ORIGINAL ARTICLE

Increasing the stability of incense gum extract (*Styrax benzoin*) with a mixture of surfactants

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J. Adv. Pharm. Technol. Res.

ABSTRACT

Styrax benzoin is a type of incense produced in North Tapanuli, Indonesia. The content of metabolite compounds provides wide use in pharmaceuticals. The occurrence of deposits in the extract makes this preparation unstable; this instability creates problems, especially in the concentration of the extract and is impractical so it needs to be redissolved. The method of using surfactants can be used as a suspending agent, a mixture of two types of surfactants gives variations in hydrophilic and lipophilic balance (HLB), by finding the optimum HLB value to create optimum solution stability and organoleptic, various mixture ratios were carried out with test parameters such as organoleptic, pH, viscosity, particle size, and components of chemical compounds. The results show that the optimum HLB of *S. benzoin* is 12.7 in surfactants Tween 80 and Span 80.

Key words: Chemical component, hydrophilic and lipophilic balance value, stability, *Styrax benzoin*, surfactant

INTRODUCTION

The Indonesian frankincense is mostly produced in North Tapanuli, Indonesia, with the largest species dominance, namely, the Toba frankincense type (Styrax paralleloneurum) and the durame frankincense type (*Styrax benzoin*). The gum obtained from *S. benzoin* has a dull reddish to grayish brown with a fragrant aroma due to the presence of cinnamic acid.[1] In contrast to other types of *S. benzoin* tends to have high cinnamic acid compounds around 20 mg/g samples with a level of purity (86%–93%), and high levels of cinnamic acid in the sap determine the good quality of the sap.[2]

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Submitted: 20-Aug-2022 **Revised:** 30-Sep-2022
Accepted: 14-Nov-2022 **Published:** 20-Jan-2023

Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/japtr.japtr_539_22

Frankincense resin has 165 bioactive compounds that can be isolated so it has many benefits, especially as pharmaceutical raw materials used traditionally or modern.[3] Frankincense resin is mostly sold in lumps and crude extracts at relatively high prices. Unfortunately, we found that the extracts sold in the market were unstable which can be observed by the appearance of unsuspend able solids within 1 month storage period. The extract decreased in its solubility due to various factors, both physically and chemically. On contrary, the stable extract is mandatory either for direct use[4] or for further use to produce any pharmaceutical dosage form.[5] Previous researches on increasing the stability of some crude extracts have shown promising results. Some methods were used such as the cosolvating.[6,7] Another method that can be applied is reducing the particle size into the nano range.[8] Previous research showed nanosized extract enhanced its bioactivity.[9]

Furthermore, the use of surfactants enables the production of optimum organoleptic properties in the pharmaceutical

How to cite this article: Susanti N, Purba J, Gopas Oetama MY. Increasing the stability of incense gum extract (*Styrax benzoin*) with a mixture of surfactants. J Adv Pharm Technol Res 2023;14:24-8.

industry.[10] Surfactants are compounds that have hydrophilic and lipophilic groups that cause surfactants to be oil soluble and water soluble, and surface active compounds that have the ability to reduce surface tension and interfacial tension so that they are able to mix with each other while forming micelles that carry drug molecules to dissolve in the medium.[11] Each type of surfactant has a different hydrophilic and lipophilic balance (HLB) value, a mixture of the ratios of two different types of surfactants produces a mixed HLB value. Previous researches have used HLB mixtures for medicinal compounds and provided improvement, especially on the results of particle diameter size, viscosity, and pH.[12] Therefore, the aim of this study was to assess the stability of the extract in the several mixture ratios of surfactants in nanoparticle size.

MATERIALS AND METHODS

Materials

The instrument used in this study was the Particle Analyzer ANALYSETTE 22 Nano Tec, Gas Chromatography–Mass Spectroscopy (GC-MS) 7890B, Ostwald viscometer, and pH meter. *S. benzoin* powder 80 mesh size (harvested by farmers in Parsoburan subdistrict in North Tapanuli), ethanol, polysorbate 80/Tween 80, sorbitan monooleate/Span 80, and n-hexane and filter paper. The reference extract was obtained from the market.

