



RESEARCH ARTICLE

FORMULATION OF NANOEMULSIONS FROM *CERBERA ODOLLAM* SEEDS ESSENTIAL OIL AND THEIR LARVICIDAL ACTIVITIES AGAINST *Aedes Aegypti* LARVAE

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**Abstract.** As of September 2024, there are 322,274 and 94,807 cases due to dengue in Indonesia and Malaysia, respectively. Prompt actions must be taken to control mosquito population and mosquito-borne diseases proliferation. Larvicidal control is one of the effective strategies that are promoted by World Health Organization (WHO). Bintaro (*Cerbera odollam*) are highly available in wetlands, but they are extremely toxic, especially their seed kernels. In this study, the active compounds of *C. odollam* seed kernels were gradually extracted using Soxhlet extraction with *n*-hexane, chloroform and ethanol and were further distilled with a rotary evaporator. Nanoemulsions were formulated with Arabic gum and Triton X-100 by using both homogenisations of high-speed and ultrasonic. Gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared (FTIR) spectroscopy and scanning transmission electron microscopy (STEM) were used to characterise oils and nanoemulsions. Encapsulation efficiencies of nanoemulsions were studied using a UV-Vis spectrophotometer. Larvicidal studies of the nanoemulsions against *Aedes aegypti* were evaluated for 48 h. The presence of various compounds in different gradual extractions was confirmed by GC-MS chromatograms. The change of intensities and wavenumbers that were presented in FTIR analysis confirmed the successful encapsulation of compounds. Furthermore, spheres with diameters ranged between 8.43 and 55.6 nm were observed in STEM images. The highest encapsulation efficiency (EE) was successfully achieved at 90.3%. Based on log Probit analysis and Abbott's formula, the LC<sub>50</sub> values for extracted compounds ranged between 10.55 and 120.60 mg L<sup>-1</sup>. The highest mortality rate of larvae was exhibited with extracted compounds from ethanol based-nanoemulsion for 24 h. Overall, these results highlight the usefulness of *C. odollam* seeds essential oil nanoemulsions in mosquito control and prevention.

**Keywords:** *Cerbera odollam*, bintaro seed, nanoemulsion, *aedes aegypti*, larvicidal activities.

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## 1. INTRODUCTION

Mosquitoes are one of the deadly disease-transmitting vectors because female mosquitoes have to feed on blood to obtain the protein for reproduction. The virus enters the bloodstream of the host through saliva exchange on the proboscis of an infected mosquito. *Anopheles*, *Culex* and *Aedes* are the three most common genera that are acting as pathogen vectors and *Aedes* is the main vector to carry dengue virus. According to Centers for Diseases Control and Prevention (CDC), there are 400 million infection cases and 21,000 death cases due to dengue have been reported annually [1]. As of September 2024, there are 322,274 and 94,807 cases due to dengue in Indonesia and Malaysia, respectively [2]. Moreover, it is forecasted that dengue will infect 6.1 billion people around the world in 2080 [3]. This alarms WHO towards implementation of many strategies, particularly to work together with government and local authorities in controlling mosquito-borne diseases.

Oviposition of mosquitoes takes place at water bodies, including discarded food cans, water collection bins, ponds or puddles [4]. Eggs of mosquitoes mature to pupal stage in aquatic environment. Therefore, larval source management (LSM) is introduced by WHO as a vector control measure at larval or pupal stage. LSM is a targeted management on reduction of mosquito population by killing them at the source (breeding sites) or eliminating their breeding sites. LSM can be conducted permanently or temporarily through larviciding, biological control, habitat modification or habitat manipulation. The common larviciding methods are application of oils on water surface or application of *Bacillus thuringiensis* var. *israelensis* (*Bti*). In the aspects of biological control, mosquito fish or larvivorous fish (*Gambusia affinis*) are kept in the ponds to control mosquito larvae. Additionally, standing water area could be removed by filling depressions, draining swamps or ditching marshy areas [4-5]. However, these strategies have their challenges such as insecticide resistance, change in biting behaviour, or difficulty in maintaining stable fish populations. Therefore, it is urgent to overcome these issues.

