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Suspension Stability Ethanol Extract Of Frankincense Gum (Styrax Benzoin)

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ABSTRACT

Sap is a very important commodity in the industrial pharmacy, perfumes and medicines contain abundant active compounds of frankincense making resin widely used. Extraction and solvation processes play an important role in the production of stable frankincense extract. One of the widely used preparations is suspension, which is an active compound that does not dissolve in water to become soluble. The purpose of this study was to determine the manufacture of a suspension of frankincense gum extract as an active pharmaceutical ingredient. The method used was precipitation using cosolvent, surfactant and agitation process at 1500 rpm at a temperature of 40ºC. Various comparisons were analyzed for phytochemical screening, viscosity and pH. in the most stable formula to be tested, the size of the suspension particles found that frankincense extract was soluble in distilled water with an average particle size is 4.80205 µm .

Introduction

The Benzoin Resin or kemenyan resin is a very important commodity in the pharmaceuticals, cosmetics and perfume industries, this is due to the active compound content of Benzoin, one of the largest being cinnamic acid and benzoic acid (Sharif et al. 2016). The high demand for Benzoin resin is due to the many benefits of Benzoin sap, one of which is used as wound healing (Melinda Harahap 2019). The extract is used as a topical drug in cream or ointment preparations, as an antibacterial that has been tested on Staphylococcus aureus, Escherichia coli and Streptococcus mutans, Propionibacterium acne (Siregar, Suryanto, and Yurnaliza 2019). and a mixture of other ingredients as active ingredients in the form of ethanol extract. It is widely used as fragrances, incense, religious events and customs and has a high antioxidant content so that it can be used as a source of natural antioxidants (Hidayat et al. 2018).). In the ethanol extract of incense gum there are volatile compounds ranging from 30-40% such as vanillin, benzoic acid, cinnamic acid, benzly cinnamate and 60-70% nonvolatile compounds such as coniferyl benzoate, cinnamyl cinnamate, and p-coumaryl cinnamate. the area of frankincense gum obtained (Burger et al. 2016).

Benzoin has a high solubility in organic solvents such as 95 percent ethanol but is difficult to dissolve in water (Jayusman

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2014). Cinnamic acid and benzoic acid are a class of carboxylic compounds that are weak acids. In its pure form, this compound is soluble in organic solvents such as ethanol, ethyl acetate, acetone and others. However, these carboxylic compounds or organic acids will not ionize in water, which makes Benzoin gum difficult to dissolve in water. Until now, Benzoin resin sold in bulk is also in the form of ethanol extract, but in the market, Benzoin sap ethanol extract is found to be less stable or there is a decrease in stability where there are dispersed solids that settle or can be called flocculation. Research on improving the stability of Benzoin extract has been carried out by using a cosolvent technique or mixing two or more solvents to obtain a suitable solvent, using the ethyl acetate fraction of the ethanol extract of Benzoin gum mixed with propylene glycol solvent (Susanti, Purba, and Simatupang 2021). and the Isopropyl alcohol fraction. with glycerol (P Simatupang, Susanti, and Purba 2021). Increasing the stability of drug solutions can be done in various ways such as cosolvency, hydrotropy, supercritical fluid process, use of buffers, nanosuspension and etc.

Suspension is a drug preparation in liquid form which has the dispersed phase which is an active compound that is insoluble in water and evenly dispersed in the solvent. The dispersed substance must be smooth, should not settle quickly, and if shaken slowly, the precipitate must be re-dispersed (Suena 2020). Generally large suspension particles are 0.1-10 m, one of the methods in making suspensions is precipitation where the drug particles will be dissolved with organic solvents to dissolve, then mixed with water solvent where the solution is first dissolved and then added a suspending agent so that the particles will be suspended. in aqueous solvents (Pujiharti, Dewi, and Dhiasa 2015). Surfactants as suspending agents can cause electrostatic or steric stabilization of the dispersed system, having 2 groups namely lipophilic and hydrophilic groups so that they can bind polar and nonpolar compounds such as fats and form micelles. Solvents and Stabilizers are the determining factors in the manufacture of suspensions there are various stabilizers that can be used such as cellulose, poloxamers, polysorbate, lecithin and povidone, additives are sometimes used such as buffers and salts (Nagare et al. 2012). in this study used precipitation techniques with solvents its cosolvent is ethanol and propylene glycol and the surfactant is tween 80.