Methods

Extraction

S. benzoin powder sample size of 80 mesh as much as 400 g was extracted by maceration method using ethanol with a ratio of 1:2. The extraction was carried out in parallel where stirring was carried out periodically for 3 × 24 h at room temperature. Then, the solution was filtered, the filtrate obtained would be thickened and washed with n-hexane, the polar fraction was collected and then evaporated again. The fraction was screened for its secondary metabolite content.

Addition of surfactants mixture

The addition of Tween 80 and Span 80 was 30% v/v with various ratios in the formula, namely, 1:2 (F1); 2:1 (F2); 1:1 (F3); 3:1 (F4); 4:1 (F5); 1:0 (F6). After obtaining the semisolid incense gum extract, its stability was tested with organoleptic parameters, pH, viscosity, particle size, and GC-MS and compared with the reference.

RESULTS

Benzoin extraction

S. benzoin used from the Parsoburan area was extracted by maceration method for 3 × 24 h. The results of the study showed that the ethanolic extract of frankincense contained 40% volatile compounds and 70% nonvolatile compounds.

Mixture of surfactants

The mixed HLB result is shown in Table 1.

Organoleptic and viscosity

In formula 1–3, there is a fairly clear phase difference, whereas formula 4–6 does not experience a significant difference through observation.

pH

The pH of the formula was demonstrated in Figure 1.

Particle size

The particle size of the formula was demonstrated in Figure 2. Chromatograms for F4, F5 and F6 can be seen on Figures 3-5, respectively.

DISCUSSION

S. benzoin has a solubility of 85% w/w in pure ethanol. The extract was then washed with n-hexane. Washing with nonpolar solvents will not have much effect on polar compounds in the molecule because n-hexane solvent cannot break covalent bonds and the electrolyte is ionized because nonpolar solvents belong to the aprotic solvent group, and cannot form hydrogen bridges with nonelectrolytes, but for compounds, nonpolar molecules can dissolve under the same pressure through induced dipole interactions and solute such as oils or fats will remain soluble due to the Van der waals-london. Hence, it is expected that nonpolar compounds of oil or fat can be separated. From the results of washing with a solution of n-hexane, the color of the n-hexane becomes clear yellow which indicates the dissolution of nonpolar

Table 1: Mixed hydrophilic and lipophilic balance results of each surfactant mixture ratio

Formula	Mixture HLB
F1	7.5
F2	11.2
F3	9.4
F4	12.1
F5	12.7
F6	15

HLB: Hydrophilic and lipophilic balance

Table 2: Viscosity and organoleptic test result

Formula	Organoleptic			Viscosity (cP)
	Phase	Precipitation	Odor	
F1	2	NC	NC	19.9550
F2	2	NC	NC	29.5912
F3	2	NC	NC	30.9220
F4	1	NC	NC	25.5476
F5	1	NC	NC	37.2721
F6	1	NC	NC	24.7501
Reference sample	1	C	NC	44.455

