Characterization of Encapsulated Ginger Essential Oils and its Antimicrobial Properties

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ABSTRACT

Ginger essential oils (GEO) are natural products with antibacteria properties consisting of many different volatile compounds have high potential to be used in many applications. In this study, the ginger GEO was successfully encapsulated in chitosan as a carrier agent using a spray drying technique. The extraction of Zingiber officinale (ginger) essential oil is performed by steam distillation method. The GEO was encapsulated in chitosan as a carrier agents at 1:3, GEO:chitosan ratio by using spray drying technique. GEO together with encapsulated GEO were further assayed for antimicrobial activity by disc-diffusion method. For characterization of encapsulated GEO, Fourier transform infrared spectroscopy (FTIR) and Field emission scanning electron microscopy (FESEM) were used. FTIR analysis revealed that there was no existence of a new functional group in the encapsulated GEO showing that there is only physical interaction between GEO and chitosan. Besides, FESEM analysis showed the encapsulated GEO were in micro in sizes and possessed spherical shape with smooth and porous surface. Furthermore, Both GEO and encapsulated GEO showed in vitro antimicrobial activity against Escheriachia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella typhi with encapsulated GEO possessed higher in the activities for all studied bacteria compared to GEO. The encapsulated GEO demonstrated a superior performance against Salmonella typhi with the inhibition zone of 22.5 mm compared to GEO only 13.5 mm. The results obtained indicated that due to the volatility and instability of the GEO when exposed to environmental factors, its encapsulation considerably improve and enhanced its performance.

Keywords: Ginger Essential Oil, Chitosan, Encapsulation, Antimicrobial, characterization

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INTRODUCTION

Ginger is generally use in Malaysian food as spice due to their aromatic and pungent properties (Maizura *et al*. 2011). Ginger plant (Zingiber officinale) is one of the important plant that are rich in phytochemicals has been widely used in medicinal to treat sore throats, muscular aches, pains, vomiting, hypertension, indigestion and infectious diseases (Islam *et al.* 2014). Based on previous studies, many researchers have found that the main compounds exist in ginger essentially involve monoterpene and sesquiterpene hydrocarbons groups (Rahmani, Al, & Aly, 2014; Sharma, Singh, & Ali, 2016). Previous studies also mentioned that some of the compounds for example α-terpineol, linalool and geraniol contribute to the antimicrobial properties of ginger essential oil (Sivasothy et al., 2011).

Essential oil consists of greatly aromatic substances which mainly a multi-component of bioactive compounds presence in plants (Lucchesi et al., 2004). However, essential oil has certain drawbacks which degrade at higher temperature, easily oxidized and lost during storage. One of the new advanced technologies that have been well developed to overcome this problem is encapsulation. Encapsulation is one of the techniques that have been implemented to protect volatile compound against oxidation, degradation and evaporation. Chitosan is an example of wall material that offer a low cost, abundance, biodegradability and antimicrobial properties attribute excellent protection for volatile essential oil *(Souza*

et al. 2014). Therefore, the aim of this study is to encapsulate ginger essential oil in chitosan and characterize their morphology and chemical composition by using Thermogravimetric analysis, Fourier transform infrared spectroscopy and Field emission scanning electron microscopy. Finally, the antimicrobial properties of encapsulated ginger essential oil will be investigated.

METHODOLOGY

Extraction of Ginger Essential Oils Using Steam Distillation

The volatile compounds of ginger rhizomes were extracted using steam distillation process. A 500 g of sample was placed on the grating inside the distillation chamber with the setting temperature is 300 °C and the collection process took about 5 hrs. The essential oil obtained was collected in a sealed vial and stored in the chiller at temperature -10 °C for further analysis.

Extraction of Ginger Essential Oils Using Steam Distillation

The The process of emulsion formation in this study refers to previous research by Jayanudin & Rochmadi, (2017) with minor modification. An oil-in-water emulsion was prepared using ginger essential oils as the core material and chitosan as the wall material. 1 g of chitosan was dissolved in 250 ml of 0.5% acetic acid to form chitosan solution with continuous stirring for 24 hours at room temperature using magnetic stirrer. In order to remove the impurities, the chitosan solution was filtered through a nylon cloth. Ginger essential oil was added to the chitosan solution with essential oil-to-chitosan ratios of (GEO:CS): 1:3. Then, 2 ml of Tween-80 was added into the mixture solution and stirred it using a high-speed homogenizer at 290 rpm for 15 min to form an emulsion. The emulsions were fed into the spray drying. The settings of spray dryer were operated with inlet air temperature at 180 °C while the outlet air temperature automatically maintained at 137 °C, feed rate was adjusted to 1 L/h. The encapsulated powder form was collected, sealed and stored at 4 °C for further analysis.

