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ENCAPSULATION OF LEMONGRASS (Cymbopogon citratus) OLEORESIN WITH β-CYCLODEXTRIN: PHASE SOLUBILITY STUDY AND ITS CHARACTERISATION

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***Abstract*—Lemongrass oleoresin was successfully extracted from lemongrass (*Cymbopogon citratus*) using Pressurised Liquid Extraction. The biological active constituent in lemongrass oleoresin is citral or its isomers; geranial and neral is more than 75% by weight of essential oil. Geranial was used as marker compound for this study. The solubility of geranial increased with the addition β-cyclodextrin until it reached the solubility limit at 7 mM β-cyclodextrin, with no further improvement after that. The phase solubility diagram obtained was characterised as B_S. Inclusion complex of geranial was prepared by using co-precipitation (CP) and kneading method (KM). Based on phase solubility study, the stability constant, $K_{1:1}$, it indicated that molar ratio obtained was 1:1 β-cyclodextrin (β-CD):geranial for complexation. The complexes formed were characterised by using Fourier transform infrared spectrometry (FTIR) and differential scanning calorimeter (DSC). The shift of C-H stretching to higher wavenumber and the reduction of intensity of band C=O and C-H band indicated the formation of new solid phase; β-CD-lemongrass oleoresin. The DSC thermogram showed the new solid phase formed using co-precipitation and kneading methods. The peak intensity of co-precipitation shows the highest compared to kneading. The results obtained from FTIR analysis showed that co-precipitation and kneading methods were able to produce inclusion complex. However, DSC indicates that co-precipitation method able to produce inclusion complex. Therefore, co-precipitation was the chosen method for the formation of inclusion complex between lemongrass oleoresin and β-cyclodextrin.**

Keywords: lemongrass; Pressurised Liquid Extraction; encapsulation; β-cylodextrin; geranial; phase solubility

I. INTRODUCTION

Lemongrass or known as *Cymbopogon citratus* is a tall perennial grass. It is a genus of about 55 species of grasses, native to warm temperature and cultured in almost all tropical and subtropical countries [8]. It is an herb that belongs to the genus *Cymbopogon* of aromatic grasses and contains essential oil with fine lemon flavour. The biological active or active agent of lemongrass constituent is citral which is more than 75% by weight of essential oil [6]. Lemongrass is widely used as essential ingredient in Asia cuisine due to its sharp lemon flavour. In India, a tea prepared from lemongrass is used as sedatives for the central nervous system [1]. The essential oil from lemongrass has also been used to treat a wide variety of health condition such as acne, oily skin, scabies, flatulence, excessive perspiration, antimicrobial and antibacterial [9].

Despite the numerous benefits of lemongrass oil, its active agent known as citral or its isomers geranial and neral are unstable compounds. Lemongrass oil can suffer oxidation and volatilisation or react with other formulation component that may cause skin irritation. However, some of researcher reported that encapsulation is a feasible alternative way to increase the stability of this compound [3] [13]. Besides that, the physical form of lemongrass oleoresin is liquid and sticky forms make it difficult for storage and transportation, so it will increase in production cost. Lemongrass oleoresin also has limited usage because of its low water solubility.

Cyclodextrin (CD) has a crown-like structure which is cyclic (α-1, 4) linked oligosaccharide. It produces from starch by enzymatic conversion. CD is an unstable compound. Therefore, it usually combined with other chemicals to form a stable aqueous compound. Typical CDs are constituted by 6-8 glucopyranoside units, it represents with the larger and the smaller openings exposing to the solvent secondary and primary hydroxyl groups respectively. Because of this arrangement, the interior of the CD is not hydrophobic, but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic to impart CDs (or their complexes) water solubility. β-cyclodextrin (β-CD) has been on the GRAS (Generally Recognize as Safe) list since 1998, as a flavour carrier and protector, at a level 2% in numerous food products. Based on the previous researches [12][13], they commonly used β-CD and its derivatives to form a complex with other compounds due to its ability to produce a complex with comparable quality as aroma, colour and appearance. Usually, β-CD been used as an encapsulation agent. Several researchers had encapsulated complex materials like oleoresin, essential oil (*Salvia sclarea L.* essential oil, *Lippia sidoides* oil and lemon oil) and fatty acid compounds (lineoleic acid and cholesterol) with CD [3][12] [13].

