Preparation and Characterization of Encapsulated *Cymbopogon citratus* Essential Oils in Alginate/Chitosan Complexes Using Ion-Gel Technique

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Received: November 30, 2023 Accepted: August 5, 2024

DOI: 10.22146/ijc.91251

Abstract: In this study, the alginate/chitosan complexes were prepared to encapsulate lemongrass essential oils (Cymbopogon citratus). Essential oils are secondary metabolites that are easily changed under environmental influences (temperature, pH, light). This research opened a new line in protecting essential oils from adverse effects from the surrounding environment. The ion-gel encapsulation technique combined with alginate/chitosan complexes has been applied to encapsulate C. citratus essential oil. The factors were surveyed including homogenization time (10-20 min), concentration of Tween 80 (0-5% w/w), concentration of sodium alginate (1.5-3.5% w/v), concentration of essential oils (20-40% w/w), concentration of CaCl₂ (1.0-2.5% w/v), concentration of chitosan (0.5–2.0%), and pH of chitosan solution (4–6). The properties of products have been determined including moisture content as 80.39%, encapsulation yield as 98.79%, encapsulation efficiency as 88.74% with homogenization time as 15 min, concentration of Tween 80 as 1.5%, concentration of sodium alginate as 2.5% (w/v), concentration of essential oils as 30% (w/w), concentration of $CaCl_2$ as 2.5% (w/v), concentration of chitosan as 2.0% (w/w), and pH chitosan solution as 5. The main chemical compositions of essential oils before and after encapsulation, such as citral, myrcene, and limonene, have still remained.

Keywords: lemongrass essential oil; alginate/chitosan complexes; encapsulation

INTRODUCTION

Lemongrass is a type of grass belonging to the rice family, scientifically known as *Cymbopogon citratus* [1]. The whole plant has a light lemon-like scent because its main ingredient is citral essential oil, which has many medicinal uses [2]. Lemongrass essential oil is extracted from leaf and root parts [3]. Lemongrass essential oil is colorless or pale yellow, has a strong lemon scent, and is often extracted by direct distillation using water solvent [4]. The chemical composition of lemongrass essential oils varies depending on the variety, growing conditions, climate, soil, and the part used to extract the essential oil [5]. The main ingredients in lemongrass essential oils, such as citral, geraniol, citronellol, nerol, limonene, geranyl, acetate, linalool, and citronellal, account for a high proportion [6]. The two isomers of citral (geranial and neral) are the main components in the lemongrass essential oils and have high biological value [7]. However, they are easily changed during the storage process.

Encapsulation is a technique of encapsulating solids, liquids, or gases into a thin shell with particle size in the micrometer range [8]. This shell would protect the active compounds from changing, reducing quality (for temperature-sensitive substances) or limiting loss (for volatile substances) [9]. The active substance is only released under certain special conditions. Encapsulated particles varied in size over a wide range from nanometers to micrometers, depending on the technique used. Currently, many methods are used to encapsulate biologically active compounds, such as iongel, spray drying, sublimation drying, fluidized bed drying, and extrusion. Among them, ion-gel is one of the most commonly used techniques because of its uniform particle size, simplicity, and low cost.

Currently, many studies conducted have encapsulation of essential oils using the ion/gel method. Specifically, Ferreira et al. [10] conducted microencapsulation of ginger oil using the droplet condensation method by creating a gelatin/ginger oil emulsion on a Gum Arabic solution. The results showed that the diameter of the microcapsules varied from 57.00 to 85.00 µm, the encapsulation efficiency went from 89.74 to 98.70%, and the encapsulation yield ranged from 21.00 to 88.00%. Zhang et al. [11] studied the development of nanocapsules using gelatin/sodium carboxymethyl cellulose complexes to encapsulate zeaxanthin extracted from Lycium barbarum L. The results showed that the capsules can enhance the thermal stability of zeaxanthin.

Lemongrass essential oil is extracted mainly from lemongrass stems and leaves [12]. In liquid form, it is very volatile, causing loss, making it easy to oxidize, and changing composition when exposed to oxygen and light [13]. As a flavoring, lemongrass essential oil is used in tiny amounts, so mixing the liquid essential oil with other ingredients during food production is difficult [14]. Therefore, it is necessary to use an appropriate encapsulation technique to preserve and maintain the quality of the essential oil inside. The purpose of this study is to apply the ion-gel technique to encapsulate lemongrass essential oil through the use of the alginate/chitosan complexes. In this study, the ion-gel technique was chosen to encapsulate lemongrass essential oil because of its suitability for laboratory scale, relatively uniform particle size, and saving on chemical costs.

