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Original Article

THE EFFECT OF DIFFERENT CONCENTRATION OF β-CYCLODEXTRIN AND GUM ARABIC ON THE MICROENCAPSULATED MORINGA SEED OIL BY USING THE SPRAY DRYING METHOD

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ABSTRACT

Objective: The aim of this study was to determine the impact of various concentrations of β-cyclodextrin and gum arabic on the characteristics of Microencapsulated Moringa Seed Oil (MSO).

Methods: The soxhlation method was used to extract MSO. The resulting MSO was microencapsulated employing a spray dryer. The variations in of β-cyclodextrin: Gum arabic concentrations were made to determine the coating material suitable for this formula. The characterization includes organoleptic tests, FTIR, encapsulation efficiency, morphology, particle size and moisture content of microencapsulated MSO.

Results: The results obtained from the particle size for F1, F2, F3, F4, and F5 were 5.42; 4.29; 4.23; 4.34; 5.15 µm, respectively. Then the percentage of encapsulation efficiency obtained was 74.42±0.13; 78.81±0.12; 82.27±0.07; 93.94±0.09; 71.50±0.11, respectively. The IR spectra shows no chemical interactions that occurred in the formulation of microencapsulated MSO.

Conclusion: In conclusion, microencapsulated MSO formulated with β-cyclodextrin (40% w/v) was recommended as the most optimal formula with a smaller particle size (4.34 µm) among others and exhibited the highest microencapsulation efficiency.

Keywords: Moringa seed oil, Microencapsulation, β-cyclodextrin, Gum arabic, Physico-chemical characteristics

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INTRODUCTION

Moringa oleifera L. seeds are a part of the Moringa plant that contains high levels of vegetable oil, particularly unsaturated fatty acids [1]. They have shown several health benefits in nutritional and health consumption [2-4]. Indeed, Moringa oil could be a viable alternative for olive oil in the diet [5]. Previous studies have established that Moringa Seed Oil (MSO) contains>70% unsaturated fatty acids, specifically oleic acid (omega-9) [6, 7]. Tocopherols, phenolic compounds, sterols and carotenoids are inherent natural anti-oxidants which are also found in MSO [1, 8]. MSO can be extracted by maceration with n-hexane [9] and or chloroform: methanol (1: 1) [10]. MSO also could be obtained by soxhletation using n-hexane solvent with an oil yield of 41.47% [11].

One of the main drawbacks associated with oils rich in unsaturated fatty acids is their susceptibility to oxidation, which leads to the formation of free radicals known as hydroperoxides [12]. Therefore, the application of encapsulation techniques is carried out to prevent oxidation reactions thereby increasing storage time during handling and processing. The encapsulation process involves transforming the liquid core substance into a solid state, which facilitates handling and prevents the core substance against flavor deterioration [13]. When considering the encapsulating procedure, it is important to take into account the specific type of coating utilized as the wall materials in microcapsules.

Several microencapsulation procedures of essential oil, such as spray drying [14], spray chilling [15], fluidized bed coating [16], extrusion [17], lyophilization [18], and liposome adsorption [19], are available. The spray drying technique is commonly employed to produce commercially available microencapsulation products. The spray drying method for microencapsulation is cost-effective and allows for multiple coating agent. The resulting microcapsules exhibit desirable qualities, such as a particle size of 10 µm and an encapsulation efficiency of up to 90%. Study examined how various coating combinations (maltodextrin, gum arabic, and whey protein concentrate) affected the properties of the emulsion using the spray drying method. Their findings showed that the emulsion developed had increased viscosity, reduced droplet size, improved stability, and a smaller oil surface area, leading to the highest encapsulation efficiency [20].

This study used both single and combination of β-cyclodextrin and gum arabic as wall materials. The choice of β-cyclodextrin in this research is because β-cyclodextrin has been widely used in the encapsulation of essential oils by providing high encapsulation results, forming a molecular inclusion complex between the oil and the polar β-cyclodextrin cavity [21]. This inclusion complex protects oil efficiency during storage with low thermal stability [22, 23]. Then, gum arabic possesses the ability as a texture former, film former, binder and emulsifier due to its protein components. In previous research, Surini *et al.* reported that microencapsulation of grapeseed oil was successfully carried out by cross-linking emulsification method with gum arabic and 50% glutaraldehyde [24]. Moreover, gum arabic can preserve the taste of food that has been dried using the spray drying method by forming a protective layer, which can prevent it from destructive changes [25]. β-cyclodextrin with gum arabic coating led to a high % encapsulation efficiency of 91% for kenaf seed oil, along with a reduced particle size [26]. Based on the description above, this study aims to investigate the impact of varying the concentration of wall materials in microencapsulated MSO formulation, specifically β-cyclodextrin and gum arabic using the spray drying method to the morphological characteristics of microcapsules, particle size, microencapsulation efficiency and FT-IR analysis.

