons) pp 191–210
[21] Ghyczy M 1989 Synthesis and modification of Phospholipids Lecithins Sources: Manufacture & Uses, ed B F Szuhaj (Champaign: AOCS Press) pp 131–44
[22] Hudiyanti D, Raharjo T J, Narsito N and Noegrohati S 2012 A gri tech 32 23–6
[23] Hudiyanti D, Raharjo T J, Narsito N and Noegrohati S 2015 Orient. J. Chem 31 435–9
[24] Hudiyanti D, Triana D and Siahaan P 2017 Jurnal Kimia Sains dan Aplikasi 20 5–8
[25] McIntosh T J 1979 Biochim. Biophys. Acta. 513 43–58
[26] Ivanov D S, Lević J D and Sredanović S A 2010 Journal of the Institute for Food Technology in Novi Sad. 37 65–70
[27] Rosenkrantz V and Wink M 2008 Molecules 13 2462–73
[28] Wildman S A and Crippen G M 1999 J. Chem. Inf. Comput. Sci. 39 868–73