EXPERIMENTAL SECTION

Materials

Lemongrass essential oils were supplied by AOTA Ltd company. The chemicals including Tween 80 ($C_{64}H_{124}O_{26}$, 98%), chitosan ($C_8H_{13}O_5N$, 98%), sodium alginate (Na $C_6H_7O_6$, 99%), calcium chloride (Ca Cl_2 , 95%), trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, 99%), acid acetic (CH₃COOH, 99%), *n*-hexane (C_6H_{14} , 99%), and sodium hydroxide (NaOH, 99%) were purchased at Xilong Chemical Company, China.

Instrumentation

The homogenizer used was an IKA T 25 digital ULTRA-TURRAX^{*}. The composition of lemongrass essential oil was determined by gas chromatographymass spectrometry (GC–MS, Agilent 6890N GC with an inert MS 5973 with an HP5-MS column), and column head pressure was set to 9.3 psi.

Procedure

Optimizing the encapsulation process of C. citratus essential oil using alginate/chitosan complexes

First, sodium alginate solution was prepared with concentrations of 1.5, 2.0, 2.5, 3.0, and 3.5% (w/v). Next, emulsifier Tween 80 (concentrations from 0.0, 1.5, 2.5, 3.5, and 5.0% w/w) and lemongrass essential oil (concentrations of 20, 30, and 40% w/w) were added to the sodium alginate solution. The solution was homogenized at different times (10, 15, and 20 min) and at a homogenization speed of 6000 rpm to obtain the emulsion. The emulsion was dripped into the CaCl₂ bath (concentrations from 1.0, 1.5, 2.0, and 2.5%) using a 25G \times 1" syringe to form into capsules. The CaCl₂ tank was stirred during the droplet process, and the produced capsules were kept in it for 30 min. In parallel, chitosan solution was also prepared with concentrations of 0.5, 1.0, 1.5, and 2.0% (w/v) in 1.0% acetic acid and kept stable overnight. Then, the pH of the sodium alginate and chitosan solution was adjusted with 5.0 M NaOH solution and 0.5 M acetic acid solution. The pH of the chitosan solution was adjusted to levels (pH 4-6). After the droplet process, the capsules were taken out, washed with distilled water, and stirred in the prepared chitosan solution. After stirring for 60 min, the capsules in solution were taken out and then analyzed for encapsulation yields and encapsulation efficiency.

Determination of moisture content

The moisture content of the sample was determined according to the method of Abbasiliasi et al. [15]. As many as 5 g of capsule was dried at 105 °C until the weight remained constant. Capsule moisture contents were displayed as mean \pm SD.

Identification of encapsulation yields

In the study, spectrophotometric methods were

suitable for current laboratory conditions to identify encapsulation yield (EY) and encapsulation efficiency (EE) indexes. EY% was the amount of essential oil in the capsules over the total amount of initial essential oil in the solution. EY% was determined using spectroscopy, referring to Soliman et al. [16]. The procedure was as follows: 0.5 g of capsules was added to 2.5 mL of acetic acid 1.0% and 2.5 mL of 0.055 M trisodium citrate solution and then ground until the capsules broke. Then, 15 mL of *n*-hexane was added to the solution and stirred for 30 min. The solution was moved into a Falcon tube, shaken for 1 min, and then centrifuged for 10 min at 4000 rpm. The supernatant was collected and measured by spectrophotometry. The EY% index was identified, followed by the Eq. (1):

$$EY(\%) = \frac{F \times V \times m_1}{m_0} \times 100 \tag{1}$$

where F is essential oil concentration (mg/mL), V is the volume of hexane (mL), m_1 is the mass of encapsulated particles obtained (g), and m_0 is the initial mass of essential oil added to the solution (g). The essential oil concentration was calculated based on the calibration curve of lemongrass essential oil dissolved in hexane at different concentrations from 20 to 100 mg/mL. The absorption was identified at 400 nm. The equation of the obtained calibration curve of was y = 0.0034x - 0.0078 ($R^2 = 0.9977$).

