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Posted Date: 9 February 2026

doi: 10.20944/preprints202602.0658.v1

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Article

Improved Method of Recrystallization of *Zingiber zerumbet* Extracts to Obtain Zerumbone

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Abstract

The oil of the *Zingiber zerumbet* has been gaining traction amongst the researchers due to its phytochemical benefits mainly zerumbone. Due to the presence of a complex mixture of terpenoids in the oil, recrystallization is an effective method to obtain the active compound, zerumbone**. Objective**: This study was to optimized the recrystallization via decanting with hexane and evaporation method to produce the most yield that contain purified zerumbone crystals. **Materials and methods:** Ground dried and intact dried *Zingiber zerumbet* were compared to determine the highest yield of zerumbone crystals. A yield comparison between ground and intact dried *Zingiber zerumbet* was carried out through 6 hours of hydrodistillation followed by decanting with hexane. HPLC qualitative analysis was done on the purity of zerumbone crystals from ground and intact material respectively at wavelength 280nm. **Results:** After 6 hours of hydrodistillation, intact dried crude plant material produced 0.29%$^{w/w}$ more zerumbone crystals than the ground dried *Zingiber zerumbet*. HPLC qualitative analysis done on the purity of zerumbone crystals from ground dried and intact plant material were 98.51% and 99.68% respectively at wavelength 280nm. **Discussion:** A yield comparison between ground and intact dried *Zingiber zerumbet* that was carried out through hydrodistillation, exhibited significant difference ($p<0.05$). The low yield of oil from the ground crude plant material, which in turn produced lesser zerumbone crystals can be contributed to the heat emanated by the blades of the grinder resulting in melted oil from the compound sticking to the surface of the grinder. It is also lamented that only 1% of the energy transmitted to the grinder is used for size reduction, the balance of the energy is converted into heat. Also, the colour of the compound from traditional grinding also intensified due to the heated compound. Besides that, the method of recrystallization that produced a higher yield of zerumbone crystals can be performed for future research. **Conclusion:** Intact dried *Zingiber zerumbet* produced higher yield of zerumbone crystals through an improved method of recrystallization.

Keywords: zerumbone; hydrodistillation; recrystallization; purification; decanting

1. Introduction

Plant foods, spices and herbs have ruled for generations as traditional medicines, ayurvedic, perfumes, preservatives, aroma enhancers for food and beverages, seasonings and flavourings and will continue to partake in our daily lives as they contain a barrage of phytochemicals (Shahrajabian et al., 2019). In this era, an increasing number of people are using herbs and spices as home remedies to alleviate simple ailments and to promote one's health. In addition, with burgeoning metabolic syndromes, complementary and alternative medicines are being sought after to manage such diseases and to prevent worsening of these syndromes. Furthermore, there are a number of

 doi:10.20944/preprints202602.0658.v1

publications in the pharmaceutical industry that have considered these phytochemicals beneficial to human health.

Zingiberaceae is the largest monocotyledonous family of the plant kingdom found in tropical and subtropical regions of the world. The members of this family, such as *Zingiber officinale* (ginger), *Curcuma longa* (turmeric), *Zingiber zerumbet* (bitter ginger) and *Elettaria cardamomum* (cardamom), are used in complementary medicine, agriculture, in cuisines and for ornamental purposes (Zakaria et al., 2011). The genus *Zingiber* contains approximately 85 species. This medicinal plant is cultivated extensively because of its low planting costs. Besides its wide usage as a spice, ginger which can be taken raw or cooked has been used traditionally as treatment for indigestion, flatulence, gastritis, gastro-esophageal reflux, dyspepsia, nausea, morning sickness, hangover, common cold, sinusitis, sore throat, fever, arthritis, rheumatoid arthritis, colic, stomach cramps, toothache, tension headache, migraine headache, asthma and many more (Ghasemzadeh et al., 2017; Singh et al., 2014; Li et al., 2020; Silva et al., 2018; Noor and Sirat, 2016; Rahman et al., 2014). Even products these days have added brown sugar with ginger for a more sweetness to spiciness taste and with that, making it more palatable to consume.

Zingiber zerumbet (L.) Smith, one of the most cultivated *Zingiberaceae* family which is native to SouthEast Asia, especially India, Indonesia and Malaysia, has made its way into our lives for generations as a spice and most widely used condiments in various cuisines and beverages throughout Asia (Ghasemzadeh et al., 2016; Rahman et al., 2014; Shahrajabian et al., 2019). Due to its many types of phytochemicals found *in Zingiber zerumbet,* it is regarded as one of the spices in preventing or managing certain medical conditions (Ghasemzadeh et al., 2017). It is also well spread throughout tropical and subtropical regions of other countries such as Sri Lanka, Nepal, China, Japan and other parts of the world (Rahman et al., 2013). *Zingiber zerumbet* is a warm-season perennial, tuberous root plant adapted for growth in damp and shaded parts of lowlands or hill slopes (Khalid et al., 2011; Shahrajabian et al., 2019). It is observed that they tend to have the best growth spurts at temperatures of 25-28 °C (Tan et al., 2018).

There are various names being used in different countries with regards *to Zingiber zerumbet* such as bitter ginger, wild ginger, shampoo ginger, bitter ginger, awapuhi, lempoyang as it is commonly known as in Malaysia and Indonesia; parsu kedar, ghatian, and yaiimu in India, hong qui jiang in China and the list goes (Sachin et al., 2017; Shahrajabian et al., 2019; Singh et al., 2019). All the plant parts such as the flower, leaves and rhizomes respectively carry multiple health promoting effects and have been researched by many as they contain medicinal benefits (Padalia et al., 2018; Sachin et al., 2017). Briefly, the fragrant leaves are used to enhance the taste, colour and odour of cuisines and the red pine cones which is the flower is used for softening and bringing shininess to the hair (Jalil et al., 2015). Rhizomes with a light brown skin have a fibrous and scaly root and a pungent scent (Singh et al., 2014). Powdered rhizome is used to treat ear infections and toothache as the stinging nature of ginger tends to curb pain (Ghosh et al., 2011). Rhizomes are the ones that play a part in food flavouring in cuisines and traditional medicine amongst the people across the globe (Nik et al., 2009; Singh et al., 2019).

