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Gum Arabic Microencapsulation of *Zingiberaceae Family* Rhizome Extracts (*C. longa, C. xanthorriza and Z. officinale*) Using Spray Drying Processing and Its Characterization

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Abstract: *Zingiberaceae family* rhizomes contain bioactive compound that contains high antioxidant, antimicrobial and anti-inflammatory properties. Due to its instability, it cannot be used extensively in the sector. Therefore, spray drying was utilized in conjunction with microencapsulation to stabilize the labile chemicals. The input temperature was 180 °C, the blow rate was 40 m^3/h, and the pump speed was 5 rpm. This study has been conducted to microencapsulate *Zingiberaceae family* rhizome extracts (*C. longa, C. xanthorriza and Z. officinale*) using spray drying processing method and to analyse the physiochemical properties of the microencapsulated *Zingiberaceae family* rhizome extracts based on the physical, structural and chemical properties. The effectiveness of the extracts in terms of its anti-inflammatory of the plant extracts were also evaluated. Total phenolic content is calculated using the Folin-ciocalteu technique, whereas BSA assays is used to measure anti-inflammatory of the extracts. This study determines that among those three extracts turmeric has the highest yield (32.77%) and lowest moisture content (2.23%). The FTIR spectrum indicates that all three samples have alcohols group with -OH bond. The SEM image show that all three samples have the same shape and structural. The phenolic content of turmeric (14.201 mg GAE/g) is much higher than temulawak (11.342 mg GAE/g) and ginger (9.997 mg GAE/g). BSA assays shows that the *Zingiberaceae family* rhizomes does possess anti-inflammatory activity, temulawak and turmeric has same of anti-inflammatory value at 96.30% that higher than ginger (92.59%) at lowest concentration of 125 µg/mL. At a concentration of 250 µg/mL and 500 µg/mL, the anti-inflammatory activity was achieved in the range

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of 81.48% to 90.74% and 94.44% to 98.15% respectively. The result shows that the active compound found in *Zingiberaceae family* rhizomes have been proven to be effective anti-inflammatory agents.

Keywords: Temulawak, Turmeric, Ginger, *Curcuma Sp.*, *Zingiberaceae Family* Rhizome, Spray Dry, Microencapsulation, Arabic Gum

1. Introduction

Most medicinal plant such as *Zingiberaceae family* rhizomes have their own bioactive compounds. *Zingiberaceae family* rhizomes which is made up of over 120 different species, has a long history of medical use [1]. Among those three *Zingiberaceae family* rhizomes have been chosen which are *Curcuma longa, Curcuma xanthorrhiza,* and *Zingiber officinale*. These plants consists higher antioxidant, antimicrobial and anti-inflammatory in bioactive compound.

Therefore, this study was conducted to microencapsulate *Zingiberaceae family* rhizome extracts and the physiochemical properties were analyzed in terms of physical, chemical, structure, and anti-inflammatory were evaluated. However, the phenolic pigment is photosensitive, unsteady at pH more than 7, and degrades oxidatively. It cannot truly be widely employed in the business because of its lack of stability. The bioactive compounds such as curcumin is also water insoluble, but soluble in organic solvents [2]. In order to increase bioactives stability and boosting turmeric solubility in aqueous media, microencapsulation of *Zingiberaceae f*amily rhizome extract may be able to overcome these shortcomings [3]. In addition, the use of wall material such as gum Arabic, xanthan gum, and maltodextrin in the spray drying will improve the stability of plant extract. The microcapsules created at the end of the procedure will be examined for their physicochemical characteristics, including their chemical, physical, and structural compositions, as well as their anti-inflammatory properties.

Spray drying is a process that we used to microencapsulate the bioactive compound in the *Zingiberaceae family* rhizome extracts. Spray-dying has the benefit of being a physical approach of microencapsulation that does not need the use of organic solvents and produces microparticles in the form of a dried powder at the end of the process. Powdery food components are far easier to manage than liquid ingredients, as they are easier to handle, maintain throughout storage, and take up less space [2]. Starting with an aqueous polymeric solution in which the active ingredient had been dissolved, disseminated, or emulsified, it is quite easy and permits the formation of microspherical structures with the core material embedded within a continuous matrix of protective biopolymers [3].

2. Materials and Methods

2.1 Materials

In this research, temulawak, ginger and turmeric were extracted using subcritical water extraction (SWE) at AM Zaideen Ventures Sdn. Bhd. Then it will be microencapsulated by using spray drying method and will be encapsulated with Arabic gum. Folin-Ciocalteu reagent, standard substance including gallic acid, bovine serum albumin (BSA) and aspirin were used. Chemical used in this research were methanol, ethanol, potassium buffer saline (PBS) and sodium carbonate were used for determination of TPC and anti-inflammatory.

