Prospect of Patch Design From Chrystal Etil P-Methoksisinamat of Kencur As An Alternative Drug Delivery System Antiinflamation

Hesti Riasari*[1], Revika Rachmaniar[1]

[1]Department of Pharmaceutical Biology, Indonesia School of Pharmacy Jl. Soekarno Hatta 354, Bandung 40266, West Java, Indonesia

Email*: hmm_riasari@yahoo.com

ABSTRACT

Aim: The aim of the study was to obtain gauze tape and gel containing suspension extracts and crystal kencur rhizome (*Kaempferia galanga* L.) as anti-inflammatory through ionic gelation method

Method: Kencur rhizome extracted and then evaporated naturally until crystals are formed, nanosuspension extracts and crystals are formed, then tested with PSA and made plaster preparations. The plaster preparation from the crystal suspension and suspension extract was tested for anti-inflammatory activity.

Result: The particle size of the crystal suspension was 831.6 nm, the potential zeta value of the suspension of the crystal suspension of 31.72 mV. The results showed that the gauze and gel compartment compartments containing crystal rhizome suspension of ionic gelation product result have anti-inflammatory activity. Gel patch of kencur rhizome extract has the largest anti-inflammatory activity among other forms of plaster dosage.

Conclusion: Plaster gel extract and crystals from the kencur rhizome have the greatest anti-inflammatory activity compared to the form of gauze tape.

Keyword : Kencur rhizome, ethyl p-methoxycinnamate crystal, anti-inflammation, Patch

INTRODUCTION

The result of nature is a source of medicinal material which until now is still developed to be made into new drug ingredients[1]. Over the last ten years, research has been done by Hu, et al and Kawabata, et al., They are focusing on the development of drug delivery systems / Novel Drug Delivery system (NDDS) especially for those derived from nature in other words herbal remedies.

One of the medicinal herbs is kencur (*Kaempferia galanga* L.). Kencur (*Kaempferia galanga* L.) includes tribe findings (Zingiberaceae) [2]. Empirically, kencur efficacious as a drug for anti-inflammatory[3]. The chemical substance in the anti-inflammatory gum rhizome is ethyl p-methoxycinnamate. Ethyl p-methoxycinnamate has the ability to inhibit the inflammatory process by inhibiting the activity of cyclooxygenase-2 [4]. Based on the results of the study [5] ethanol extract of kencur rhizome can be used as anti-inflammatory plaster and can reduce inflammation in rat test animals.

Based on [6] kencur rhizome into indigenous plants of Indonesia that must be developed as raw materials of traditional medicine. Based on some of the above considerations, the research conducted in the form of patching of ethyl p-methoxycinnamate crystals from kencur (*Kaempferia galanga* L.) using gauze and gauze compartments, which are expected to provide good anti-inflammatory effect and become an alternative anti-inflammatory preparation for the community.

MATERIALS AND METHOD

Material

Kencur rhizome from Balitro, Manoko, District Lembang, Bandung regency. Solvent for maceration of 96% ethanol (Brataco), ethyl acetate (Brataco), n-hexane (Brataco), ammonia 25% (Merck,105432), chloroform 2,5 L (Merck CAT 1.02445.2500), HCl (Merck 1.00317,2500), NaOH (Meck 1.06498.1000), dragendrof reagents, FeCl3 reagents, liberman burchard, gelatin, vanilin sulphate, diethyl ether (Merck, 1.00921.2500), formic acid (EMSURE® ACS,Reag. Ph Eur), silica gel 60 F254(plate 20x20 cm/ pk Merck 105554), carrageenan 1%, glycerin (Merck 104094.1000), sterile gauze, and adhesive plaster, aquadest, polyvinyl alcohol (Sigma Aldrich), polyvinyl pyrrolidine(72000 merck), and propylen glycol (Sigma Aldrich). Test animals used white male rats Wistar strain with a weight of 140 -210 grams.

