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Production of Curcumin-loaded Nanoemulsion from *Curcuma longa* (Turmeric) Extract and Its Antimicrobial Activity

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Abstract: Microorganisms are capable of causing variety of diseases in humans, most particularly skin infections. This is because skin acts as the first shield, protecting our bodies from foreign organisms. Antimicrobial topical creams may be used to treat wound infection caused by these microbes. The majority of topical medications available on the market to treat skin-related diseases are formulated via synthetic processes that involve the use of chemicals and may result in adverse effects. Thus, this research aims to investigate the potential of curcumin-loaded nanoemulsion as natural antimicrobial agents in formulated creams, with the hope of advancing the pharmaceutical industry using Malaysia herb plants. Based on this study, nanoemulsion was developed via oil-in-water method using Tween 20 and black pepper oil with different ratio and temperatures (70 °C and 75 °C). Then, the particle diameter size, pH and colour were evaluated. Next, the antimicrobial analysis was conducted using disk diffusion method against *E. coli*, *S. aureus*, and *A. niger*. Findings shows that, the sample heating at 75 °C shows the best characterization with the smallest particle diameter size (320 nm), and suitable pH (5.76) for skin. The results also shows that the nanoemulsion with *Curcuma longa* extract has a great potential to inhibit the growth of microorganism. Nanoemulsion heating at 75 °C shows greater zone of inhibition against *E.coli*, *S. aureus*, *A. niger* (11.5 ± 0.7, 13.5 ± 0.7, 11.5 ± 0.7) mm. In conclusion, *Curcuma longa* was more susceptible against *S. aureus* compared with *E. coli* and *A. niger*. Findings from this research can contribute to the development of a safe and greener alternative with plant ingredients in the formulation of cream. Some recommendation include using ultrasonic to get fine emulsions and undergo storage and stability study. Then, conduct the antimicrobial test using variation of microbe species and MIC test to determine the lowest concentration that prevents visible growth of microorganisms.

Keywords: Turmeric, Nanoemulsion, Black Pepper Oil, Antibacterial, Antifungal

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1. Introduction

Over the last three decades, the use of medicinal products and supplements based on herbal plant has exploded, with at least 80% of people around the globe depending on them for several aspects of primary healthcare. Malaysia is also a country in South East Asia (SEA) that is well-known for its medicinal herb resources. Due to the numerous positive effects provided by the herb plants, consumers and experts are paying interest to the medicinal and culinary uses of herbs. Additionally, the Malaysian government recognises the herbal industry's potential and the consumer demand for high-quality herbal products. In September 2009, Malaysia launched the Economic Transformation Programme (ETP) that recognises 12 new key economic areas (NKEAs) and act as catalysts for economic activity and contribute wealthness to Malaysia's growth [1]. The Entry Point Project (EPP) High-Value Herbal Products, which falls under the NKEA agriculture subsector of herbs, has been identified to focus on the development of herbal products with nutraceutical claims and high-value botanical drugs.

Curcuma longa from the *Zingiberaceae f*amily, is a perennial herb known in Malaysia as "kunyit" and is extensively planted throughout Asia, primarily in India and China [1]. *Curcuma longa* has been use as a medicinal herb, particularly in Asian state, due to its biological functions such as antimicrobial, and antioxidant [2]. *Curcuma longa*, the source of curcumin, is being used in Chinese and Ayurvedic medicine for thousands of years. Curcumin, alternatively referred to as diferuloylmethane, is the primary natural polyphenol discovered in the rhizome of *Curcuma longa* and other *Curcuma* species. Curcumin is widely recognised and been using in a variety of forms throughout the world for its numerous potential health benefits. Curcumin is now available in a variety of forms on the market like tablets, capsules, ointments, beverage, soaps, and cosmetics.