2.2 Spray Drying Process

The spray drying process were carried out using a lab-scale spray drier, equipped with the spray-drying chamber, high speed atomizer, cyclone separator, a hot air blower, an air compressor, and a standard 0.7 mm spray nozzle. The homogenized sample mixed with Arabic gum was fed into the chamber with the inlet temperature of 180 °C, atomized by the hot air at the blow rate of 40 m^3/h, and the pump speed at 5 rpm for each different sample which is temulawak, turmeric and ginger.

2.3 Physiochemical Analysis

2.3.1 Determination of Product Yield

To calculate the yield were divided the mass of powder from the spray drier by the mass of the solid raw materials we put into the solution at the beginning [2]. By using Eq. 1, product yield of the sample will be calculated:

$$Product\ yield\ (\%) = \frac{Mass\ of\ powder\ obtained\ at\ spray\ dryer}{Amaount\ of\ solid\ materials\ in\ the\ initial\ fed\ solution} \times 100 \quad Eq.1$$

2.3.2 Determination of Color

Color analysis was carried out using a colorimeter (Hunter Lab), color values of samples of curcumin microparticles were measured. The color was measured in terms of the L* (lightness), a*(redness and greenness) and b*(yellowness and blueness) [4]. 0.6g of powder sample for each sample was placed in the sample port above the light source. Make sure to standardize the calorimeter before use it.

2.3.3 Determination of Moisture Content

For the moisture content of *Zingiberaceae family* rhizome extracts samples were determine by employing a direct drying procedure that was modified slightly to ascertain from Guo, J. et al. and Sahin Nadeem, H. et al. In depth, aluminum pans that were dry and clean were weighed and loaded with 0.6 g of microparticle powder. The samples were dried for 2 hours at 105°C in drying oven. The moisture content was calculated by:

$$Moisture\ content = \frac{W - W_1}{W_1} \times 100 \quad Eq.2$$

Where, W = weight before drying process

W_1 = weight after drying process

2.3.4 Determination of Functional Group

The analysis of functional group was determined with a modified version method from Rohman, A. et al., 200 mg of KBr IR grade and a 2 mg ethanolic extract of *Curcuma longa, Curcuma xanthorriza,* and *Zingiber officinale* were combined, then the mixture was homogenized in a mortar. With a resolution of 4 cm^{-1} and 32 scans, FTIR spectra were examined in the mid-infrared range of 4000-650 cm^{-1}. The samples were positioned with the HATR attachment in proximity [5]. On top of an air spectrum background, all spectra were proportioned.

2.3.5 Determination of Morphology

The sample of *Zingiberaceae family* rhizome extracts (*C. longa, C. xanthorriza and Z. officinale*) were dried first by using dry oven at the temperature of 110°C for 24 hours. Before being gold coated, microparticle samples were adhered to a brass stub using double-sided adhesive tape [2]. Then, it will be coated with the gold by using sputter coating and analyze the morphology by using SEM.

2.3.6 Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) analysis in *Zingiberaceae family* rhizome extracts use Folin-Ciocalteu reagent in each extract which the previous study Nisar, T. et al., (2015) was used to determine the phenolic compound, with some modification. 10 ml of folin-ciocalteu was put into a 100ml volumetric flask and the distilled water was added up to the mark on the volumetric flask. The volumetric flask was shaken gently to homogenize the solution and wrap the volumetric flask with aluminium foil because folin-ciocalteu is light sensitive. for sample in form of powder, 0.05g sample was dissolved in 10ml of distilled water. Test tubes containing 250 µL of Folin-Ciocalteu reagent, 750 µL of 20% sodium carbonate solution, and 520 µL of each produced extract are added individually for this purpose. The final volume is brought up to 5 mL with distilled water. After two hours, absorbance is measured using a UV/visible light spectrophotometer at 765 nm in comparison to control with all reaction reagents present except sample extract. The methanol was used as a blank. The calibration curve was produced using gallic acid as a standard. Good linearity of gallic acid was obtained (R^2 = 0.9917). The TPC was calculated as:

$$y = 840.46x - 19.786 \qquad Eq.3$$

Where y = absorbance

x = concentration from calibration curve

$$Total\ phenolic\ compound = \frac{cV}{m} \qquad Eq.4$$

Where c = concentration from calibration curve (mg/ml)

V = volume of the sample (ml)

m =mass of the sample (mg)

2.3.7 Determination of Anti-Inflammatory

With the technique used from Khatun, M. et al. (2021), protein denaturation inhibition was assessed. To achieve a concentration of 1 mg/mL for each experiment in this investigation, the obtained extracts are dissolved in 100% ethanol. With 0.1 M phosphate buffer solution (pH-7.4), the stock solution of test sample extracts and standard samples is diluted to various quantities (125– 500 µg/mL) in various test tubes. The reaction mixture (5 mL) is made up of 0.02 mL of extract, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL of 1 percent bovine albumin [6]. The reaction mixture is blended and heated for 5 minutes at 70°C after 15 minutes of incubation in a water bath at 37°C. A UV/VIS spectrometer will be used to quantify turbidity at 660 nm after cooling the reaction mixture [6]. Equation below is used to compute the percent inhibition of protein denaturation:

$$Inhibition\ of\ denaturation(\%) = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100 \quad Eq.6$$

3. Results and Discussion

3.1 Analysis of Spray Dry for *Zingiberaceae Family Rhizome Extracts*

3.1.1 Product Yield

Spray dryer have been used to produce microparticles for a different type of *Zingiberaceae family* rhizome extracts at the same temperature and same wall material which is Arabic gum. Table 1 shows the product yield of *Zingiberaceae family* rhizome extracts at temperatures 180 °C for turmeric is 32.765% which is the highest among the sample, while the lowest product yield is temulawak 30.39%.

