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**Original Article** 

# PHYSICOCHEMICAL PROPERTIES AND NUTRITION OF *MORINGA OLEIFERA* LAM. LEAF EXTRACT: A PRELIMINARY STUDY ON PREPARATION PHYTOSOMES AS HERBAL SUPPLEMENT FOR CHILDREN

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### ABSTRACT

**Objective:** The study aimed to evaluate the physicochemical properties and nutrition of Moringa leaf extract. In addition, the preliminary study for the preparation of Moringa leaf extract-loaded phytosomes for a supplement.

**Methods:** Extraction of Moringa leaf made using microwave-assisted extraction, followed by evaluation of proximate analysis (water, total ash, acidinsoluble of ash contents, and residual n-hexane), phytochemical screening, and nutrition such as crude protein, amino acids, and minerals (iron, zinc, and calcium). The phytosomes were prepared by the anti-solvent precipitation method and assessed for the morphology, particle size, zeta potential, polydispersity index (PDI), entrapment efficiency (EE), and Fourier-transform infrared spectra.

**Results:** The nutrition contents of crude protein, iron, zinc, and calcium were 19.61±0.07%, 3.47±0.00 mg/100g, 5.46±0.05 mg/100g, and 747.40±4.89 mg/100g, respectively. The amino acids with the highest concentrations were glutamic acid, phenylalanine, aspartic acid, alanine, and arginine in the extract. The best preparation using sonication 10 min by morphology was a spherical included particle size, PDI, zeta potential, and EE of arginine was 87.16±1.73 nm, 0.22±0.04, -23.07±0.76 mV, and 108.94±0.52%, respectively.

**Conclusion:** These preliminary results provide evidence of the nutritional benefit of Moringa leaf extract-loaded phytosomes as a promising supplement to prevent stunting in children.

Keywords: Moringa oleifera, Stunting, Supplement, Phytosome, Amino acid

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# INTRODUCTION

Stunting refers to  $\geq -2$  SD Z-score of height for age below the median child growth standard established by the World Health Organization. Stunting affects approximately 144 million children worldwide, commonly 54.3% of cases occurring in Asia [1]. The incidence of stunting in Indonesia was 30.8% in 2018 [2]. Stunted children of age <5 y also experience delays in the development of fine motor skills (2.86%), gross motor skills (1.90%), and social independence (2.10%) incidences, which are slightly below the respective global estimated rate of 3% childhood developmental delays [3]. Good nutrition in complementary food for the first 1000 d of life is considered critical for the physical growth of children for supporting brain development as maturation of myelination to reduce cognitive deficits in children [4, 5]. Essential amino acids (i.e., arginine, glutamine, leucine, lysine, isoleucine, glycine, serine, and valine) correlated to the linear growth of children [6]. The comparison of stunting children had serum concentrations of arginine, glutamate, serine, glycine, glutamine, and asparagine which were significantly lower than non-stunting children [7].

Moringa oleifera Lam. leaf contains various nutrition such as mineral, vitamin, and vegetable protein has a function to increase enzyme activities has a role in protein production as a ligand for DNA sequence promoter for transcription of DNA in development growth children [5]. Suppressing stunting by supplementation from Moringa leaf powder mixed in porridge by enhancing the nutritional and phytochemical, by increasing approximately 11% protein, 45% iron, 40% phenolics, 139% flavonoids, and 10% antioxidant activity [8]. Daily supplementation of 5-10 g of Moringa leaf powdered leaf could increase the appetite of children aged <5 y and the body weight by 0.53 kg [9]. Moringa leaf extract contains 9.88% bioaccessible iron, 70.1% insoluble protein, 3.5% glutelin, 2.2% prolamin, and 0.3% globulin [10, 11]. Previous studies have reported the biochemical substances of Moringa leaf powder to have low absorption of amino acids because of its anti-nutrient substances (i.e., phytic acid, tannins, and saponins). The fibers and protease inhibitors have favorable effects on digestion [11]. The side effects consumed supplementation of Moringa leaf powder 20 g daily were reported causing nausea and for safety used consumption 70 g/day as maximum to avoid the toxicity of harmful elements and prolong consumption causing cumulative toxicity [12, 13].