Encapsulation Efficiency

Encapsulation efficiency (EE %) was determined using previous methods suggested by Jayanudin & Rochmadi, (2017). The oil content is divided into two type which are total oil and surface oil. Total oil is the internal and surface oil content of the powder, whereas surface oil is the free oil at the surface of particles. The total oil is represented as encapsulated powder while surface oil referring to un-encapsulated powder. The concentration was calculated by calibration curve and equation 1 below shows the formula to determine the encapsulation efficiency.

$$\text{Encapsulation Efficiency}\ (\%) = \frac{\text{Total oil} - \text{Surface oil}}{\text{Total oil}} \times 100\ \% \qquad (Eq\ 1)$$

Total Oil Determination

The method to determine the total oil by solvent extraction refers to previous study with minor modification (Li et al., 2013). Spray-dried powder (0.1 g) was combined with 10 g of 1% acetic acid in a 50 mL centrifuge tube and stirred until the powder was completely dissolved. The ginger oil released from the microcapsules was extracted with 50 mL of n-hexane for 10 min three times. The extracts were collected in a 25 mL volumetric flask. The content of ginger oil in the extract was measured at wavelength 280 nm in triplicate by a UV spectrophotometer.

Surface Oil Determination

The method to determine the total oil refers to previous study with minor modification (Li et al., 2013). Spray-dried microcapsules (0.1 g each) were suspended in a 10 mL of n-hexane and extracted for 10 min three times. The total extracts were collected by centrifugation at 289 rpm. The content of ginger oil in the

extract was measured at wavelength 270 nm (the maximum absorbance for ginger oil in n-hexane by full wave scanning) in triplicate.

Characterization of Encapsulated Ginger Essential Oil

The encapsulated GEO was characterized using Fourier transform infrared spectroscopy (FTIR) and Field emission scanning electron microscopy (FESEM). FTIR is used to identify the functional group and chemical composition that exists in each sample. The samples were measured at ambient temperature in the range of 640 to 4100 cm^{-1} using Perkin Elmer Spectrum GX FTIR. While, FESEM was used to study the morphology of encapsulated ginger essential oil. The best result of encapsulated ginger essential oil that identified in encapsulation efficiency was tested at an accelerating voltage of 5 kV with × 1.50 K and × 10.00 K magnification using Zeiss/Merlin FESEM.

Antimicrobial Study

The antimicrobial study of the essential oil of ginger were evaluated against one strains of Gram-positive bacteria (Staphylococcus aureus) and two strains of Gram-negative bacteria (Salmonella typhirium and Pseudomonas aeruginosa). The bacteria strains were obtained from Microbiology Laboratory, Faculty of Science and Technology, Universiti Sains Islam Malaysia. All the bacteria strains were cultured at 37 °C on nutrient broth medium for overnight. Then, the bacterial inoculum was diluted to the concentration of 106 cell/mL. Each bacteria culture at a concentration 1×10^7 cell/ml was spread onto different nutrient agar plates by using sterilized cotton swabs. The two wells per plate were made in the set agar containing the bacterial culture by using a sterile paper disc of 6 mm diameter. Then, a sterile disc will be impregnated with 10 µL of the ginger essential oil and placed on the test plate. The plates were subsequently incubated at 37 °C for overnight. The diameter of zone of inhibition in three different directions was measured and the mean diameter was recorded in mm.