Table 1: Product yield of *Zingiberaceae family* rhizome extracts at temperatures 180 °C

Sample	Weight sample + Arabic gum (g)	Weight of spray dried powder	Product yield (%)
Temulawak	20	6.078	30.39
Ginger	20	6.16	30.80
Turmeric	20	6.553	32.77

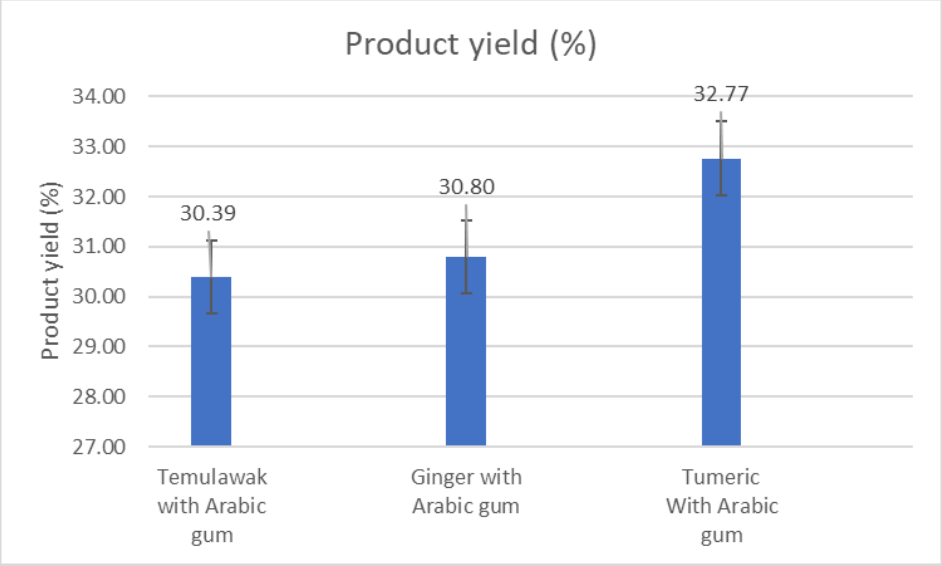

Figure 1: Product yield of *Zingiberaceae family* rhizome extracts at temperatures 180 °C

Carrier materials is very important for the spray drying of the *Zingiberaceae family* rhizome extracts and any other extract sample. According to the Tontul, I. et al. (2017), high glass transition temperatures are achieved by adding high volumes of carrier materials to fruit juices. Maltodextrin and gum Arabic are the two most often utilized carrier substances. Thus, glass transition temperatures are lower than drying temperatures. When spray-drying at high temperatures, the material surface becomes viscoelastic and sticks to the chamber.

3.1.2 Color

Table 2 shows the colour value of each different powder sample obtained after spray dry at temperature 180°C. The value of L*, a* and b* is known as CIE Lab colour space, L* a* b* color values

enable us to identify and convey colors, just like the geographical coordinate's longitude, latitude, and altitude. The value of L* value represents lightness, the a* value represents red or green, and the b* value represents blue and yellow [7]. Based on table 2, the value of L* for temulawak, ginger and turmeric when inlet temperature of 180°C was 84.19, 83.39 and 84.13. For a* value at inlet temperature 180 °C for temulawak, ginger and turmeric were 1.6, 1.23 and 1.4, while b* value for those samples were 7.95,6.86 and 8.11.

Table 2: Physiochemical properties in terms of colour for each different powder sample at temperature 180 °C

Sample	Colour		
	L*	a*	b*
Temulawak	84.19	1.6	7.95
Ginger	83.39	1.23	6.86
Turmeric	84.13	1.4	8.11