NC: No change, C: There is a change

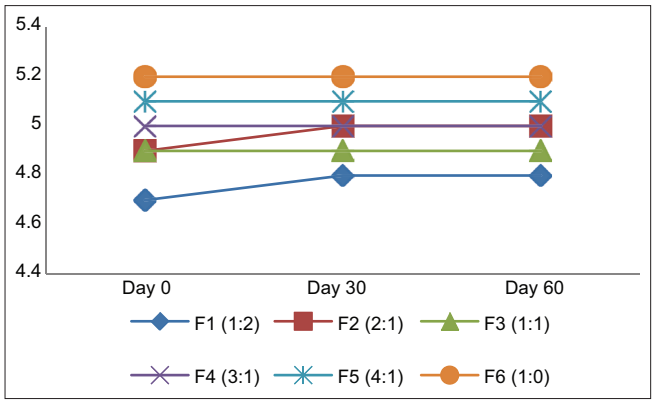

Figure 1: Graph of sample pH

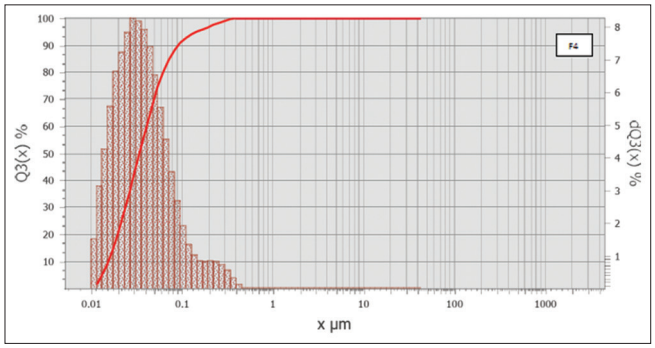

Figure 3: F4 particle size distribution chromatogram

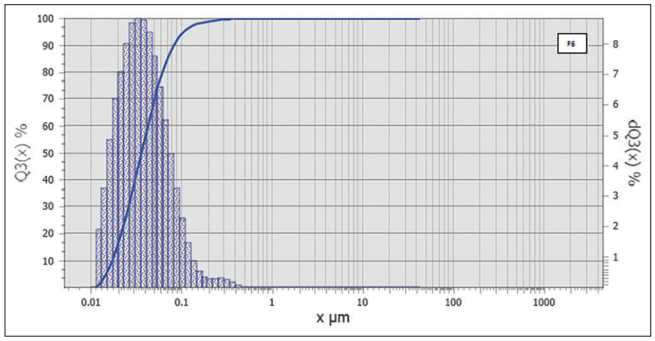

Figure 5: F6 particle size distribution chromatogram

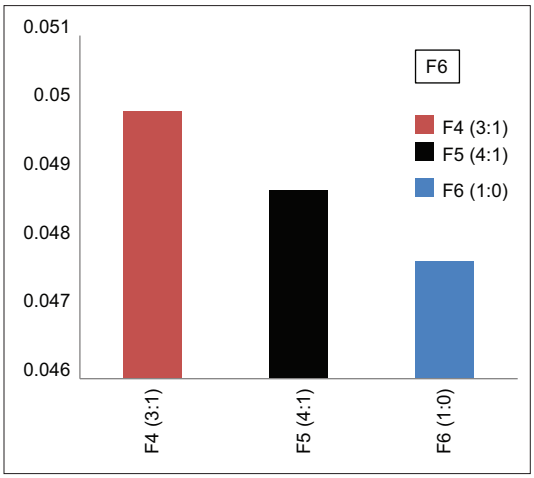

Figure 2: The average particle size of each formula

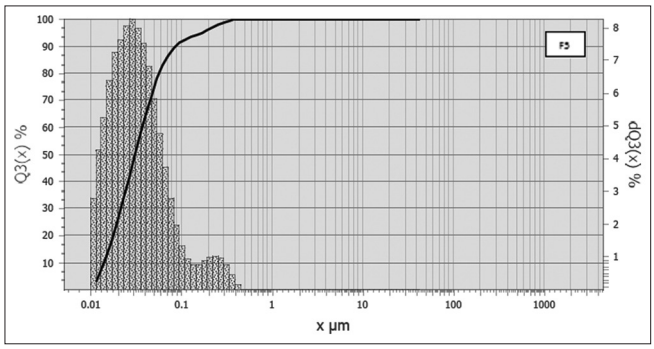

Figure 4: F5 particle size distribution chromatogram

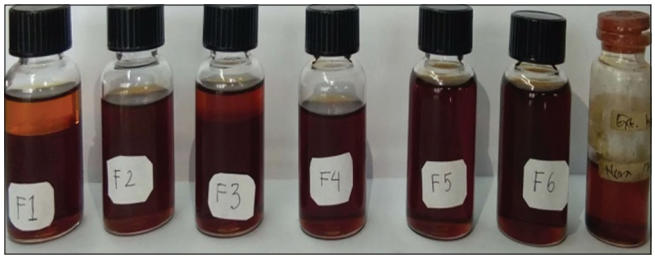

Figure 6: The organoleptic of each formula and reference

compounds. This step was aimed at reducing impurities and increasing the purity of the ethanol fraction.