The oil of the rhizome has attracted the most attention from researchers for its potential human health benefits as it is a complex mixture of terpenoids, comprising the largest subgroup sesquiterpenoids; mainly zerumbone the most active constituent followed by humulene, humulene oxides, βcaryophyllene and α-caryophyllene and varying proportions of monoterpenoids; camphene, sabinene, myrcene, amongst others (Akhtar et al., 2019; Azelan et al., 2018; Kiyama, 2021; Koga et al., 2016; Singh et al., 2019; Zhang et al., 2018). Recrystallization of the oil of *Zingiber zerumbet* with an inorganic solvent is an effective way to produce the pure active compound, zerumbone. Zerumbone has three double bonds, two conjugated and one isolated, as well as a double conjugated carbonyl group in the 11-membrane ring structure (Kitayama et al., 2001). The boiling and melting point of zerumbone are 321–322◦C at 760 mm Hg and 65.3◦C, respectively (Kitayama et al., 1999). Zerumbone (2E,6E,10E)-2,6,9,9- tetramethyl cycloundeca-2,6,10-trien-1-one), a monocyclic sesquiterpene ketone that has reported to act as an anti-inflammatory, antimicrobial, anticancer,

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antioxidant, antipyretic, analgesic, cyclooxygenase-2 suppressant properties, antibacterial, anti-allergic, anti-ulcer, antioxidant, antinociceptive, antiplatelet aggregation, and hepatoprotective (Foong et al., 2018; K. Kalantari et al., 2017; Md et al., 2018; Padalia et al., 2018; Sulaiman et al., 2009)

According to Khalid et al. (2011), upon testing zerumbone on models of nociception, found that the extract of *Zingiber zerumbet* acquires central and peripheral antinociceptive activity. It is also further reported by Zakaria et al. (2010), that the involvement of zerumbone in inhibition of prostaglandin, histamine and opioid-mediated among others manages to exhibit anti-inflammatory and antinociceptive activities. Furthermore, Chia et al. (2020), suggested that due to the mode of action that zerumbone plays in the antiinflammatory receptors pathway, zerumbone is beneficial in managing neuropathic pain in combination with conventional therapy. However more studies are needed to improve the current approaches in the treatment of neuropathic pain. As demonstrated by Somchit et al. (2012), the extract of *Zingiber zerumbet* showed lower anti-inflammatory activity as compared to the pure active compound, zerumbone. This also goes to show that zerumbone has a potent analgesic effect which is comparable to that of a non-steroidal anti-inflammatory drug (NSAID). *In vitro* and *in vivo* studies by Chien et al. (2016) and Tian et al. (2020) have concluded that zerumbone extracted from *Zingiber zerumbet* exhibited an anti-inflammatory effect on induced arthritis in mice as it involves the downregulation of overly-secreted prostaglandins which are responsible for inflammation. In addition to the benefits of the pure active compound of *Zingiber zerumbet*, various preclinical studies have indicated that zerumbone managed to repress the IL-6/JAK2/STAT3, PI3K/AKT/mTOR pathways and downregulate the expression CXCR4, activation of NF-κB, and other oncogenic proteins (Girisa et al., 2019). Various cancers such as brain, breast, colon, liver, and lung can be managed as cancer cell proliferation will be inhibited due to induction of cell cycle arrest and apoptosis (Girisa et al., 2019). However, Jalili-Nik et al. (2020) also lamented that more preclinical trials need to be undergone for a better understanding of the anti-cancer properties of zerumbone.

Since the extracts of *Zingiber zerumbet* possess many pharmaceutical benefits, it is only necessary to delve in its benefits by optimizing the extraction of its rhizomes. Due to the presence of various mixtures of terpenoids in the oil of rhizomes as mentioned previously, it is of utmost importance to recrystallize to obtain the pure active compound of zerumbone crystals.

2. Materials and Methods

2.1. Materials

Crude material, fresh rhizomes of *Zingiber zerumbet*, were procured from a local market in Chow Kit, Malaysia. Fresh rhizomes were identified by the Dr. Mohd Firdaus Ismail, Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Malaysia (MFI 0180/20). Analytical grade hexane was used for decanting of extraction compound. Distilled water from filtration system in Physiology Lab, Universiti Putra Malaysia (UPM), Serdang was used with the solvent to prepare the extraction compound. HPLC was done at Natural Products Division, Forest Research Institute Malaysia (FRIM), Selangor. All other equipment used was from Physiology Lab, Universiti Putra Malaysia (UPM).

2.2. Preparation of Extraction

Firstly, 6 kg rhizomes of *Zingiber zerumbet* were cleaned and cut into pieces approximately 1 cm lengths. The cut rhizomes were then oven-dried for three days at 50°C and weighed thereafter. Two batches of oven-dried cut rhizomes, each weighing 1 kg were prepared. The first batch of 1 kg of oven-dried cut rhizomes were ground into powder by using a commercial grade grinder (Model EBM-9182, ELBA Malaysia) and weighed again after being ground. And the second batch of 1 kg of oven-dried cut rhizomes were extracted as intact.

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2.3. Extraction of Zingiber zerumbet

Both forms of dried rhizomes were extracted by hydrodistillation method using a Clevenger-type apparatus (Noor et al., 2016). Briefly, the crude materials were added into a round bottom flask seated in a heating mantle. Then, 3L of distilled water was added into the flask, to immerse the crude materials. The round bottom flask was connected to the distiller and finally to the condenser. The pipes were connected to the running filtered tap water and the cooler was set at 9°C. The contents were boiled at 100°C. Hydrodistillation was stopped when no pale yellowish oil crude extracts were seen at the collecting tube. Hydrodistillation was conducted for 6 hours for both forms of crude plant materials respectively. Pale yellowish crude extracts, a mixture of oil and water that were collected from both forms of dried materials were stored in conical tubes at 4°C respectively.