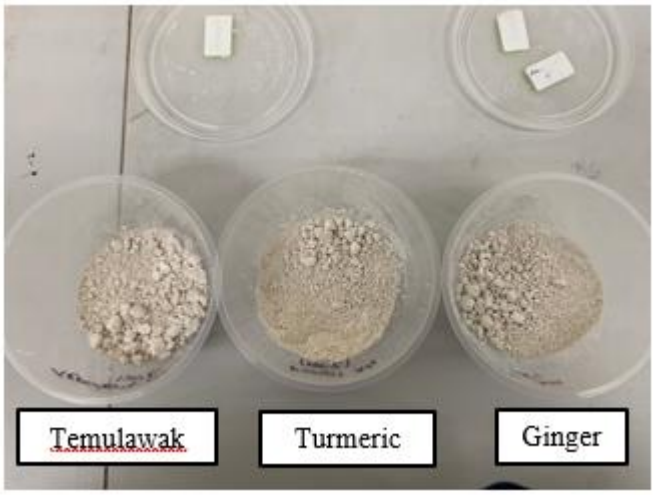

Figure 2: The color of different sample powder at inlet temperature of 180°C

The amount of curcumin in the *Zingiberaceae family* rhizomes has a significant impact on the finished product's color intensity. But there are additional components in the powder that have a major impact on *Zingiberaceae family* rhizomes color. Based on the previous study by Şahin Nadeem, H. et al., (2011) the author reported that the a* value of the samples was unaffected by the input air temperature, but it had a considerable impact on the powders' L* and b* values. According to the Guo, J. et al (2020), the majority of the a* values were just above 0, showing a modest inclination toward redness, whereas the high b* values above 0 showed a noticeable tendency toward yellowness because to their curcumin concentration. Among those three samples, turmeric has the highest b* value. There could have been a possibility that there was curcumin trapped in the coating material which influenced the carrier and curcuma species.

3.1.3 Moisture Content

The table 3 shows moisture content of *Zingiberaceae family* rhizomes at temperature 180 °C range in between 2.23% until 11.90%. Based on the figure 3, the highest moisture content is ginger at 11.9% and the lowest moisture content is turmeric at 2.23%. According Şahin Nadeem, H. et al., (2011) to due to the constant exit air temperature for all of the items in this investigation, the input air temperature had no discernible impact on the moisture contents of the powders. This was accomplished by adjusting the feed solution's processing flow rate. On the other hand, it was noted that as the

temperature of the input and outlet air rises while the feed flow rate remains constant, the moisture content of the spray-dried powders drops.

Table 3: Moisture content of *Zingiberaceae family* rhizomes at temperatures 180 °C

Sample	Mass of empty container (g)	Mass of container with sample (g)	Mass of container with dry sample (g)	Moisture content (%)
Temulawak	0.644	1.245	1.228	3.00
Ginger	0.675	1.277	1.213	11.90
Turmeric	0.652	1.272	1.258	2.23

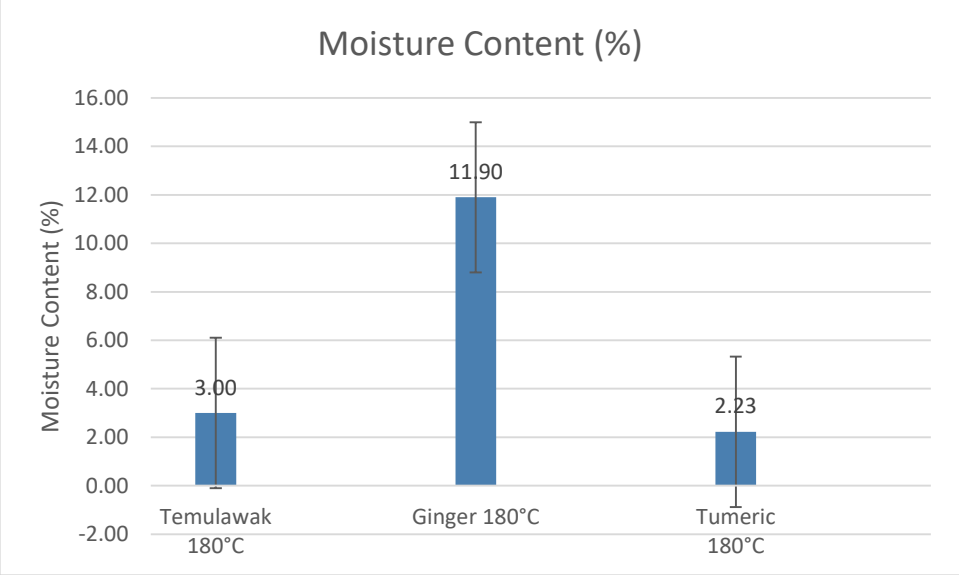

Figure 3: Moisture content of *Zingiberaceae family* rhizomes at temperatures 180 °C

The spray-dried powder was typically less than 5% in moisture content, making it microbiologically safe and suitable for long-term storage. Additionally, because spray dried powders have lower moisture contents, less water can function as a plasticizer, which has an impact on the powder caking during storage [8]. Based on the result that obtained, turmeric has the lowest moisture content among all the extracts. According to Şahin Nadeem, H. (2011), at a constant feed flow rate, the spray-dried powders' moisture content goes down as the inlet and outlet air temperatures go up. Other research also state that the temperature and humidity of the air leaving the drying chamber usually control how much moisture is in the product.