Mixing two types of nonionic surfactants, Span 80 and Tween 80 with various ratios gives different results. These two surfactants were first mixed with the solvent to produce a clear yellow homogeneous solution and completely dissolved. The purpose of this mixing is to obtain HLB values so that optimum characteristics are obtained as can be seen in Table 1.

In each sample formula and reference, organoleptic tests were carried out using the senses of sight and smell, where the sample was in the form of a viscous liquid with a characteristic frankincense odor as can be seen in Table 2.

In the interaction, surfactants will interact on the surface of the drug particles with hydrophilic and the medium with lipophilic groups. On observation after 2 months, it can be seen that each formula has differences, especially in phase differences or separating, which occurs in formulas with more span of 80%. Researchers tend to see the optimum HLB of *S. benzoin* extract in the range of 12.7.

In the use of a mixture of surfactants, the sample has a brighter color than a single surfactant, and the ratio of the surfactant mixture affects the viscosity of the sample. The formula 5 sample provided the closest viscosity to the reference sample [Table 2]. 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 redistribute. Precipitation was observed in the reference sample after a 30-day storage period [Figure 6]. On contrary, precipitation was not observed in any of the samples even after a 6-month storage period.

From the results of pH measurements, it can be seen that the effect of the type of surfactant is increasing high concentration of Tween 80 gave an increase in the acidity of the solution, on the other hand, an increase in Span 80 gave a decrease in the acidity value.

Among the six formulas, the particle test was only carried out on F4, F5, and F6. The previous three formulas did not meet the criteria due to the presence of immiscible two layers of solution in the sample.

In the histogram above, it can be concluded that there is an effect of the ratio of the surfactant mixture to the particle diameter of a solution. The Span 80-Tween 80 tends to have a smaller diameter with a diameter of 1 µm. The use of Span 80-Tween 80 in this study has a value at F4 = 49.90 nm, F5 = 48.74 nm, and F6 = 47.71 nm experienced a decrease in the average diameter as the number of Span 80 decreased. The use of a single surfactant Tween 80 tended to produce a smaller average diameter but not too significant. Through Stokes' law approach if all factors are held constant by reducing the particle size of the dispersed phase, the researcher assumes that the settling rate will be slower so that stability increases.

An analysis of content using GC-MS was performed on the sample and the reference obtained a chromatogram containing the peaks for each compound.

Incense gum powder in the GC-MS test found many compounds with varying retention time and percent area (% area). The components that have the largest % area in the compound were selected, namely, benzoic acid, vanillin, and cinnamic acid.

Table 3 indicates all benzoic acid, vanillin, and cinnamic acid are the most abundant metabolites found in the extract. The difference was found in the stereoisomer of cinnamic acid as can be seen in Table 4.

The (Z)-3-Phenyl-2-propenoic acid found in sample [Figures 7 and 8] is a stereoisomer of *trans*-Cinnamic acid found in reference [Figures 9 and 10]. In general, compounds that are often found in plants in the transform, this is due to the transform having lower energy so it is more stable. The researcher assumes that the rotation of the functional groups due to the conditions when making the sample which is in

