Characteristics of Nano Zn-Fitogenik (NZF) made by green synthesis process using guava leaves (*Psidium guajava*) for feed additives

C Hidayat¹, Sumiati², E Wina¹, and A Jayanegara²

¹ Indonesian Research Institute for Animal Production, Ciawi Bogor 16720, Indonesia
² Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia

Corresponding author e-mail: hidayat_c2p@yahoo.com

Abstract. The main objective of this study was to characterize (chemical, biological, and physical) of Nano Zn-Fitogenik (NZF) synthesized using guava leaves (*P. guajava*) extracted using water. Among the stages conducted in the study were extraction of guava leaves, NZF biosynthesis using green synthesis method, and evaluation of NZF characteristics. Parameters detected in the evaluation of the chemical and biological characteristics of the NZF used a completely randomized experimental (CRD) design using 2 main factors (unheated water versus hot water), where each experimental unit was repeated 3 times. The results of this study revealed that chemical characterization study showed that NZF contained 0.15% dry matter (DM) of total phenol, 12.68% DM Zn, and 22.12 mg ml⁻¹ of antioxidant activity (IC50). NZF had the ability as an antibacterial agent against *Escherichia coli* and *Salmonella enteridis*. FTIR analysis showed that NZF contained phyrogenic compounds sourced from guava leaf extract (*Psidium guajava*). It can be concluded that NZF is a nanoparticle containing Zn and phyrogenic compounds (total phenol) which function as an antioxidant and antibacterial. Therefore, NZF has the potential to be used as a feed additive.

Keywords: Nano Zn-Fitogenik, Guava leave, Feed additive

1. Introduction

Nanotechnology exists as a field that is rapidly developing its application in the fields of science and technology. There are several methods in the nanoparticle synthesis process, i.e., biological, chemical, physical, hydrothermal, and electrochemical methods. The green synthesis method (using phytochemicals/phytogenics) is an environmentally friendly method [1]. In this process, metal nanoparticle synthesized using plant phyogenic elements (terpenoids, alkaloids, flavonoids, and total phenolics) as main actor in this method [2]. Phyogenic compounds in plant extracts can act as reducing agents and stabilizers for ion bioreduction reactions in the process of metal nanoparticles synthesis [3]. Nanotechnology application is currently starting to develop in the field of animal nutrition, including the development of minerals in the size of nanoparticles. Zn was reported as crucial trace element needed by poultry for numerous functions of biology [4]. Zn is one of the essential micro-minerals known as antioxidants with several functions because Zn reported has important role as a cofactor for more than 300 enzymes, including the essential ones, in the metabolic system [5]. Phyogenic compounds from plant extracts that become bioreductors and biostabilizers in the process of forming metal nanoparticles are actually plant secondary metabolites which at certain doses can be used to support metabolic
processes in livestock [6]. Nano Zn-Fitogenik is a combination of nano Zn with phytogenic elements which function as a source of organic Zn in the size of nanoparticles containing phytogenic compounds from plant extracts which can be used as feed additives for livestock.

One type of plant extract that is widely reported to have potential that can be used in green nano particle synthesis are guava leaf extract (Psidium guajava). Guava leaf extract (P. guajava) contains high levels of many type of phenolic compound [7]. Guava leaf extraction process produced the highest phenolic yields was using water, compared to using ethanol and methanol [8]. Accordingly, this study was carried out with the aim of evaluating the characteristics (chemical, biological, and physical) of NZF synthesized using guava leaf (P. guajava) extracted using water.

2. Methodology
This study was approved by the ethical clearance from the Animal Welfare Commission of the Agricultural Research and Development Agency with the ethical clearance number: Balitbangtan/Balitnak/A/01/2019. The guava leaves used were those of the local red guava (Psidium guajava, Linn) which was widely available in Indonesia. Guava leaves were used from those located at the top of the stalk to the tip of the stem (including shoots). The following is an overview of guava leaves used.

2.1. Extraction of guava leaves
One kg of guava leaves was prepared and washed clean. Each leaf was desiccated in dryer (oven) at 60°C for ± 1 day. Desiccated leaves were pounded to form a powder, then filtered with a sieve measuring of 0.300 mm. Guava leaf flour was extracted with distilled water using a sonicator for 30 minutes. The centrifuged extract was then stored in a separate container from the residue. In this study, there were two types of guava leaf extraction processes used, namely extraction using distilled water (unheated water) and distilled water that was heated up to 100°C.