Methods

Preparation of Raw Materials and Plant Determination

Kencur was determination, then it is wet, washed, drained, cross-sectioned with a thickness of 2-5 mm, and dried in an oven with a temperature of 40-50 ° C to dry (moisture content ≤ 10%) then ground.

Characterization of simplicia

Characterization of simplicia aims to determine the quality and quality of simplicia compared with the literature. Characterization that is done include ash content, acid soluble ash content, water soluble extract, ethanol soluble content, moisture content, and drying rate.

Phytochemical Screening

Phytochemical screening was performed to determine the secondary metabolite content found in kencur, ie testing of alkaloids, flavonoids, tannins, polyphenols, monoterpenes and sesquiterpenes, steroids, terpenoids, saponins. Phytochemical screening is an early stage to detect compounds in plants based on their compounds based on Farnsworth (1966) conducted on simplicia and ethanol extract of kencur rhizome.

Preparation of Kencur Crystal

The simplicia of kencur rhizome is macerated by using 96% ethanol solvent for three days with three times the solvent replacement. The liquid extract collected was evaporated by aerated at room temperature for three weeks until the crystals were obtained.

Monitoring Thin Layer Extract Chromatography

Thin Layer Chromatography (TLC) method was performed on liquid extract and kencur crystals to find out what kind of secondary metabolites contained in the ethanol extract of kencur rhizome. With the mobile phase: n-hexane P, ethylacetate P (9: 1) and stationary phase: silica gel 60 GF254

Kencur Crystal Washed

The kencur rhizome crystal formed on the extract is separated, then washed using n-hexane to be clean and then monitored by using thin layer chromatography until obtained pure ethyl p-methoxycinnamic crystals.

Manufacture of Antiinflammatory Patches

A. Patch of Gauze Compartments

Gauze cushion was applied to kencur rhizome crystals which have been dissolved in ethanol until saturated and then aerated. After that, the wound pad is affixed to the adhesive tape.

B. Patch Gel Compartment

The making of patch is done by technique of making layer of cassette with length 2,5 cm and width 2,5 cm. The patch mixture was prepared by preparing a mixed polymer solution of PVA and PVP at a ratio of 7: 3 in hot aquadest, stirred to homogeneous, then incorporating rhizome crystals and then added propylene glycol and aquadest to the required amount. Stirring is done using a magnetic stirrer until homogeneous, then the mixture is poured onto the glass mold and left to dry. After that, the compacted gel is affixed to the adhesive tape.

Antiinflammatory Patch Test

Antiinflammatory testing of kencur crystals patch was conducted in 2 groups of test animals, rat feet marked with markers to get the foot into the mercury every time always the same. The rat foot volume was measured before (Vo) and after (Vt) induced 1% carrageenan solution on the sole of the foot subcutaneously and attached patch. Foot volume is recorded every 1 hour until 5 hours. The experimental group I was given patch with gel compartment, group of animal II test was given patch with gauze compartment, group III as negative control, group

IV as positive control patch with gel compartment, and group V as positive control patch with gauze compartment, where test attached the filing of ethical commissions.

Result and Discussion

Simplicia Processing

Kencur (Kaempferia galanga L.) that has been harvested from the dirt by washing with running water then drained, then thinly sliced to facilitate the process of drying the rhizome kencur. Rhizome rhizome that has been in the iris then dried to room temperature so as not to damage the secondary metabolites contained in the rhizome kencur. The purpose of the plant in the dry to reduce water content so that plants are not easily overgrown by the fungus. After dry rhizome kencur called simplicia rhizome kencur. The simplicia is then milled to expand the surface of the simplicia with the solvent in order for the solvent diffusion process into the cell to be better so that the secondary metabolite in the cell is perfectly attracted to the solvent.

Characterization of Simplicia

Characterization of simplicia include determination of water content, total ash content, water soluble extract, ethanol soluble extract, and drying shrinkage. The results of characterization can be seen in Table 1.