This study significantly endeavors in microencapsulating of lemongrass oleoresin. It can be useful, especially in food industry and any other field including pharmaceutical and medical areas. Besides, this study can be used as a model study for future research on inclusion complex of any plant materials that contain geranial.

II. METHODOLOGY

A. Materials

Fresh lemongrass was obtained from a local market in Shah Alam, Selangor. Only stem of lemongrass was used in analysis. Prior to extraction, lemongrass was cut into 3 mm and air-dried at room temperature.

β-cyclodextrin (Wacker-Chemie GmbH, Munchen, Germany), Ethanol and n-hexane (ACS, Reag. PhEur. MERCK, Germany), KBr powder (BDH, UK) for FTIR analysis.

B. Extraction of Lemongrass Oleoresin

1) Hydrodistillation: About 900 g sample of lemongrass was weighed in a 500 ml flask and was submitted to hydrodistillation for 12 hours. The distillate was saturated with sodium chloride and added with n-hexane. Then, the ether layer and hydro layer were separated by funnel. After dehydrated by anhydrous sodium sulphate, the n-hexane layer was further dried at 40°C in a rotary evaporator to make oil to be more concentrated [4].

2) Soxhlet: Lemongrass oleoresin was extracted from fresh plant with n-hexane as a solvent, for 16 h using a Soxhlet extractor, following the AACC Method 30-25 [7].

3) Pressurissed Liquid Extraction: Extraction was done by using an ASE 200 accelerated solvent extractor (Dionex Ltd. Camberley, Surrey, UK). About 3 g of sliced lemongrass stem and 1 g of diatomaceous earth was accurately weighed and mixed together before being placed into the 22 mL cells with cellulose filter at the bottom end. The sample cell was closed to finger tightness before being placed into the carousel of the ASE 200 system. Sample was extracted using n-hexane, using standard method as proposed by [14], which were a temperature of 100°C, pressure of 1000 psi and a 30 minutes time.

C. Phase Solubility

Phase solubility studies were carried out following the method used by [5]. An excess amount of lemongrass oleoresin (20 mg) was added to screw-capped vials containing β-CD in 5.0 ml of ethanol: water (25:75 v/v) solution at various concentrations, ranging from 0 to 9 mM for β-CD. The vials then were shaken at 30°C for 48 hours in a water bath (Memmert, Germany) until reached equilibrium. The samples were centrifuged at 3000 rpm for 10 minutes. After attainment of equilibrium, the contents of the tube were filtered through Whatman filter paper (type 42). The extract solutions were determined from the absorbance at 549 nm using UV visible spectrophotometer model Perkin Elmer Lambda 35. The wavelength of absorbance of curcumin in ethanol solution was reported by [2]. The duplicate absorbances were made for each assay. To nullify the absorbance due to the presence of cyclodextrin, the apparatus was calibrated with ethanol as blank.

The appearance stability constant, K_c of lemongrass oleoresin and β-CD inclusion complex was calculated from the slope and intercept of the linear segment of phase solubility line according to the following equation:

$$K_c = \frac{k}{S_o (1-k)} \quad (1)$$

S_o = intrinsic solubility of lemongrass oleoresin in ethanol: water solution (25:75)

k = slope of the straight line

D. Inclusion Complexes

The inclusion complex of lemongrass oleoresin:β-CD was prepared by using co-precipitation and kneading methods; following the method reported by [15] [11].

E. Co-precipitation

Lemongrass oleoresin was added to screw capped vials containing β-CD in ethanol: water (25:75 v/v) mixture of 5 ml. The vials were shaken at 30°C until equilibrium reached; this is done in water bath for 48 hours (Memmert, Germany). The samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted to form the complex as microcrystalline precipitate. The product obtained was dried in oven at 40°C for 48 hours. The dried mass was sieved through 150 μm mesh (Endecotts Ltd., England).