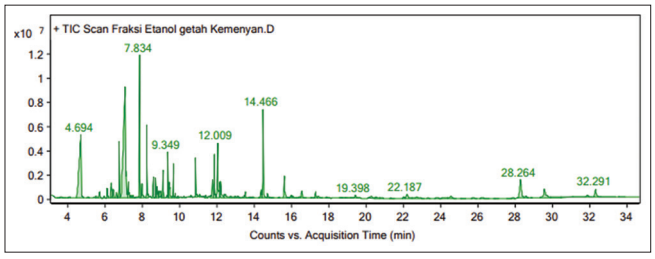

Figure 7: Chromatogram of reference GC-MS test sample

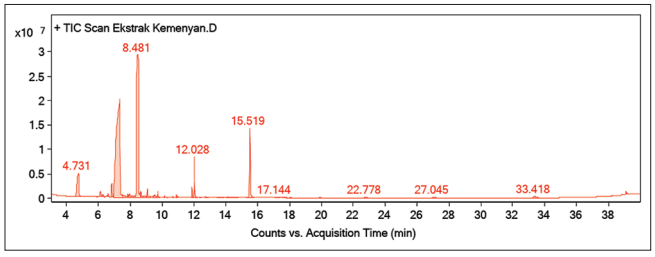

Figure 9: Chromatogram of reference GC-MS test analysis results

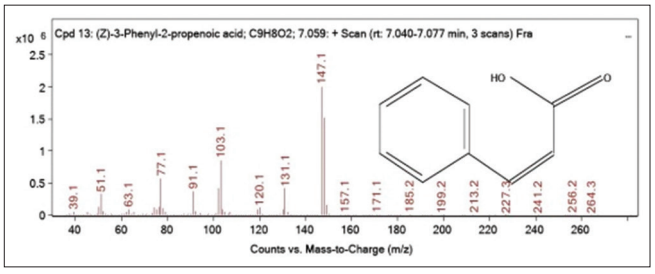

Figure 8: The GC-MS spectra of (Z)-3-Phenyl-2-propenoic acid

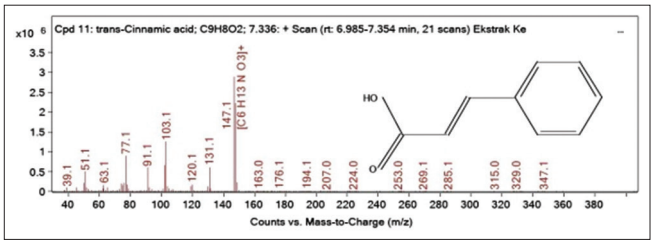

Figure 10: GC-MS spectra of *trans*-Cinnamic acid

Table 3: Gas chromatography–mass spectroscopy analysis of chemical components in the sample

Peak	Retention time	Percentage area	Chemical components	Qual
1	4.694	50.87	Benzoic acid	97.4
2	6.745	13.48	Vanillin	96.89
3	7.059	100	(Z)-3-Phenyl-2-propenoic acid (Cis cinnamic acid)	99.14
4	7.834	24.68	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)	97.13
5	8.222	14.67	Eugenol	87.79

Table 4: Gas chromatography–mass spectroscopy analysis of chemical components in reference

Peak	Retention time	Percentage area	Chemical components	Qual
1	4.731	14.01	Benzoic acid	98.83
2	6.837	9.43	Vanillin	99.29
3	7.336	100	*Trans*-Cinnamic acid	98.94
4	8.481	88.59	Diethyl phthalate	98.97
5	8.666	1.11	n-propyl cinnamate	85.75

direct contact with ultraviolet light causes the energy given by the light. This theory is supported by[13] *trans*-Cinnamic isomerization to cis in the presence of light where the C–C pi electrons are excited into antibonding orbitals that allow rotation of the pi bonds.

CONCLUSION

The use of two types of surfactants has an effect on the sample, especially on organoleptic, pH, and particle size, and the most optimal results are assessed at F5 with HLB value = 12.7 which has the closest organoleptic reference with particle size = 48.74 nm, pH = 5,1, and a viscosity of 37.2721 cP. The resulting extract exhibited better stability while maintaining the major content of the original extract.

Financial support and sponsorship

State University of Medan Research and Community Service Institute 2022.

Conflicts of interest

There are no conflicts of interest.

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