The explore supplementation derived from local biodiversity to improve safety against malnutrition is required. *Moringa oleifera* plant is beneficial as a supplement for suppressing stunting, but the absorption of amino acids is inadequate owing to polar phytoconstituents and antinutrient substances. Various solutions to overcome this problem included the preparation of phytosomes as promising strategies to increase absorption. Phytosomes are the complexion of extract and phospholipid to transport the outer membrane wall to reach circulation systemic. Thus, phytosomes are more easily absorbed [14]. The study aimed to evaluate the physicochemical properties and nutrition of Moringa leaf extract as a preliminary study to prepare Moringa leaf extract-loaded phytosomes for a supplement.

# MATERIALS AND METHODS

### Materials

*Moringa oleifera* leaf purchased from CV Herbal Anugrah Alam, Bantul, Yogyakarta, Indonesia, and verified by The Indonesian Institute of Sciences, Bogor Botanical Gardens (West Java, Bogor, Indonesia) with the authentication number B-1579/IPH.3/KS/XII/2020, phospholipid (Lipoid GmbH Ludwigshafen am Rhein, Germany), alpha-aminobutyric acid (AABA), yttrium, dichloromethane, n-hexane (Merck KGaA, Darmstadt, Germany), water for injection (PT. Ikapharmindo Putramas, East Jakarta, Indonesia), in addition to analytical-grade of all reagents and solvents.

### Preparation and extraction

The extract prepared using 3 kg of Moringa leaf powders were macerated in 5 l of n-hexane for 24 h at 25  $^{\circ}C$  and then filtered. The

powdered residue was re-macerated nine times until produced clear liquid. Next, the solvent was filtered, and the powder dried in a drying cabinet. Extraction used microwave (Buono-MV 3002, modified; MODENA, Jakarta, Indonesia) for 8 min and 70% tool power equivalent of 630 W to obtain ratio 1:8 (sample:solvent 50% ethanol), which was subsequently filtered. The filtrate was concentrated using a rotary vacuum evaporator (Buchi LabortechnikAG, Swiss) and dried in a vacuum oven at 40 °C (Memmert GmbH+Co. KG, Büchenbach, Germany) to produce a viscous extract [15].

#### **Physicochemical properties**

The extract was determined organoleptically and proximately analysis. Extraction yield by calculation dividing the extract weight obtained by the weight of the powder. The water content was determined using the distillation of the toluene method. The total ash and acid-insoluble ash contents were analyzed using the gravimetrical method. The residual n-hexane was analyzed using gas chromatography, which separated the mixture, and the components, then evaporated at 400 °C using gas as the mobile phase [16, 17].

#### **Phytochemical screening**

The phytochemical screening identifies alkaloids, tannins, saponins, flavonoids, triterpenoids, steroids, glycosides, and anthraquinones. The test used special chemical reagents was analyzed by following the standard method [18, 19].

# **Nutrition contents**

#### Crude protein and amino acids

The nutrition of crude protein content was determined using the Kjeldahl method [20]. The amino acids profile was analyzed using Ultra-Performance Liquid Chromatography (UPLC). The standard amino acids solution made with 40 µl amino acid solution added 40 µl internal AABA and 920 µl double-distilled water. Preparation of sample using 1 g was placed in a vial, hydrolyzed by adding 5 ml hydrochloric acid, and homogenized by vortexing for 5 min. The samples were heated in an oven at 110 °C for 22 h. afterward, it was diluted in 50 ml double-distilled water, then filtered using a 0.2  $\mu$ m syringe filter. Then, 500 µl of the filtrate pipetted mixed with 40 µl of AABA and 460 µl of double-distilled water. Next, a 10 µl aliquot of standard amino acid or the sample was added with 70 µl of AccQ-Tag Ultra borate buffer and homogenized by vortexing. Afterward, 20 µl of the AccQ-Tag Ultra-reagent was added and homogenized, let stand for 1 min, and then incubated at oven 55 °C for 10 min. A total of 1 µl standard amino acid or the sample was injected into the UPLC column (Waters Corporation, Milford, USA) with a Photo Diode Array (PDA) detector and a C18 column. The mobile gradient phase included 5% AccQ-Tag ultra-borate buffer (A) and AccQ-Tag ultrareagent (B) at 0.70 ml/min at temperature 49 °C [21].