2.2. NZF biosynthesis using green synthesis method
The synthesis process of NZF was carried out in the following manner: Zinc sulfate heptahydrate (ZnSO₄·7H₂O) was put in 2 Erlenmeyer flasks, each of 300 ml, then added with 300 mL of 2 types of guava leaf extract (guava leaf extract using unheated water and heated water). The initial pH of the mixture was checked using a pH meter (initial pH ranges from 4.90 - 5.20), then increased with NaOH to pH 7. Each mixture was heated while stirred with a magnetic stirrer. The heating solution was then stirred with a magnetic stirrer at room temperature. The sediment was separated by a centrifugator for 15 minutes. The liquid was separated from the sediment. After that, solution was thrown away, then the sediment was dried in dryer (oven). The dried sediment was then weighed to determine total sediment of NZF product. The NZF product from drying was then ground.

2.3. Evaluation of Nano Zn-Fitogenik characteristics
Nano Zn-Fitogenik products that had been formed were then measured for their chemical characteristics (dry matter content, Zn content, total phenol, total tannins, antioxidant activity), biological characteristics (mortality percentage of E. coli and S. enteridis bacteria). Furthermore, the NZF physical characteristic test was carried out, where the two types of dried NZF were compared (oven-dried NZF versus freeze-dried NZF). Particle Size Analyzer (PSA) test used to analyze particle size average, distribution of particle size and index of polydispersity. Furthermore, SEM (Scanning Electron Microscopy) tests were also carried out to see the structure of the reaction products formed. After that, FTIR (Fourier Transform Infrared Spectroscopy) test used to evaluate the comparison of functional groups in NZF dried by freeze-drying method and oven-drying method, and their comparison with functional groups in guava leaf extract, the main ingredient in making NZF which was the source of organic components in NZF.
2.4. Data analysis
Parameters observed in the evaluation of the chemical and biological characteristics of the NZF used a completely randomized experimental design (CRD) with 2 main factors (unheated water versus heated water) and 3 replications. Furthermore, data were analyzed using ANOVA test. Further test was conducted by using the Duncan test with the help of SAS 19.3 software.

3. Results and discussions

3.1. Chemical characteristics
There are two types of extraction used in preparing guava leaf extract used as an ingredient in the preparation of NZF in this experiment, i.e., extraction with unheated water and heated water. The influence of the type of extraction on the total phenol content and total tannins of guava leaf extract is showed in Table 1.

| Type of extraction   | Total phenolic (%) | Total tannins (%) |
|----------------------|--------------------|-------------------|
| Unheated water       | 8.78               | 0.117             |
| Heated water         | 9.63               | 0.09              |
| SEM                  | 0.96               | 0.0078            |
| p-value              | 0.687              | 0.175             |

As presented by Table 1, it can be seen that the type of extraction does not affect the total phenol content and total tannin content for extract of guava leaf. The effects of the type of extraction on the total NZF deposit is showed by Table 2.

| Type of extraction   | Total NZF deposit |
|----------------------|-------------------|
|                      | DM¹ (g)           | g 100g⁻¹ guava leaf | g 100g⁻¹ ZnSO₄ |
| Unheated water       | 37.31             | 45.30               | 15.40           |
| Heated water         | 44.85             | 64.68               | 18.52           |
| SEM                  | 2.78              | 4.46                | 1.15            |
| p-value              | 0.201             | 0.201               | 0.201           |

¹) Dry Matter

According to Table 2, it can be noticed that the type of extraction does not affect (p>0.05) the total weight of NZF deposits, the percentage of NZF weight to the weight of guava leaves used in the biosynthesis process, and the percentage of NZF weight to the weight of Zn sulfate used in the biosynthesis process. The effects of extraction type treatment on the total phenol content, Zn content, and NZF antioxidant activity is showed in Table 3.

| Type of extraction   | Total phenol (% DM) | Zn (%DM) | IC₅₀ (mg ml⁻¹) |
|----------------------|---------------------|----------|----------------|
| Unheated water       | 0.156A              | 12.68b   | 22.12          |
| Heated water         | 0.099B              | 14.69a   | 24.79          |
| SEM                  | 0.011               | 0.54     | 13.45          |
| p-value              | 0.0051              | 0.044    | 0.335          |

Similar letters or superscripts in the similar column indicate insignificant differences (p>0.05).