Surfactant tween 80 or polysorbate 80 or Polyoxyethylene 20 sorbitan monooleate is a type of nonionic surfactant which is a polymer used as a suspending agent to control flocculation in insoluble drug solutions. non-toxic and can be used for oral preparations, can increase solubility and decrease aggregation in preparations, increasing the concentration of tween 80 can increase the area of drug particle size (Saeedi et al. 2015). Through the microemulsion approach, propylene glycol and ethanol can also be called cosurfactants where the cosurfactant will interact with the surfactant and the ratio of surfactant and cosurfactant affects the value of physical properties (Hendradi and Yuwono 2016). With the many benefits of Benzoin resin, the aim of this research is to make Benzoin gum in a stable suspension preparation by the precipitation method with the help of cosolvents and surfactants which can later be used as pharmaceutical raw materials as well as research developments in the field of Benzoin resin.

Materials and Methods

Material

The materials used in this study were incense gum powder size 80 mesh from the Parsoburan area, Ethanol, propylene glycol, tween 80, filter paper, aluminum foil, Aquades, Dragendorff reagent, Iron (III) Chloride (FeCl3), Ethanol (pa), propylene glycol, ethyl acetate (pa), Hydrochloric acid (HCl 2N)

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Method

Sampel Preparation

Bezoin resin sampel were ground to 80 mesh as much as 250 g were extracted by reflux method with ethanol solvent 1: 3 for 2 hours at 65ºC. Then the solution was filtered using filter paper with the help of Buchner and a vacuum pump, the filtrate was separated and concentrated with a rotary evaporator at a temperature of 78ºC with a time of 4-5 hours the results were in the form of a concentrated thick extract then the extract was carried out with a phytochemical screening test to determine the secondary metabolite group contained in the sample. Benzoin sap. The thick extract was poured thinly into the container and then in the oven at 105ºC for 2 hours until the solvent was used up and then ground and sieved with a size of 200 mesh.

Formulation and Preparation of Suspensions

Suspensions are made by the precipitation method, precipitation is used from previous studies (C., Dipali, and Pratiksha 2020) by dissolving the sample in an organic solvent with the addition of a surfactant then homogenized with rapid stirring, this is also supported (Agrawal and Patel 2011) which describes the technique For precipitation of poorly soluble drugs, 2 solutions are made which will be combined at a rotating speed(Jadhav, Gawandar, and Vitore 2018). Performed at 1500 rpm with a temperature of 40ºC.

Table 1. Suspension Formulation of Benzoin Ethanol Extract

Ingredients	F1	F2	F3	F4
Ethanol	2 ml	-	2 ml	-
Propilen Glikol	-	2 ml	-	2 ml
Tween 80	1 ml	1 ml	2 ml	2 ml
Aquades	9 ml	9 ml	8 ml	8 ml
Ethanol Ekstrak Powder	102 mg	102 mg	102 mg	102 mg

Organoleptic Test

Performed by observing the color, shape and odor of the suspension. Pipette 5 ml of the suspension preparation into the bottle and observe for color, odor and precipitate by observing and inhaling the air above the vial.

pH test

Determination of pH using a universal pH where the sample is smeared and compared to the color of the indicator that matches the color on the available pH trajectory.

Viscosity Test

Measurement of viscosity or viscosity using an Ostwald viscometer where by measuring the time it takes for the liquid to pass through two predetermined points on a tube capillary, then the density of the suspension was calculated using a pycnometer, the viscosity was calculated by the formula and the Aquades reference was used as a comparison(Regina, Sudrajad, and Syaflita 2019).

PSA Test (Particle Size Analyzer)

This test is carried out to see the size of the suspension particles produced in the formulation that has the best visual preparation which will be tested with the Analysette 22 NanoTec/MicroTech Plus.

Results and Discussion

Extraction of Benzoin Resin

Sap The incense sap used came from the parsoburan area of North Sumatra, It was observed to have a strong characteristic odor of Benzoin, light brown in color. Samples were extracted using the reflux method to obtain a reddish-brown extract.

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a b

Figure 1. a. Benzoin gum powder size 80 mesh b. filtrate of Benzoin extract

Through the observations of researchers, Benzoin resin powder is slightly hygroscopic, and the ethanol extract will react with water to form a white precipitate.