Past several years, plants have been utilised as active ingredients of new drug formulation, food additives or green synthesis of nanomaterials. Various volatile organic compounds such as terpenoids, fatty acid derivatives, benzenoids and alkaloids that are produced by plants have been proven to be effective in controlling mosquito larvae [6]. Thus, phytochemistry has emerged as a new study for mosquito management because bioactive chemicals are proven to be effective in controlling mosquito larvae. There are several studies reported about the great potential of plant extracts to control *Aedes aegypti* larvae. Narayanan et al. [7] tested the feasibility of aqueous extract of *Ocimum americanum* to kill 3<sup>rd</sup> and 4<sup>th</sup> instars *A. aegypti* mosquito larvae. They reported that 35 mg L<sup>-1</sup> is the optimum concentration to achieve 100 % of larvicidal activity against 3<sup>rd</sup> instars larvae in 24 h. On the other hand, Mbatchou and his teams [8] investigated the larvicidal effect of *Cassia tora* seed extracts towards 3<sup>rd</sup> and 4<sup>th</sup> instars of *Anopheles gambiae* (Mbita strain), and it resulted in 100 % of larval mortality achieved with only 100 ppm of the seeds extracts after 24 h of exposure. These studies proved that the plant extracts possess the potential larvicidal activities against mosquito larvae.

*Cerbera odollam* trees grow in coastal forests, and they belonged to *Apocynaceae* family. *C. odollam* Gaetn. *Apocynaceae* is the species that can be found in Malaysia. The tree can reach 12 m high and bearing round green fruits with poisonous seeds. When the fruit is ripe, the fruit turns to reddish purple and fall onto ground. They have many common names in Malaysia and Indonesia, and they are “bintaro”, “sea mango”, “pong pong”, “wood octopus” or “*buah tikus kering*”. Almost all parts of the bintaro trees contain cerberin, and cerberin is a toxin that blocks human calcium ion channels, thereby disrupt heartbeat and can cause fatality. The fruits look like apples or mangoes and are misunderstood to be edible. Thus, there are many accidentally poisoning cases happening. Its fruit kernel can kill a person with only one serving. Hence, they are commonly known as “suicide trees” [9]. Although the tree is poisonous, the locals still use them as biopesticides against rodents. Additionally, there are many studies proved the capabilities of seed kernels as larvicidal agents towards many mosquito species. For instance, 2000 ppm crude extracts of *C. odollam* seeds is effective against *Culex quinquefasciatus* larvae [10]. Aziz and the team [11] extracted *C. manghas* seeds with hexane and ethanol. The higher mortality rate of *A. aegypti* larvae accomplished with ethanol extracts but at a dosage of 104,360 ppm. Hence,

lower concentration of *C. odollam* seeds must be utilised without affecting the larvicidal activities and life in aquatic environments. In this study, an innovative nanoemulsion formulation has been developed against *A. aegypti* larvae to sustain aquatic environment and to extend the activity of larvicidal effect in lower concentration.

## 2. MATERIALS AND METHODS

### 2.1 Materials

*C. odollam* fruits were collected from the trees along Jalan Parit 5 in Sekinchan, Selangor, Malaysia (3.503°N, 101.114°E). Arabic gum was obtained from Trade Serve Resources (Malaysia). Ethanol (99.8%), *n*-hexane (99%) and chloroform (99+%) were purchased from Thermo Scientific (United States of America). Triton X-100 was purchased from Sigma-Aldrich (United States of America).

### 2.2 Extraction of *C. Odollam* Seeds

The *C. odollam* fruits were dried under sunlight until they were totally dried. Next, they were soaked overnight in tap water to remove any dirt off the kernel before they were cut into half to get seeds from the fruits. The seeds were then dried at 60°C and pulverised using an electric pulveriser. The ground samples were kept in an airtight container at room temperature. Next, 20 g of fine powder of *C. odollam* seeds underwent an extraction process with *n*-hexane, chloroform and ethanol. The extraction was conducted 24 h in a Soxhlet extraction system using 250 mL of solvent. Each solvent extract was then transferred to a vacuum rotary evaporator (BUCHI Rotavapor R-200, Switzerland) set at 60°C for moisture removal. The extract yield obtained was kept at 4°C until ready for analysis.

In the present study, liquid-solid extraction was done using a Soxhlet extractor because the interested organic compounds in *C. odollam* seed have different solubilities. Soxhlet extraction is an atmospheric system that utilises solvents at boiling temperatures and ambient pressures. This system is refluxed continuously through a porous cellulose thimble that hold sample [12].

