Original Research Article

Assessment of Commercial Camellia Seed Extract for Reducing Visible Benzyl Acetate Separation in Water

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ABSTRACT

In modern society, many different types of surfactants fall into one of four categories: anionic surfactants, cationic surfactants, non-ionic surfactants, and amphoteric surfactants. Out of all the surfactants, only a small portion are natural, meaning more than 90% are synthetic surfactants. For this reason, we wanted to investigate natural surfactants that could be derived from plants. Since the commercial camellia seed extract used in this study was labeled as containing at least 90% saponin, we hypothesized that concentrated ethanol-processed commercial camellia seed extract (CEPCCSE) could improve the visible dispersion of benzyl acetate in water. Benzyl acetate is poorly soluble in water; thus, it is useful as a nonpolar compound model for testing visible dispersion in water. We tested our hypothesis by mixing benzyl acetate and CEPCCSE at different ratios, in order to find the minimum concentration of CEPCCSE necessary to reduce the visible separation of benzyl acetate. We tested CEPCCSE concentrations of 0%, 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v) and found that the benzyl acetate layer was no longer visible at concentrations of 15.87% (w/v) and higher. Thus, we concluded that a higher concentration of CEPCCSE is needed to reduce the visible separation of benzyl acetate under our tested conditions.

Keywords: saponin; benzyl acetate; natural surfactant; concentrated ethanol-processed commercial camellia seed extract (CEPCCSE); benzyl acetate dispersion

INTRODUCTION

The term “surfactant” is derived from “surface-active agent,” and it refers to amphiphilic molecules that have a hydrophobic tail and a hydrophilic head (1). Generally, surfactants are able to reduce surface or interfacial tension by breaking strong cohesive forces between polar molecules, such as dipole-dipole forces. This process is typically achievable by the surfactant’s hydrophobic tail interacting with nonpolar molecules through micellization. In contrast, the hydrophilic head interacts with polar molecules via hydrogen bonds or dipole-dipole forces (2, 3). Surfactants are used across various industries today, and they are mainly used in

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cosmetics and cleaning products as an ingredient to disperse nonpolar compounds in aqueous systems.

Benzyl acetate is an ester formed from benzyl alcohol and acetic acid. It is often used as a fragrance or flavoring ingredient in foods, drinks, soaps, and other products (4). It also occurs naturally in some plants, including jasmine. Benzyl acetate can irritate the eyes, mucous membranes, and respiratory tract when exposure occurs at sufficient levels (4). Despite these concerns, it is used in the cosmetic, food, and pharmaceutical industries (5). Reported exposure estimates from household products range from 0.0003 mg/kg/day for products such as shampoo to 0.0451 mg/kg/day for products such as eau de toilette fragrances. Its use, reported hazards, and low water solubility led us to test whether a plant-derived commercial extract could reduce its visible separation in water.

Saponins are naturally occurring compounds that are found in many plants, including soapwort and legumes, and in some marine organisms (6, 7). Many saponins have surfactant properties. In this study, we selected a commercial camellia seed extract labeled as containing 90% saponin at minimum. Because saponins are plant-derived compounds with reported surface activity, researchers have studied them as possible natural surfactants in the past (8, 9). Saponins can also form stable, soap-like foams in water and include a chemically diverse group of compounds. These properties made commercial camellia seed extract a suitable material for testing whether a saponin-containing product could reduce the visible separation of benzyl acetate in water (6, 10, 11).

Due to saponins' reported surfactant behavior, we hypothesized that CEPCCSE could reduce the visible separation of benzyl acetate in water. We tested various concentrations of the CEPCCSE and evaluated whether a separate benzyl acetate layer remained visible.