Due to growing awareness on standard of living of human life, it is crucial to control the consequences of harmful microorganisms. While a diverse array of microorganisms coexist biologically with the human body and its surrounding environments, uncontrolled and rapid growth of microbes can result in certain serious challenges. This has created into a significant health problems in a number of developing and under-developed countries especially in densely populated places with high humidity and unsanitary conditions. An antimicrobial agent is a material that kills or inhibits microorganisms from growth. Antimicrobial agents are classified into two categories which is synthetic or chemical antimicrobials and natural-derived antimicrobials. Turmeric, ginger, and garlic are all herbal antimicrobial agents that also act as free radical scavengers and thus inhibit the creation of reactive oxygen species (ROS) in the human body [3], [4].

Antimicrobial activity encompasses both antibacterial and antifungal properties. Bacteriostatic/fungistatic inhibiting the growth of bacteria or fungal, whereas bactericidal/fungicidal kill bacteria or fungal. Humans require antimicrobial activity to protect themselves from numerous types of disease that caused by microbes such as ringworm or other life-threatening infectious disease. *S. aureus* is the most common bacterial infection in human especially skin and soft tissue infections and is the causative agent of multiple human infections [5]. As for fungal infections, particularly *A. niger*, seemed to be the most prevalent causative agent for otomycosis (fungal ear infection).

Thus, this research was performed to produce a nanoemulsion incorporated with *Curcuma longa* extract and to analyse its antimicrobial activity. However, the low bioavailability of curcumin is a significant impediment to its clinical efficacy. The formulation of curcumin-loaded nanoemulsions is further enhanced by using black pepper oil to increase the bioavailability of curcumin. This research aims to investigate the potential of curcumin-loaded nanoemulsion as natural antimicrobial agents to substitute synthetic antimicrobials in formulated creams, with the hope of advancing the pharmaceutical industry using Malaysia herb plants.

2. Methods

2.1 Collecting, processing, and extracting turmeric sample

The sample was taken from AM Zaideen Ventures Sdn. Bhd. as shown in Figure 2.1. The turmeric comes from Pahang, Malaysia. Sub-critical water extraction (SWE) was used to get the turmeric extract. This method used water as a solvent to make it natural and safer to use. It used a 70 litre scale. There is a 1:13 ratio between the sample and water. During the extraction process, 10 bar and 120 °C were used for 10 minutes. After extracting the sample with SWE, the sample was weighed and put in the cell. Finally, the sample was let to cool down so that it could be stored in the freezer at 4 °C for further used.

Figure 1: Pure extract of turmeric

2.2 Preparation of curcumin-loaded nanoemulsion

The curcumin-loaded nanoemulsion was prepared following oil-in-water (O/W) emulsion method from a published study [6], with some modification. To make nano-curcumin using the method, black pepper oil (Plant Therapy, India) and surfactant (Tween 20(Merck Darmstadt, Germany)) were mixed together at a 1:9 w/w ratio [6]. The mixture was continuously stirred for 30 minutes at 1000 rpm using magnetic stirrer (Thomas Scientific, USA) to form the oily phase. 100 mg of the curcumin extract was added to the oily phase, and the mixture was agitated for 1 hours at room temperature using a magnetic stirrer at 500 rpm. After that, the final nanoemulsion was created by adding deionized water to the oily phase (ratio 5:1 w/w) and stirred for 30 minutes at 500 rpm (heating at 70 °C or 75 °C for 30 min and then rapidly cooled down at 5°C for 15 min), as shown in Table 1.

Table 1: Recipe of the formulated nanoemulsion

Ingredients	Weight (g)
Black pepper oil	0.5
Surfactant (Tween 20)	9.5
Turmeric extract	0.1
Deionized water	50.0

In this work, different ratio (w/w) of oil and surfactant and two different heating temperature were employed as shown in Table 2.