Identification of encapsulation efficiency

EE% was the amount of essential oil encapsulated in the core of the capsules over the total amount of encapsulated essential oil (core and surface essential oil). EE was also determined using spectrophotometry methods, according to Soliman et al. [16]. The procedure was as follows: 0.5 g of capsules were added to 5 mL of hexane to remove surface essential oil. Then, capsules were dried at 50 °C for 20 min to evaporate hexane. Next, the capsules were added to 2.5 mL of 1.0% acetic acid, 2.5 mL of 0.055 M trisodium citrate solution, and ground until the capsules were broken. An amount of 15 mL *n*hexane was supplied to the solution and stirred for 30 min. The solution was moved into a Falcon tube, shaken for 1 min, and centrifuged for 10 min at 4000 rpm. The supernatant was collected and measured by spectrophotometry. The EE% index was identified, followed by the Eq. (2):

$$EE(\%) = \frac{F'}{F} \times 100 \tag{2}$$

where F' is the essential oil concentration after removing the surface essential oil of capsules (mg/mL) and F is the concentration of essential oil (mg/mL) of both core and surface essential oil of capsules. The F' and F values were determined based on the calibration curve of lemongrass essential oil.

The chemical compositions of lemongrass essential oil

GC–MS was used to determine the composition of lemongrass essential oil (before and after encapsulation). The essential oil sample (25 μ L) was mixed in 1.0 mL of *n*-hexane. The GC–MS system operated under the following conditions: carrier gas as He; flow rate as 1.0 mL/min; split line as 1:100; injection volume as 1.0 μ L; injection temperature at 250 °C. The temperature cycle was set up as follows: kept the initial temperature of the oven at 50 °C for 2 min, then an increase of 2 °C/min to 150 °C, and then increased to 200 °C at 10 °C/min and finally rose to 300 °C at 20 °C/min for 5 min.

Statistical analysis

Each experiment was repeated three times. Experimental data were processed using Microsoft Office Excel 2016 software. Results are displayed as mean \pm SD. Analysis of variance (ANOVA) and least significant difference (LSD) analysis for each experiment with 95% confidence level were performed through Statgraphics XV software.

RESULTS AND DISCUSSION

Chemical Compositions of Lemongrass Essential Oils

Table 1 presents the components of lemongrass essential oil based on GC-MS analysis. The lemongrass essential oil sample contained about 64 identified compounds. Lemongrass essential oil has many peaks with great intensity and was the main ingredient with a high proportion. Based on the peaks on the chromatogram, the main chemical components of lemongrass essential oil are identified in Table 1.

No.	Compounds	Molecular formula	Percentage (%)
1	citral	$C_{10}H_{16}O$	32.73
2	2,6-octadienal (Z-citral)	$C_{10}H_{16}O$	21.98
3	beta-myrcene	$C_{10}H_{16}$	8.69
4	d-limonene	$C_{10}H_{16}$	3.78
5	linalool	$C_{10}H_{18}O$	0.50
6	alpha-pinene	$C_{10}H_{16}$	0.12
7	6-methyl-5-hepten-2-one	$C_8H_{14}O$	0.32
8	2,6-octadienoic acid (<i>E</i> -citral)	$C_{10}H_{16}O$	0.11
9	caryophyllene oxide	$C_{15}H_{24}O$	0.10
10	cyclohexanemethanol	$C_{10}H_{20}O_2$	0.04

Table 1. The chemical compositions of lemongrass essential oil

In particular, the main chemical compositions included citral (32.73%), 2,6-octadienal (21.98%), beta-myrcene (8.69%), *d*-limonene (3.78%), and so on. The results obtained were higher than the report of Clery et al. [17], who reported that citral (32.15%), 2,6-octadienal (16.24%), beta-myrcene (6.09%), and *d*-limonene (1.76%). This research also indicated that citral was the main ingredient and accounted for the highest proportion of the Citrus genus essential oils. Citral is the main active ingredient of lemongrass essential oil; it could account for up to 70–80% of the chemical composition of lemongrass essential oil. Furthermore, it could help create a scent that reduces stress and anti-inflammatory, anti-bacterial, and repellent insects [18].