RESULTS AND DISCUSSION

From the experimental data, the yield percentage of the GEO was calculated to be 0.52 % and the encapsulation efficiency was found to be 92.35%. FTIR data showed the resulting spectrum corresponds specifically to the chemical bonds that present in the molecules. Therefore, in this study, the FTIR analysis were carried out to identify the interaction of major functional groups involved in the encapsulation of ginger essential oil. The FTIR spectra of raw GEO, raw chitosan and encapsulated GEO were illustrated in Fig 1 (a, b and C), respectively. Based on Figure 1(a), the FTIR spectrum of raw ginger essential oil illustrates the presence of an OH-H group at wavenumber 2932 cm^{-1}. Then, the bond of C=C appear at wavenumber of 1661 cm^{-1}. In previous research, it had been reported that ginger essential oil bands tend to have C=C and C=C–C=C stretching bond at 1740 and 1640 cm^{-1}, respectively (Fernandes et al., 2017). Meanwhile, the spectrum of chitosan in Fig 1(b) possess to have O-H stretch group on the wavenumber of 3384 cm^{-1}. Another important vibrational mode associated to NH groups is presented in the raw chitosan spectrum at 1560 cm^{-1} which was the bending vibration of the absorption peaks of the amine group and the low intensity of CH groups in the wavenumbers at 2910 cm^{-1}.