F. Kneading

The β-CD and lemongrass oleoresin with molar ratio 1:7 was added in mortar and kneaded for 45 minutes. During the kneading, 40% of ethanol: water (25:75 v/v) mixture was added to the mixture to maintain proper consistency. The products were dried in oven at 40°C for 48 hours. The dried mass was sieved through 150 μm mesh (Endecotts Ltd., England).

G. Fourier Transform Infrared Spectroscopy (FTIR)

The KBr disk method was used. In this procedure, the pellets were prepared by mixing the samples and KBr a pestle and on agate mortar and compacted with a hydraulic press. Fourier Transform Infrared Spectroscopy (FTIR) spectra of the samples were obtained in the range of 450-4000 cm^{-1} using a Perkin Elmer Model Spectrum One FTIR spectrophotometer. The resolution was 1.0 cm^{-1} and the spectra were results in averaging 4 scans.

H. Differential Scanning Calorimeter (DSC)

Samples (1-5 mg) were weighed and placed in aluminum pans with pinhole lid and it followed by heating at rate of 10°C/min in temperature range of 140°C to 250°C. The measurements were carried out under dry nitrogen at the flow rate of 50 ml/min. DSC curves of pure materials and all system was recorded on a Mettler Toledo differential

scanning calorimeter (model DSC 1 STAR^e System). An empty pan of aluminium pan was used as reference.

III. RESULTS AND DISCUSSION

A. Extraction of Lemongrass Oleoresin

Lemongrass oleoresin that was used in this analysis was extracted from fresh lemongrass using 3 different extraction techniques. Oleoresin obtained from PLE contained the highest concentration of both neral and geranial which were 786.05±1.4 and 2842.44±5.5 mg/L, respectively (Table 1). Eventhough, the yield obtained was higher for Soxhlet extraction, the quality of oleoresin was better with PLE. Therefore, oleoresin obtained using PLE will be used for further analysis..

TABLE 1 COMPARISON OF VOLATILE COMPOUNDS (PPM) AND YIELD USING PLE, HYDRODISTILLATION AND SOXHLET EXTRACTION.

Extraction methods	Marker compounds (ppm)		Yield (%)
	Neral	*Geranial*	
PLE[a]	786.05±1.4	2482.44±5.5	2.90±0.44
Soxhlet extraction[b]	533.28±4.2	828.30±2.7	3.81±1.12
Hydrodistillation[c]	70.94±5.7	123.15±3.0	0.01±0.00

[a] Pressurised liquid extraction conditions: sample, 3g; solvent, *n*-hexane; temperature, 100°C; pressure,1000 psi; static time, 30 min.
[b] Soxhlet extraction conditions: sample, 2g (air-dried); solvent, *n*-hexane; time, 16 h.
[c] Hydrodistillation conditions: sample, 900g (fresh); time, 12 h.

B. Phase Solubility

The phase solubility diagram of lemongrass oleoresin was obtained by plotting the dissolved geranial, as a function of β-CD concentrations. Although addition of solvent (ethanol) can affect the solubility constants, mixtures of ethanol and water were used to increase the solubility of lipophilic geranial in water. A phase solubility study of turmeric oleoresin with cyclodextrin using similar method was reported by [14]. In order to simulate encapsulation of lemongrass oleoresin, geranial was used as model. The phase solubility diagrams for the complex formation between lemongrass oleoresin and β-CD are shown in Figure 1, the solubility curve can be classified as B_s type as described by [5]. B_s denotes complexes with limited solubility. Solubility curve had been plotted for geranial from 0 to 13 mM β-CD. This plot shows that there was an increment in the solubility of geranial up to 7 mM β-CD due to formation of more soluble inclusion complex. At this point, no more geranial was available for the formation of soluble inclusion complex. Addition of β-CD above 7 mM resulted in formation of precipitate of the less soluble complex. At this stage, two distinct types of precipitate were obtained, which is the less soluble inclusion complex (greenish precipitates) and excess CDs (white precipitates).