3.2 Functional Group

Figure 4 shows the FTIR spectra of extract temulawak, ginger and turmeric. Each peak and shoulders are coming from the absorption of functional group in each curcuma species extract. From the figure we can observed that, each of curcuma species shows a similar peak due the similarities in chemical components that contained in them. However, using close examination, it can be shown that the peak intensity (absorbance) of the various curcuma species varies somewhat due to variations in the component concentrations. The phenolic chemicals known as curcuminoids, which are responsible for turmeric's yellow colour, are found in the majority of curcuma species. As a result, it is not unexpected

that peaks from curcuminoids' functional groups predominate in FTIR spectra of extracts from curcuma species.

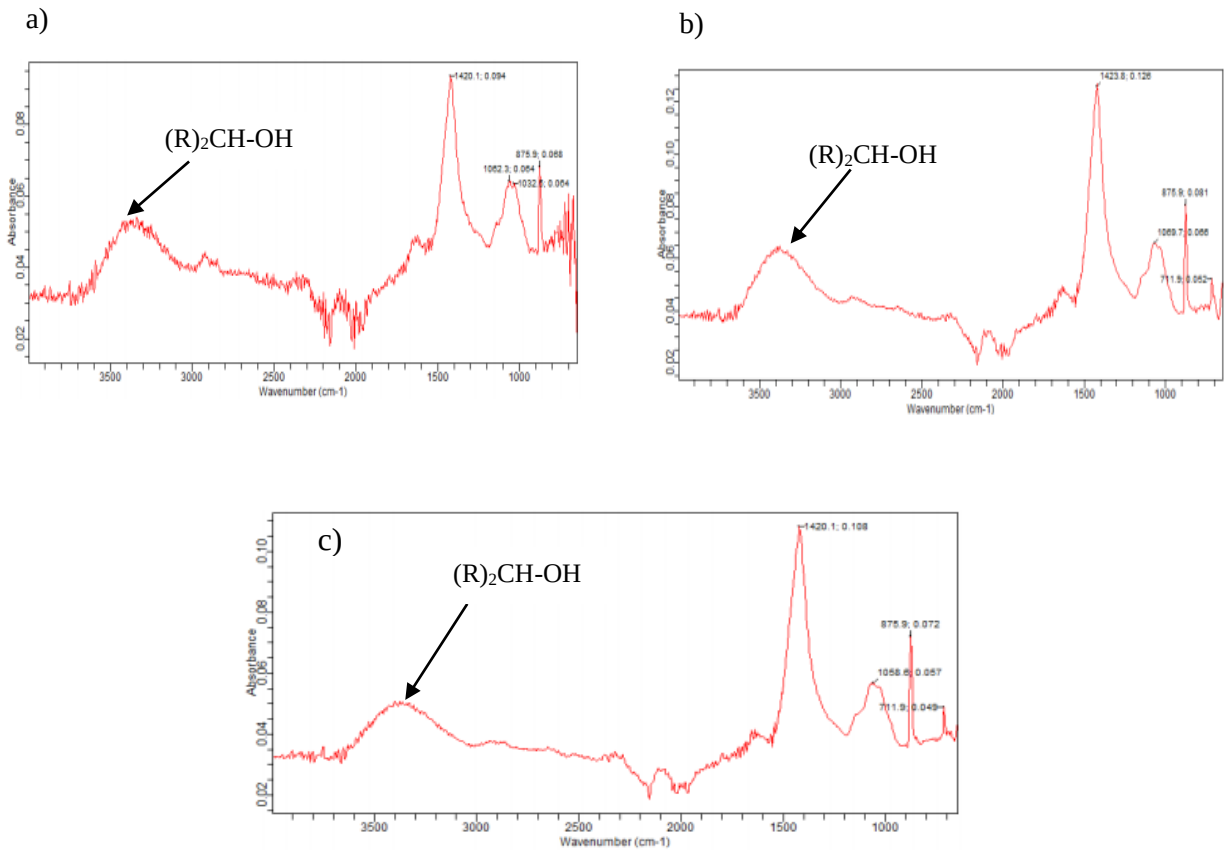

Figure 4: FTIR spectra of *Zingiberaceae family* rhizome extracts a) temulawak, b) ginger and c) turmeric

Table 4: Functional group and range for *Zingiberaceae family* rhizomes sample

Sample	Classification	Functional group	Range
Temulawak	Alcohols	$(R)_2CH$-OH	3400-3200
Ginger	Alcohols	$(R)_2CH$-OH	3400-3200
Turmeric	Alcohols	$(R)_2CH$-OH	3400-3200

From figure 4 there are a few peaks of functional group in each *Zingiberaceae family* rhizomes samples. The highest peak was known as alcohol classification where -OH bond have been identifying in the sample. The range value for the -OH bond is between 3400 until 3200 as shown in the table 4 above. According to Sapturi, Y. et al. (2019), the turmeric powder sample's absorbance spectrum displays many peaks and valleys that indicate the vibration of the chemical characteristics of the molecular bonds as based on O-H linked to moisture content, C-H-O related to starch and carbohydrates, and C-H related to curcumin concentration.