The tryptophan was analyzed using High-Performance Liquid Chromatography (HPLC). The L-tryptophan standard solution used 25 mg standard L-tryptophan mixed with 10 ml double-distilled water and 0.3 ml hydrochloric acid and sonicated for 15 min then diluted in 25 ml of double-distilled water. Stock solution 1 ml was added with 10 ml of double-distilled water and mixed well. The working standard solutions made using eight concentrations levels were prepared from the stock solution for the calibration curve. A total of 1 g samples into a vial and added 10 ml sodium hydroxide and vortexed immediately. The sample was hydrolyzed in an oven at 110 °C for 22 h. Let cool at 25°C. Hydrolysate added 3 ml citrate buffer solution and adjusted with hydrochloric acid or sodium hydroxide until pH 4.25. The mixture was diluted with 50 ml of double-distilled water and mixed well. Transfer to 2 ml of centrifugal tube and centrifuge at 14000 rpm for 3 min. Supernatant filtered by using a 0.45 µm syringe filter into a vial. A total of 15 µl of standard or the sample was injected into the HPLC column (Shimadzu Prominence-i LC 2030, Germany) with a PDA and a C18 reversedphase column. The mobile isocratic phase included sodium acetate (A) and methanol (B) at 1.0 ml/min at 37 °C [22].

#### Minerals

The nutrition of minerals included iron, zinc, and calcium analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). A total of 10 mg/l of standard solutions were prepared in 100 ml volumetric flasks from the minerals standard stock solutions that contained 1000 mg/l for each element. Stock solution 1 ml added with 10 ml of double-distilled water, mix well. The working standard solutions made using eight concentrations levels were prepared from the stock solution with double-distilled in concentrated nitric acid for the calibration curve. A total of 1 g samples was added to 10 ml of concentrated nitric acid in a digesting vessel, then heated in a microwave digestion system at 150 °C for 10 min and allowed to digest for 15 min. After cooling, the digested solution was moved to a 50 ml flask and mixed with 0.5 ml of internal standard diluted to 100 mg/l in double-distilled water. The filtered by using a filter paper Whatman no. 42, the samples were determined using ICP-OES (Agilent 7500 Agilent Technologies, Inc., USA) [21].

#### **Preparation of phytosomes**

The preparation of phytosomes using the anti-solvent precipitation method [23]. The ratio phospholipid and the Moringa leaf extract used 1:1 (w/w). The phospholipid was dissolved in dichloromethane in a beaker by gentle swirling. After the phospholipid dissolved, it was airdried in a fume hood in 2 h to obtain a thin film, which was then further dried and dissolved in 10 ml of n-hexane by continuously swirling. Afterward, the film air-dried under a fume hood. Moringa leaf extract solution was added in dried film and sonic by using a probe sonicator on an ice bath to keep the temperature to <8 °C at an amplitude (60%) at 15-s pulses. Optimization sonication at a different time (i.e., 5, 7, 10, and 15 min). The sonicated sample was stored in a bottle at 2–8 °C until further analysis.

#### **Evaluation of phytosomes**

#### Morphology

The morphology of phytosomes was analyzed using a transmission electron microscope (Jeol JEM-1400 Flash Electron, Japan). The sample was dissolved and placed on a copper-coated grid and dried, then added with 1% phosphotungstic acid solution, and observed under the microscope.

### Particle size, PDI, and zeta potential

The particle size, PDI, and zeta potential were analyzed using a particle size analyzer and zeta sizer (Malvern Instruments, Ltd., Malvern, UK). The analysis using Dynamic Light Scattering (DLS) method range of-2 00 to+200 mV. The samples were prepared by dilution (1:10) in demineralized water and measured in disposable cuvettes at a temperature of 25 °C.