Table 2: Different ratio of oil and surfactant and two different heating temperature prepared

Heating Temperature (°C)	Sample	Oil (Black pepper) / g	Surfactant (Tween 20) / g
70	Cur. 1A	0.5	9.5
	Cur. 2A	1.0	9.0
	Cur. 3A	1.5	8.5
	Cur. 4A	2.0	8.0
75	Cur. 1B	0.5	9.5
	Cur. 2B	1.0	9.0
	Cur. 3B	1.5	8.5
	Cur. 4B	2.0	8.0

2.3 Analysis of particle size of curcumin-loaded nanoemulsion

The particle size distribution of the curcumin-loaded nanoemulsion was found measured using an inverted microscope (Olympus IX-HOS, Japan), that had a fluorescence. After a small amount of emulsion was put on the glass slide, the cover slip was put on it. To determine the size of the particle, the sample was looked at a magnification of 4x. Prior to microscopic imaging, this procedure was repeated at 10x, 20x, and 40x magnifications to obtain the best image for the sample.

2.4 pH of formulated nanoemulsion

The nanoemulsion's pH was checked using pH meter (Hanna Instruments, Malaysia) at 20 ± 1˚C according manufacturer's procedure.

2.5 Colour of the emulsion

Emulsion stability affects the products' appearance. The emulsion instability can be observed directly by visual observation to assess the gravitational separation of the emulsion without expensive analytical instruments [7].

2.6 Subculturing of microorganism

Subculturing is a technique used by a microbiologist to maintain a fresh batch of bacterial and fungal strains that are viable for study. For this subculturing in agar plate process, gram-positive and gram-negative bacteria strain was used which is *S. aureus* and *E. coli* while fungal strain used was *A. niger*. The growth of bacteria and fungi on agar was made by the streaking plate method. The plates are incubated at 37 ℃ for 24 hours. Subculturing in broth was performed by dipping the loopful of bacteria into the 10 mL broth.

2.7 Preparation of McFarland standard and microbe suspension

McFarland was prepared by diluting a 1.00 % solution of anhydrous barium chloride (BaCl2) in 0.05 mL with a 1.00 % solution of sulphuric acid (H_2SO_4) in 9.95 mL to obtain the correct McFarland turbidity standard of 0.5 (equivalent to 1.5 x 10^8 CFU/mL). The bacterial suspension was prepared by transferring the bacteria using a sterile loop into the broth in the universal bottle. As with fungal spore suspensions, a loopful of fungal spores was added to a 10 mL 0.85 percent saline solution [8]. Both bacteria and spores suspension was used within 30 minutes.

2.8 Preparation of media

Mueller Hinton Agar (MHA) was a type of media used in antibacterial testing, whereas Potato Dextrose Agar (PDA) was used in antifungal testing. 23.7 g. MHA Powder was dissolved in 625 mL distilled water, whereas 24.4 g of PDA powder is dissolved in 625 mL of distilled water. The solution

was heated to 121 °C and autoclaved for 15 minutes before cooled down to room temperature. The media was poured into a sterile petri dish and allowed it to solidify. The process was repeated to create broth media.

2.9 Assessment of antibacterial activity

100 µL of *S. aureus* bacterial suspension was added on the Mueller Hinton Agar (MHA) by using a 20-200 µL micropipette (Eppendorf, Germany) and spread uniformly through all surfaces of the MHA by a glass L-shaped spreader. 20 µL of each curcumin-loaded nanoemulsion samples (labelled Cur. 1 until Cur. 4) with different concentration and was dried for 15 minutes to prevent introducing excess liquid onto the inoculated agar plate [9]. Ampicillin (10 µg) was used as positive control, and sterile distilled water as negative control. The soaked disc was air-dried before placing on the agar to prevent introducing excess liquid onto the inoculated agar plate. The disc and agar plates were incubated at a temperature of approximately 37 ℃ for 24 hours [9]. After that, zone of inhibition was measured in millimetres (mm). The experiment was repeated using a different bacteria culture, *E. coli*.

2.10 Assessment of antifungal activity

100 µL of *A. niger* fungal suspension was added on the Potato Dextrose Agar (PDA) by using a 20-200 µL micropipette (Eppendorf, Germany) and spread uniformly through all surfaces of the PDA by a glass L-shaped spreader. 20 µL of each curcumin-loaded nanoemulsion samples (labelled Cur. 1 until Cur. 4) with different concentration and was dried for 15 minutes to prevent introducing excess liquid onto the inoculated agar plate [9]. Fluconazole (0.1 g/mL) was used as positive control, and sterile distilled water as negative control. The soaked disc was air-dried before placing on the agar to prevent introducing excess liquid onto the inoculated agar plate. The disc and agar plates were incubated at a temperature of approximately 37 ℃ for 24 hours [9]. After that, zone of inhibition was measured in millimetres (mm).