METHODS AND MATERIALS

Materials

The materials for this experiment included 500 g of commercially available camellia seed extract (Saponin Powder, MarkNature, CAS No. 8047-15-2), beakers of 50 mL, 100 mL and 250 mL (ULAB, ASIN: B0BXSJX7ZL), 500 mL of ethanol (Ethyl Alcohol, TCI America, ASIN: B0B35918CG), magnetic stirrer hot plate with 1000 mL capacity (Vevor, ASIN: B07FP8J8YV), 15 cm qualitative filter paper (Ahlstrom, ASIN: B00AZ3AV1K), digital precision weight scale (Weigh Gram, ASIN: B0C4JV8MZJ), stainless steel lab spatula set (Aozita, ASIN: B07XJPJJM3), 500 g of benzyl acetate (Mystic Moments, ASIN: B07DVXT8XS), disposable 3 mL pipettes (DIYChem, ASIN: B0C4JV8MZJ), 20 mL capped glass vials (Kesell, ASIN: B0CSSV48P2), 100 mL plastic graduated cylinder (EISCO, ASIN: B00AITZUMQ).

Preparation of CEPCCSE:

Commercial camellia seed extract (Saponin Powder, MarkNature containing at least 90% saponin) was used as the source for preparing CEPCCSE for experiments designed to improve benzyl acetate dispersion in an aqueous environment. To select the ethanol-based processing conditions, ethanol volume, heating temperature, and heating time were tested separately while the other conditions were kept constant (including commercial camellia seed extract mass, cooling time, filtering method, ethanol washing volume, and temperature and time for ethanol reduction).

For the ethanol-volume test, 5.00 g of commercial camellia seed extract was mixed with 1, 5, 10, 15, or 20 mL of ethanol and heated at 65 °C for 5 minutes. For the temperature test, 5.00 g of extract was mixed with 10.0 mL of ethanol and heated at 25, 45, 65, or 75 °C for 5 minutes. For the heating-time test, 5.00 g of extract was mixed with 10.0 mL of ethanol and heated at 65 °C for 1, 3, 5, 10, or 20 minutes. For each test, the extract and ethanol were mixed in a 100 mL beaker with a stainless-steel laboratory spatula until those were fully blended. The mixture was then heated under the selected conditions and cooled for 10 minutes. It was filtered into a clean 250 mL beaker, and the material remaining on the filter paper was washed with an additional 2 mL of ethanol. The collected liquid was heated at 50 °C for 20 minutes to reduce the amount of ethanol. The resulting material is referred to as CEPCCSE. Its recovered mass was determined by subtracting the empty-container mass from the combined mass of the container and CEPCCSE.

Three independently prepared trials were conducted for each condition. The results were compared using the mean recovered material mass and relative standard deviation (RSD). RSD was calculated by dividing the standard deviation by the mean and multiplying by 100%. Recovered material was reported as mass in grams rather than percent yield (Figures 1-3). Based on these tests, the selected preparation conditions were 5.00 g of commercial camellia seed extract, 10.0 mL of ethanol, 65 °C, and 5 minutes. These conditions were then used to prepare material for the benzyl acetate dispersion test.

Because the product was only labeled as containing at least 90% saponin, all concentrations were calculated using the mass of the CEPCCSE, not the starting powder mass or an assumed mass of pure saponin.

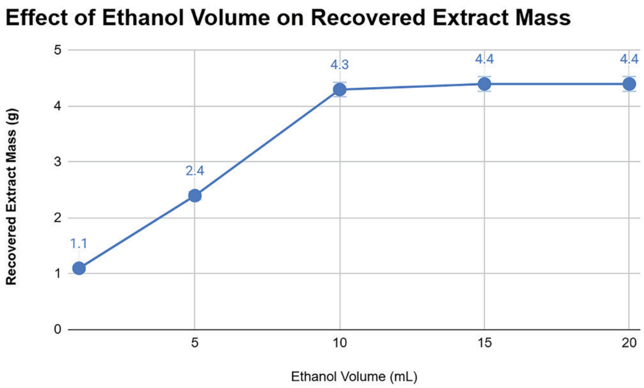


Figure 1. Five grams of commercial camellia seed extract were processed at 65 °C for 5 minutes using 1, 5, 10, 15, and 20 mL of ethanol. With the rise in volume of ethanol, there was an increase in recovered CEPCCSE mass up to 4.3 g when 10 mL of ethanol was used, and it remained relatively constant thereafter. All five data points in the graph above show the average mass recovered in three separate experiments with a relative standard deviation of 3%.