MATERIALS AND METHODS

Materials

The Moringa seeds used in this research were 7 mo old and were collected from Sibedi village, Sigi Regency, Indonesia in August 2018. The samples were collected by selecting the brown fruit and subsequently removing the outer layer to obtain the white seeds. The collected seeds underwent a drying process and were subsequently ground to acquire the Moringa seed powder. Gum arabic (food grade), and aquadest were bought from Jaya Kimia Co. β-cyclodextrin (Sigma-Aldrich, USA) and n-hexane (Merck, Germany). All reagents used in this experiment were analytical grade.

Solvent extraction of MSO

The Soxhlet apparatus was used for the extraction of MSO. A 50 g sample of Moringa seed powder was wrapped in filter paper and

placed into a thimble. The round-bottomed flask was filled with 300 ml of n-hexane solvent, which was thereafter heated up to a temperature of 65 °C for 4 h. After the extraction is complete, the extract obtained was then separated from n-hexane by using an rotary evaporator (RE100 S DLAB) at a temperature of 69 ˚C to obtain MSO [11].

Formulation of microencapsulated MSO

The encapsulation of the MSO with β-cyclodextrin and gum arabic as wall materials was carried out by simple coacervation technique. In this study, we used MSO at a fixed concentration of 30% w/v because, according to research by Athikomkulchai *et al.*, at this concentration, MSO can inhibit more than 50% of DPPH free radicals [27].

Microencapsulation was made by mixing β-cyclodextrin and gum arabic in distilled water at 50 °C using T25 digital Ultra-Turrax homogenizer (IKA T18B, Germany). The dispersion was left overnight to ensure full hydration. MSO was added slowly at a fixed concentration of 30 % w/v under magnetic stirring at 1000 rpm for 30 min. The solution was stored at ambient temperature for 12 h then subjected to spray-drying (BÜCHI, Switzerland). The microcapsules were afterward placed to a desiccator for further analysis.

Characterization of microencapsulated MSO

Organoleptic observations

Organoleptic observation involves evaluating preparations through the use of the five senses. Organoleptic analysis includes the evaluation of visual appearance of color and odour.

Moisture content

The moisture content of microencapsulated MSO was measured gravimetrically by using moisture balance equipment (Sartorius MA 35, Germany).

Table 1: Formula of microencapsulated moringa seed oil (MSO)

No	Composition	Concentration (% w/v)				
		F1	F2	F3	F4	F5
1	Moringa seed Oil (MSO)	30	30	30	30	30
2	β-cyclodextrin	10	20	30	40	-
3	Gum arabic	10	15	20	-	20
4	Aquadest	50	35	20	30	50

Particle size distribution

Particle size was measured using the Fraunhofer method using a PSA (Particle Size Analyzer), where the sample was dissolved in water at a temperature of 25 °C. The particle size distribution was determined using dynamic light scattering.

Particle morphology

The particle morphology was examined using the IK morphological testing method (IK-4) and IK element analysis (IK-6) using a SEM (Hitachi Model SU3900). The sample was placed in the sample holder and then examined and seen for its morphology at an intensity of 7 kV and a spot intensity of 50. Particle morphology were observed at magnifications of 10,000x, 5,000x, 2,500x, 1000x and 500x.