# **Entrapment efficiency**

The entrapment efficiency was determined using the direct method. The phytosomes 1 ml was poured into a 15 ml centrifuge filter tube (Amicon Ultra, Merck, Germany) and ultracentrifuged (Hitachi CP100WX, Japan) at 13.000 rpm in 5 h at 4 °C. The sediment was collected and analyzed to estimate the amino acid (AA) contents. The amino acids (AA) in the Moringa leaf extract were glutamic acid, arginine, glycine, and serine selected as the biomarkers. The calculation of entrapment efficiency using the following equation:

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Entrapment \ Efficiency \ (\%) = \frac{AA \ concentration \ measured}{AA \ concentration \ in \ teoritical} \ x \ 100\%
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# Fourier-transform infrared spectroscopy

The molecular interactions were analyzed using a Fouriertransformed infrared spectrometer (FTIR) (Nicolet<sup>M</sup> iS50; Thermo Fisher Scientific). Infrared scans of the extract, phospholipid, physical mixture (extract-phospholipid), and phytosomes. Each 40 mg of samples was mixed with potassium bromide and then dried in an oven at 40 °C in 3 d to remove moisture. Determination of infrared scans at a wavelength of 4000–500 cm<sup>-1</sup>.

#### **RESULTS AND DISCUSSION**

#### Preparation and extraction

Preparation of dried Moringa leaf powder macerated in n-hexane to remove the non-polar compounds may interfere identification of the active substances and eliminate the odors. The odor comes from volatile components of aldehydes (i.e., 2-hexenal and benzaldehyde) that reduce consumer acceptance [24]. The yield of n-hexane maceration was 89.62%. The extraction used a modified microwave with a double condenser to prevent evaporation solvent and reduced pressure. The previous study reported extraction yield using microwave was 26% which is lower compared to our result. Microwave-assisted extraction based on electromagnetic radiation at 0.3 to 300 GHz provides extraction efficiency by shorting extraction times due to increasing the analyte solubility in the extraction solvent [25].

# **Proximate analysis**

Determination of organoleptic evaluation, proximate analysis, and residual n-hexane is urgent for safety considerations presented in table 1. All results met the requirement [16]. The water, total ash, and acid-insoluble ash contents are indices to demonstrate the quality and purity of extract. Based on a previous study of water content 1.06%, total ash 12.76%, and acid-insoluble ash 1.69%, our study was higher in water content and low in acid-insoluble ash contents. The low water content

prevents contamination by microorganisms. The acid-insoluble ash contents were low in extract, which indicated the small content of the contaminant. Residual of n-hexane did not exceed 290 ppm owing to inherent toxicity [15].

### **Phytochemical screening**

The phytochemical screening to identify secondary metabolites shows in table 2. Moringa leaf extract contained alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. The previous study found similar to saponins, alkaloids, and flavonoids in very much amounts but different in the presence of steroids, terpenoids, and anthraquinones. Several functions of flavonoids include protecting against inflammatory disorders, diarrhea, microbes invasion, and ulcers. The presence of epicatechin, quercetin, and luteolin in flavonoids plays vital roles in diarrhea [26]. Alkaloids have functioned as a muscle relaxant and antispasmodic for relieving intestinal spasms in diarrhea. Moriginine as an alkaloid prove fatal side effect ingestion which has potentially nerve-paralyzing properties when consumed in high doses [27].

### Table 1: Physicochemical properties of Moringa oleifera leaf extract

| Parameters (Units)      | Result            | Requirement       |  |
|-------------------------|-------------------|-------------------|--|
| Organoleptic evaluation |                   |                   |  |
| Form                    | Viscous           | Viscous           |  |
| Color                   | Brown             | Brown             |  |
| Taste                   | Bitter            | Bitter            |  |
| Odor                    | Distinctive aroma | Distinctive aroma |  |
| Proximate analysis      |                   |                   |  |
| Extract yield (%)       | 27.23±1.40        | Not less than 9.2 |  |
| Water content (%)       | 7.33±1.15         | Not more than 10  |  |
| Total ash (%)           | 8.36±0.60         | Not more than 9.0 |  |
| Acid-insoluble ash (%)  | 0.26±0.09         | Not more than 0.9 |  |
| Residual n-hexane (ppm) | Not detected      | Not detected      |  |

Values expressed as mean $\pm$ SD (n = 3)

# Table 2: Phytochemical screening of Moringa oleifera leaf extract

| Metabolites    | Result |  |
|----------------|--------|--|
| Alkaloids      | +++    |  |
| Saponins       | +++    |  |
| Tannins        | ++     |  |
| Flavonoids     | +++    |  |
| Terpenoids     | +      |  |
| Steroids       | -      |  |
| Anthraquinones | -      |  |
| Glycosides     | ++     |  |