Phytochemical Screening Test

Test was carried out using a thick extract from the concentrated filtrate. Screening was carried out on five types of secondary metabolites, namely alkaloids, flavonoids, saponins, terpenoids and tannins. The screening results can be seen in the following table:

Table 2. Phytochemical Screening Test Results of Benzoin Ethanol Extract

Secondary Metabolites	Reagent Test	Results
Alkaloids	Dragendroph	+++
Flavonoids	FeCl3 5%	+++
Saponins	Aquades and HCl	+
Tannins	FeCl3 1%	++ +
Terpenoids	Acetic Acid	+

Description: +++ indicates a high content of secondary metabolites in the extract which is indicated by a high color density from the reaction product while the + sign indicates a low secondary metabolite content indicated and a weak color intensity from the reaction results. The active compound content of benzoin depends on the type, place and solvent used where benzoin also has various types, one of which is in North Sumatra (Kiswandono et al. 2016).

Addition Surfactant and Cosolvent

In the manufacture of suspensions, 2 different types of cosolvents are used, namely ethanol and propylene glycol, ethanol is a common solvent used in pharmaceuticals other than distilled water, ethanol as a solvent can dissolve cinnamic acid and benzoic acid compounds (Zhu et al. 2018) in mixing The cosolvent of the ethanol extract dissolves immediately because ethanol is the initial solvent, while the use of propylene glycol as a cosolvent requires stirring time to dissolve homogeneously. Propylene glycol can be used as a solvent, soluble in water, ethanol and glycerin can be used as a stabilizer. The addition of propylene glycol cosolvent can increase solubility exponentially and can increase drug penetration and is safe for oral and topical use with a dielectric constant of 32 cosolvents can also reduce the chemical potential of the solution by lowering the hydrogen bond density of water so as to create a less polar environment so that the drug solution is more concentrated. much soluble (Nayak and Panigrahi 2012). Surfactants are compounds that have hydrophilic and lipophilic groups that cause surfactants to be oil soluble and water soluble, surface active compounds that have the ability to reduce surface tension and interfacial tension so that they are able to mix with each other or diffuse like an emulsion in water (Muhammad Suhail et al. 2019). In the use of tween 80 on its solubility, it can produce 3 types of solubility, namely micelles, solutions and emulsions (Wang et

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al. 2020).

Organoleptic Test

From The results of the suspension were carried out by organoleptic tests using the senses of sight and smell, where the samples were in liquid form with a distinctive odor derived from cinnamic acid and benzoic acid compounds.

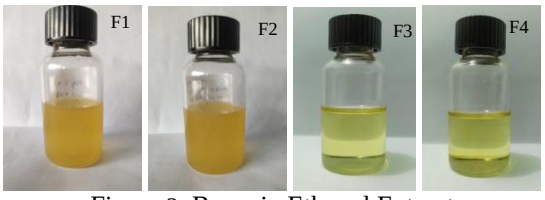

Figure 2. Benzoin Ethanol Extract Suspension

It is seen that there are quite significant differences in the suspensions of F1, F2 to F3, and F4. This difference is thought to be influenced by the concentration of tween 80 which tends to result in a clearer color. The clearer the suspension, the smaller the suspended particles, which can reduce the rate of deposition of the suspension and tend to be more stable (Badiah et al. 2019).

Table 2. Analysis Results of Organoleptic Observations

Storage	Formula	Week			
		1	2	3	4
Odor	F1	-	-	-	-
	F2	-	-	-	-
	F3	-	-	-	-
	F4	-	-	-	-
Color	F1	-	-	-	-
	F2	-	-	-	-
	F3	-	-	-	-
	F4	-	-	-	-
Sediment	F1	+	+	+	+
	F2	-	+	+	+
	F3	-	-	-	-
	F4	-	-	-	-