### 2.3 Preparation of Nanoemulsion

Extract nanoemulsions were formulated based on Wong and the research team [13] with some modification. On a stirring hotplate (Favorit HS070V2, Italy), 0.1 mL of *n*-hexane extract and 1.0 mL of Triton X-100 were mixed for 2 h. An exact amount of 15 mL of Arabic gum solution (1.0% (w/v)) was added dropwise into the mixture (*n*-hexane and Triton X-100) and left to stir for another 1 h. Then, the emulsion underwent homogenisation processes, namely high-speed homogenisation for 15 mins using FSH-2A (China) and an ultrasonic homogenisation for 5 mins using Biosafar 150-96 (China). The nanoemulsion was stored in a chiller ( $4 \pm 1^\circ\text{C}$ ) until further analysis. The procedure was repeated for extracts from chloroform and ethanol.

### 2.4 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

An Agilent 5975C VL MSD with Triple Axis Detector gas chromatography coupled with a signal electron impact mass spectrometer was used to determine the components in the extract. Extracts were diluted in ethanol, chloroform and *n*-hexane, separately at 1:50 ratio. They were filtered through 0.45 µm nylon 66 filters. Approximately 1 µL of sample was injected into GC through a HP-5 ms column (30 m × 250 µm i.d. × 0.25 µm film thickness) set at 250°C using an Autosampler 7693. Helium served as a carrier gas with a flow rate set at 1 ml min<sup>-1</sup>. The oven was allowed to heat at 80°C for 1 min, followed to 200°C by 12°C min<sup>-1</sup> ramp, before a 10°C ramp to 255°C, held for 7 mins. MS was performed at 230°C in positive mode. The ionising energy, CID gas pressure and detector voltage were set at 70 eV, 9.4 psi and 2600 V, respectively. The mass range for mass spectra was 50-550 *m/z* [14].

## 2.5 Fourier Transform Infrared (FTIR) Analysis

The extracts and their respective nanoemulsions were analysed on a Perkin Elmer FTIR Spectrum 3 (Waltham, Massachusetts) by utilising an attenuated total reflectance (ATR) technique [15]. The wavenumber range, resolution and number of scans were set at 4000 to 400  $\text{cm}^{-1}$ , 4  $\text{cm}^{-1}$  and 16 scans. The background of ATR-FTIR spectrophotometer was air.

## 2.6 Scanning Transmission Electron Microscopy (STEM) Analysis

STEM is one of the most powerful techniques to observe the internal structures of colloidal system [16]. A Hitachi SU 8020 UHR FESEM (Japan) equipped with tungsten filament was employed to study the internal morphologies of prepared nanoemulsions that dehydrated on lacey carbon copper grids (300 mesh). Then, they were left on a qualitative filter paper for air drying at  $24 \pm 2^\circ\text{C}$ . The samples were analysed with accelerating voltage of 20.0 kV.

## 2.7 Encapsulation Efficiency (EE) and Kinetic Release Mechanism

The EE of nanoemulsions were evaluated with an Agilent Cary 60 ultraviolet-visible spectrophotometer. The maximum wavelength ( $\lambda$ ) was determined at 264 nm by identifying the characteristic peak of the extract. An exact amount of 5 mL of nanoemulsion was homogenised ultrasonically for 10 mins and then underwent centrifuge process at 4,000 rpm (5 mins) for separation. Subsequently, the supernatant was collected, and a quartz cuvette was used during the analysis. EE was calculated by using the formula as below (Equation 1):

$$\text{EE (\%)} = \frac{\text{Amount of encapsulated extract}}{\text{Total amount of extract}} \times 100 \quad (1)$$

In this study, 5 ml of extract nanoemulsions were prepared in cellulose dialysis tubes and were allowed to diffuse through 100 mL of rainwater. Afterwards, the rainwater (5.0 mL) in the system was collected in a specified time interval up to 48 h, and then analysed by using an Agilent Cary 60 ultraviolet-visible spectrophotometer. Zero order (Equation 2), first order (Equation 3), Higuchi (Equation 4), and Korsmeyer-Peppas (Equation 5) kinetic models were applied to fit kinetics release data. The  $R^2$  values for each kinetic models were obtained from the graph plots. Regression analysis obtained from the graph provided the release exponent ( $n$ ) of Korsmeyer–Peppas model [17].