3. Results and Discussion

3.1 Curcumin-loaded nanoemulsion

The oil-in-water emulsion technique was used to create the nanoemulsion. Table 3.1 summarises the diameter size, pH, and colour of each sample with varying temperature and concentration.

Table 2: Several characterizations that were identified from the nanoemulsion

Heating Temperature (°C)	Sample	Ratio (Black pepper oil : Tween 20)	Particle Diameter Size (nm)	pH	Colour
70	Cur. 1A	0.5:9.5	530	5.42	Yellow (Clear)
	Cur. 2A	1.0:9.0	590	5.10	Yellow (Clear)
	Cur. 3A	1.5:8.5	670	4.75	Yellow (Cloudy)
	Cur. 4A	2.0:8.0	720	4.34	Yellow (Cloudy)
75	Cur. 1B	0.5:9.5	320	5.76	Yellow (Clear)
	Cur. 2B	1.0:9.0	410	5.24	Yellow (Clear)
	Cur. 3B	1.5:8.5	460	4.82	Yellow (Cloudy)
	Cur. 4B	2.0:8.0	550	4.55	Yellow (Cloudy)

Based on characterization of particle diameter size with heating temperature. Particle diameter size of the Cur. 1B heating at 75 °C was smaller (320 nm) while Cur. 4A nanoemulsion heating at 70 °C was the biggest (720 nm) as shown in Figure 2. Accordingly, as the heating temperature increased, hence the diameter size of the particle will be decreased. The data collected also correlated with previous research, which is smaller in diameter size (9.7 nm) when fabricated at heating temperature of 75 °C because the temperature is closer to the phase inversion temperature (PIT) and better facilitates the

spontaneous emulsification [10]. Thus, the diameter size of the particle will become smaller when heating at higher temperature that was closer to the PIT.

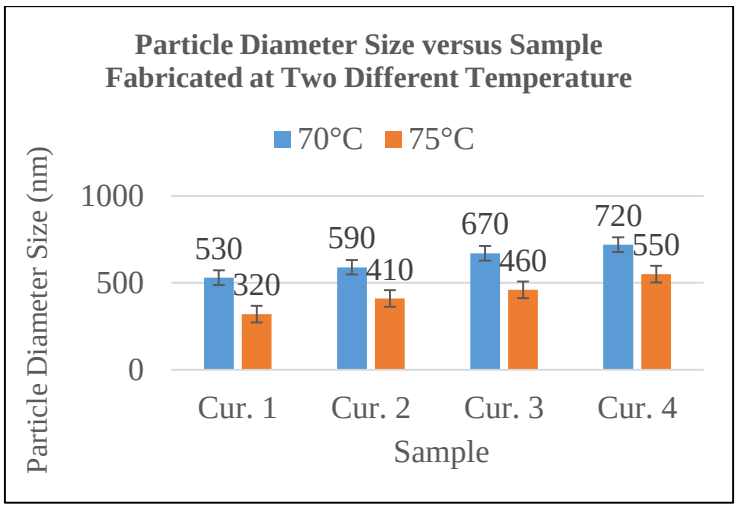

Figure 2: Comparison of particle size of formulated nanoemulsion fabricated at two different temperature

Next, the oil-surfactant ratio will affect the particle diameter size of nanoemulsion. From Table 2, Cur. 4A shows the largest particle diameter size (720 nm) with oil-surfactant ratio was 2.0:8.0. However, Cur. 1B shows the smallest particle diameter size (320 nm) with oil surfactant ratio was 05:9.5. As the surfactant concentration is increased, the diameter of the nanoemulsion decreased. Similar to other published research, the size of droplets is extremely decreased (23.7 nm) by increasing the surfactant content, oil-surfactant ratio (0.5:9.5) [6]. The addition of an emulsifier is critical for the creation of small sized droplets as it decreases the interfacial tension including the surface energy per unit area, between the oil and water phases of the emulsion [11]. Thus, the composition of the oil phase was critical in the preparation of the nanoemulsion as decreasing the surfactant content in the formulated nanoemulsion significantly increased the particle diameter size.

