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The Use of Sonication Method For Reducing Size of Liposomes In Papaya Leaf Extract *(Carica Papaya Linn)* Preparations As A Candidate In Treatment of Cervical Cancer

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Abstract
Liposomes are vesicle-shaped particles whose walls are composed of lipid molecules (the main constituent is a double layer phospholipid which wraps the liquid compartment inside. Liposomes can be made from natural ingredients in the form of natural phospholipid derivatives mixed with fat chains (*eg phosphatidylcholine*) by dispersing. Liposomes from papaya leaf extract (*Carica Papaya Linn) are* made with the aim of increasing the effectiveness of the drug and biocompatible work, as well as protecting healthy tissue from the toxic effects of the drug. The process of making liposom papaya leaf extract using the sonication method by mixing soybean lecithin with papaya leaf extract. Then do the heating and sonication. This method is done to reduce particle size. The aim of this study was to produce an extract of liposomes from papaya leaf phospholipid formulations using a combination of heating and sonication.The method to produce liposomes with a size of <150 nm using a combination of heating 40°C for 40 minutes and stirring using ultraturrax at a speed of 15,000 rpm for 30 minutes, then stirring with a sonicator for 30 minutes.The results showed the liposome nano papaya leaf extract formulation from soybean lecithin by the PSA (*Particle Size Analyzer*type 1090 / Cilas) method. The liposome product was in the form of liquid, light green and milk white and odorless. Particle size distribution is obtained in the range of 0.04 µm - 500.00 µm / 100 Classes and an average pH of 5.45.

Keywords: Cervical Cancer, Liposomes, Papaya Leaf Extract, Sonication

I. INTRODUCTION

Cancer is one of the main causes of death worldwide and accounts for 8.2 million deaths (22% of all non-communicable diseases) in 2012. In Indonesia, the number cancer deaths reached 111 per 100,000 population [1]. The type of cancer that is currently being suffered by cancer patients in the world including cancers of the breast, cervix, lung, colon, stomach, liver, ovary, esophagus, pancreas, blood, and skin. Cervical cancer in Indonesia ranks number two in the world. In 2014, more than 92 thousand Indonesian women died of cancer with 10.3 percent of them due to cervical cancer [1]. Cervical and breast cancer is a cancer with the highest prevalence in Indonesia in 2013, namely cervical cancer by 0.8 ‰ and breast cancer at 0.5 ‰. Behavioral factors and eating patterns have an important role to play in cancer. In general, the lack of consumption of vegetables and fruit is the highest risk factor in all age groups. The proportion of people who smoke, obesity, and often consume fatty foods is highest in the age group 25-34 years, 35-44 years, and 45-54 years. Meanwhile, the habit of consuming burnt / baked foods and consuming preservative animal foods tends to be higher in younger age groups. Therefore, because there are differences in behavior and diet in each age group, prevention and health promotion efforts are needed [2]. Cancer Can Be Treated With Surgery, Radiation, Chemotherapy, Hormones, and Immunotherapy [3]. Chemotherapy is a type of therapy for cancer that is aimed at killing or slowing growth.

Cancer cells, which grow and divide rapidly. However, this chemotherapy can also damage the normal dividing body cells, such as cells in the mouth, intestines, and hair. Damage to Normal Cells Can Cause Unwanted Side Effects in Patients with Cancer [1]. To Minimize the Side Effects of Drugs in the Body, The Use of Natural Extracts as Anticancer Compounds Has Been Researched Quite Much, Such as Broccoli Extract of Mangosteen Extract, Turmeric Extract and Many Other Plant Extracts [4].

Papaya Leaves Have Latin Names (*CaricaPapaya*L) Is One Of The Types Of Vegetables That Are Processed When Still Young To Be A Food That Is Delicious And Highly Nutritious. Besides being able to be processed into a delicious food, papaya leaves can be used as medicine for several types of diseases. Papaya Leaves Are One Of The Plants That Are Known As Anticancer. The Biggest Anti-Cancer Properties of Papaya Concentrated on Leaf Extracts. According to Research Conducted in the Ethnopharmacology Journal**,** Papaya Leaf Juice Contains Enzymes that Have Cancer-Fighting Properties, such as Cervical Cancer, Breast Cancer, Liver Cancer, Lung Cancer and Pancreatic Cancer Without Toxic Effects on the Body. Thus, Papaya Leaf Extract is also often recommended in some countries as part of chemotherapy. By Adjusting Cells, Papaya Leaf Extract Will Improve Immune System Response to Cancer [5], [6]. Methanol Extract (*Carica Papain L*) Has Inhibiting Activity Against DNA Topoisomerase II Enzymes, Enzymes That Play Important Roles In The Process Of Replication, Transcription, DNA Recombination, And Cancer Cell Proliferation. Papaya Leaf Extract Can Inhibit Cell Proliferation And Improve Cancer Cell Apoptosis [6], [7].