2.4. Optimization of Recrystallization and Isolation of Zerumbone

After hydrodistillation, using a glass separatory funnel with stopcock, a liquid-liquid extraction using hexane as a solvent was performed for both pale yellowish crude extracts that were obtained from both forms of crude plant material (Al-Amin et al., 2019). Briefly, crude extracts were washed with distilled water twice, each with 10 ml. Then, 10 ml of hexane was added into the separatory funnel and secured with a stopper. The funnel was shaken a few times to release the pressure that had formed in the funnel and it was allowed to stand for a few seconds for the separation to be settled. The aqueous phase was drained into a flask. In a separate beaker, the remaining liquid was collected. The funnel was flushed with an additional 5 ml of hexane and the step was repeated twice. To evaporate the hexane, the liquid was boiled to isolate the oil. It is noticed that the liquid boiled at 67.5°C. The liquid was poured in another beaker and the previous beaker was washed with a further 5 ml of hexane twice. Heating process repeated. The liquid was again poured in another beaker and the previous beaker was washed with a further 5 ml of hexane twice and heating process continued thereafter. On low heat, when the thermometer that was placed in the beaker showed 90°C and no evaporation activity was seen, the heating process and evaporation of hexane was stopped. After decanting, the extracts from both forms were left in the fume hood for a day, to dry and to spontaneously crystallize respectively.

After which, pure zerumbone crystals was further dried by vacuum filtration. Using a Buchner funnel, zerumbone crystals were placed on a wet filter paper which was cut to fit into the funnel. The end of Buchner funnel was then placed into the rubber funnel followed by snugging it tightly onto the mouth of the filtration flask. The vacuum hose was connected to the filtration flask and the vacuum was switched on for 30 minutes to remove any moisture from the crystals. Pure zerumbone crystals from respective forms of dried *Zingiber zerumbet* were weighed in vials, sealed in aluminium foil and kept at 4°C, respectively. The percentage of yield of pure active compound, zerumbone was calculated based on weight to weight ($^{w/w}$) as shown in Equation 1

$$Yield\ (\%) = \frac{W_{PE}(g)}{W_T(g)} \times 100$$

Where W_{PE} is the weight of pure extract after recrystallizat [Equation 1] veight of dried/ and ground *Zingiber zerumbet*.

2.5. Identification of Zerumbone

Preparation of samples were done by adding 2 ml of methanol to dissolve 1 mg of sample in a vial. The mixtures were then placed in an ultrasonic machine for 15 minutes. The resulting solutions were filtered using a PTFE 0.45 μm cartridge prior to analysis. Samples were then analyzed to ascertain the purity of Zerumbone with the high-performance liquid chromatography (HPLC) system (Waters 2535 quaternary gradient using a PTFE 0.45 μm cartridge prior to analysis. Samples were then analyzed to ascertain the purity of zerumbone with the High-Performance Liquid Chromatography (HPLC) system (Waters 2535 quaternary gradient pump, Waters 2707 autosampler and Waters 2998 PDA). A HPLC Phenomenex Luna PFP (2) (5 μm, 250 mm x 4.6 mm) was used and

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for elution, an isocratic solvent consisting of 2 types of solvent which were 25% A (0.1% aqueous formic acid) and 75% B (acetonitrile) were used (Foong et al., 2018). The flow rate used was 1.0 ml/min and the injection volume was 10 μl. The samples were analyzed by ultraviolet detection at a wavelength of 280 nm (Rahman et al., 2013). The retention times and UV spectra of the major peaks were determined and compared with the standard zerumbone.

3. Statistical Analysis

The data represents three independent experiments, and the results were expressed in mean ± SD. The differences between two groups were analyzed using independent t-test. Graph pad version 8.0.2 was used to perform all statistical data. The significance level was set at $p<0.05$.

4. Results

4.1. Extraction of Zingiber zerumbet

After being dried in the oven for three days, the weight of 6 kg of freshly cut rhizomes was reduced to 3.03 kg. Only 1 kg of dried cut rhizomes was taken to prepare two batches, one ground and the other left intact. The weight of powdered dried *Zingiber Zerumbet* was reduced to 900 g from the initial 1 kg, after grinding and had to be made up to 1 kg separately to compare the yield with intact *Zingiber zerumbet* which was 1 kg. Figure 1(a) shows the plant material, *Zingiber zerumbet* and (b) freshly cut *Zingiber zerumbet*.

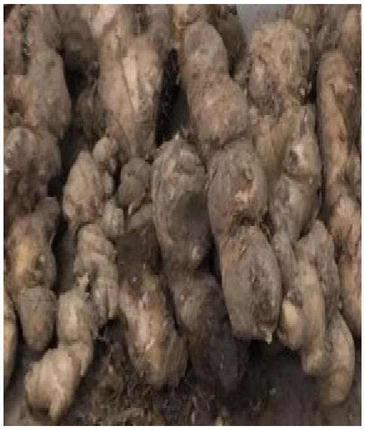
a)  b) 

Figure 1. (a) Fresh rhizomes, *Zingiber zerumbet* (b) Cut *Zingiber zerumbet.*

After 6 hours of hydrodistillation, pale yellowish crude extracts that were collected from dried intact rhizomes and ground dried rhizomes of *Zingiber zerumbet* were 57.50 g/kg and 30.0 g/kg respectively. Significant difference ($p<0.05$) by independent t-test was exhibited in terms of yellowish crude extracts collected between ground dried rhizomes and dried intact rhizomes after hydrodistillation. Figure 2(a) depicts the apparatus used for hydrodistillation and the example of (b) pale yellowish crude extracts obtained after extraction. Crude extracts from both powdered dried and intact dried *Zingiber Zerumbet* had a mixture of oil and water.

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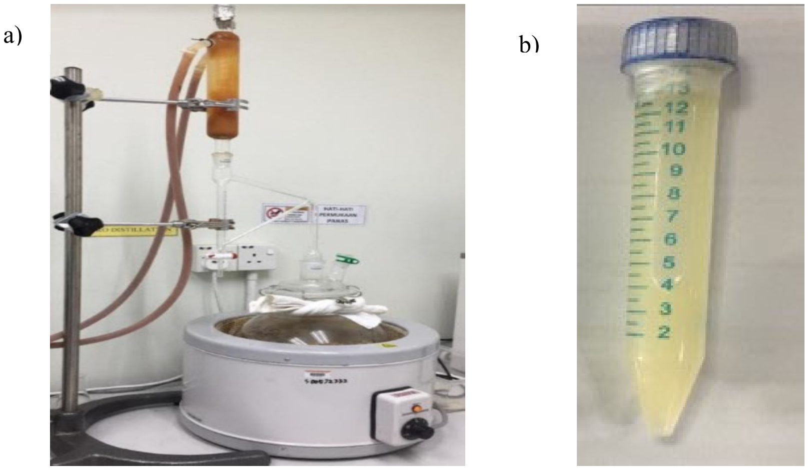

Figure 2. (a) Hydrodistillation apparatus used to extract dried *Zingiber zerumbet* and (b) Pale yellowish crude extracts collected after hydrodistillation.