As for the pH of nanoemulsion, Cur. 1B nanoemulsion shows a higher pH value which is 5.76 while Cur. 4A nanoemulsion shows a lower pH of 4.34 (refer Table 3.1). As the surfactant concentration of the formulated nanoemulsion increased, hence the pH was also increased. The data found in this study correlated with previous study conducted by Anjali et al., (2012). Nanoemulsion formulations with increasing of oil-surfactant ratio 1:3 shows the higher pH value (5.60) compared with ratio 1:0.3 which shows lower pH value (4.85), i.e. more acidic [7]. The addition of a non-ionic emulsifier, such as Tween 20, as a co-surfactant could possibly enhance the system by preventing aggregation and improving its pH stability [12]. The optimal pH of human skin is 5.5, while the pH of a suitable nano-cream is between 5 and 6 [13]. Thus, Cur. 1 and Cur. 2 heating at both temperatures were suitable for cream formulation and application on human skin.

For colour visual observation, Cur. 1 and Cur 2 were clear in colour while for Cur. 3 and Cur. 4, a cloudy yellowish colour developed due to the decreased of surfactant-oil ratio as shown in Figure 3 and 4. Thus, when the ratio of oil-surfactant increased, the colour of the nanoemulsion will be clearer. The results indicated the same trend with Moghaddasi et al., (2018) in which the colour of the nanoemulsion shows a clear yellowish if the surfactant-oil ratio increased while cloudy yellowish when the surfactant-oil ratio decreased [6]. Depending on the size of particles, the emulsion can be transparent, translucent or milky. The smaller the particle size, the more translucent the colour is. Nanoemulsions can be transparent, translucent or milky. Therefore, nanoemulsion is very useful in the formation of cream as it has good transparent visual aspect [14]. Thus it can be said that Cur. 1 and Cur 2 were suitable in the formulation of cream due to their colour which is clear yellowish.

Figure 3: The colour of nanoemulsion sample prepared at temperature 70 °C

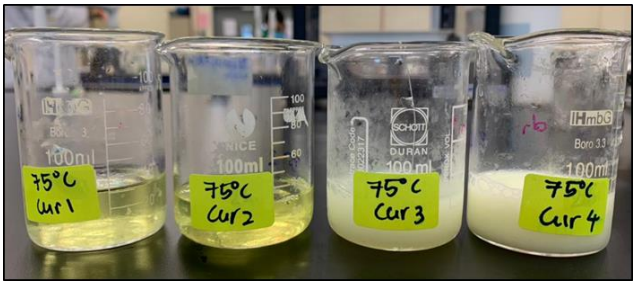

Figure 4: The colour of nanoemulsion sample prepared at temperature 75 °C

In summary, all these characteristics are related to each other. Firstly, the particle diameter size is influenced by two factors such as heating temperature, and also composition of oil-surfactant. When increased in heating temperature (75 °C**)** and oil-surfactant ratio (0.5:9.5), the particle diameter size of the nanoemulsion was at the smallest, 320 nm. Then, the oil-surfactant ratio also affecting the pH value and colour of the formulated nanoemulsion. As the oil-surfactant ratio increased (0.5:9.5), the pH value was higher (5.76), and the colour was clearer (Cur. 1 and 2). Thus, it can be concluded that the nanoemulsion fabricated at 75 °C heating temperature shows a better characterization compared to the heating temperature at 70 °C.