$$\text{Zero order} \quad Q_0 - Q_t = K_0 t \quad (2)$$

$$\text{First order} \quad \frac{dc}{dt} = -Kc \quad (3)$$

$$\text{Higuchi model} \quad f_t = Q = A\sqrt{D(2C-2Cs)Cs.t} \quad (4)$$

$$\text{Korsmeyer-Peppas model} \quad \frac{M_t}{M_f} = kt^n \quad (5)$$

where,  $Q_0$  is the initial amount of extract;  $Q_t$  is the cumulative amount of extract release at time  $t$ ;  $K_0$  is the zero order release constant;  $c$  is the concentration of extract in the nanocomposite;  $K$  is the first order release constant;  $Q$  is the amount of extract release in time  $t$  per unit area  $A$ ;  $C$  is the initial concentration of extract;  $C_s$  is the solubility of permethrin in the matrix media;  $D$  is the diffusion coefficient of extract molecules in the matrix media;  $M_t$  is the amount of extract release at time  $t$ ;  $M_f$  is the the maximum amount of extract release;  $k$  is the constant that indicates the release rate of extract; and  $n$  is the release mechanism of extract.

## 2.8 Larvicidal Studies

The 4<sup>th</sup> instar *A. aegypti* larvae were bred in a rectangular polypropylene container with dimensions of 50 cm × 30 cm × 10 cm. Sample containers consist of 50 mL of rainwater (pH 5.5) and 20 larvae were tested with 20 drops of respective nanoemulsions (0.5 mL; 0.062 %). The larvicidal effect on *A. aegypti* mosquito larvae were conducted in triplicates for 48 h of exposure time. Rainwater was used as a negative control. Log probit analysis (Equation 6) and the Finney's table were used to calculate the median lethal concentration (LC<sub>50</sub>) of the extract and nanoemulsions [18]. Probit regression analysis was conducted using Excel 365 software.

$$P = c + m [\log_{10} \text{dose}] \quad (6)$$

where P is Probit (5 + inverse normal transform of the response rate), c is y-intercept of the graph, and m is slope of the graph.

## 3. RESULTS AND DISCUSSION

This study began with GC-MS and FTIR analyses to study the active compounds in the *C. odollam* extracts from *n*-hexane, chloroform and ethanol. After the *C. odollam* extracts from *n*-hexane, chloroform and ethanol were prepared into respective nanoemulsions, the blending of extracts with the chosen materials (Arabic gum and Triton X-100) were confirmed by FTIR. The internal morphologies of those respective extract nanoemulsion were observed under STEM with 10,000× magnification. Other than that, encapsulation efficiencies (EE) of extract nanoemulsions were studied using UV-Vis analysis. The kinetic release mechanism of extract nanoemulsions in rainwater were also investigated through a few mathematical models. Lastly, the larvicidal activities from extracts and respective extract nanoemulsions were examined with fourth instars *A. aegypti* larvae.

### 3.1 GC-MS Analysis

The extraction of *C. odollam* seeds with *n*-hexane, chloroform and ethanol yielded 0.70 ± 0.043 (m/v), 0.23 ± 0.1 (m/v) and 1.26 ± 0.21 (m/v), respectively. Transparent yellow colours were observed in all extracts, and they have slight nuts aroma. Table 1 lists the major compounds in extracts from *n*-hexane, chloroform and ethanol.