Linear host-guest correlation with slope less than 1, indicates stoichiometry of 1:1 (β-CD:geranial) with this assumption, the stability constant, K_c was calculated. The value of small K_c indicate a weak interaction, while value of stability constant within the range of 100-1000 M^{-1} are consider ideal [10].

The K_c value calculated for geranial:β-CD inclusion complex was 835 M^{-1}. [14] described the phase solubility studies of turmeric oleoresin, ar-tumerone with β-CD and γ-CD. They reported the apparent stability constant for it was 468 M^{-1} and 865 M^{-1}, respectively which were similar to the result obtained in this study. These indicate strong interactions of guest compounds with β-CD. The solubility of geranial was enhanced in the presence of β-CD due to the formation of inclusion complexes. Therefore, based on solubility limit obtained from phase solubility diagram, 20 mg lemongrass oleoresin needs 7 mg of β-CD to form soluble inclusion complexes.

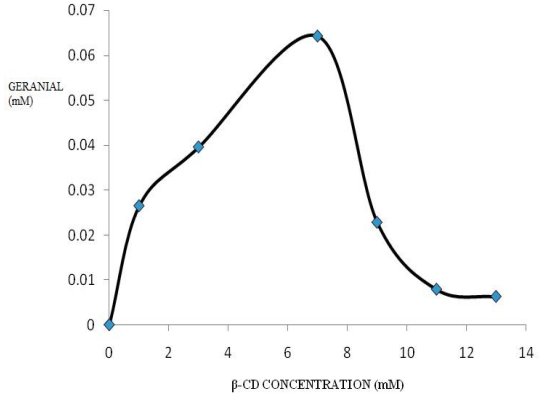

Figure 1. Solubility of geranial as a function of β-cyclodextrin in ethanol:water (25:75 v/v) solution at 30oC. Each data point is the mean of three measurements.

C. Fourier Transform Infrared Spectroscopy (FTIR)

The complexation between lemongrass oleoresin and β-CD was investigated by using FTIR. The FTIR spectra for lemongrass oleoresin, β-CD and the inclusion complexes were shown in Figure 2. The prominent spectrum of lemongrass oleoresin are as follows; 3394, 2849, 1738, 1463, 1108 and 720cm^{-1}.

Generally, the stretching region of hydroxyl group, O-H was shown at the band range of 3600-3200 cm^{-1}. As shown is Figure 2, the band at 3400 cm^{-1} indicates the presence of hydroxyl group in the lemongrass oleoresin. The presence of water in β-CD resulted in the presence of broad peak of O-H which masks the presence of O-H in lemongrass oleoresin.

A few bands of alkanes (C-H) are shown at 2930-2800 cm^{-1}. The peak at 2913 cm^{-1} and 2849 cm^{-1} can be observed in the lemongrass oleoresin and 2928 cm^{-1} for β-CD. Inclusion complex bands formed by co-precipitation and kneading were shift to higher wavenumbers indicate that the conjugation in lemongrass oleoresin was reduced in the

presence of β-CD. These results indicate C-H been used for inclusion complex.

The band for carbonyl group (C=O) peaks appeared at the band range of 1650-1620 cm^{-1}. The major peak at 1738 cm^{-1} was important characteristic of lemongrass oleoresin. The FTIR studies indicated that a cyclodextrin complex was formed with guest molecules possessing carbonyl group. As shown in Figure 2, the intensity of this band appeared reduced for co-precipitation and kneading methods. This indicated that carbonyl group been used for complexation.

A few bands of alkanes (C-H) were shown at 1300-1512 cm^{-1}. The wavenumber for lemongrass oleoresin was noticed at 1463 cm^{-1} and 1393 cm^{-1} for β-CD. The intensity of this band appeared reduced for co-precipitation and kneading. This indicated that C-H group also been used for complexation.

Stretching bands at 1260-1000 cm^{-1} indicated the presence of ether group (C-O). As can been observed in all spectra appeared for co-precipitation and kneading, suggesting did not contribute to the complexation process. Several intense bands in the 500-900 cm^{-1} region correspond to the out-of-plane bending of aromatic C-H bonds. As can be seen in Figure 2, these bands be seen in the spectra of β-CD, lemongrass oleoresin, co-precipitation and kneading which indicate this band also does not contribute for complexation.