II. MATERIAL AND METHODS

Liposomes Are Pharmaceutical Preparations Developed In The Pharmaceutical World Because Liposomes Have Advantages, Including Increasing Efficacy And Therapeutic Index And Increasing Stability Of Drugs With Encapsulation System [8]. Soy Lecithin Contains Unsaturated Fatty Acids That Have High Compatibility In The Body And Good Penetration. Soy Lecithin Is Widely Used In Making Liposomes [9].

Experimental Desaign:
In this study using descriptive exploratory method, which uses papaya leaf samples that have been extracted using methanol to make Liposomes. Making Liposomes Can Be Done In Conventional Ways And Novel Methods Until Now Still Developed. These Methods Require Long Time In Making. Apart from that, the method requires organic solvents which, if leaving residues can be toxic [9][22] . Heating Method *(Mozafari Method*) Is One Of The Novel Methods Developed In Making Liposomes Without Organic Solvents. Heating Methods Can Be Used To Make Liposomes Containing Enzymes, Vaccines, Or Other Compounds That Are Sensitive To Organic Solvents [10][21]. Particle Size Is A Physical Properties Parameter That Needs To Be Looked For In Making Liposomes. One Effort That Can Be Done To Reduce Particle Size is Sonication [8]. The Diameter of Liposomes Produced by Sonication Method is Affected by Temperature and Duration of Sonication Process [11][19]. The Sonication Length Can Also Affect The Particle Size Produced [8], [9][20]. Factors Affecting Physical Properties of a Liposome Shows That the Composition of Soy Lecithin, Mixing Speed, Mixing Duration and Mixing Temperature Affect the Various Physical Properties of Liposomes [13]. Making Liposomes Using Conventional Methods Has Many Constraints, Among Others Is Quite Complex, Requires Long Time, And Uses Toxic Organic Solvents.

Meterial:

The materials used in this study were soybean lecithin phospholipids (labware), aquades (hydrobatt), papaya leaf extract 100 ml. The tools used in this research are ph meters, electric scales, measuring cups, vials, watch glass, magnetic stirrer, ultra turrax, soltec bath, *particle size analyzer* (1090 / cilas).

Methods:
Liposomes were made by dispersing 1.0024 grams of soy lecithin phospholipids in 11.5 ml of distilled water. a mixture of lecithin and distilled water was added with papaya leaf extract0.5012 grams. comparison of lecithin and papaya leaf extract 100: 50. Then stirring was carried out using a magnetic stirrer with a speed of 700 rpm, a temperature of 40^{o}C for 40 minutes. Liposome formulation homogenized using ultraturrax at a speed of 15,000 rpm for 30 minutes and sonicated using a bath sonicator for 30 minutes at 60°C. The liposome formulation was made in 4 formulas, namely 1 sample 200 mg liposomes, 2 400 mg samples, 3 doses samples 800 mg, a sample of 4 doses of 1600 mg without the addition of the active Liposome substance produced then particle size measurements were carried out using a particle size analyzer (PSA) using the Cilas 1090 liquid method. And in the pH it uses a pH meter.

III. RESULT

Products are conducted at the Instrumentation UPT of the Department of Chemistry FMIP Universitas Brawijaya Malang, using the PSA (*Particle Size Analyzer*) Type 1090 / Cilas method.

a. The results of liposome products are liquid, light green and milk white and odorless.
b. Particle size distribution with the 1090 Cilas liquid method obtained range: 0.04 µm - 500.00 µm / 100 Classes.

Table 1: pH and PSA Test Results of Liposome

No	Sample	pH	PSA (µm)
1	Liposome Papaya leaf extract sample 1	4.8	14.62
2	Liposome Papaya leaf extract sample 2	4.72	12.36
3	Liposome Papaya leaf extract sample 3	5.95	16.32
4	Liposome Papaya leaf extract sample 4	6.35	18.56
	Average	5.45	15.46

Liposome Particle Size Distribution Curve Shown In Figure 1 And Figure 2.

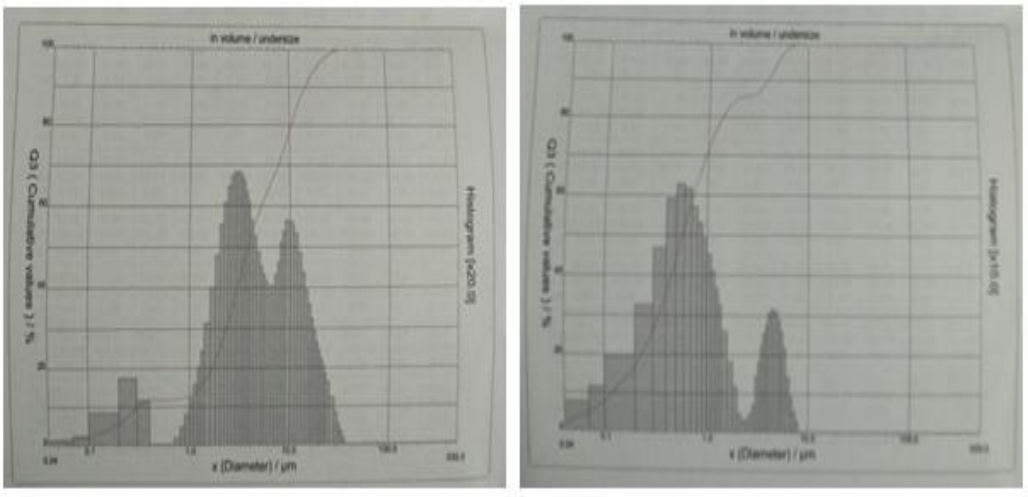

Fig. 1 and 2: liposome particle size distribution curve of papaya leaf ekstak formula 1 and 2 using