In order to remove the water from the crude extracts, separatory funnel was used to drain the oil and water in a separate beaker. Distilled water was also added to completely wash the extracts and to make sure only the oil was collected. Upon repeated decanting with hexane, a method also suggested by Noor and Sirat, (2016) and Rahman et al. (2013), a dark yellowish oil which had a warm aromatic scent was produced and both the forms crystallized rapidly. After vacuum filtration to completely remove any moisture from the crystals, off-white zerumbone crystals of 9.87 g/kg rhizomes (yield,0.99%$^{w/w}$) and 6.97 g/kg (yield, 0.70%$^{w/w}$) rhizomes was obtained from dried intact rhizomes and ground dried rhizomes respectively. Independent t-test showed significant difference ($p<0.05$) of weight of zerumbone crystals from dried intact rhizomes and dried grround rhizomes. Figure 3 depicts the pure zerumbone crystals obtained after vacuum filtration.

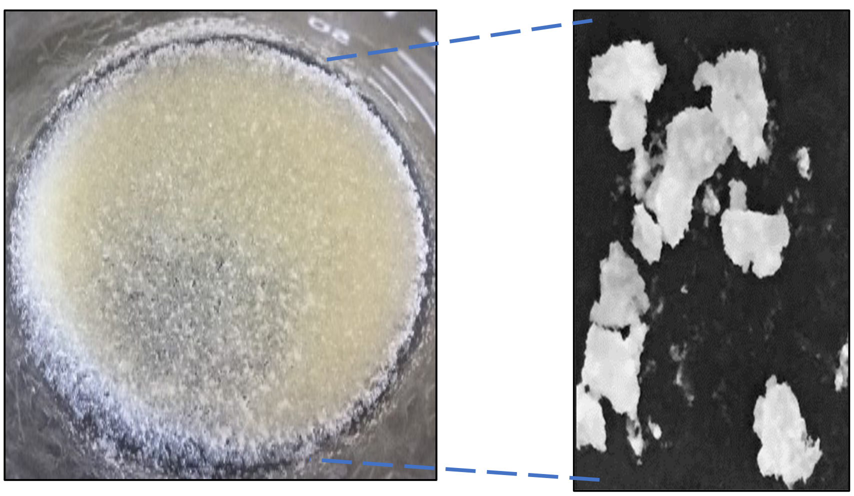

Figure 3. Pure zerumbone crystals after recrystallization.

The purity of zerumbone determined by the HPLC analysis for ground and intact samples were 98.51% and 99.68% respectively demonstrating a major peak with retention time of 6.126 minutes from ground sample and 6.133 minutes from intact sample with an insignificant difference. Figure 4 and Figure 5 illustrates the respective HPLC analysis of zerumbone.

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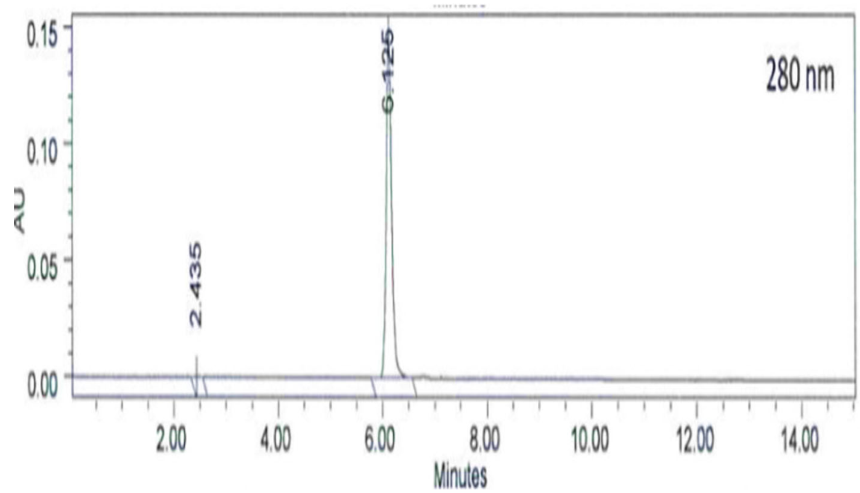

Figure 4. HPLC analysis of zerumbone crystals from dried ground *Zingiber zerumber*t at a major peak at 6.126 minutes' retention time.

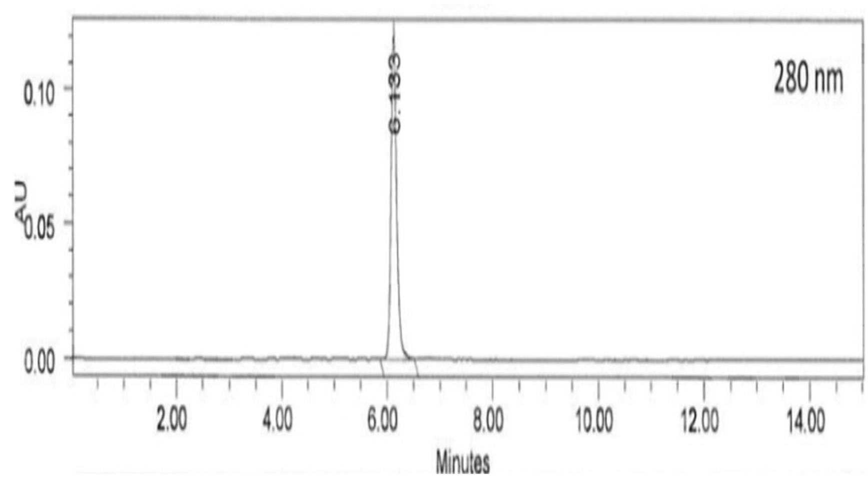

Figure 5. HPLC analysis of zerumbone crystals from dried intact *Zingiber zerumber*t at a major peak at 6.133 minutes' retention time.