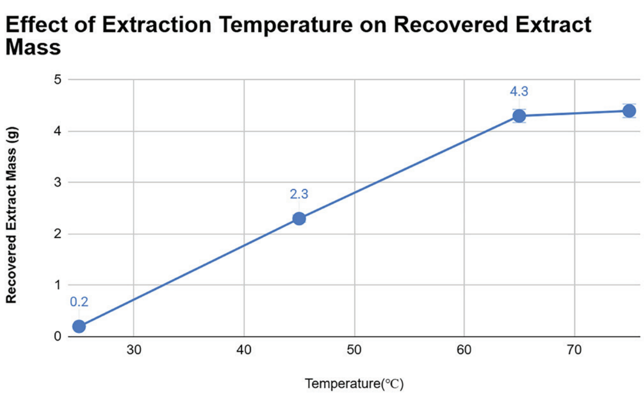


Figure 2. Five grams of commercial camellia seed extract were processed at separate temperatures of 25, 45, 65, and 75 °C using 10 mL of ethanol. As the temperature on the hot plate increased, the CEPCCSE mass increased up to 4.3 g and showed minimal change thereafter. All four points plotted on the graph represent the mean of three trials, with a relative standard deviation of approximately 3%.

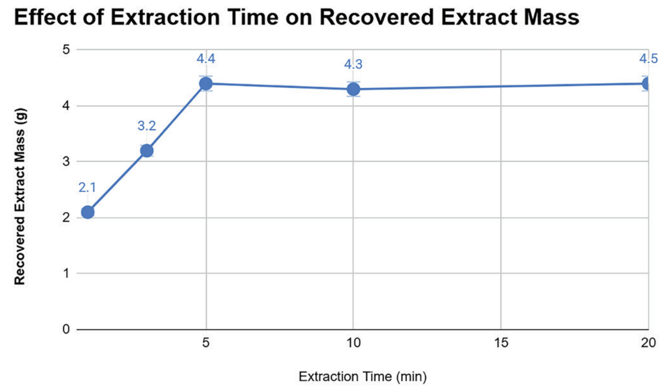


Figure 3. Five grams of commercial camellia seed extract were processed using 10 mL of ethanol at a temperature of 65 °C for extraction times of 1, 3, 5, 10, and 20 minutes. Recovered CEPCCSE increased up to 5 minutes and had a minimal change with longer extraction time. All five points plotted on the graph represent the mean of three trials, with a relative standard deviation of approximately 3%.

Benzyl Acetate Dispersion Evaluation

The independent variable was the concentration of CEPCCSE. Concentrations were reported as percentages (% w/v), calculated as grams of CEPCCSE per 100 mL of the measured final mixture volume. The concentration was calculated using this equation: concentration (% w/v) = CEPCCSE mass (g) / measured final volume (mL) × 100. Seven nonzero CEPCCSE concentrations were tested: 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v). A 0% negative control contained only 10.0 mL of water and 10 drops (0.48 g) of benzyl acetate dispensed with a disposable 3 mL pipette. The dependent variable was whether a separate benzyl acetate layer was visibly present after mixing and settling.

The main outcome was identifying the lowest tested CEPCCSE concentration where a separate benzyl acetate layer was no longer visible after mixing and settling. Each of the different eight conditions was tested in three independently prepared trials. Every mixture contained 10.0 mL of distilled water and 0.48 g of benzyl acetate before CEPCCSE was added. The final mixture volumes were measured using a graduated cylinder. The measured final mixture volumes were 10.4, 10.5, 10.6, 10.8, 11.0, 12.5, 12.6, and 15.5 mL for CEPCCSE masses of 0.00, 0.01, 0.10, 0.30, 0.50, 1.00, 2.00, and 5.00 g, respectively. Controlled variables included the water volume, benzyl acetate mass, beaker type, mixing time, settling time, and visual observation criteria. In summary, the