+++= very much; ++= much; += little; -= none

### **Nutrition contents**

The UPLC and HPLC method identified in extract and phyosomes of amino acids, the chromatograms can be seen in fig. 1 and fig. 2. The nutrition contents of Moringa leaf extract (100 g) as shown in table 3. The essential and non-essential of 18 amino acid profiles such as glutamic acid, phenylalanine, aspartic acid, alanine, arginine, serine, valine, threonine, proline, glycine, leucine, isoleucine, tryptophan, tyrosine, histidine, lysine, cysteine, and methionine. The amino acids of extract such as glutamic acid, phenylalanine, and aspartic acid were present at the highest concentrations also found cysteine and methionine were at the lowest concentrations. These results are similar to a previous study that found the lowest was cysteine and glutamic acid at the highest concentration as acidic amino acid has good potential of an antioxidant [21]. The previous study reported crude protein, iron, zinc, and calcium reported by 9.4%, 4 mg, 0.60 mg, and 185 mg, respectively found low nutrition comparison with our results, which is a suitable protein source for a supplement. Differences proximate analysis and nutrition contents because of the variety of plants, geographical location, methods of analysis, and growth conditions. Plant protein has a direct impact on soil type presents nitrogen content. Nitrogen has a role in synthesizing amino acids and proteins, storage of protein in all plant parts such as leaf [28]. *Moringa oleifera* Lam. plant used to overcome protein and minerals deficiency with small molecules of Fe, Zn, Ca storage proteins of amino acids histidine and arginine contributed to combating the child stunting in developing countries. The leaf contains crude protein, amino acids, and minerals in higher amounts compared with spinach. The Moringa leaf could be an alternative to vegetarian or poor people unable to consume protein from meat [27, 29].

### Table 3: Nutrition contents of Moringa oleifera leaf extract

| Nutrition             | Result       |  |
|-----------------------|--------------|--|
| Crude protein (%)     | 19.61±0.07   |  |
| Amino acids (mg/100g) |              |  |
| Glutamic acid         | 2155.75±4.14 |  |
| Phenylalanine*        | 998.91±0.95  |  |
| Aspartic acid         | 951.11±4.37  |  |
| Alanine               | 748.64±2.11  |  |
| Arginine**            | 729.19±6.56  |  |
| Serine                | 545.34±1.25  |  |
| Valine*               | 398.40±1.10  |  |
| Threonine*            | 395.48±0.91  |  |
| Proline**             | 382.31±1.65  |  |
| Glycine**             | 348.32±0.80  |  |
| Leucine*              | 344.82±2.15  |  |
| Isoleucine*           | 251.44±0.67  |  |
| Tryptophan*           | 212.74±2.05  |  |
| Tyrosine**            | 195.30±1.03  |  |
| Histidine*            | 164.46±1.12  |  |
| Lysine*               | 102.30±2.71  |  |
| Cysteine              | 75.55±0.10   |  |
| Methionine*           | 6.67±0.01    |  |
| Minerals (mg/100g)    |              |  |
| Iron                  | 3.47±0.00    |  |
| Zinc                  | 5.46±0.05    |  |
| Calcium               | 747.40±4.89  |  |

Data represented as mean $\pm$ SD (n = 2), \*Essential amino acids; \*\*Semi-essential amino acids.

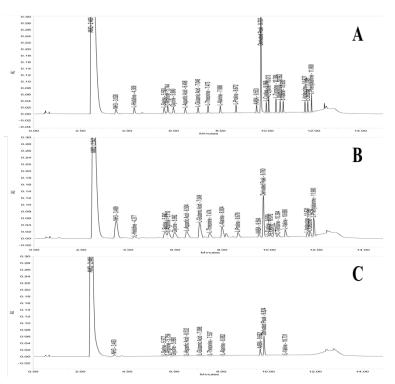


Fig. 1: UPLC chromatograms of amino acids in (A) reference standard (B) Moringa leaf extract and (C) Moringa leaf extract-loaded phytosomes