Encapsulation efficiency

The encapsulation efficiency was determined by calculating the ratio of the surface oil to the total oil content of the microcapsules. The surface oil content was determined by combining 2 g of dehydrated microcapsules with 20 ml of n-hexane, followed by stirring for 10 min at ambient temperature. The suspension was then filtered through a Whatman no. 1 filter paper and the remaining solid was rinsed again with 20 ml of hexane 3 times. The residual solid was then dried at a temperature of 60 ˚C until the weight was constant. The amount of surface oil was calculated by the difference in the weight of the microparticles before and after washing (Equation 1). Total oil was determined by using Soxhlet apparatus. 2 g of microcapsules were extracted using 120 ml of hexane for 6 h to ensure complete oil extraction. Following the extraction process, the powder, which had been completely drained of oil, was subjected to drying until the weight was constant (Equation 2). The following equations were used to determine encapsulation efficiency [28].

$$\text{Surface oil} = \text{Initial weight} - \text{Final weight of microcapsules} \quad \ldots\ldots (1)$$

$$\text{Total oil} = \text{Initial weight} - \text{Weight after extraction with soxhlet} \quad \ldots (2)$$

$$\%\ \text{Encapsulation efficiency} = \frac{(\text{total oil} - \text{surface oil})}{\text{total oil}} \times 100\% \quad \ldots\ldots (3)$$

FT-IR analysis

Microencapsulated MSO, β-cyclodextrin and gum arabic were observed for infrared absorption using a FT-IR spectroscopy (Thermo Scientific, Waltham, MA, USA). The samples were mixed with KBr at a ratio of 1:10. The mixture was then crushed and flattened to form a plate. The plate was placed on a transparent disk and analyzed using an FT-IR instrument (resolution 4 cm^{-1}). A total of twenty scans were conducted, and the resulting values were averaged and graphed to show the transmission percentage as a function of wave number (cm^{-1}) [29].

RESULTS AND DISCUSSION

Extraction of MSO

The Soxhlet extraction method yielded MSO of 35.51±2.22%, which is slightly lower than the previous study by Ogbunugafor *et al.*, which obtained a yield of 41.47 % with the same method and solvent used [11]. The difference in yield percentage is influenced by the oil content of the Moringa seeds and the place where the Moringa tree grows. The solvent evaporation process is carried out using a rotary evaporator to separate the solvent and Moringa oil. This apparatus was chosen because it can evaporate the solvent below the boiling point so that the substances contained in the oil are not damaged by high temperatures [30].

Formulation of microencapsulated MSO

This study utilized five formulations consisting of carbohydrate-based encapsulating agents. These agents were composed of a combination of β-cyclodextrin and gum arabic at different ratios (1:1, 4:3, and 3:2), as well as a single use of β-cyclodextrin and gum arabic, respectively as shown in table 1. The concentration of gum arabic as an emulsifier is in the range of 10-20% w/w [31, 32].

β-cyclodextrin has a truncated cone or a hollow cone shape with an empty middle space. This empty central space (cavity) allows β-cyclodextrin to capture or confine a hydrophobic molecule, which is referred to as an inclusive process. Previous research shows that 10% β-cyclodextrin can encapsulate 12-25% essential oil [33]. However, increasing the amount of β-cyclodextrin will increase the free β-cyclodextrin filled with oil constituents; therefore in this study, we used a β-cyclodextrin concentration range of 10-40% [34].

Microencapsulation of MSO was carried out using the spray drying method. The spray drying process includes dispersing the core material into the wall material by homogenizing and spraying the core-wall material dispersion into an environment by compacting the coating in spray drying, which is influenced by rapid evaporation of the wall material solvent [17]. In this study, β-cyclodextrin and gum arabic were used as wall materials, which have the potential to

form a microcapsule matrix. The β-cyclodextrin matrix network in the wall system and the good properties of gum arabic as an emulsifier that can form a film layer help to maintain the oil's condition despite the fact that the viscosity of the emulsion tends to decrease [35, 36].

Characterization of microencapsulated MSO

Organoleptic observations and moisture content

Organoleptic assessment, including color and odour, are essential determinants of a product's overall excellence. Organoleptic assessment offers useful insights for optimising the composition of microencapsulated MSO to enhance consumer satisfaction in consuming the products. The organoleptic observation of all formulations of microencapsulated MSO produced by spray-drying shows that they are ivory-white powders with a mild distinctive odour.

Water content is a critical factor affecting the stability of a product during storage. The lower water content in microcapsule powder helps minimize the proliferation of bacteria and microorganisms, as well as undesirable chemical reactions like hydrolysis and oxidation in fats and oils. Consequently, the end product will have an extended shelf life. The moisture content of MSO microcapsules is 7.05±1.48, which is considered favourable. Microcapsule products obtained from spray drying generally have a water content of less than 6% [37].