3.3 Determination of Morphology

Figure 5 SEM picture of 5,000-times-amplified microparticles of turmeric, temulawak, and ginger with gum Arabic. The figure shows the size of *Zingiberaceae family* rhizome extract microparticles. ImageJ software measured the size of each microparticle. Turmeric, ginger, and temulawak microparticles have 2.596, 3.885, and 3.81 μm sizes, respectively. Turmeric is the largest at 3.885 μm, whereas temulawak is the smallest at 2.596 μm. The figure shows the gum Arabic-encapsulated rhizome extract lumps. All of the microparticles are the same size and form since they were made in a spray dryer, which uses a spherical or regular particle shape.

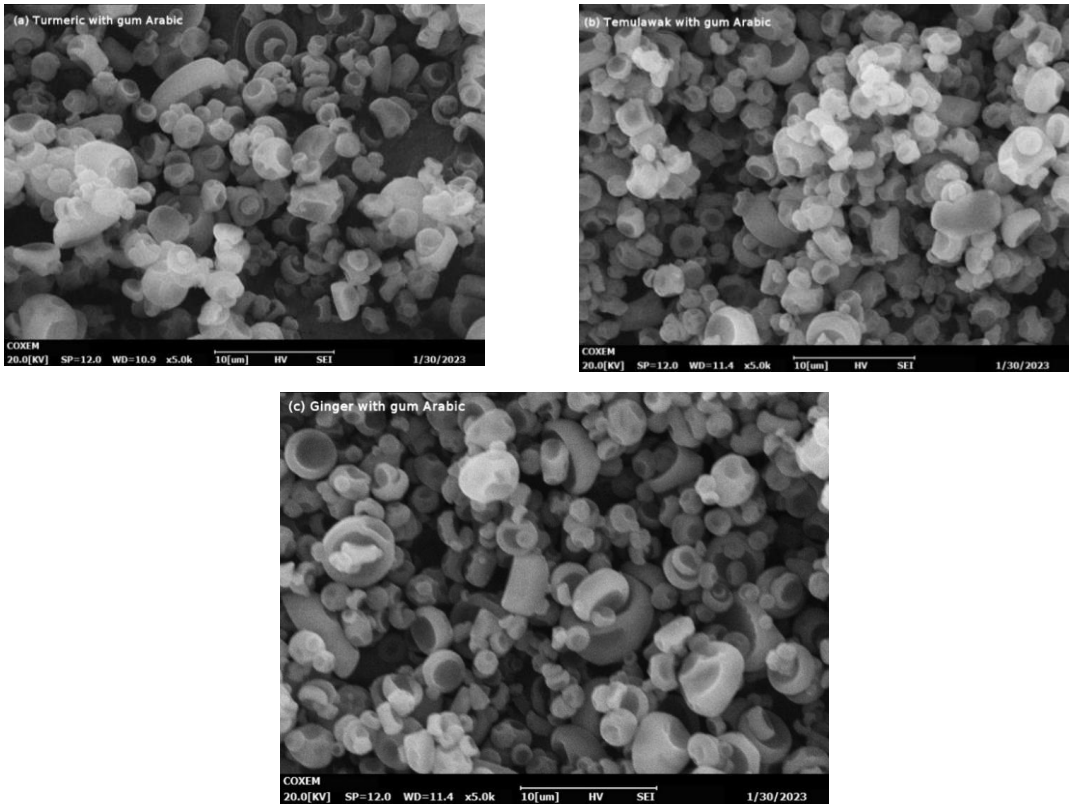

Figure 5: SEM image for microparticle of (a) Turmeric with gum Arabic, (b) Temulawak with gum Arabic, (c) Ginger with gum Arabic, that has been amplified for 5,000 times

Table 5: Mean size of Zingiberaceae family rhizome extracts microparticles produced by spray drying with gum Arabic using imageJ software

Sample	Size distribution (μm)
Temulawak	2.596
Ginger	3.885
Turmeric	3.810

Bucurescue, A. et al. (2018) reported that other authors generated curcumin/turmeric microparticles with different encapsulating agents and procedures. Bucurescue, A. et al. (2018) found that gum Arabic concentration does not affect curcumin microparticle morphology. Encapsulating agents gave microparticle surfaces different textures, according to Lucas, J. et al. (2020). Gum Arabic and alginate particles have a rough surface. Modified chitosan particles were highly polished. Gou, J. et al. (2020) examined microparticle morphology after spray drying and freeze drying. Spray-dried microparticles were smoother and more spherical than freeze-dried ones, which had more cracks [2].