PSA*(ParticleSize*Analyzer)Type1090 / CILAS

Discussion:

One of the parameters that need to be considered in the formulation of the liposome is the size of the particles produced. One parameter that needs to be considered in the formulation of liposomes is the size of the particles produced. Some studies use small vesicles with a diameter range of 50-150 nm with consideration of capsulation efficiency, stability and distribution in drug delivery systems [12]. The nanoliposome formulation is still being developed today. Many attempts have been made to reduce the size of the liposome. Sonication energy is often used to produce liposomes with small diameters of up to 15-50 nm. The method of making liposomes used in this study is a combination of 60 ○ C heating method and sonication energy to produce liposome preparations with a range of sizes 50-150 nm [12].

The length of sonication used in this study is 30 minutes. The heating method is one of the novel methods for making liposomes without using organic solvents, so they are not toxic [13]. The development of the method of making liposomes used in this study was the use of a combination of heating methods of 60 ° C and sonication for 30 minutes. The advantage of the combination of the two methods is the faster duration of manufacture and not using organic solvents during manufacture. The solvent used during the liposome formulation is bidestilata water so that it does not leave any organic solvent residues that might be toxic. The nanoliposome formulation in this study was made in 4 formulas, namely liposome sample 1 at a dose of 200 mg, sample 2 doses of 400 mg, sample 3 doses of 800 mg, sample 4 doses of 1600 mg without the addition of active substances.

The active substance commonly used is 4-n-butylresorcinol. The 4-n-butylresorcinol compound has a hypopigmentation effect by inhibiting tyrosinase enzyme activity and suppressing the synthesis of tyrosinase which triggers the synthesis of skin melanin [13], [14]' This compound was developed in the treatment of melasma skin. The activity of this compound is to inhibit the tyrosinase enzyme found in the basal stratum layer so that a formulation in the form of nanoliposomes is needed to be able to deliver the active substance to the basal layer of the skin layer. The size of the liposomes produced in this study is presented in table 8.1 with the results of the average liposome size of the four formulas between 12.36 to 18.32 µm with an average pH of 4.72 to 6.35 gr%. The results of liposome measurements (Table 1) showed that the liposome formula without the addition of active substances produced liposomes with a size range of 12.36 to 18.32 µm. The formed liposomes usually have a heterogeneous particle size distribution. The size of the liposomes also changes with time of storage [9]. The liposome particle size produced in this study was also heterogeneous with a size distribution of 12.36 to 18.32 µm. Description of Liposome Size Distribution Curve Without Addition of Formula 1 Active Substance (Figure 1), and Formula 2 (Figure 2) Shows the Results of Distribution of Normal Particle Size Distribution. Polydispersity index (PI) Used in Observing Particle Size Using *Dynamic Light Scattering* (DLS) Meets Requirements [15] For Polydisperse Solutions That Is For Particles Size 100-300 Nm PI Requirements Are <0.3. In observing Liposomes without Active Substance Addition the PI value was 0.204. The Size of Liposomes Produced in This Study Is Similar to Research [16]. Which Produces Liposomes with Average Size of 98 ± 48nm. Differences with Research [17], [18] Is the Formulation Method Used. The Formulation Method Used In This Research Is A Combination Of Heating And Sonication Methods While Research [18]. Using the Dehydration-Rehydration Method So That It Takes a Longer Time and Uses Organic Solvents in the Formulation Process. Nanoliposomes with 100 Nm Size Containing Active Materials Have Stability As a cosmetic preparation of ingredients and a topical drug delivery system [18]

IV. CONCLUSION

Formulasisediaannanoliposomdarifosfolipiddariekstrakdaunpepayadenganpenambahanlesitinkedelai (soy lecithin) dapatdilakukandenganmenggunakankombinasimetodepemanasan 60○C dan lama sonikasi 30 menit. Saran daripenelitianiniadalahperludilakukanpenelitianlebihlanjutterkaitoptimasisuhu dan lama sonikasidalampembuatannanoliposom dan pengamatanmorfologibentukliposom yang

dihasilkandenganmenggunakan Transmission Electron Microscopy(TEM). ProdukakhirLiposamdariekstrakdaun papaya menunjukkanDistribusiPartikel size denganmetodeCilas 1090 liquid didapatkan range : 0,04 µm - 500.00 µm / 100 Classes. Rerata pH 5.45 danrerata PSA 15.46 µm.

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