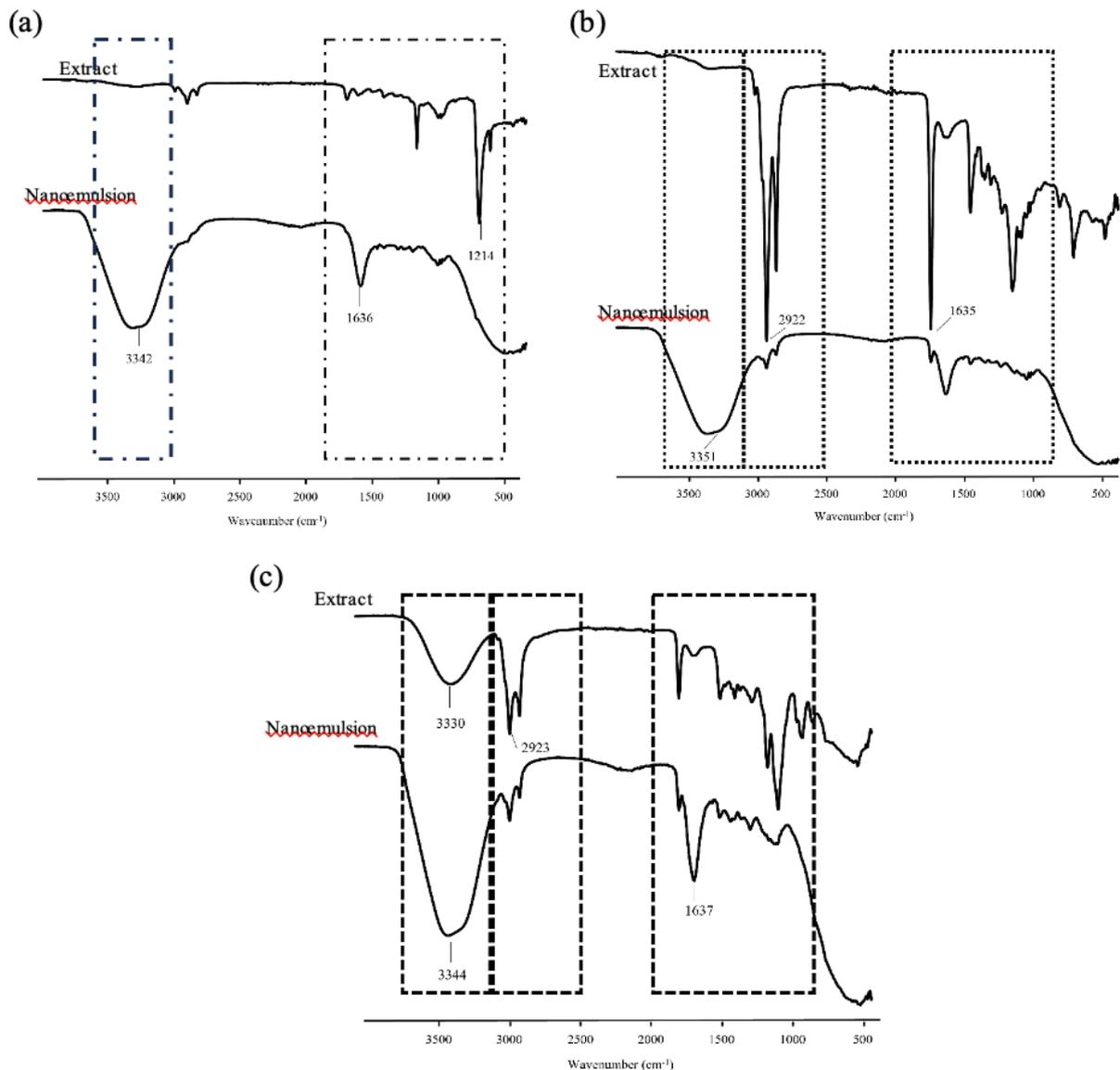
In this study, three organic solvents were chosen for extraction because they are from different polarities that can extract the active compounds from different polarities from seeds. The least polar solvent is *n*-hexane, followed by chloroform and then ethanol. From Table 1, it is obvious that the most active compounds were found in ethanol extract of *C. odollam* seeds. There were two compounds found in hexane extract of *C. odollam* seeds and they were isomers of triphenylphosphine oxide and bis(2-ethylhexyl) phthalate. Triphenylphosphine oxide is a neurotoxin, and the toxicity of bis(2-ethylhexyl) phthalate is proven against *Culex quinquefasciatus* larvae [19]. Furthermore, there are four compounds found in *C. odollam* seeds extract using chloroform that have the RT of 5.961 min, 13.573 min, 15.338 min and 15.391 min. The highest number of compounds have been found in *C. odollam* seeds extract using ethanol and most of them are unsaturated fatty acids. The findings are in line with Kuddus et al. [20] on the steroid content in Bintaro. Additionally, the discrepancies in the RT for compounds extracted with different solvents could be ascribed to variances in polarity of plant chemicals and type of solvents used for extraction.