Table 1. Results of Characterization

Characterization	Obervation Result	
	Simplicia	**Reference (FHI)***
Water Content (%) w/v	4	≤ 10
Total Ash (%) w/v	6.5	< 8.7
Water Soluble Extract (%) w/v	16	>14.2
Ethanol Soluble Extract (%) w/v	20	> 4.2
Shrinkage Drying (%) w/v	14.4	≤ 10

Famakope Herbal Indonesia

Ethanol Extraction Kencur Rhizome (*Kaempferia galanga* L.)

The extraction process used maseration method with 96% solvent or 96% ethanol. Maseration is chosen in the extraction process of kencur rhizome because the content of ethyl p-methoxycinnamate compounds that efficacious in kencur rhizome has thermolable properties. Ethanol 96% is chosen because it can dissolve almost all secondary metabolites contained in the simplicia. From 711.57 grams of kencur rhizome simplicia extracted with 6.25 liter of 96% ethanol solvent, an extraction yield of 8.4% was obtained in accordance with the literature in Herbal Indonesia Farmasiopean rendeman more than 8.3%. The yield of crystals of kencur extract was 7.69%.

Phytochemical Screening

Phytochemical screening is an early stage for identifying secondary metabolites present in the simplicia. The results of phytochemical screening can be seen in Table 2.

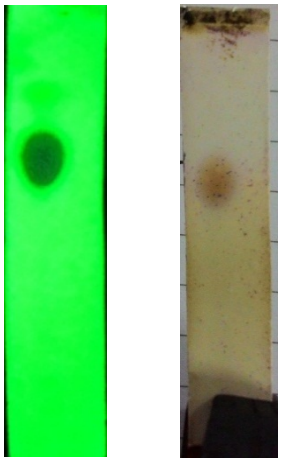
Table 2. Results of Phytochemical Screening

Phytochemical Screening	Simplicia	Ethanol Extract
Alkaloid	+	+
Flavonoid	+	+
Tanin	+	+
Phenolat	+	+
Monotherpen dan Sesquiterpen	+	+
Steroid dan Triterpenoid	-	-
Quinon	+	+
Saponin	-	-

The results obtained from the phytochemical screening performed on dry simplicia and viscous simplicia rhizomes of kencur showed that the process of extraction using the maceration method did not remove the compounds contained in the simplicia. This is evidenced by the results of phytochemical screening which shows that the content of the compound in the extract is the same as the compound content in the simplicia.

Separation of Kencur Rhizome Crystal Extracts

The evaporation process of kencur rhizome extract produces crystals. The formed crystals are separated from the rhizome extract of kencur and washed using n-hexane solvent to remove impurities still attached to the crystal. Washing is done until the crystal is yellowish white. Kencur produced white to yellowish white and typical aromatic smell. Measurement of the melting point of the crystals is carried out and yields a range of melting point crystals of 45-50 ° C. The crystals were analyzed by means of thin layer chromatography to identify the compounds in the crystals. Eluen used was n-hexane: ethyl acetate with a ratio of 9: 1 and obtained Rf value of 0.636. Comparison of TLC extract and crystal results can be seen in Figure 1.



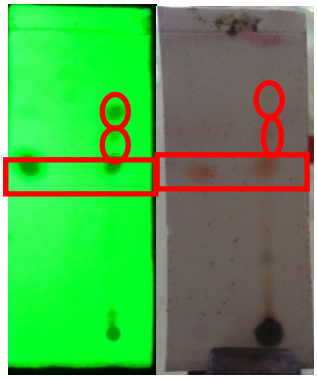
a. b.