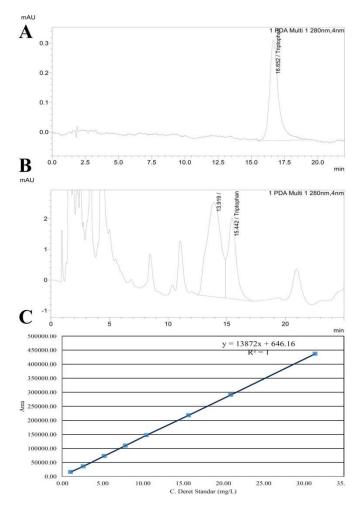


Fig. 2: HPLC chromatograms of tryptophan in (A) reference standard (B) Moringa leaf extract and (C) calibration curve of tryptophan

### **Preparation of phytosomes**

The preparation of phytosomes at an optimal weight ratio of phospholipids and the extract was 1:1 by an anti-solvent precipitation method using dichloromethane as the medium of reaction an n-hexane used to precipitate phytosomes complex as anti-solvent from the medium solvent [23]. The phytosomes confirmed by organoleptic were light brownish, distinctive odor, and formed sediments easily redispersed. The optimal time of probe sonic was achieved at 10 min and gave the best characteristics and entrapment efficiency of the phytosomes. The optimized sonic time was shorter than the previous study reported sonication time in 15 min due to different compositions of phytosomes [30].

### **Evaluation of phytosomes**

# Morphology

The morphology of phytosomes was spherical in shape with the size range of 200 nm can be seen in fig. 3. This result has similar reported by previous studies confirmed as a single spherical shape and self-closed structures of the vesicle commonly ranged 50 nm to 100  $\mu$ m. The aggregation between vesicles was not visible and observed in mostly spherical vesicles, relatively uniform sizes, and well-distributed small cells such as micelles seen as a dispersed collection [23, 31].

#### Particle size and zeta potential

The best characteristics of particle size, PDI, and zeta potential 87.16 nm, 0.22, -23.07 mV, respectively, are presented in table 4 and fig. 4. The previous study reported particle size was larger 198 nm because of the composition of cholesterol and polysorbate 80. The nanoparticles at sizes lower than 100 nm show high direct

absorption at the mucus [30, 32]. The PDI showed <0.5 for homogenous particle size. A lower PDI indicates better homogeneity because the large particle size distribution indicates extracts were physically bonded to phospholipid and gave well distributed and homogenous phytosomes suspension [33]. The zeta potential is a measurement of electrostatic attraction or force repulsion between suspension particles to predict stability during storage [34]. The zeta potential was-23.07 mV, which is lower than the standard around +25 mV or below25 mV is essential for colloidal solutions to prevent aggregation and ensure stabilization [35].

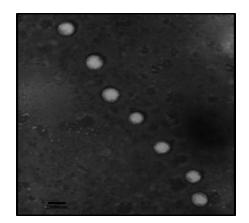


Fig. 3: The morphology of phytosomes with magnification 20.000x

#### Table 4: The characteristics of phytosomes

| Sonication time | Zeta potential (mV) | Particle size (nm) | Polydispersity index |
|-----------------|---------------------|--------------------|----------------------|
| 5 min           | -21.93±0,78         | 95.16±2,37         | 0.41±0,02            |
| 7 min           | -21.90±0,26         | 80,04±0,71         | 0.24±0,02            |
| 10 min          | -23.07±0,76         | 87.16±1,73         | 0.22±0,04            |
| 15 min          | -21.87±1,29         | 197.53±12,17       | 0.36±0,01            |

Values are mean±SD (n = 3)

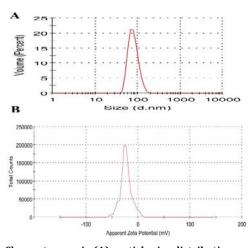


Fig. 4: Chromatogram in (A) particle size distribution and (B) zeta potential

### **Entrapment efficiency**

The entrapment efficiency of amino acids included glutamic acid. arginine, glycine, and serine selected as the biomarkers of infant nutrition and abundance in Moringa leaf extract shown in table 5. Glutamic acid, glycine, and serine correlated with the weightfor-height and the length velocity for infants aged 6-12 mo. Arginine has important rules for infants who are still unable to provide it for their growth requirements [6, 27]. EE of arginine is 108.94% which becomes conditionally essential in stunting conditions due to inflammation state needs to balance for increasing linear growth [4]. Nutrition substances in Moringa leaf powder reduce inflammatory processes that occur specifically in stunting. Arginine is a precursor for synthesis molecules of creatine, nitric oxide, ornithine, and proline stimulates the secretion of growth hormone, prolactin, insulin, and glucagon. Arginine plays in the detoxification of ammonia by the urea cycle and has a high turnover in growing infants. The requirement of arginine in infants is very high because used in the high amount of arginine in protein tissues and the multiple utilization pathways for synthesis [5, 36].