3.4 Determination of Total Phenolic Content (TPC)

Table 6 shows the total phenolic content of *Zingiberaceae family* rhizomes at temperatures 180 °C. the color of Folin-ciocalteu reagent that is used in TPC analysis was changed from yellow to blue in the presence of phenolic compound in the *Zingiberaceae family* rhizomes sample as shown in figure 6. Gallic acid was used as a standard curve to be determining the total phenolic content in this study.

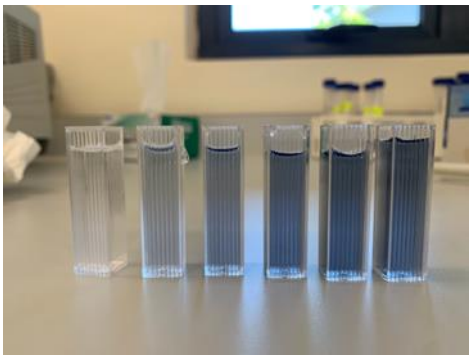

Figure 6: The presence of total phenolic compound in the sample

Table 6: Total phenolic content of *Zingiberaceae family* rhizomes at temperatures 180°C

Sample	Absorbance	Concentration (µg/mL)	Total phenolic content (mg GAE/g)
Temulawak	0.091	56.712	9.997
Ginger	0.083	49.987	11.342
Turmeric	0.108	71.003	14.201

Figure 7 shows the total phenolic content for each *Zingiberaceae family* rhizomes sample, the range of total phenolic content is 9.997 mg GAE/g until 14.201 mg GAE/g. From the graph in figure 6, we can observe that turmeric has the highest phenolic content which is at 14.201 mg GAE/g, while the lowest one is temulawak at 9.997 mg GAE/g after being spray dry at temperature 180 °C. The previous study showed that temperature have an influenced toward the TPC of mulberry powder. The findings clearly show that TPC decreased from 36.99 mg GAE/g DW to 34.35 mg GAE/g DW when the input air temperatures increased from 120 °C to 150 °C. Since phenolic compounds are often said to be particularly sensitive to heat, thermal processing may cause oxidative degradation and a substantial loss of natural antioxidants. Spray-dried blackberry powder and amla juice powder produced similar results [11]. Researchers outside of Do, H. T. et al. have shown that higher temperatures speed up the drying process, decreasing the amount of time heat-sensitive components are exposed to high temperatures. Furthermore, the polymerization and release of polyphenols from bound form while temperatures were raised to 200 °C may be the cause of the rise in TPC with temperature [12].

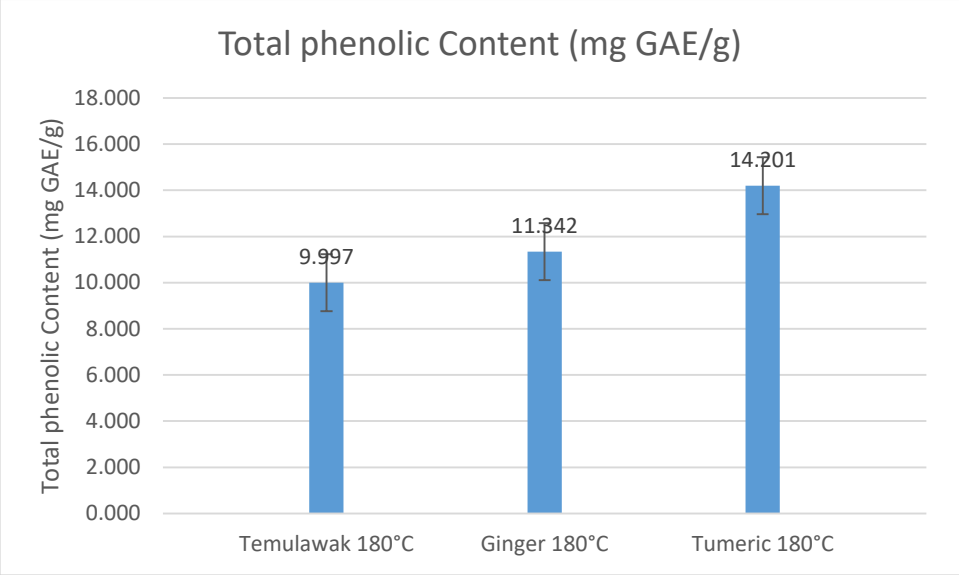

Figure 7: Total phenolic content of *Zingiberaceae family* rhizomes at temperatures 180 °C

3.5 Determination of Anti-Inflammatory

The inhibition of denaturation of different concentration of *Zingiberaceae family* rhizomes on protein denaturation is shown in table 7 with temulawak, ginger, turmeric and aspirin at concentration range of 125, 250, 500 and 1000 µg/mL. Aspirin was used as a positive control, while phosphate buffer saline (PBS) was used as negative control in this research.