Particle size distribution and morphology

Particle size is a significant variable in determining the quality and application of microcapsules. According to research by Zhao *et al.*, smaller particle sizes have better temperature stability. Microcapsules with large particle sizes at high temperatures will twist, collapse, break, and expose more of the core material. Meanwhile, the smaller microcapsules showed they were still in an accelerated release state [38]. Then, smaller particle sizes have significantly thinner capsule shell thicknesses than larger capsules due to the larger specific surface area, thus containing more MSO [39]. Furthermore, microcapsules with larger particles generally have poor dispersion ability in the final product [40]. However, powders that are too small may be hollow particles with no embedded core material, and the microencapsulation efficiency is very low. Therefore, the optimal particle diameter and encapsulation efficiency must be considered.

The particle size of microcapsules may differ based on the manufacturing method and application requirements. The results of this study indicated that the diameter of microencapsulated MSO was between the range of 4.23-5.42 µm (table 2). All microcapsules formed fall within the microcapsule size range of 0.2-60 µm [35]. In manufacturing microcapsules, stirring speed is a crucial factor in determining the size of microcapsules. Slow stirring will form large microcapsules; on the other hand, fast stirring will form non-spherical microcapsules with a very small size. Thus, a stirring speed of 3000 rpm was employed to achieve optimal microcapsules. The spray drying process also influences particle size of microencapsulated MSO. A previous study has demonstrated that increasing the inlet air temperature results in larger particles, which is attributed to the pre-formed microcapsule structure [41]. At elevated temperatures, the particles reduce shrinkage and maintain their size. This is due to the rapid drying rate in the spray drying chamber, which leads to the generation of high temperatures that facilitate the early formation of particle structure. As a result, the particles are prevented from shrinking throughout the drying process. The working temperature can affect the particle size. When the inlet air temperature is high and the difference is low between the inlet and outlet air temperatures, the resulting particles are slightly larger than those produced by drying [41].

Table 2: Characterization of microencapsulated MSO

Formulation	Water content (%)*	Particle size (µm)
F1	7.6050±0.035	5.42
F2	8.0850±0.12	4.29
F3	8.1000±0.14	4.23
F4	6.0650±0.38	4.34
F5	7.8900±0.12	5.15

*The data is shown as mean±SD (n = 3)

Morphologies of microencapsulated MSO using various coating concentrations were analyzed under scanning electron microscope (SEM) (fig. 1). The morphology of microencapsulated MSO formed showed indentations caused by uneven shrinkage during drying and cooling. SEM image of microencapsulated MSO using β-cyclodextrin displays irregular and non-uniform crystal shapes of relatively large size. Meanwhile, the microencapsulated MSO using β-cyclodextrin and gum arabic as the wall material exhibited a wrinkled morphology. Some microcapsules formed aggregates, while others were uniformly distributed. Previous studies reported that indentations on microcapsules can be attributed to the rapid evaporation of water during the spray drying process [42, 43].

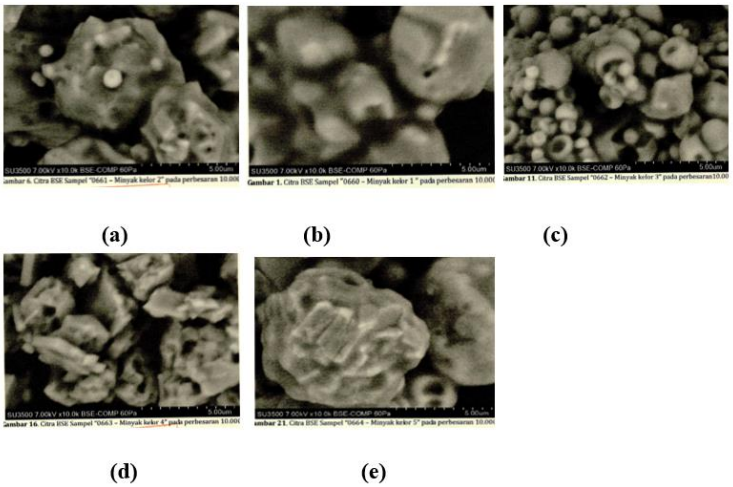

Fig. 1: Morphology of microencapsulated MSO microcapsules using SEM at 10,000x magnification (a) F1, (b) F2, (c) F3, (d) F4, (e) F5

Encapsulation efficiency

The encapsulation efficiency of microcapsules refers to the percentage of the core material (MSO) that is entrapped or encapsulated within the microcapsule structure during the encapsulation process. It is a critical parameter as it directly affects the performance and functionality of the microcapsules. A high encapsulation efficiency is desirable because it indicates that a larger percentage of the core material has been successfully encapsulated, leading to a more effective and economical use. Different properties of the core and wall materials have varying impacts on encapsulation efficiency.