**Table 1:** Phytochemical compounds of *C. odollam* extracted with n-hexane, chloroform and ethanol

Extract	No.	RT (min)	Compounds	Molecular formula	Molecular weight (g/mol)	Percentage (%)
n-hexane extract	1	18.273	Triphenylphosphine oxide	C <sub>18</sub> H <sub>15</sub> OP	278.3	48.7
	2	19.231	Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.6	25.7
	3	19.865	Triphenylphosphine oxide	C <sub>18</sub> H <sub>15</sub> OP	278.3	3.2
Chloroform extract	1	5.961	1H-Indene,1-methylene-	C <sub>10</sub> H <sub>8</sub>	128.2	7.6
	2	13.573	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.4	5.5
	3	15.338	9,12-octadecadienoic acid (Z, Z)-methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.5	3.552
	4	15.391	11-octadecenoic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O	296.5	17.719
Ethanol extract	1	4.254	Benzene,1,3-dichloro-	C <sub>6</sub> H <sub>4</sub> Cl <sub>2</sub>	147.0	16.4
	2	5.398	Benzene, (dichloromethyl)-	C <sub>7</sub> H <sub>6</sub> Cl <sub>2</sub>	161.0	2.4
	3	6.788	Benzene, (1,2-dichloroethyl)-	C <sub>8</sub> H <sub>8</sub> Cl <sub>2</sub>	175.1	5.6
	4	7.435	Phthalic anhydride	C <sub>8</sub> H <sub>4</sub> O <sub>3</sub>	148.1	6.1
	5	7.885	Phenol, 2,4,6-trichloro-	C <sub>6</sub> H <sub>6</sub> Cl <sub>3</sub> O	197.5	2.3
	6	8.102	Dichloroxylenol	C <sub>8</sub> H <sub>9</sub> ClO	156.6	2.4

### 3.2 FTIR Analysis

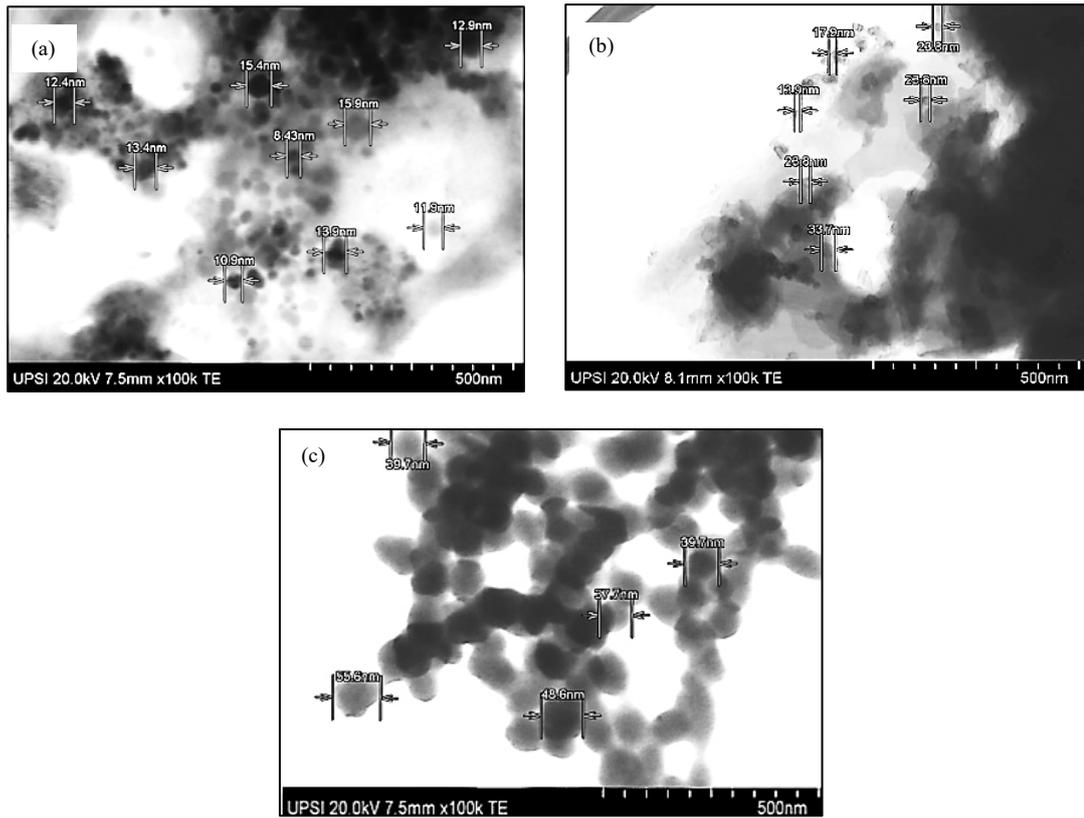
Phytochemicals in the extracts and the respective nanoemulsions have been confirmed through identification of functional groups via FTIR analysis. The FTIR spectra of extracts and nanoemulsions are depicted in Figure 1. Figure 1(a) shows that the broad OH stretching ( $3342\text{ cm}^{-1}$ ) became prominent after incorporating with Arabic gum. Other than that, the peaks at  $1636\text{ cm}^{-1}$  and  $1214\text{ cm}^{-1}$  could be assigned to the C=C and C-O, respectively and they correlated to the interaction between compounds from n-hexane extract and Arabic gum, which is rich with free carboxyl groups. In Figure 1(b), sharp peaks in the region of  $2922\text{ cm}^{-1}$  ascribed to asymmetric stretching vibration of CH(CH<sub>2</sub>) [21]. However, this peak disappeared after the preparation of nanoemulsion. It is interesting to note that another sharp peak at  $1635\text{ cm}^{-1}$  (C=C vibration) has the same phenomena. After chloroform extract nanoemulsion is prepared, a new broad O-H peak emerged at  $3351\text{ cm}^{-1}$ . For ethanol extract in Figure 1(c), a broad peak emerged at  $3330\text{ cm}^{-1}$ , indicating the stretching of hydroxyl groups in the compounds. Another sharp peak is observed at  $2923\text{ cm}^{-1}$  and this can be assigned to C-H stretching of alkyne group. The O-H peak of nanoemulsion became more significant and shifted to  $3344\text{ cm}^{-1}$ , and this could be due to resemblance of hydroxyl groups in the nanoemulsion. C=C stretching contributed the prominence of sharp peak at  $1637\text{ cm}^{-1}$  [18]. Overall, results from FTIR suggest that successful encapsulation of extracts into Arabic gum.