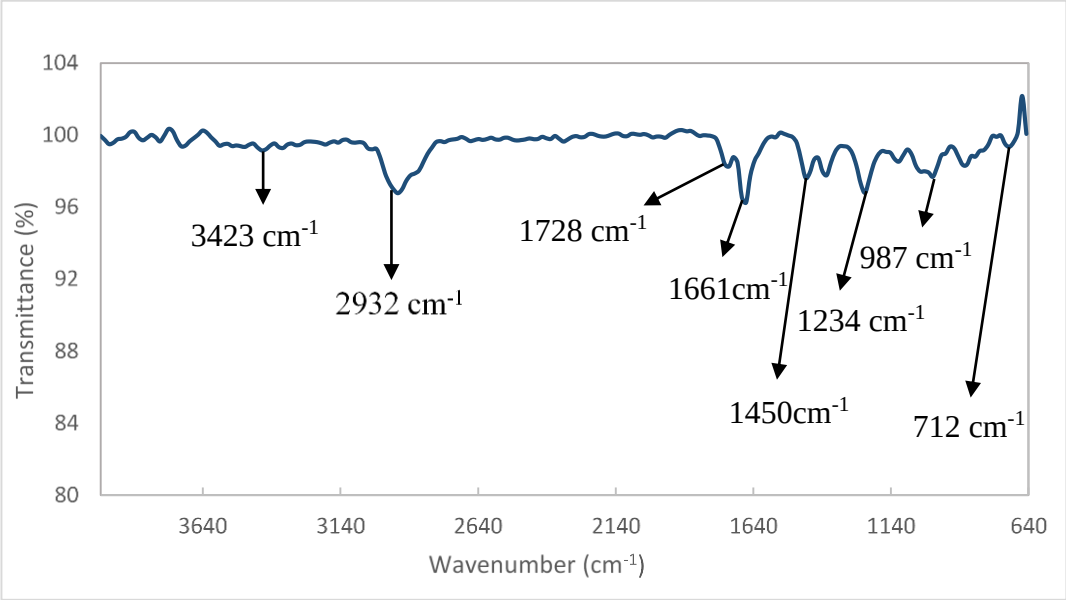

Fig 1 (a) FTIR spectra of raw ginger essential oil

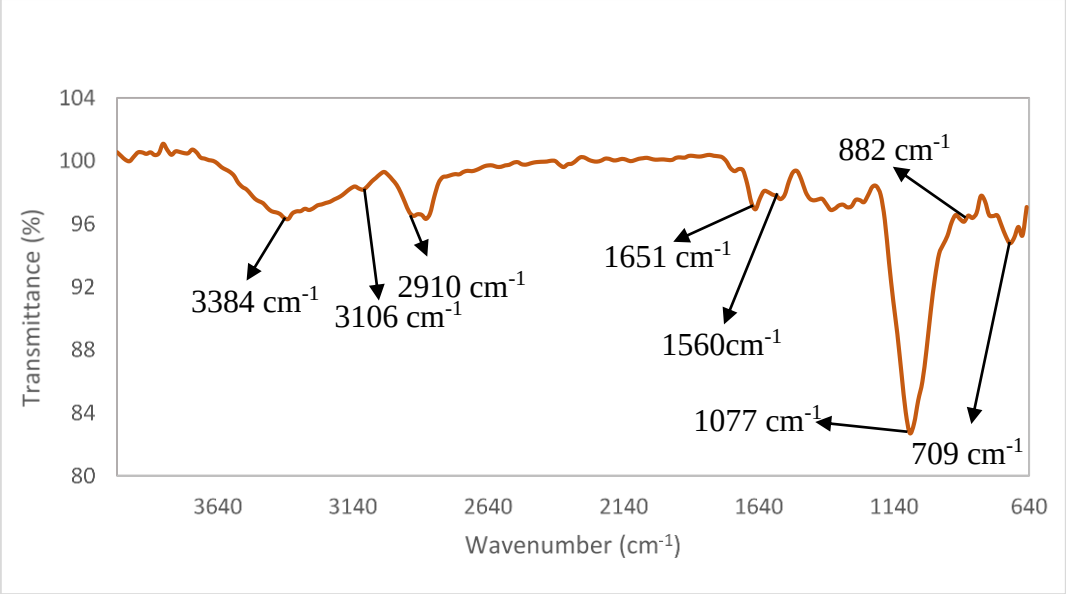

Fig 1 (b) FTIR spectra of raw chitosan

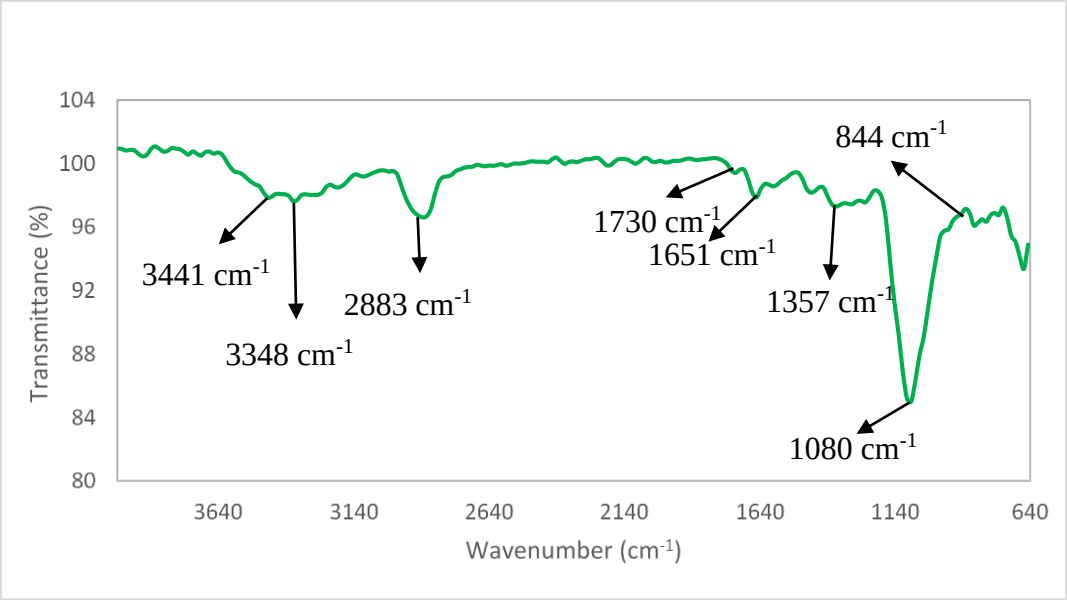

Fig 1 (c) FTIR spectra of encapsulated GEO with chitosan

Field Emission Scanning Electron Microscopy was used in this research in order to visualize the details on the surface morphology of encapsulated GEO sample at a high resolution. Based on the experimental analysis, the FESEM results revealed that the encapsulated particles exhibited an uneven spherical shapes. As illustrated in Fig 2, FESEM images clearly shows very small particles which were found to adhere to large particles of the microcapsules and irregular shape was identified for low loading of core material (ginger essential oil). Somehow, the microcapsules containing chitosan only showed a large agglomerate formation among the spray dried particles which was illustrated in Figure 2(a). So, from the images revealed, it can be deduced that there might be contamination and shrinking due to empty core during the early stage of spray dried process. In Figure (b), it clearly shows stable spherical size of microcapsules with just slightly agglomeration which indicate that the amount of oil loading used was very well protected by the wall material (chitosan).