### Table 5: The entrapment efficiency of phytosomes

| Sonication time | Glutamic acid (%) | Arginine (%) | Glycine (%) | Serine (%) |  |
|-----------------|-------------------|--------------|-------------|------------|--|
| 5 min           | 49.07±0,66        | 103.20±0,51  | 65.38±0,18  | 70.06±1,63 |  |
| 7 min           | 43.82±0,54        | 96.93±0,20   | 60,51±0,55  | 54.85±0,12 |  |
| 10 min          | 54.84±1,72        | 108.94±0,52  | 83.58±0,48  | 77.60±0,58 |  |
| 15 min          | 42.08±0,22        | 102.67±0,97  | 72.33±0,64  | 71.01±2,01 |  |

Data represented as mean $\pm$ SD (n = 2)

### Fourier-transform infrared spectroscopy

The molecular interaction identified in FTIR spectra of phytosomes, extract, phospholipids, and physical mixture showed differences in fig. 5. The spectrum of extracts revealed specific peaks O-H stretching vibration of the aromatic ring around 3600 cm<sup>-1</sup>and the N-H group of amines at 1550 cm<sup>-1</sup>. The other spectra of phospholipids revealed C-H stretching and C=O stretching related in the chain of fatty acid peaks at 3000 cm<sup>-1</sup> and 1800 cm<sup>-1</sup>. The physical mixture spectra contained peaks specific to individual components at 3600 cm<sup>-1</sup>, 3000 cm<sup>-1</sup>, 1800 cm<sup>-1</sup>, and 1550 cm<sup>-1</sup>. The phytosomes spectra showed significantly

decreased intensity and shifting of-O-H peak at 3354 cm<sup>-1</sup> and appearance of new peak N-H stretching groups of amines at 1600 cm<sup>-1</sup>. Our result study similar reported decreased intensity and shift of bands-O-H peak at range 3200–3600 cm<sup>-1</sup> and N-O at ranged 1250-1650 cm<sup>-1</sup> as the new peak. These interactions may be due to ionic bonds and or Van der Waals forces. The interactions formed between –O-H groups of the extract and the amine phosphate groups of phospholipid [31, 37]. Phytosomes absorbs by endocytosis or paracellular transport from gastrointestinal tissue through enterocytes [38]. Thus, phytosomes can be increased absorption and reduce the required daily dose of supplementation.

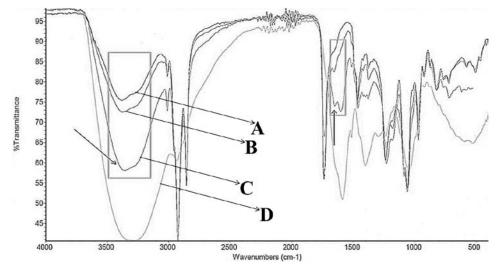


Fig. 5: FTIR spectra of (A) phospholipid (B) physical mixture (C) phytosomes (D) Moringa leaf extract, the boxes, and small arrows showed intensity changes also new peaks in the spectra of Moringa leaf extract-loaded phytosomes

### CONCLUSION

The *Moringa oleifera* Lam. plant is beneficial as a supplement for suppressing stunting incidence, but the absorption of amino acids is inadequate owing to polar phytoconstituents and anti-nutrient substances. The study aimed to prepare Moringa leaf extract through microwave extraction, followed by an evaluation of physicochemical properties and nutrition of Moringa leaf extract. A preliminary study for the preparation of Moringa leaf extract-loaded phytosomes then characterized the physicochemical properties. The result data presented indicate that Moringa leaf extract is a promising source of protein. Preparation phytosomes used sonic 10 min had the best properties particle size, zeta potential, polydispersity index, and entrapment efficiency is a promising supplement to prevent stunting in children.

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### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

# **CONFLICT OF INTERESTS**

The author reports no declarations of interest.

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