Table 7: Comparison Inhibition of Denaturation of *Zingiberaceae family* Rhizomes and Aspirin (%) With Exposure Concentration

Concentration (µg/mL)	Inhibition of denaturation (%)		
	Temulawak	Ginger	Turmeric
125	96.30	92.59	96.30
250	90.74	88.89	81.48
500	96.3	98.15	94.44

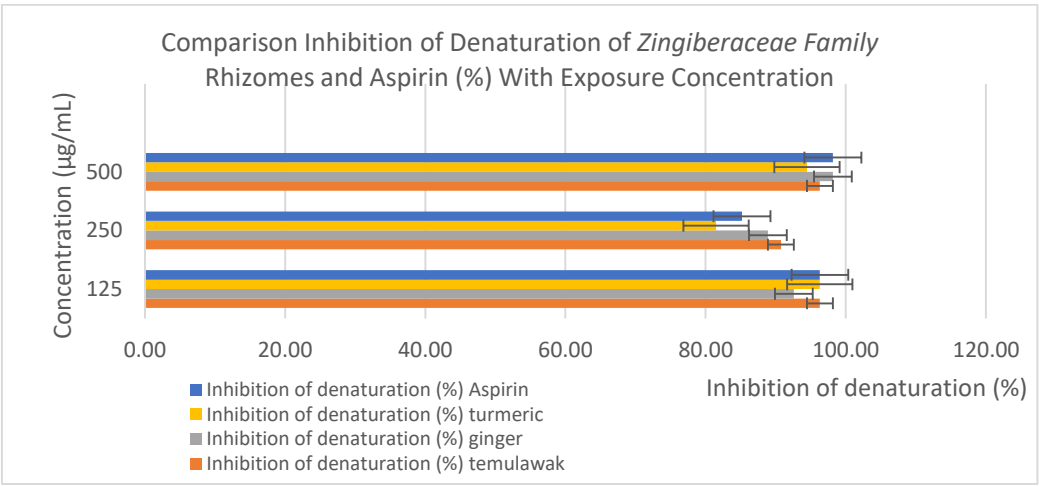

Figure 8: Comparison inhibition of denaturation of *zingiberaceae family* rhizomes and aspirin (%) with exposure concentration

Figure 8 demonstrates the comparison of anti-inflammatory effect of *Zingiberaceae family* rhizomes and aspirin (%) with different concentration (µg/mL) of exposure. Based on the data collected, the *Zingiberaceae family* rhizomes showed a remarkable inhibition of protein denaturation in a manner that depends on concentration. As a positive control, aspirin exhibits the greatest percentage value of the anti-inflammatory action in comparison to curcuma species. On the concentration of 500 µg/mL aspirin and ginger shows higher percentage value of anti-inflammatory effect which is 98.15% while temulawak and turmeric shows 96.3% and 94.44%. at concentration 250 µg/mL, temulawak show higher percentage value of anti-inflammatory effect which is 90.74%, while aspirin, ginger and turmeric shows 85.19%, 88.89% and 81.48%. On concentration of 125 µg/mL, temulawak, turmeric and aspirin have the same anti-inflammatory effect which 96.30%. From the data given, we can see that *Zingiberaceae family* rhizomes is able to exhibit anti-inflammatory effect comparable to those marketable drugs of aspirin.

Based on Khatun, M et al. (2021) study, The BSA denaturation experiment was used to investigate the ethanolic extract from the four distinct sources of turmeric powder's potential anti-inflammatory properties. The turmeric sample prepared at home provides the strongest anti-inflammatory effects. All four preparations strongly prevented BSA denaturation, according to anti-inflammatory data, although the homemade version dominated [6]. This suppression was equivalent to the aspirin-based conventional anti-inflammatory drug's inhibition of BSA denaturation. It can be because the homemade sample has a lot more bioactive chemicals than the other samples, or because it contains a unique component [6].

4. Conclusion

To conclude, the three *Zingiberaceae family* rhizome extracts (*C. longa, C. xanthorriza, and Z. officinale)* were successfully microencapsulated using spray drying process and gum Arabic as the wall materials. Next, the physical, structural, and chemical properties of *Zingiberaceae family* rhizome extracts were successfully conducted. Turmeric has the highest yield (32.77%) and the lowest moisture content (2.23%) than the other *Zingiberaceae family* rhizomes. For functional group analysis, all three samples are having alcohols with -OH bonds, according to the FTIR spectrum. SEM images demonstrated that all three samples are having the same shape and structure. TPC was measured. When compared to the other two rhizomes, turmeric's phenolic content of 14,201 mg GAE/g is the highest. Finally, BSA assays shows that the *Zingiberaceae family* rhizomes does possess anti-inflammatory activity, temulawak and turmeric has same of anti-inflammatory value at 96.30% that higher than ginger (92.59%) at lowest concentration of 125 µg/mL. At a concentration of 250 µg/mL and 500 µg/mL, the anti-inflammatory activity was achieved in the range of 81.48% to 90.74% and 94.44% to 98.15% respectively. The result shows that the active compound found in *Zingiberaceae family* rhizomes have been proven to be effective anti-inflammatory agents.

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