Description : + : There is a Change, - : No Change

Each Suspension Formula Produces Foam due to the influence After stirring for 90 minutes, the foam disappeared. In F1 and F2 it had a cloudy yellow color with a characteristic odor of incense which was not concentrated at a concentration of 10% v/v tween 80, while at F3 and F4 it produced a transparent yellow suspension at a concentration of 20% v/v. v tween 80 is due to the nature of tween 80 as a surfactant which is used as a foaming agent, wetting agent, and can reduce surface tension. The effect of the concentration of tween 80 under optimum conditions was successful in reducing the particle size of the drug . This occurs as a result of the surfactant's ability to change the surface and interparticle forces. (Hong et al. 2017). On day 5, F1 flocculated or started to precipitate particles of Benzoin gum extract with cosolvent , on day 9 on F2 there was a slight precipitate, in organic solvent Propylene glycol. Propylene glycol than ethanol is less volatile and insoluble in paraffin, fatty oils and mineral oils but soluble in some essential oils (Malkin 2006). so that when making solvent 1 Propylene glycol does not immediately dissolve with the sample. However, propylene glycol is a

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semi-polar semi-polar solvent that can act as an intermediate solvent that can cause polar and non-polar liquids to mix so that it can increase the solubility and have a smoother texture. In the use of ethanol cosolvent which is the initial solvent for the suspension, it tends to flocculate more quickly. However, the use of surfactants has a significant impact when the concentration is increased.

Viscosity and pH Test

Viscosity of the Benzoin extract suspension is also an indicator that can be used to show the stability of the suspension. Thickening and formation of sediment indicates an unstable suspension. The results of the measurement of viscosity or pH of the incense extract suspension in this study can be seen in the following table.

Table 4. Results of Analysis of Viscosity and pH Suspension of Benzoin Ethanol Extract

Formula	Density (g/cm3)	Viscosity (cP)	pH
F1	1.1862	1.7289	4
F2	1.234	1.5204	4
F3	1.1966	3.1768	4
F4	1 ,2458	4.3559	4

The sedimentation rate can be reduced considerably by increasing the viscosity of the dispersion medium and within certain limits this is practically possible, but a product having a high viscosity is generally undesirable because it is difficult to flatten or homogenize again. At a concentration of tween 80 20% v/v has a thicker liquid, due to surfactant tween 80 which has a thickener function but is still easy to shake or pour.

PSA Test (Particle Size Analyzer)

For the PSA analysis test, the best formulation according to the researchers was F3 using Laser Diffraction, namely Analysette 22 Nano Tec, Germany. To see the size of the drug particles, there are peaks with a size range from 0.01µm - 42, 30 µm with an average particle size is 4.80205 µm.

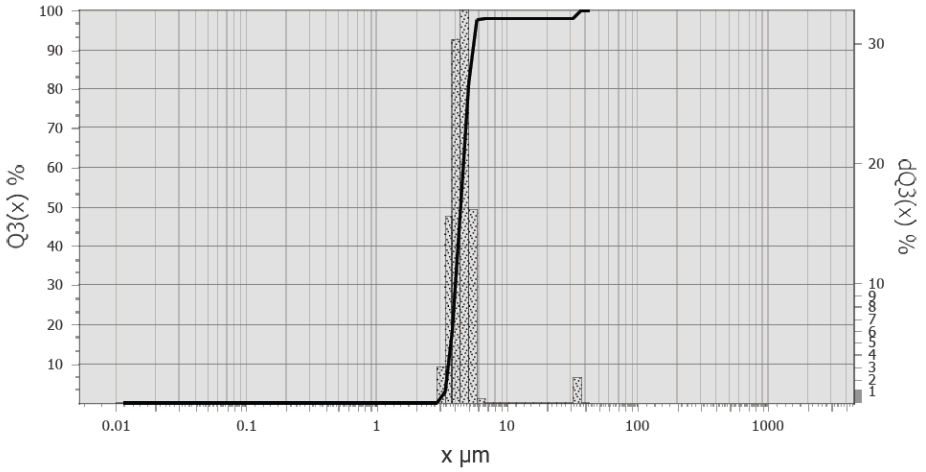

Figure 3. Particle Size Graph of
Benzoin Ethanol Extract Suspension

From the distribution chromatogram, it can be seen that the peak area is quite narrow giving information that the particle size distribution is not diverse or it can be said that the drug particle size is almost homogeneous.

Conclusions

Benzoin Ethanol Extract Suspension was successfully dissolved in water by precipitation method with solvent and surfactant tween 80 as suspending agent in

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the optimal formulation, namely F3 with a particle size of 4.80205 μm.

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