CEPCCSE masses were 0.00, 0.01, 0.10, 0.30, 0.50, 1.00, 2.00, and 5.00 g, and their corresponding final mixture volumes were 10.4, 10.5, 10.6, 10.8, 11.0, 12.5, 12.6, and 15.5 mL, and these produced CEPCCSE concentrations of 0%, 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v), respectively. Each mixture was stirred with a stainless-steel laboratory spatula for 5.0 minutes and then left undisturbed for 10.0 minutes. The mixtures were photographed and examined visually. A separate benzyl acetate layer was recorded as present if a floating droplet or oily layer remained on the surface. Otherwise, the layer was recorded as absent. After the experiment, all solutions were taken to a local household hazardous-waste facility for proper disposal.

RESULTS

We first tested how ethanol volume affected the recovered mass of CEPCCSE. For each condition, 5.00 g of commercial camellia seed extract was processed at 65 °C for 5 minutes using 1, 5, 10, 15, or 20 mL of ethanol. Recovered mass increased as the ethanol volume increased from 1 to 10 mL, with only small additional changes at 15 and 20 mL (Figure 1). Therefore, 10.0 mL was selected as the lowest tested ethanol volume that produced a high recovered mass. Using 10.0 mL of ethanol, we then tested temperatures of 25, 45, 65, and 75 °C. Recovered mass increased from 25 to 65 °C, with only a small additional change at 75 °C (Figure 2). Therefore, 65 °C was selected because it produced a high recovered mass while remaining below ethanol's boiling point. Finally, heating times of 1, 3, 5, 10, and 20 minutes were tested at 65 °C using 10.0 mL of ethanol. Recovered mass increased up to 5 minutes and changed little at longer times (Figure 3). Therefore, 5 minutes was selected as the shortest tested heating time that produced a high recovered mass.

Three independently prepared trials were completed for each ethanol-volume, temperature, and heating-time condition. In Figures 1-3, each point represents the mean recovered mass from three trials, and the RSD was approximately 3%. We then tested CEPCCSE concentrations of 0%, 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v), with three independently prepared trials per concentration. At CEPCCSE concentrations ranging from 0% to 8.00% (w/v), a floating oily benzyl acetate layer remained visible. No floating oil layer was visible at 15.87% or 32.26% (w/v) (Figure 4). Visual observation alone cannot determine how much benzyl acetate was dissolved or dispersed.

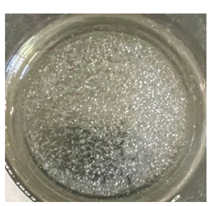
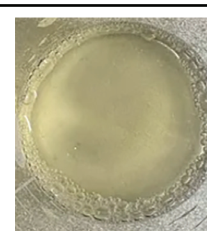
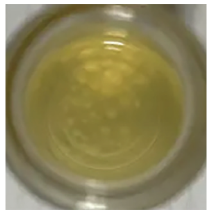
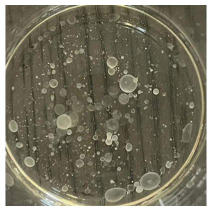
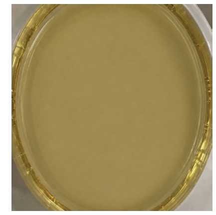
CEPCCSE concentration (% w/v)	Visible benzyl acetate layer after mixing and settling
0%	
0.095%	
0.943%	
2.78%	

Figure 4. Visual effect of CEPCCSE concentration on benzyl acetate dispersion in water. *Images show mixtures containing 10 drops of benzyl acetate, 10.0 mL of water, and CEPCCSE concentrations of 0%, 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v). Mixtures were photographed after mixing for 5 minutes and settling for 10 minutes to compare visible changes of the solution as the CEPCCSE concentration increased. The separate benzyl acetate layer became less visible at higher concentrations, while the mixtures became more opaque, and the layer was recorded as present if floating oil remained on the surface. Thus, we recorded the benzyl acetate layer as present for 0%, 0.095%, 0.943%, 2.78%, 4.55%, and 8.00% (w/v) and absent at 15.87% and 32.26% (w/v).*