3.2 Analysis of antibacterial activity

According to Reddy et al., (2013), the existence of methoxyl and hydroxyl groups is believed to be responsible for the antimicrobial activity of curcumin [15]. Table 3.2 indicates the zone of inhibition (ZOI) for pure extracted turmeric sample at 50.00 % to 100.00 % of concentration respectively for the bacteria *of E. coli and S. aureus*.

Table 3: Zone of inhibition against bacteria for pure extracted turmeric sample

Sample	Concentration (%)	Zone of Inhibition (mm)	
		E. coli	***S. aureus***
Pure extracted turmeric	50	3.0 ± 4.2	3.0 ± 4.2
	70	6.0 ± 0.0	6.5 ± 0.7
	100	7.0 ± 0.0	7.0 ± 0.0

From the analysed data, minimum ZOI of 3.0 ± 4.2 mm and maximum ZOI of 7.0 ± 0.0 mm for both bacteria species, *E. coli* and, *S. aureus*. Based on previous study, ZOI of the turmeric against *E. coli* was 10 ± 0.38 mm [16]. The ZOI of turmeric against *S. aureus* was 6.0 ± 0.0 [17]. Thus, as the concentration of the sample increased, hence the ZOI also increased. Based on the analysed data, shows that the pure extracted turmeric have low effectiveness in reducing and killing the bacteria.

Next, the pure extracted turmeric was formulated in the form of nanoemulsion with black pepper and Tween 20 as shown in Table 3.3. As for positive control, ampicillin shows a greater ZOI of 11.5 mm and 14.5 mm against *E. coli* and *S. aureus.*

Table 4: Zone of inhibition for antimicrobial test for formulated nanoemulsion

Heating Temperature (℃)	Sample	Concentration (%)	Zone of Inhibition (mm)	
			E. coli	*S. aureus*
70	Cur. 1A	50	7.5 ± 0.7	10.5 ± 0.7
		70	10.0 ± 1.4	11.0 ± 0.0
		100	11.0 ± 0.0	13.5 ± 0.7
	Cur. 2A	50	6.5 ± 0.7	8.5 ± 0.7
		70	9.5 ± 0.7	9.5 ± 0.7
		100	10.5 ± 0.7	11.5 ± 2.1
	Cur. 3A	50	6.0 ± 0.0	7.5 ± 0.7
		70	8.0 ± 0.0	8.5 ± 0.7
		100	9.0 ± 0.0	11.0 ± 1.4
	Cur. 4A	50	6.0 ± 0.0	7.0 ± 0.0
		70	8.0 ± 1.4	8.5 ± 0.7
		100	8.5 ± 0.7	10.5 ± 0.7
75	Cur. 1B	50	9.5 ± 2.1	10.5 ± 2.1
		70	10.0 ± 1.4	11.5 ± 2.1
		100	11.5 ± 0.7	13.5 ± 0.7
	Cur. 2B	50	9.5 ± 0.7	7.5 ± 0.7
		70	10.0 ± 1.4	8.5 ± 0.7
		100	10.5 ± 0.7	11.0 ± 1.4
	Cur. 3B	50	9.0 ± 1.4	6.5 ± 0.7
		70	9.0 ± 1.4	7.0 ± 0.0
		100	10.5 ± 2.1	9.5 ± 0.7
	Cur. 4B	50	7.5 ± 0.7	6.5 ± 0.7
		70	8.0 ± 0.0	7.0 ± 1.4
		100	9.0 ± 0.0	8.5 ± 0.7

Figure 5 demonstrates an example of the results obtained from the disc diffusion method used to determine the sample's antibacterial activity. For sample prepared at heating temperature (70 ℃), ZOI of nanoemulsion sample as shows in Table 4 For minimum sample concentration (50%), Cur. 1A exhibit the highest ZOI against *E. coli* and *S. aureus* at 7.5 ± 0.7 mm, and 10.5 ± 0.7 mm respectively. However, Cur. 4A shows the lowest ZOI against both species 6.0 ± 0.0 mm, and 7.0 ± 0.0 mm for 50% of concentration. As for maximum sample concentration (100%), Cur. 1A possessed the highest ZOI against *E. coli* and *S. aureus* at 11.0 ± 0.0 mm, and 13.5 ± 0.7 mm. The lowest ZOI recorded at Cur. 4A for both species (*E. coli* and *S. aureus*) with value of 8.5 ± 0.7 mm, and 10.5 ± 0.7 mm respectively.