Effect of Sodium Alginate and Essential Oil Concentrations

The result of the effect of sodium alginate solution and essential oils concentrations is shown in Fig. 1. ANOVA analysis showed that essential oil and sodium alginate concentrations had a statistically significant effect on EY and EE indexes at the 95% confidence level (p < 0.05). The interaction between the two factors was also statistically significant (p < 0.05). LSD analysis showed that with different essential oil concentration values, there was a difference between the 30.00% treatment and the remaining treatments. There was a difference between the 2.5% treatment and the other treatments at sodium alginate concentrations. Fig. 1 shows that the EY and EE indexes reached the highest value when the essential oil concentration was 30.00% and the sodium alginate concentration was 2.50% (43.57%). At the same sodium alginate concentration, the EY and EE indexes grow when the concentration of essential oil is increased. Still, when increasing the concentration of sodium alginate, these indexes tend to decrease. Increasing the sodium alginate concentration led to increased EE values due to the formation of a dense network structure with voids that trap essential oil droplets.

On the contrary, the decrease in EE values with an increase in alginate concentration could be explained by the increased space occupied by alginate, causing a reduction of free volume in the polymer matrix and decreasing the amount of essential oil trapped in the pores [19]. At high essential oil concentrations, the concentration of sodium alginate was not enough to completely trap the oil droplets and prevent them from clumping together. This would be lost during encapsulation, thus, decreasing EE [20].

From the results of this experiment, a sodium alginate concentration of 2.5% and an essential oil concentration of 30% were chosen for the following experiment. This result is similar to the report of Chan [21], but the concentration of this sodium alginate was higher than that reported by Soliman et al. [16].

Effect of Tween 80 Concentrations

The effect of Tween 80 concentration on the indicators was shown in Fig. 2. According to the ANOVA analysis results, Tween 80 concentration had a statistically significant effect on the EY index at the 95%



Fig 1. The effect of sodium alginate and essential oil concentrations in (a) encapsulation yield and (b) encapsulation efficiency

confidence level (p < 0.05) but has no statistical significance on the EE index at the confidence level of 95%. LSD test on the effect of Tween 80 concentration on the EY index showed that the treatments were different. Fig. 2 showed that when the concentration of Tween 80 increased from 0.0% to 2.5%, the EY values increased (from 15.23% to 56.66%), but when continuing to increase to 5%, the microencapsulation efficiency decreased (15.1%). Surface tension plays a vital role in ensuring the

stability of particles. Surfactants reduce surface energy and increase EE and EY values [22]. As the emulsifier concentration increased, the surface tension decreased but more slowly because most of the surface was occupied. Furthermore, the surfactant concentration continued to grow beyond the concentration at which it had sufficiently adsorbed onto the surface layer; it tended to leave the surface into the liquid [23]. This led to a decrease in EY values. In this experiment, the EY



Fig 2. The effect of Tween 80 concentration on the indicators

values had the highest value at the Tween 80 concentration of 1.5%. Velderrain-Rodríguez et al. [24] confirmed that EY values increased when using emulsifiers with high HLB (HLB > 15), and Tween 80 tended to stabilize O/W emulsions firmly. Hence, using an emulsifier as Tween 80 was consistent with the above report.

Effect of CaCl₂ Concentration

The effect of CaCl₂ concentration on the indicators was shown in Fig. 3. ANOVA analysis showed that CaCl₂

solution concentration had a statistically significant effect on EY values at the 95% confidence level (p < 0.05) but had no statistical significance on EE values at the 95% confidence level. LSD test analysis of the effect of CaCl₂ solution concentration on EY values showed differences between treatments. When CaCl₂ concentration was increased from 1.0% to 2.5%, EY values rose from 48.41% to 97.09%. The EE values insignificantly increasing changed with CaCl₂ concentration. The highest EY values reach the highest