**Figure 1:** FTIR spectra of (a) *n*-hexane extract and its nanoemulsion, (b) chloroform extract and its nanoemulsion, and (c) ethanol extract and its nanoemulsion

### 3.3 STEM Analysis

Detailed information about internal structure of a colloid system or an emulsion can be obtained under a STEM or a TEM. Spherical droplets were observed in Figures 2(a)-(c). In Figure 2(a), the sizes of the spherical droplets of *n*-hexane nanoemulsion are between 8.43 nm and 15.9 nm, and some irregular shapes were spotted due to aggregation phenomena. At the same time, there are more grey areas observed in chloroform extract nanoemulsion (Figure 2(b)) and these might be caused by agglomeration of Arabic gum. It is interesting to note that the ethanol extract nanoemulsion showed uniform monodisperse spheres and the average size is 39.7 nm. Shi et al. [22] studied the optimisation parameters of synthesising a nanoemulsion, including the ultrasonication time. They found that 23.42 mins is the best ultrasonic operating time for emodin loaded nanoemulsion to reduce the size of nanoemulsion.



**Figure 2:** STEM Images of (a) *n*-hexane extract nanoemulsion, (b) chloroform extract nanoemulsion, and (c) ethanol extract nanoemulsion at 100,000 × magnification

### 3.4 Encapsulation Efficiency (EE) and Kinetic Release Mechanism

The percentage EE of extract nanoemulsions were reported in Table 2. The percentage EE of *n*-hexane, chloroform and ethanol extract nanoemulsions were  $65.2 \pm 0.12\%$ ,  $79.0 \pm 3.44\%$  and  $90.3 \pm 1.56\%$ , respectively. Those high %EE suggested that the nanoemulsion system prepared in this study were successful to encapsulate large amounts of extracts in such small droplets of nanoemulsion. An appropriate amount of surfactant, a suitable biopolymer type contributed to the stable nanoemulsions. Results from %EE are important to know because a specific amount will be able to release into the aquatic environment, and this could ease the job for monitoring the suitable concentration for formulation when applied in real field larvicidal studies.

**Table 2:** Encapsulation efficiencies (EE) of extract nanoemulsions and respective release kinetic models

Nanoemulsion	EE (%) *	Kinetic model			
		Zero order	First order	Higuchi	Korsmeyer-Peppas
		R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup> <i>n</i>
<i>n</i> -hexane extract	$65.2 \pm 0.12$	0.564	0.932	0.945	0.962    0.842
Chloroform extract	$79.0 \pm 3.44$	0.652	0.804	0.834	0.983    0.824
Ethanol extract	$90.3 \pm 1.56$	0.735	0.943	0.745	0.974    0.867

\* Values represent mean of three replicates ± standard deviation

The kinetic release data for those three formulations were best fitted to the Korsmeyer-Peppas kinetic model. Furthermore, all three formulations established a transport exponent value ( $n$ ) above 0.5, suggesting that their release are driven by non-Fickian diffusion [23]. The results of release mechanism indicate the use of nanoemulsion could be a good strategy to improve the controlled release of compounds to be a more durable and efficient larvicidal formulation.