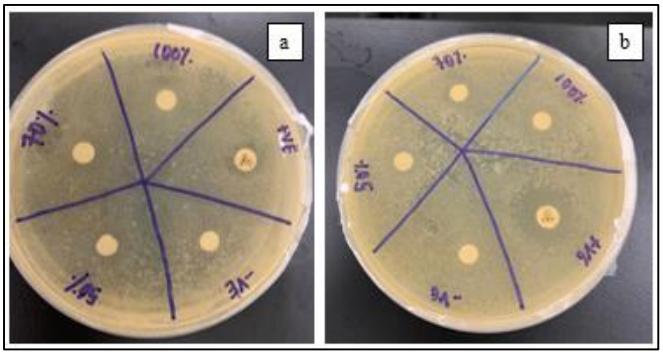

Figure 5: Disk diffusion method conducted against (a) *E. coli* (b) *S. aureus*

Then, at heating temperature 75 ℃ for 50.00 % of concentration, Cur. 1B shows the highest ZOI against *E. coli* and *S. aureus* which were 9.5 ± 2.1 mm, and 10.5 ± 2.1 mm while Cur. 4B shows the lowest ZOI at 7.5 ± 0.7 mm, and 6.5 ± 0.7 mm respectively. Next, for 100.00 % of concentration, Cur

1B shows the highest ZOI against *E. coli* and *S. aureus* at 11.5 ± 0.7 mm, and 13.5 ± 0.7 mm whereas Cur 4B possessed the lowest ZOI at 9.0 ± 0.0 mm, and 8.5 ± 0.7 mm.

Based on the analysis, the ZOI against gram-positive bacteria were significantly greater than those against gram-negative bacteria. Indeed, gram-positive bacteria were found to be more susceptible to plant extracts than gram-negative bacteria [18]. The fact that selected gram-positive bacteria are more sensitive than selected gram-negative bacteria may explain their differences in cell membrane constituents and structure [19]. Gram-negative bacteria's relative resistance is due to their lipopolysaccharides layer and periplasmic space [18].

3.3 Analysis of antifungal activity

As for fungal, the maximum ZOI was 6.5 ± 0.7 mm. At minimum concentration of 50.00 %, there was no activity recorded. Thus, as the concentration of the sample increased, hence the ZOI also increased. Based on Table 5, pure extracted turmeric have low effectiveness in inhibiting fungal.

Table 5: Zone of inhibition of antifungal for pure extracted turmeric sample

Sample	Concentration (%)	Zone of Inhibition (mm)
		A. niger
Pure extracted turmeric	50	NA
	70	6.0 ± 0.0
	100	6.5 ± 0.7

The pure extracted turmeric was formulated in the form of nanoemulsion as shown in Table 6.

Table 6: Zone of inhibition of antifungal test for formulated nanoemulsion

Heating Temperature (℃)	Sample	Concentration (%)	Zone of Inhibition (mm)
			A. niger
70	Cur. 1A	50	8.5 ± 0.7
		70	9.5 ± 0.7
		100	10.5 ± 0.7
	Cur. 2A	50	8.0 ± 0.0
		70	9.5 ± 0.7
		100	10.0 ± 1.4
	Cur. 3A	50	7.5 ± 0.7
		70	9.0 ± 0.0
		100	10.0 ± 1.4
	Cur. 4A	50	7.5 ± 0.7
		70	8.5 ± 0.7
		100	9.5 ± 0.7
75	Cur. 1B	50	8.5 ± 0.7
		70	10.0 ± 0.0
		100	11.5 ± 0.7
	Cur. 2B	50	8.0 ± 0.0
		70	9.5 ± 0.7
		100	10.0 ± 1.4
	Cur. 3B	50	8.0 ± 0.0
		70	9.0 ± 0.0
		100	10.0 ± 1.4
	Cur. 4B	50	7.0 ± 1.4
		70	8.5 ± 0.7
		100	9.5 ± 0.7