Fig 3. The effect of CaCl₂ concentration on the indicators

concentration value of 2.5%. Increasing the CaCl₂ concentration increased the Ca2+ ion content in the solution, which created more cross-links between the alginate chains and formed a densely interconnected structure that helped retain higher amounts of essential oil. However, an excessive amount of CaCl₂ reduces the mechanical strength of the capsules. The essential oil would escape from the microcapsules, reducing EY values; the contraction of the alginate chains explained this, which reduced the pore size formed in alginate chains leading to reduced EY of essential oil [25]. In the case using the low concentration of CaCl₂, there would not be enough crosslinking between the alginate ions and Ca²⁺ on the droplet surface when dropping emulsion into a CaCl₂ bath, leading to the Ca-alginate hydrogel structures becoming loose [26]. The results showed that EY values reached the highest with a CaCl₂ concentration of 2.5%. The increase in CaCl₂ concentration leading to the rise in EY values was considered consistent with the report of Soliman et al. [16]. Still, the CaCl₂ concentration used in this study was higher than that previously reported [27].

Effect of Time Homogenization

The effect of time homogenization on indexes is shown in Fig. 4. ANOVA analysis results show that emulsion homogenization time statistically affected EY and EE values at the 95% confidence level (p < 0.05). The

LSD test shows the difference between different homogenization times in both EY and EE. Fig. 4 shows that when increasing the homogenization time from 10 min to 15 min, the EY values rise from 85.59% to 91.20%; however, when continuing to grow the homogenization time to 20 min, the EY values decrease (81.01%). The EE values changed insignificantly when increasing the homogenization time. EY values peaked at a homogenization time of 15 min. This could be explained as follows: the essential oil particles were broken into smaller particles during homogenization. Therefore, when the homogenization time increased, the particle size and viscosity of the solution increased, and the system became more stable. However, the longer homogenization time, the more significantly the particle size decreased, leading to the agglomeration of particles due to van der Walls force [28].

Additionally, increasing the homogenization time provided more energy to break down the particles, making them smaller. However, after a specific time, larger particles would start to form, and extending the homogenization time would reduce the initial amount of essential oil due to evaporation. Increasing the homogenization time was beneficial for creating a smooth emulsion as long as the critical time was not exceeded. However, the homogenization time must be



homogenization treatments

Fig 4. The effect of time homogenization on the indicators

large enough to make a stable emulsion system, avoiding phase separation, essential oil loss, and reduced EE [29]. From the experimental results, a homogenization time of 15 min was chosen for the following experiment. The above results are consistent with the reports of Bamba et al. [30], Wang et al. [31], and Thuy et al. [32].

Effect of Concentration and pH of Chitosan Solution

The effect of the concentration and pH of the chitosan solution is displayed in Fig. 5. ANOVA analysis showed that the concentration and pH of chitosan

solution had a statistically significant effect on EY values at the 95% confidence level (p < 0.05); the interaction between the two factors was also statistically significant (p < 0.05). The LSD test showed that there was a difference between the concentration of 2% chitosan solution and the remaining concentrations. In addition, the pH value of the chitosan solution was 5, which was different from the remaining pH values. Based on Fig. 5, EY and EE values reached the highest when combining chitosan solution with a 2% concentration and pH of 5 (98.79%). Encapsulation yield values tended to increase



*Note: The letters 1, 2, 3, 4 represent the differences between chitosan concentration treatments. The letters a, b, c, d, e represent differences between pH treatments

Fig 5. The effect of concentration and pH of the chitosan solution: (a) encapsulation yield and (b) encapsulation efficiency

when increasing the pH value from 4 to 5 and decrease when advancing from 5 to 6; this could be explained by the fact that the pH value of 5 was close to the pKa value of chitosan, at which charge density of chitosan molecules decreased significantly. The decrease in EY values could be due to a charge imbalance between the concentration and pH of the chitosan solution. When the pH of the chitosan solution was higher than its pKa value, chitosan became insoluble in water and clumped together. In contrast, when the pH dropped below pKa, it also led to the loss of its charge. It reduced the electrostatic attraction of the particles, causing the loss of encapsulated essential oil [33]. In this study, the highest EY values were 98.79% at a pH value of 5 and a chitosan concentration of 2%. This result was similar to the report of Bastos et al. [34], with an appropriate pH in the range of 4-5. However, this value was higher than the results of Kulig et al. [33], with a proper pH value of 3.92.