5. Discussion

The most used extraction method of plant materials has been hydrodistillation as it isolates non-water-soluble compounds with high boiling points (Akhtar et al., 2019; Huong et al., 2019; Katayoon Kalantari et al., 2017). The powdered dried rhizomes produced a lesser yield of yellowish crude extracts as compared to the dried intact rhizomes. That is because during the process of grinding, only 1% of the energy transmitted to the grinder is used for size reduction whilst 99% of energy is converted into heat (Jung et al., 2018; Murthy et al., 1999; Rossi et al., 2020). The elevated temperature causes not only a significant loss of its volatile oil but also intensified the colour of the compound. Moreover, with every size reduction, more surface area is being increased and more cells are being damaged resulting in the availability of the oil to be at the surface (Liu et al., 2018; Murthy et al., 1999). It was also observed that the powdered dried rhizomes were easier to extract, and it produced oil extracts faster than the dried intact rhizomes. This is due to the oil pockets in dried rhizomes breaking down during grinding which makes oil from powdered rhizomes to be easily extracted (Saxena et al., 2018). After grinding the 1 kg of dried cut rhizomes, the weight was reduced to 900 g and the powdered rhizomes appeared warm to touch and exuded a strong smell. The process of grinding creates high temperatures which in turn melts the fat or oil present in the compound

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resulting in it sticking to the surface of the grinder (Saxena et al., 2014; Sharma et al., 2016). This further explains the loss of weight of the compound after grinding. Therefore, these reasons conclude a lesser yield of powdered dried rhizomes as compared to dried intact rhizomes.

Based on the GC-MS conducted by Tian et al. (2020) as depicted in Figure 6, there are thirty-six compounds identified representing 98.8% of essential oil from dried *Zingiber zerumbet* consists mainly zerumbone (41.9%), followed by α-humulene (29.4%), humulene oxide I (6.0%), humulene oxide II (3.9%), camphene (3.9%), β-caryophyllene (2.5%), camphor (2.4%), caryophyllene oxide (2.1%), and 1,8-cineole (1.6%). And as mentioned previously, only zerumbone that exerts pharmaceutical benefits is needed. Therefore, recrystallization with hexane is an effective method to obtain pure zerumbone off-white crystals (Zhang et al., 2018). The apparatuses were flushed with hexane at least three times to remove any traces of crude oil extracts to maximize yield of crystals. As hexane easily evaporates into air and has a boiling point of 69°C, heating of the oil extracts was continued to 90°C until no evaporation was seen to ensure only zerumbone was acquired (Karthikeyan et al., 2019; Perez-Hurtado et al., 2017).

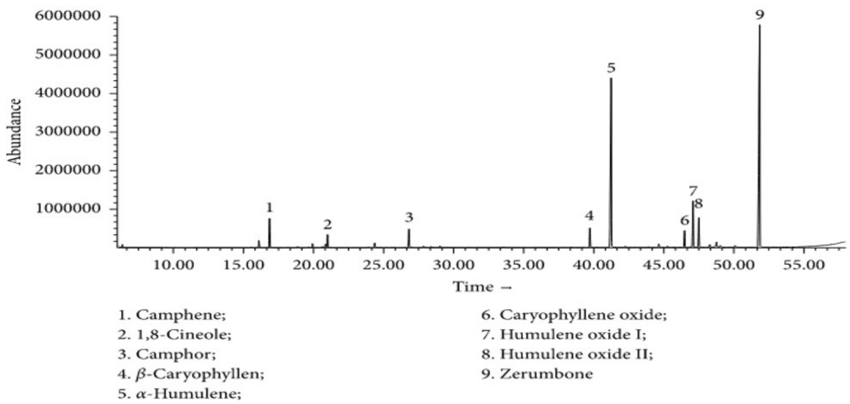

Figure 6. GC-MS chromatogram of dried *Zingiber zerumbet* oil. (Taken from Tian et al., 2020).

Even though, previous authors did not disclose the method of recrystallization in depth but in this study, the curated recrystallization steps in this study have indeed produced more yield of zerumbone crystals as compared to 1.30 g/kg as reported by Albaayit et al. (2020) and Rahman et al. (2013) respectively. Akhtar et al. (2019) produced 87.40 mg of zerumbone crystals from 1.75 kg of *Zingiber zerumbet* rhizomes. On the contrary, the closest yield to our study is the one reported by Al-Amin et al. (2019) who produced 9.10 g of zerumbone from 1.30 kg of dried rhizomes, through vacuum liquid chromatography using 5 types of solvent through 5 fractions. However, in our study, the use of only one solvent and its simple method of recrystallization renders the proposed method to be cost effective and applicable for large scale. As also discussed previously, due to the high temperature that emanates during grinding, plant compounds are damaged resulting in low volatile oil which in turn produces a lesser yield of active compound crystals (Cirri et al., 2012; Liu et al., 2018; Saxena et al., 2014). Therefore, oil from dried intact rhizomes produces more white crystals of zerumbone as opposed to the oil from ground dried rhizomes.

6. Conclusions

This study concluded that ground dried *Zingiber zerumbet* produced significantly lesser yield of zerumbone compared to the intact dried *Zingiber zerumbet*. Most importantly, the optimization of recrystallization of essential oil which is an effective method in acquiring significantly more yield of pure active compound of zerumbone crystals also showed satisfactory purity of 99.68% and 98.51% and from intact and ground and dried *Zingiber zerumbet* respectively. The zerumbone crystals obtained through the recrystallization method employed in this study were utilized throughout this research.

 doi:10.20944/preprints202602.0658.v1

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgments: This study was supported by Geran Putra Berimpak (UPM/800-3/3/1/GPB/2018/9659000) by Universiti Putra Malaysia. The authors are appreciative of the support given by the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia and the Physiology Laboratory. This invention is registered and patented under file reference NSB/NN/PATENT/UPM/MZZ/2020.