Figure 1. (a.) TLC crystal KLT results seen at UV λ 245 nm, (b) TLC results of crystal kencur after sprayed H_2SO_4

After the identification, the compound contained in the kencur rhizome crystal is thought to be the ethyl p-methoxycinnamate compound because the melting point range of the pure ethyl p-methoxycinnamate compound is present at 47-52 ° C, whereas the melting point of the crystals obtained is at 45-50 ° C. Rf of ethyl p-methoxycinnamate compound of 0.58, while Rf of crystals obtained by 0.63. The analysis of ethyl p-methoxycinnamate is yellowish white and has a distinctive aromatic odor just as the crystals obtained. Ethyl p-methoxycinnamate compound is one of the many compounds found in the rhizome kencur and is a compound marker on the rhizome kencur. Umar (2012) mentioned in his research that ethyl p-methoxycinnamate is effectively used as anti-inflammatory.

Thin layer chromatography

The thin layer chromatography method was carried out on ethanol extract and kencur rhizome crystal to detect the ethyl p-methoxycinamate and flavonoid compounds contained in the sample. In thin layer chromatography used n-hexane motion phase: ethyl acetate with ratio 9: 1. Comparison of extract and crystalline TLC results can be seen in Figure 2.



(a) (b)

Figure 2 (a) Crystalline TLC and extract on lamp λ 254 nm, (b) Crystalline TLC and extract after sprayed H2SO4

Based on the spots on TLC extract and kencur rhizome crystal showed that the extract and kencur rhizome crystals have one spot of spots with the same Rf value of 0.547 which is suspected of ethyl p-methoxycinnamic compound. In TLC extract there are 3 spot spots with Rf value of 0.547, 0.667 , and 0.762 which is thought to be the first spot is ethyl p-methoxycinnamate, while the other two spots are flavonoids. The suspension formed after 24 hours of stirring is then characterized using PSA (Particle Size Analyzer).

Table 3. Particle Size Characterization Results

Sample	Partikel Size(nm)
Crystal	831.6
Extract	1286.7

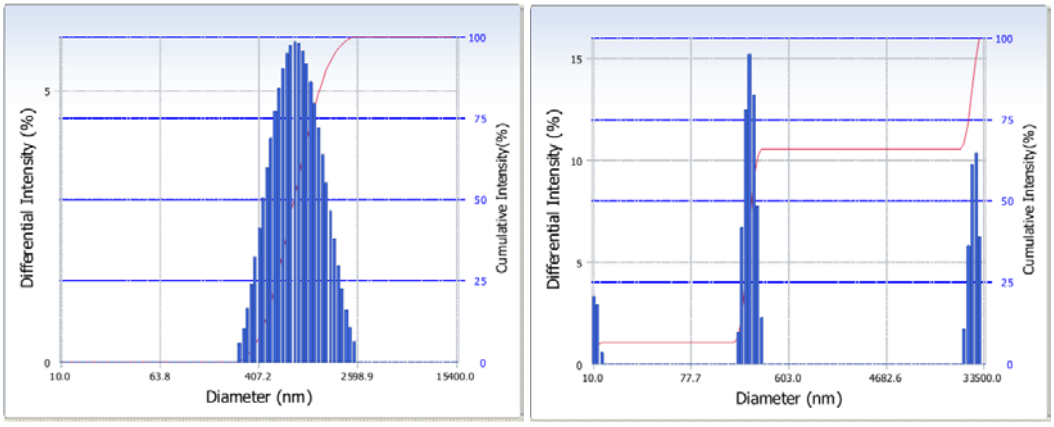
Based on the characterization using PSA, the particle size of the kencur galvanizing crystal after the ionic gelation process enters the nanometer size range, while the particle size of the extract is still within the micrometer size range. This is because the amount of extract that is used more than the crystal because the factors that affect the formation of nanoparticles is the ratio of the amount of material used between chitosan, TPP, and crystals or extracts. Comparison of TPP materials should always be more than any other material. The amount of chitosan and crystals or extracts should not be more than TPP because TPP serves as a crosslinking linking agent with chitosan. The concentration of TPP is smaller than that of other materials, so the cross linkage formed will be weaker. The cross link link is weak to allow for re-joining of particles.