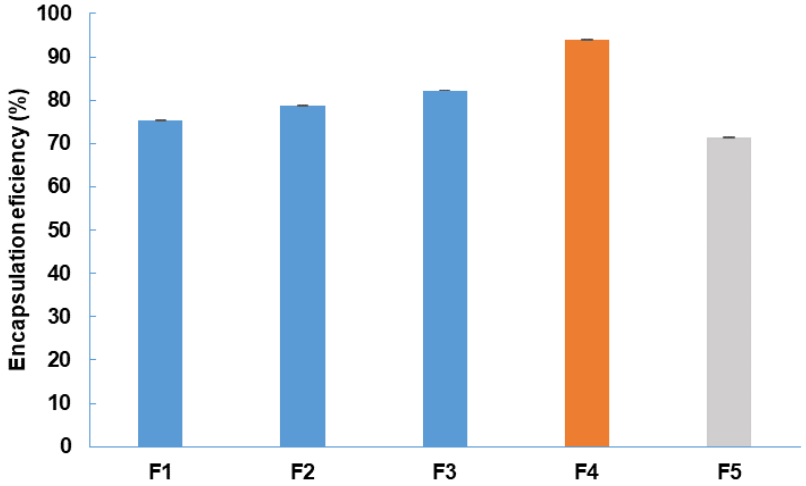

Fig. 2: Encapsulation efficiency (%w/w) of microcapsules loaded with MSO at different concentrations of wall materials. *Data represents mean±SD (n=3)

The result of encapsulation efficiency of microencapsulated MSO using β-cyclodextrin, gum arabic, and their combination as coating material are presented in fig. 2. The results indicate that there is a positive correlation between the concentration of the wall material and the encapsulation efficiency value. The greater the concentration of the coating material, the greater the encapsulation efficiency value. The largest encapsulation efficiency value was seen in F4, specifically 93.94±0.09%, when using β-cyclodextrin as wall material. The coating of β-cyclodextrin exhibits the capacity to form a macrocycle structure, including hydrophobic cavities, enabling the encapsulation of lipophilic substances. According to Gong *et al.*, the interaction between β-cyclodextrin and the lipophilic compound MSO forms an inclusion complex. This complex strengthens the walls of the microcapsules, ensuring that the lipophilic compounds are effectively coated and do not diffuse out during the drying process in the spray dryer [44]. The smallest encapsulation efficiency was found in F5 with gum arabic as the wall material, which was 71.5±0.11%. This is due to the absence of β-cyclodextrin as a coating as a coating. The insufficient amount of gum arabic used as a coating caused a decrease in the encapsulation efficiency value of MSO, as it fails to encapsulate it comprehensively. The same results were also obtained, where gum arabic, a hydrocolloid, could not coat lipophilic substances when used as a single coating material [45]. As a result, the encapsulation efficiency value decreased. However, the encapsulation efficiency value in this study was greater than the previous study which used the cross-emulsification method between gum arabic and 50% glutaraldehyde as a coating material [24].