Evaluation of the Physicochemical Properties of Lemongrass Essential Oil Encapsulated Capsules

Table 2 presents the properties of lemongrass essential oil encapsulated capsules. The resulting product was spherical, uniform in size, unbroken, and white. With the optimal parameters in experiments from 4.1 to 4.6 sections, the obtained particles had a moisture content of 80.39%, EY of 98.79%, and EE of 88.74%. This result was higher than the report of Truong et al. [35], with an EY value of 74.52%, EE of 86.87%, and moisture content of 80.02%. The moisture content of the product was high,

which could be explained by the prolonged soaking time in the chitosan solution, leading to the diffusion of water in the solution into the product's structure.

The chemical compositions of after-encapsulated lemongrass essential oil are displayed in Table 3. Using GC-MS, lemongrass essential oil, after encapsulating, had approximately 69 compounds with different contents. The GC spectrum of lemongrass essential oil had many peaks with great intensity, mainly some of the compounds accounting for high proportions. The results of comparing the components of lemongrass essential oil before and after encapsulation are shown in Table 3.

Comparing the GC spectrum of two lemongrass essential oil samples before and after encapsulation, it was found that the main active ingredient content in lemongrass essential oil was citral with two isomers as Zcitral and E-citral, which were well preserved through the encapsulating process. The E-citral content decreased from 32.73% to 25.62%, while the Z-citral content increased from 21.98% to 29.64% when encapsulating; this could be explained by the partial transformation of the *E*-isomer into the *Z*-isomer form after the encapsulation process [36]. In the encapsulating process, essential oil components were lost, such as beta-myrcene content decreased from 8.69% to 0.15%. In addition, due to the effects of the drying process, some of the components have changed, such as d-limonene content increasing from 3.78% to 4.42%, linalool content rising from 0.5% to 10.44%, and

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No.	Parameter	Result				
1	Encapsulation yield (%)	98.79 ± 0.000				
2	Encapsulation efficiency (%)	88.74 ± 0.355				
3	Moisture content (%)	80.39 ± 2.342				
4	Visualization					

Table 2. The properties of capsules

No.	Compound	Molecular formula	Before encapsulation (%)	After encapsulation (%)
1	citral	$C_{10}H_{16}O$	32.73	25.62
2	2,6-octadienal (Z-citral)	$C_{10}H_{16}O$	21.98	29.64
3	beta-myrcene	$C_{10}H_{16}$	8.69	0.15
4	<i>d</i> -limonene	$C_{10}H_{16}$	3.78	4.42
5	linalool	$C_{10}H_{18}O$	0.50	10.44
6	alpha-pinene	$C_{10}H_{16}$	0.12	0.11
7	6-methyl-5-hepten-2-one	$C_8H_{14}O$	0.32	0.79
8	2,6-octadienoic acid (<i>E</i> -citral)	$C_{10}H_{16}O$	0.11	0.71
9	caryophyllene oxide	$C_{15}H_{24}O$	0.10	0.03
10	cyclohexanemethanol	$C_{10}H_{20}O_2$	0.04	0.10

Table 3. The chemical compositions of before and after encapsulated lemongrass essential oils

so on. The results of lemongrass essential oil compositions were quite similar to the study of Tran et al. [37].

CONCLUSION

The ion-gel technique was successfully applied to encapsulate *C. citratus* essential oil using the alginate/chitosan complexes at 2.5% (w/v) sodium alginate, 30.0% (w/w) essential oil, 1.5% (w/w) Tween 80, 2.5% (w/v) CaCl₂, homogenization time as 15 min, 2.0% (w/v) chitosan, pH chitosan solution as 5 with EY% value as 98.79% and EE% as 88.74%. The composition of lemongrass essential oil before and after the encapsulation still retained the main ingredients, such as citral, betamyrcene, and *d*-limonene. The moisture content of encapsulated capsules was 80.39%. Encapsulated lemongrass essential oil capsules showed potential for broad application in the pharmaceutical chemistry and food technology industries.

ACKNOWLEDGMENTS

This study was supported by Nong Lam University, Ho Chi Minh City, Vietnam.

CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Huynh Mai Pham: Writing – original draft, Methodology, Investigation, and Conceptualization. Thuong Nhan Phu Nguyen: Writing – original draft, Investigation, Formal analysis, and Conceptualization. Chi Khang Van: Writing – review & editing, Supervision, Resources, and Conceptualization. Huynh Cang Mai: Writing – review & editing, Validation, Software, and Formal analysis.

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