References

Akhtar, N. M. Y., Jantan, I., Arshad, L., and Haque, M. A. (2019). Standardized ethanol extract, essential oil and zerumbone of *zingiber zerumbet* rhizome suppress phagocytic activity of human neutrophils. *BMC Complementary and Alternative Medicine*, *19*(1), 1–12. https://doi.org/10.1186/S12906-019-2748-5

Al-Amin, M., Salhimi, S., Eltayeb, N. M., Siddiqui, M. A., Ruma, S. A., Nurun Nahar Sultana, G., Salhimi, S. M., and Faiz Hossain, C. (2019). Antimicrobial activity of the crude extract, fractions and isolation of zerumbone from rhizomes of *zingiber roseum*. *Journal of Research in Pharmacy*, *23*(3), 559–566. https://doi.org/10.12991/jrp.2019.163

Albaayit, S. F. A., Rasedee, A., and Abdullah, N. (2020). Zerumbone-loaded nanostructured lipid carrier gel facilitates wound healing in rats. *Revista Brasileira de Farmacognosia*, *30*(2), 272–278. https://doi.org/10.1007/S43450-020-00023-7

Azelan, N. A., Aziz, R., and Hasham, R. (2018). Optimisation of essential oil yield and zerumbone content in *zingiber zerumbet* extract using hydrodistillation process. *Chemical Engineering Transactions*, *63*, 595–600. https://doi.org/10.3303/CET1863100

Chia, J. S. M., Izham, N. A. M., Farouk, A. A. O., Sulaiman, M. R., Mustafa, S., Hutchinson, M. R., and Perimal, E. K. (2020). Zerumbone modulates α2A-adrenergic, TRPV1, and NMDA NR2B receptors plasticity in CCI-induced neuropathic pain *in vivo* and LPS-induced SH-SY5Y neuroblastoma *in vitro* models. *Frontiers in Pharmacology*, *11*, 1–24. https://doi.org/10.3389/fphar.2020.00092

Chien, T. Y., Huang, S. K. H., Lee, C. J., Tsai, P. W., and Wang, C. C. (2016). Antinociceptive and anti-inflammatory effects of zerumbone against mono-iodoacetate-induced arthritis. *International Journal of Molecular Sciences*, *17*(2), 249. https://doi.org/10.3390/ijms17020249

Cirri, M., Bragagni, M., Mennini, N., and Mura, P. (2012). Development of a new delivery system consisting in "drug - In cyclodextrin - In nanostructured lipid carriers" for ketoprofen topical delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*(1), 46–53. https://doi.org/10.1016/j.ejpb.2011.07.015

Foong, J. N., Selvarajah, G. T., Rasedee, A., Rahman, H. S., How, C. W., Beh, C. Y., Teo, G. Y., and Ku, C. L. (2018). Zerumbone-loaded nanostructured lipid carrier induces apoptosis of canine mammary adenocarcinoma cells. *BioMed Research International*, *2018*(8691569), 1–18. https://doi.org/10.1155/2018/8691569

Ghasemzadeh, A., Jaafar, H. Z. E., Ashkani, S., Rahmat, A., Juraimi, A. S., Puteh, A., and Muda Mohamed, M. T. (2016). Variation in secondary metabolite production as well as antioxidant and antibacterial activities of *zingiber zerumbet (L.)* at different stages of growth. *BMC Complementary and Alternative Medicine*, *16*(1), 104. https://doi.org/10.1186/s12906-016-1072-6

Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., and Swamy, M. K. (2017). Optimization of microwave-assisted extraction of zerumbone from *zingiber zerumbet L.* rhizome and evaluation of antiproliferative activity of optimized extracts. *Chemistry Central Journal*, *11*(1), 5. https://doi.org/10.1186/s13065-016-0235-3

Ghosh, S., Majumder, P. B., and Sen Mandi, S. (2011). Species-specific AFLP markers for identification of *zingiber officinale, Z. montanum and Z. zerumbet (Zingiberaceae)*. *Genetics and Molecular Research : GMR*, *10*(1), 218–229. https://doi.org/10.4238/vol10-1gmr1154

Girisa, S., Shabnam, B., Monisha, J., Fan, L., Halim, C. E., Arfuso, F., Ahn, K. S., Sethi, G., and Kunnumakkara, A. B. (2019). Potential of zerumbone as an anti-cancer agent. *Molecules*, *24*(4), 734. https://doi.org/10.3390/MOLECULES24040734

Huong, L. T., Chinh, H. V., An, N. T. G., Viet, N. T., Hung, N. H., Thuong, N. T. H., Giwa-Ajeniya, A. O., and Ogunwande, I. A. (2019). *Zingiber zerumbet* rhizome essential oil: Chemical composition, antimicrobial and mosquito larvicidal activities. *European Journal of Medicinal Plants*, *30*(4), 1–12. https://doi.org/10.9734/EJMP/2019/V30I430197

Jalil, M., Mohamad Annuar, M. S., Tan, B. C., and Khalid, N. (2015). Effects of selected physicochemical parameters on zerumbone production of *zingiber zerumbet smith* cell suspension culture. *Evidence-Based Complementary and Alternative Medicine*, *2015*(757514), 1–7. https://doi.org/10.1155/2015/757514

Jalili-Nik, M., Sadeghi, M. M., Mohtashami, E., Mollazadeh, H., Afshari, A. R., and Sahebkar, A. (2020). Zerumbone promotes cytotoxicity in human malignant glioblastoma cells through reactive oxygen species (ROS) generation. *Oxidative Medicine and Cellular Longevity*, *2020*(3237983), 1–9. https://doi.org/10.1155/2020/3237983

Jung, H., Lee, Y. J., and Yoon, W. B. (2018). Effect of moisture content on the grinding process and powder properties in food: A review. *Processes*, *6*(69), 1–16. https://doi.org/10.3390/PR6060069

Kalantari, K., Moniri, M., Moghaddam, A. B., Rahim, R. A., Ariff, A. B., Izadiyan, Z., and Mohamad, R. (2017). A Review of the biomedical applications of zerumbone and the techniques for its extraction from ginger rhizomes. In *Molecules*, 22(10), 1–26. https://doi.org/10.3390/molecules22101645