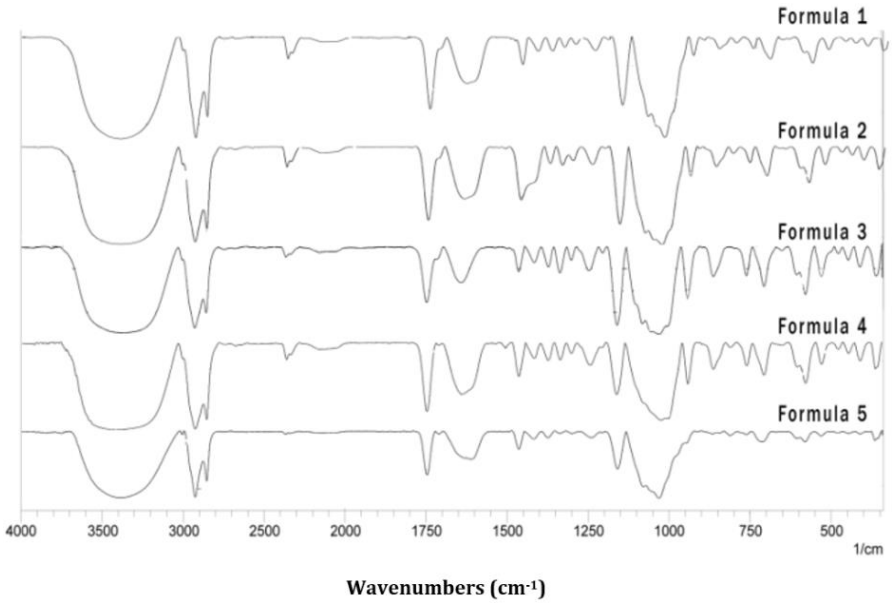

Fig. 3: FTIR spectrum of microencapsulated moringa seed MSO formulation

FT-IR analysis

Fig. 3 presents the FT-IR spectrum of microcapsules loaded with MSO. For the MSO, the main wavenumbers obtained are 705, 810, 906.54, 941.26, 1029 cm^{-1} for F1-F5, respectively. These wavenumbers indicate the presence of alkene double bond groups (-C=C-) [46]. Alkene is a group commonly found in vegetable oils with many unsaturated fatty acids. The peak at 1747 cm^{-1} is due to the stretching vibration of C= O in the ester group, indicating that there are fats in MSO [47]. The absorption bands at 2925 and 2850 cm^{-1} could be ascribed to the fatty acids because they corresponded to the symmetrical and asymmetrical stretches of the $CH\text{-}CH_2$ groups commonly found in these compounds [48]. This proves that MSO essence oil was successfully encapsulated by the β-cyclodextrin and gum Arabic. As shown in the curve, the F4 showed the presence of β-cyclodextrin, F5 showed the presence of gum arabic, and F1, F2 and F3 showed the presence of both ingredients. The strong and wide absorption peak at approximately 3379-3500 cm^{-1} was attributed to the superposition of O-H stretching vibrations in β-cyclodextrin which is generally hydrogen bonded so that the shape is widened, and the absorption bands at 1645 was ascribed to the O–H bending. The weak absorption peak at 1338 cm^{-1} was assigned to C-O stretching vibration. The peak at 1158 and 1028 was assigned to the asymmetric and symmetric stretching of the C–O–C. For gum arabic, it is seen to have a broadened and slightly tapered absorption peak at wave numbers 2900-3300 cm^{-1} corresponding to the presence of hydrogen-bonded OH group. The weak absorption peak at 1700 cm^{-1} was assigned to C-O stretching vibration. The absence of newly formed chemical bonds between the wall materials and MSO compounds is indicated by the absence of specific new peaks in the microparticles based on the resulting spectra. This result confirms that electrostatic interactions, rather than chemical reactions, are responsible for the formation of complexes.

CONCLUSION

The formulation and characterization of microencapsulated MSO prepared by spray drying method were carried out. In this study, β-cyclodextrin and gum arabic were the two wall materials used, and optimum concentration was determined to produce microcapsules loaded with MSO. The combination of β-cyclodextrin and gum arabic as the wall materials obtained smaller microparticles. This study showed the effect of a combination of β-cyclodextrin and gum arabic as the wall materials decreased in particle size compared to single gum arabic. However, the use of a single β-cyclodextrin was sufficient to obtain the desired microparticle size 4.34 µm and encapsulation efficiency (93.94±0.09%). FT-IR analysis revealed the importance of electrostatic interaction rather than chemical reactions in stabilizing microencapsulated MSO; therefore shows no chemical interactions that occurred in the formulation.

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AUTHORS CONTRIBUTIONS

NA: Collected the data, Wrote the paper; YI: Collected the data, Performed the analysis; NI: Performed the analysis, Review and Editing; MS: Conceived and designed the study, Conceptualization, Review and Editing; ES: Conceived and designed the study, Conceptualization, Review and Editing. All authors have read and approved the final version of this manuscript for publication.

CONFLICT OF INTERESTS

Declare none

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