CEPCCSE concentration (% w/v)	Visible benzyl acetate layer after mixing and settling
4.55%	
8.00%	

CEPCCSE concentration (% w/v)	Visible benzyl acetate layer after mixing and settling
15.87%	
32.26%	

Continued Figure 4. *Visual effect of CEPCCSE concentration on benzyl acetate dispersion in water.* *Images show mixtures containing 10 drops of benzyl acetate, 10.0 mL of water, and CEPCCSE concentrations of 0%, 0.095%, 0.943%, 2.78%, 4.55%, 8.00%, 15.87%, and 32.26% (w/v). Mixtures were photographed after mixing for 5 minutes and settling for 10 minutes to compare visible changes of the solution as the CEPCCSE concentration increased. The separate benzyl acetate layer became less visible at higher concentrations, while the mixtures became more opaque, and the layer was recorded as present if floating oil remained on the surface. Thus, we recorded the benzyl acetate layer as present for 0%, 0.095%, 0.943%, 2.78%, 4.55%, and 8.00% (w/v) and absent at 15.87% and 32.26% (w/v).*

Therefore, the result only shows that 15.87% (w/v) was the lowest tested concentration at which no separate oily layer was visible under the tested conditions.

DISCUSSION

Ethanol was used to process the commercial camellia seed extract because we expected it to recover components with high saponin content. However, techniques such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) were materials that were not available to be conducted at homes. Therefore, the composition and purity of the CEPCCSE cannot be confirmed, and it should not be described as purified saponin. The observed effect also cannot be attributed entirely to saponin. It is possible that other plant compounds, added ingredients, impurities, or remaining ethanol may have affected the visible dispersion.

The processing tests were used to select an ethanol volume, temperature, and heating time. An ethanol volume of 10.0 mL was selected because it was the lowest tested volume where the produced recovered mass was the highest, with only small additional increases at higher volumes. A temperature of 65 °C was selected because less material was recovered at 25 and 45 °C, while the amount recovered at 75 °C was similar to that recovered at 65 °C. Temperatures above 75 °C were not tested because ethanol boils at about 78 °C. A heating time of 5 minutes was selected because it was the shortest tested time that produced a high recovered mass, with little additional change at longer times. Using material prepared under these conditions, we found that a separate benzyl acetate layer remained visible at CEPCCSE concentrations from 0% to 8.00% (w/v). On the other hand, at 15.87% and 32.26% (w/v), no separate oil layer or floating droplets were visible.

This study was limited because the results were based only on visual observation. Neither micelle formation nor the chemical composition of the CEPCCSE was confirmed. Future studies could use GC-MS or LC-MS to measure the amount of benzyl acetate present in each part of the mixture and to characterize the CEPCCSE. These methods require laboratory equipment that was not available for this home-based study. Future work could also test the toxicity and environmental effects of saponin-containing extracts and determine whether the process could be scaled up for industrial use.

CONCLUSION

We selected ethanol volume, heating temperature, and heating time where the recovered mass was the highest with little additional increase under more demanding conditions. The selected conditions were then used to prepare CEPCCSE for testing with benzyl acetate in water. A CEPCCSE concentration of 15.87% (w/v) was the lowest tested concentration at which a separate benzyl acetate layer was no longer visible after mixing and settling. However, the study relied on visual observation and did not measure how much benzyl acetate was dissolved or dispersed. Future studies using GC-MS, LC-MS, or other analytical methods could provide quantitative results and identify the compounds in the CEPCCSE. This study did not test skin applications, rinsing performance, human safety, toxicity, environmental effects, industrial scale-up, process cost, or replacement of synthetic surfactants. Conclusions about these uses would require separate studies. Future work could also purify saponin from commercial camellia seed extract and compare its effect with that of other surfactants.

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CONFLICT OF INTEREST

The authors: Gurnho Park, Olivia Suh, Daniel Suh, Francis Hong, David Lee, and Jane Hong, declare that there are no conflicts of interest related to this work.

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