Karthikeyan, R. H., Vian, M. A., Tao, Y., Degrou, A., Costil, J., Trespeuch, C., & Chemat, F. (2019). Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly (*Hermetia illucens*) larvae. *Journal of Cleaner Production*, *238*(117861), 1–40. https://doi.org/10.1016/J.JCLEPRO.2019.117861

Khalid, M. H., Akhtar, M. N., Mohamad, A. S., Perimal, E. K., Akira, A., Israf, D. A., and Sulaiman, M. R. (2011). Antinociceptive effect of the essential oil of *zingiber zerumbet* in mice: Possible mechanisms. *Journal of Ethnopharmacology*, *137*(1), 345–351. https://scihub.wikicn.top/10.1016/j.jep.2011.05.043

Kitayama, T., Okamoto, T., Hill, R. K., Kawai, Y., Takahashi, S., Yonemori, S., Yamamoto, Y., Ohe, K., Uemura, S., and Sawada, S. (1999). Chemistry of zerumbone. Simplified isolation, conjugate addition reactions, and a unique ring contracting transannular reaction of its dibromide. *Journal of Organic Chemistry*, *64*(8), 2667–2672. https://doi.org/10.1021/jo981593n

Kitayama, T., Yamamoto, K., Utsumi, R., Takatani, M., Hill, R. K., Kawai, Y., Sawada, S., and Okamoto, T. (2001). Chemistry of zerumbone. 2. Regulation of ring bond cleavage and unique antibacterial activities of zerumbone derivatives. *Bioscience, Biotechnology and Biochemistry*, *65*(10), 2193–2199. https://doi.org/10.1271/bbb.65.2193

Kiyama, R. (2021). Nutritional implications of ginger: chemistry, biological activities and signaling pathways. *Journal of Nutritional Biochemistry*, *86*(108486), 1–15. Accessed on 29 September 2021 from https://reader.elsevier.com/reader/sd/pii/S0955286320305180?token=4D3CED86C6DDD8B32B6F67767A6A18B26FC6BE19E0DF747C3D427C2B0E637BF9BE4408D3E06E33085B7141B03D2B1D93&originRegion=eu-west-1&originCreation=20210929073719

Koga, A. Y., Beltrame, F. L., and Pereira, A. V. (2016). Several aspects of *zingiber zerumbet*: A review. *Revista Brasileira de Farmacognosia*, *26*, 385–391. https://doi.org/10.1016/j.bjp.2016.01.006

Li, L., Wu, X. H., Zhao, X. J., Xu, L., Pan, C. L., and Zhang, Z. Y. (2020). Zerumbone ameliorates behavioral impairments and neuropathology in transgenic APP/PS1 mice by suppressing MAPK signaling. *Journal of Neuroinflammation*, *17*(1), 61. https://doi.org/10.1186/s12974-020-01744-1

Liu, H., Zheng, J., Liu, P., and Zeng, F. (2018). Pulverizing processes affect the chemical quality and thermal property of black, white, and green pepper (*Piper nigrum L.*). *Journal of Food Science and Technology*, *55*(6), 2130. https://doi.org/10.1007/S13197-018-3128-8

Md, S., Kit, B. C. M., Jagdish, S., David, D. J. P., Pandey, M., and Chatterjee, L. A. (2018). Development and *in vitro* evaluation of a zerumbone loaded nanosuspension drug delivery system. *Crystals*, *8*(7), 2–13. https://doi.org/10.3390/cryst8070286

Moreira Da Silva, T., Danniel Pinheiro, C., Orlandi, P. P., Pinheiro, C. C., and Soares Pontes, G. (2018). Zerumbone from *zingiber zerumbet (L.) smith*: a potential prophylactic and therapeutic agent against the cariogenic bacterium *streptococcus* mutans. *BMC Complementary and Alternative Medicine*, *18*(1), 301. https://doi.org/10.1186/s12906-018-2360-0

Murthy, C. T., Rani, M., and Rao, P. N. S. (1999). Optimal grinding characteristics of black pepper for essential oil yield. *Journal of Food Process Engineering*, *22*(2), 161–173. https://doi.org/10.1111/J.1745-4530.1999.TB00478.X

 doi:10.20944/preprints202602.0658.v1

Nik Norulaini, N. A., Anuar, O., Omar, A. K. M., AlKarkhi, A. F. M., Setianto, W. B., Fatehah, M. O., Sahena, F., and Zaidul, I. S. M. (2009). Optimization of SC-CO2 extraction of zerumbone from *zingiber zerumbet (L) smith*. *Food Chemistry*, *114*(2), 702–705. https://doi.org/10.1016/j.foodchem.2008.09.075

Noor, N. F. M., and Sirat, H. M. (2016). Isolation, characterization and modification of zerumbone from *zingiber zerumbet*. *Journal of Teknologi*, *1*(1), 1–10. Accessed on 15 January 2021 from http://eprints.utm.my/id/eprint/68333/

Padalia, R. C., Verma, R. S., Chauhan, A., Singh, V. R., Goswami, P., Singh, S., Verma, S. K., Luqman, S., Chanotiya, C. S., and Darokar, M. P. (2018). *Zingiber zerumbet (L.) Roscoe ex Sm*. from northern India: Potential source of zerumbone rich essential oil for antiproliferative and antibacterial applications. *Industrial Crops and Products*, *112*, 749–754. https://doi.org/10.1016/j.indcrop.2018.01.006

Perez-Hurtado, P., Palmer, E., Owen, T., Aldcroft, C., Allen, M. H., Jones, J., Creaser, C. S., Lindley, M. R., Turner, M. A., and Reynolds, J. C. (2017). Direct analysis of volatile organic compounds in foods by headspace extraction atmospheric pressure chemical ionisation mass spectrometry. *Rapid Communications in Mass Spectrometry*, *31*(22), 1947–1956. https://doi.org/10.1002/RCM.7975

Rahman, H. S., Rasedee, A., How, C. W., Abdul, A. B., Zeenathul, N. A., Othman, H. H., and Yeap, S. K. (2013). Zerumbone-loaded nanostructured lipid carriers: Preparation, characterization, and antileukemic effect. *International Journal of Nanomedicine*, *8*, 2769–2781. https://doi.org/10.2147/IJN.S45313