Table 4. Value of Polisperspersity Index

Sample	Value of Polisperspersity Index
Crystal	0,262
Extract	0,521

The index value of polydispersity greater than 0.3 indicates the extent of particle size distribution which means non-uniform particle size (Yen, et al, 2008). The crystal particles after the ionic gelation process have a polydispersity index value of less than 0.3 whereas the extract particles have a polydispersity index of more than 0.3. Thus it can be concluded that the crystal suspension has a narrow particle size distribution and shows that the crystal particle size tends to be

uniform as shown in Fig. 3 (a). This is because crosslinking links between TPP and strong chitosan make it difficult for the united particles to become agrerat. The extract suspension has a wide particle size distribution and shows that the particle size tends to be uniform as shown in Figure 3. (b). This is due to the easyness of united particles into larger aggregates due to the amount of TPP that is not proportional to the amount of extract used to cause crosslink link between TPP and chitosan become weak. Graph of particle size distribution can be seen in Figure 3.



(a) (b)

Figure 3. (a) Graph of crystal particle size distribution, (b) Graph of particle size distribution of extracts .

Table 5. Potential Zeta Value

Sample	**Potential Zeta (mV)**
Crystal	31,72
Extract	-0,44

Zeta potential describes the stability of nanoparticles because of the difference in charge between the particles will cause the attraction or refuse the particles. A stable nanoparticle should have a potential zeta value of less than -30 mV or more than + 30 mV (Akhtar, et al., 2012). Based on Table 5. it can be concluded that the repulsive force between the kencur particles of kencur crystals after the ionic gelation process has a strong repulsive force, whereas the repulsive force rejects the extract particles weaker. If the repulsive forces between the particles are weak it will result in the easy aggregate formation of the particles of the particles. Therefore, the size of the extract suspension is greater than that of the crystal suspension.

Preparation of gel plasters In this gel formula, the addition of Polyvinyl alcohol (PVA) and Polyvinyl pyrrolidone (PVP) is used as adhesive so that the resulting patch may be flexible in the preparation, whereas propylenglycol is used as a humectant to obtain elastic patches, and aquades are used to mix PVA and PVP.

Testing of rat anti-inflammatory plaster rats induced by the reluctance to induce udem. Carrageenan is used as an inducer to release prostaglandins in the body of mice that will cause udem. The mouse foot was measured before induction (V0), then induced by carrageenan count of 0.1 ml.

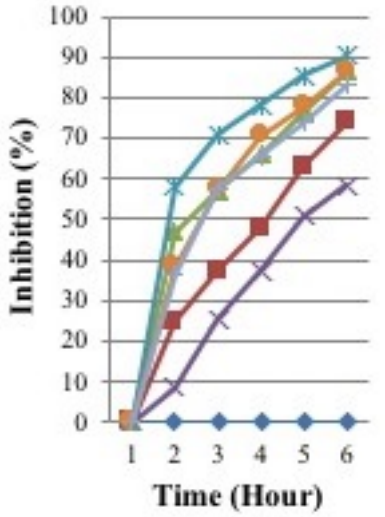


Figure 4. Graph of percent inhibition inflamatory

Based on Figure 4. it can be seen that in this study the preparation of gel suspension of kencur extract gels has the highest inhibition value between the preparation of kencur crystal suspension plaster gel, kassa plaster kenca kenca extract, and kassa cotton kassa suspension kencur because kencur extract tends to be semipolar can be dispersed well in the gel receptor compartment medium, the use of propylenglycol which serves as enhancer enhancer causes the secondary metabolite in the extract to penetrate easily into the body. This suggests that the preparation of the gel suspension plates of kencur extract has the best anti-inflammatory activity among other plaster preparations.

Conclusion: Plaster gel extract and crystals from the kencur rhizome have the greatest anti-inflammatory activity compared to the form of gauze tape.

ACKNOWLEDGMENT:
The authors thank to Kenti for technical assistance.

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