As for antifungal, fluconazole was used as positive control. However, there was no ZOI recorded as the fungi was resistant to fluconazole. As for sample prepared at heating temperature (70 ℃) for minimum sample concentration which is 50.00 %, Cur. 1A shows the highest ZOI against *A. niger* at 8.5 ± 0.7 mm while Cur. 3A and Cur. 4A shows the lowest ZOI which is 7.5 ± 0.7 mm respectively. As for maximum sample concentration (100.00 %), Cur. 1A possessed the highest ZOI against *A. niger* at 10.5 ± 0.7 mm whereas the lowest ZOI recorded at Cur. 4A with value of 9.5 ± 0.7 mm respectively.

Then, at heating temperature 75 ℃ for 50.00 % of concentration, Cur. 1B shows the highest ZOI against *A. niger* at 8.5 ± 0.7 mm while Cur. 4B shows the lowest ZOI at 7.0 ± 1.4 mm. Next, for 100.00 % of concentration, Cur 1B shows the highest ZOI against *A. niger* at 11.5 ± 0.7 mm whereas Cur 4B possessed the lowest ZOI at 9.5 ± 0.7 mm respectively. Thus, as the concentration of the sample increased, hence the inhibition of clear zone also increased. Figure 6 demonstrates an example of the results obtained from the disc diffusion method used to determine the sample's antifungal activity.

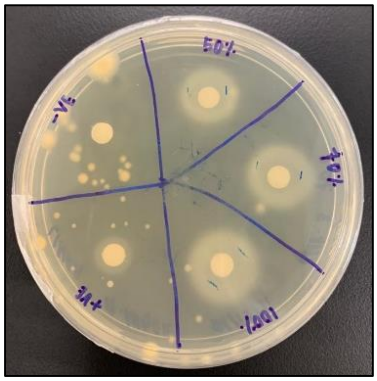

Figure 6: Disk diffusion method conducted against *A. niger*

Based on the tabulated data, it can be concluded from this antimicrobial test that turmeric in the form of nanoemulsions exhibited a greater inhibitory effect. This is because of the antimicrobial droplets sample was protected and delivered via nanoencapsulation, the antimicrobial droplets' contact area was increased. Besides, the nano-curcumin has been reported more effective against gram-positive bacteria than gram-negatives as well as fungus [20].

4. Conclusion

This study has achieved its objectives. The oil-in-water nanoemulsion formulation containing curcumin, black pepper oil, tween 20 and deionized water was successfully produced by the phase inversion method. Secondly, Cur. 1B heating at temperature 75 ℃ developed a better characteristic of the nanoemulsion in terms of particle diameter size (320 nm), pH (5.76) and clearer in colour. Thirdly, based on antimicrobial analysis, the nanoemulsion inhibit higher ZOI compared with pure extracted. *Curcuma longa* also has potential to inhibit the growth of fungal against *A. niger*. However, the extracted sample was less susceptible against *A. niger* as well as gram-negative (*E. coli*) when compared with gram-positive (*S. aureus*) which more susceptible. Therefore, this study proved that *Curcuma longa* is able to act as natural antimicrobial agents especially when prepared in the form of nanoemulsion. Thus, may also be used natural preservative that can be applied in a pharmaceutical products such as cream to treat wound infections.

The recommendation for future works based on the finding of this research include preparation of fine emulsion with diameter less than 100 nm by using ultrasonic after the coarse emulsion was produced using magnetic stirrer. Next, storage and stability study of the formulated curcumin-loaded nanoemulsion should be carried out to see how it will remain in a given timeframe. As for the antimicrobial, the minimum inhibitory concentration (MIC) testing should be carried out to determine

the lowest concentration of an antimicrobial agent that prevent visible growth of microorganism. Lastly, varying the species of microbe used against the pure extracted turmeric and formulated nanoemusion.

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