As a conclusion from FTIR results, similar result was obtained for kneading and co-precipitation.

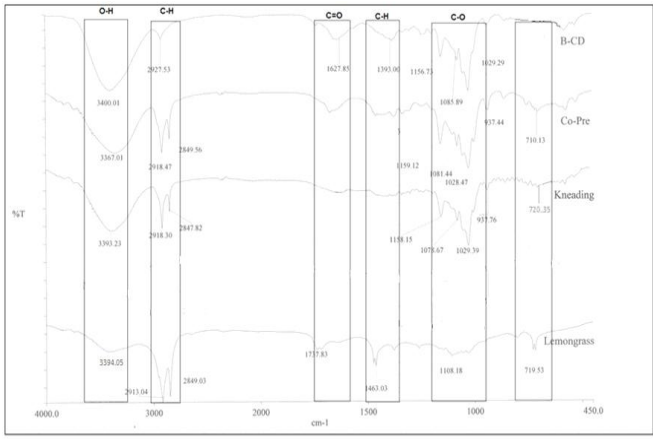

Figure 2. FTIR spectrums of β-cyclodextrin (B-CD), β-cyclodextrin-lemongrass oleoresin co-precipitation method (Co-Pre), β-cyclodextrin-lemongrass oleoresin kneading method and lemongrass oleoresin.

D. Differential Scanning Calorimeter (DSC)

Differential scanning calorimetry (DSC) was used to characterise thermal and structural properties of many compounds. DSC is a useful tool to determine the melting and crystallisation temperatures, which can provide both quantitative and qualitative information about the physiochemical state of guest inside the CD complexes. The complexation is as a result in the absence of exothermic peak or shifting to the other temperature which indicate changes in crystal lattice, melting, boiling or sublimation points. The thermogram of lemongrass oleoresin, pure β-CD, kneading and co-precipitation were represented in Figure 3.

DSC thermogram of lemongrass oleoresin shows the sharp exothermic peak that indicating the melting point of it at 78.00±0.91°C. The DSC thermograms for the lemongrass oleoresin: β-CD systems show the persistence of exothermic peak of lemongrass oleoresin in all products. The melting peak for lemongrass oleoresin was reduced in intensity for all the treatments. These results indicated there are major interaction between lemongrass oleoresin and β-CD in the inclusion complex.

DSC thermogram of β-CD shows the exothermic peak that indicates the melting point of it at 167.37±16.84°C. This exothermic peak can be also seen in kneading and co-precipitation methods with reduced intensity. This result indicates that some traces of β-CD still present in samples. However, the presence of a new peak at range between 130 to 140 °C for kneading and co-precipitation methods indicates formation of new solid phase. The peak intensity for co-precipitation was the highest, followed by kneading. These results indicate that co-precipitation was the best method for β-CD and lemongrass oleoresin complexation.

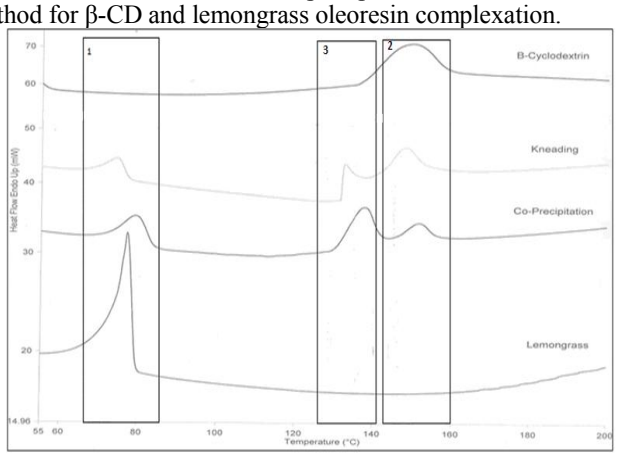

Figure 3. DSC thermograms of β-cyclodextrin, β-cyclodextrin-lemongrass oleoresin co-precipitation method, β-cyclodextrin-lemongrass oleoresin kneading method and lemongrass oleoresin.

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