Rahman, H. S., Rasedee, A., Yeap, S. K., Othman, H. H., Chartrand, M. S., Namvar, F., Abdul, A. B., & How, C. W. (2014). Biomedical properties of a natural dietary plant metabolite, zerumbone, in cancer therapy and chemoprevention trials. *BioMed Research International*, *2014*(920742), 1–10. https://doi.org/10.1155/2014/920742

Rossi, F., Sharon, M., Irudayaraj, J., Vega-Vásquez, P., and Mosier, N. S. (2020). Nanoscale drug delivery systems: From medicine to agriculture. *Frontiers in Bioengineering and Biotechnology*, *8*(79), 1–16. https://doi.org/10.3389/fbioe.2020.00079

Sachin, S. K., Ashwini, K. V, and Kawade Ashwini, C. V. (2017). Isolation and standardization of gingerol from ginger rhizome by using TLC, HPLC, and identification Tests. *The Pharma Innovation Journal*, *6*(2), 179–182. Accessed on 15 January 2021 from https://www.thepharmajournal.com/archives/2017/vol6issue2/PartC/5-8-19-645.pdf

Saxena, S. N., Barnwal, P., Balasubramanian, S., Yadav, D. ., Lal, G., and Singh, K. . (2018). Cryogenic grinding for better aroma retention and improved quality of Indian spices and herbs: A review. *Journal of Food Process Engineering*, *41*(6), 1–20. https://doi.org/10.1111/jfpe.12826

Saxena, S. N., Rathore, S. S., Saxena, R., Barnwal, P., Sharma, L. K., and Singh, B. (2014). Effect of cryogenic grinding on essential oil constituents of coriander *(coriandrum sativum L.*) genotypes. *Journal of Essential Oil-Bearing Plants*, *17*(3), 385–392. https://doi.org/10.1080/0972060X.2014.895197

Shahrajabian, M. H., Sun, W., and Cheng, Q. (2019). Pharmacological uses and health benefits of ginger (*zingiber officinale*) in traditional asian and ancient chinese medicine, and modern practice. *Notulae Scientia Biologicae*, *11*(3), 309–319. https://doi.org/10.15835/nsb11310419

Sharma, L. K., Agarwal, D., Rathore, S. S., Malhotra, S. K., and Saxena, S. N. (2016). Effect of cryogenic grinding on volatile and fatty oil constituents of cumin (*cuminum cyminum L.*) genotypes. *Journal of Food Science and Technology*, *53*(6), 2827–2834. https://doi.org/10.1007/S13197-016-2258-0

Singh, C. B., Chanu, S. B., Kh, L., Swapana, N., Cantrell, C., and Ross, S. A. (2014). Chemical composition and biological activity of the essential oil of rhizome of *zingiber zerumbet (L.) smith*. *Journal of Pharmacognosy and Phytochemistry*, *3*(3), 130–133. Accessed on 15 January 2021 from https://naldc.nal.usda.gov/download/62328/PDF

Singh, Y. P., Girisa, S., Banik, K., Ghosh, S., Swathi, P., Deka, M., Padmavathi, G., Kotoky, J., Sethi, G., Fan, L., Mao, X., Halim, C. E., Arfuso, F., and Kunnumakkara, A. B. (2019). Potential application of zerumbone in the prevention and therapy of chronic human diseases. *Journal of Functional Foods*, *53*, 248–258. https://doi.org/10.1016/j.jff.2018.12.020

Somchit, M. N., Mak, J. H., Bustamam, A. A., Zuraini, A., Arifah, A. K., Adam, Y., and Zakaria, Z. A. (2012). Zerumbone isolated from *zingiber zerumbet* inhibits inflammation and pain in rats. *Journal of Medicinal Plants Research*, *6*(2), 177–180. https://doi.org/10.5897/JMPR10.492

 doi:10.20944/preprints202602.0658.v1

Sulaiman, M. R., Perimal, E. K., Zakaria, Z. A., Mokhtar, F., Akhtar, M. N., Lajis, N. H., and Israf, D. A. (2009). Preliminary analysis of the antinociceptive activity of zerumbone. *Fitoterapia*, *80*(4), 230–232. https://doi.org/10.1016/j.fitote.2009.02.002

Tan, J. W., Israf, D. A., and Tham, C. L. (2018). Major bioactive compounds in essential oils extracted from the rhizomes of *zingiber zerumbet (L) Smith*: A mini-review on the anti-allergic and immunomodulatory properties. *Frontiers in Pharmacology*, *9*(652), 1–8. https://doi.org/10.3389/fphar.2018.00652

Tian, M., Wu, X., Hong, Y., Wang, H., Deng, G., and Zhou, Y. (2020). Comparison of chemical composition and bioactivities of essential oils from fresh and dry rhizomes of *zingiber zerumbet (L.) smith*. *BioMed Research International*, *2020*(9641284), 1–9. https://doi.org/10.1155/2020/9641284

Zakaria, Z. A., Mohamad, A. S., Chear, C. T., Wong, Y. Y., Israf, D. A., and Sulaiman, M. R. (2010). Antiinflammatory and antinociceptive activities of *zingiber zerumbet* methanol extract in experimental model systems. *Medical Principles and Practice*, *19*(4), 287–294. https://doi.org/10.1159/000312715

Zakaria, Z. A., Yob, N. J., Jofrry, S. M., Affandi, M. M. R. M. M., Teh, L. K., and Salleh, M. Z. (2011). *zingiber zerumbet (L.) smith*: s review of its ethnomedicinal, chemical, and pharmacological uses. *Evidence-Based Complementary and Alternative Medicine*, *2011*(543216), 1–12. https://doi.org/10.1155/2011/543216

Zhang, C., Liu, J., Zhao, F., Lu, C., Zhao, G. R., and Lu, W. (2018). Production of sesquiterpenoid zerumbone from metabolic engineered *saccharomyces cerevisiae*. *Metabolic Engineering*, *49*, 28–35. https://doi.org/10.1016/J.YMBEN.2018.07.010