### 3.5 Larvicidal Studies

The potential larvicidal activities of precursors and extract nanoemulsions were assessed against fourth instars *A. aegypti* larvae and the results are presented in Table 3. There was no mortality in the Arabic gum solution and Triton X-100. Yet, all three extracts and their respective nanoemulsions performed 100 % of mortality, indicating the compounds in the extract are highly toxic towards *A. aegypti* larvae. Furthermore, the  $LC_{50}$  values obtained from Probit analysis showed that 50 % of mortality rate could be achieved given the certain concentrations of extract nanoemulsions. Larvicidal study was associated with the presence of an active compound or through the synergistic action of different secondary metabolites present in the extract. In this study,  $LC_{50}$  of *n*-hexane extract, chloroform extract and ethanol extract at 24 h were 200.53, 135.42 and 80.00  $mg L^{-1}$ , respectively. After another 24 h passed, there was a slight decline observed for the  $LC_{50}$  values. When the  $LC_{50}$  values after 48 h exposure was compared with the  $LC_{50}$  values after 24 h exposure, it was as high as 2 times more potent, demonstrating a high lethal effect of the extracts against *A. aegypti* larvae.

Meanwhile,  $LC_{50}$  of *n*-hexane extract, chloroform extract and ethanol extract nanoemulsions at 24 h were 120.60, 20.56 and 60.10  $mg L^{-1}$ , respectively. At 48 h, the values decrease nearly two-folds, suggesting it is more toxic towards the *A. aegypti* larvae. Additionally, the larvicidal activities results are corroborate with the results in Section 3.4 by proving that the bioactivity of the extracts is not affected by the encapsulation, yet the dispersion in water and controlled release of the compounds have been enhanced. Furthermore, the prolonged larvicidal effect is the result of the controlled release of active compounds encapsulated in the nanoemulsion over time. Thus, it can be suggested that the larvicidal action found in the extract nanoemulsion may be related to the presence of active compounds with recognised insecticidal action, or by the synergistic action of all active compounds.

**Table 3:** Effectiveness of extracts and their respective nanoemulsions from different extract towards *A. aegypti* larvae

Extract/ Nanoemulsion	24 h		48 h	
	Probit equation	$LC_{50}$ ( $mg L^{-1}$ )	Probit equation	$LC_{50}$ ( $mg L^{-1}$ )
<i>n</i> -hexane extract	$0.052 + 0.225x$	200.53	$1.543 + 1.245x$	180.37
Chloroform extract	$0.178 + 1.264x$	135.42	$1.642 + 2.641x$	108.15
Ethanol extract	$0.645 + 2.456x$	80.00	$2.364 + 2.346x$	24.96
<i>n</i> -hexane extract nanoemulsion	$2.294 + 3.030x$	120.60	$3.074 + 2.716x$	55.50
Chloroform extract nanoemulsion	$2.756 + 2.647x$	20.56	$4.244 + 2.470x$	10.55
Ethanol extract nanoemulsion	$3.067 + 1.874x$	60.10	$3.470 + 1.858x$	30.25

## 4. CONCLUSIONS

There are significant amounts of phytochemicals in *n*-hexane, chloroform and ethanol extracts of *C. odollam* seeds. The GC-MS analysis disclosed that the compounds from extracts of *C. odollam* seeds are highly toxic towards to the larvae. Meanwhile, FTIR analysis revealed the presence of compounds after incorporated with Arabic gum into nanoemulsion. Interestingly, the spheres of varied size were observed from every extract nanoemulsion. High encapsulation behaviour of the formulations resulted in a sustained release of the compounds, demonstrating that their potential to be alternatives for delivering these compounds compared to free extracts. Following 24 h and 48 h of exposure against

4<sup>th</sup> instar *A. aegypti* larvae, treatment of ethanol extract nanoemulsion showed its outstanding larvicidal activity with the concentration range between 30.25 mg L<sup>-1</sup> and 60.10 mg L<sup>-1</sup>. Further investigation should be carried out with examination on the larvicidal activities of those nanoemulsions in real field situations and with the development of more eco-friendly plant based larvicides for vector control.

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### Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

### Disclosure of Conflict of Interest

The authors have no disclosures to declare.

### Compliance with Ethical Standards

The work is compliant with ethical standards.

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