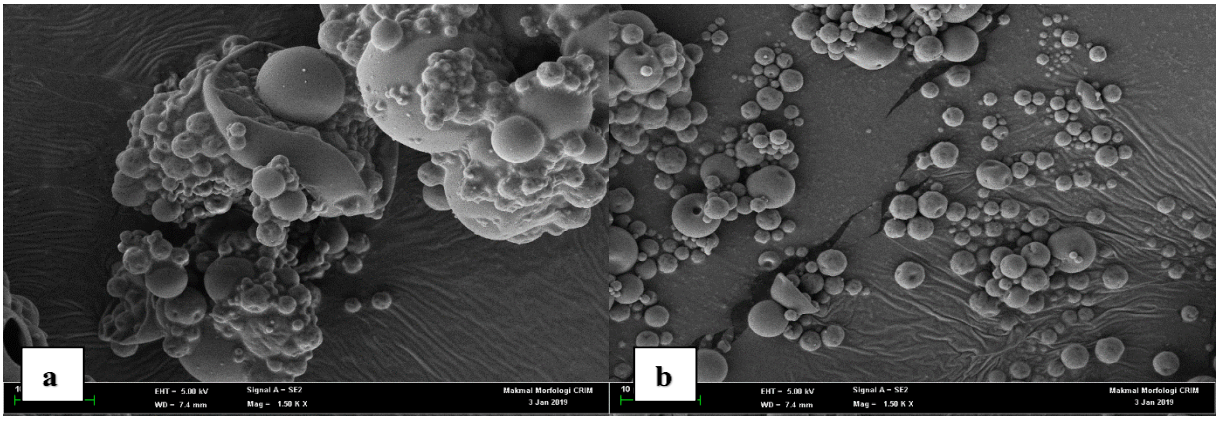

Fig 2: FESEM images of spray-dried encapsulated ginger essential oil particles operated at 5.00 kV with × 1.50 K magnifications: (a) Microcapsule containing chitosan only; (b) Microcapsule containing chitosan and ginger essential oil

For antimicrobial study, the variation of inhibition zone of GEO sample on agar plate against bacterial strains and the average mean of inhibition zones are summarized in Table 1. By referring Table 1, the volatile ginger essential oil showed potential antimicrobial activities in comparison with the encapsulated GEO:CS sample. Then, the diameter of the inhibition zone of GEO was in the range of (3.5 ± 0.1 mm) to (13.5 ± 0.2 mm) as compared to inhibition zone for chitosan in the range of (11 ± 0.1 mm) to (22.5 ± 0.3 mm) for chitosan (CS). The antimicrobial activity of the GEO was found to have highest activity against Staphylococcus aureus while lowest activity was found against Pseudomonas aeruginosa. The data for encapsulated ginger essential oil revealed to have a potent antimicrobial activity towards Staphylococcus aureus. Based on Table 1, the result indicated that the encapsulated GEO showed the highest activity towards all the bacteria strains. Thus, the encapsulated GEO was more effective as an antimicrobial agent compared to the ginger oil alone. Based on previous study, it has been proved that there is a relationship presence between the chemical components and its antimicrobial activity. It has also been reported that ginger (rich in sesquiterpenes) essential oils possessed a wide spectrum of antimicrobial activity (Kumar et al., 2016). Danijela et al. (2014) has mentioned in their research that the encapsulated thyme oil using chitosan presented to have a significant antimicrobial activity towards Staphylococcus aureus (12 mm), the outcome results was expected also due to the antimicrobial effect of chitosan.

	Diameter of Inhibition Zone (mm) ± SD (n=3)		
Microorganisms	**Ginger Oil**	**encapsulated GEO**	**CS**
Staphylococcus aureus	13.5 ± 0.2	22.5 ± 0.3	22.5 ± 0.3
Pseudomonas aeruginosa	3.5 ± 0.1	13.5 ± 0.5	10 ± 0.1
Salmonella Typhi	8.0 ± 0.1	22.5 ± 0.3	11 ± 0.1

CONCLUSION

From this research, it can be concluded that highest value of encapsulation efficiency of GEO in chitosan which is 92.35 % contribute to higher antimicrobial activity of the encapsulated GEO.

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