Based on Table 3, it can be seen that extraction with unheated water increases (p<0.05) the total phenol content of NZF. When looking at the total phenol content of guava leaf extract for the two treatments (unheated water and heated water), there is no significant difference between the two (Table 1). This shows that the total phenol content of NZF is not much influenced by content of total phenol of
guava leaf extract used in the NZF biosynthesis process. The results of testing of antioxidant activity of NZF using the DPPH method (2,2 diphenyl-1-picrylhydrazyl) showed that the type of extraction treatment has insignificant influence (p>0.05) on antioxidant activity.

The use of this type of water extraction in this experiment, apart from being more environmentally friendly, has also been previously reported by Seo et al., [8] who stated that the process of extracting guava leaves using water produces the highest phenolic content, compared to using ethanol and methanol. The absolute weight and relative weight of the NZF deposit for heated water extraction treatment resulted in a higher weight compared to the unheated water extraction treatment. This is considered to be related to the total phenol content of guava leaf extract treated with heated water extraction, whose value is higher than that of unheated water extraction (Table 1). In [2], differences in the amount of deposit occurred due to differences in the components of active compounds in each leaf extract, and proteins which play an important role in the bioreduction of metal ions and the formation of biocomplex deposits. The presence of a carbonyl group in the molecular structure of the active compound can complex the Zn cation (Zn$^{2+}$) to form Zn-Phytogenic compounds [9]. This functional group allows the metabolites to stabilize and coat the Zn particles and eventually to form Zn-Phytogenics. The reaction mechanism of NZF formation through green synthesis is same as the general formation of metal nanoparticles as described by Basnet et al., [10] where configuration of metal nanoparticles is the result of synthesis process of metal ions (Zn) which comes from the Zn solution then forms complex bonds with phytogenic elements contained in plant extracts.

Zn content of the resulting NZF products is influenced by the type of extraction (p<0.05). Zn component in NZF for heated water extraction treatment appeared to have a higher Zn content (p<0.05) compared to that extracted with unheated water. This shows that the guava leaf extract produced by extraction with heated water provides an opportunity to bind Zn ions higher during the NZF biosynthesis process. Principle of testing antioxidant activity with the DPPH method used in this experiment is that antioxidant component will react with DPPH radicals through the donation mechanism of hydrogen atoms and cause a color decay of DPPH from purple color to yellow color when calculated at 517 nm wavelength [11]. In the DPPH method as used in this experiment, the less NZF concentration needed to decrease activity of free radical at 50% (IC$_{50}$), the stronger its ability as an antioxidant. Zn nanoparticles made by the green synthesis method have antioxidant activity against DPPH that comes from the electrostatic attraction between negatively charged plant bioactive compounds (COO-, O-) and positively charged Zn nanoparticles [12]. The antioxidant activity of Zn nanoparticles made by green synthesis process method is obtained from the bioactivity of the phytogenic elements contained in plant extracts, which increases the level of bioactivity after binding with Zn [13]. The difference in the antioxidant activity of NZF for the unheated water extraction and heated water treatment in this experiment (Table 3) is caused by the total phenol content of guava leaf extract by the two treatments which are also not different (Table 1).

3.2. Biological characteristics
The effects of extraction type treatment and NZF dose level on the percentage of death of E. coli and S. enteridis bacteria treated with the type of extraction and dose of NZF is presented in Table 4.

| Treatments     | Bacterial mortality (%) |
|---------------|-------------------------|
|               | E. coli  | S. enteridis |
| Type of extraction (A) |           |             |
| Unheated water    | 70.52A  | 72.70B       |
| Heated water     | 68.88B  | 74.95A       |
| Pooled SE       | 21.98   | 22.97        |
| p-value         | 0.0012  | <0.0001      |

Table 4. Influence of NZF on percentage mortality of E. coli and S. enteridis bacteria.
Based on Table 4, there is no interaction (p>0.05) between the extraction type treatment with the NZF dose level on the percentage of death of *E. coli* and *S. enteridis* bacteria. The type of extraction affects (p<0.05) the mortality rate for *E. coli* and *S. enteridis*. NZF dose affects (p <0.05) the mortality rate for *E. coli* and *S. enteridis*. NZF produced by extraction using unheated water had a higher mortality rate for *E. coli* than the NZF produced by extraction using heated water. On the other hand, the NZF produced by extraction using heated water impacts on the higher mortality rate of *S. enteridis* bacteria compared to the NZF produced by extraction using unheated water. NZF appears to have a high ability to kill *E. coli* and *S. enteridis* as can be seen from the mortality rate of the two bacteria above in the range of 82-100% for the use of the NZF 2.5-10 mg ml$^{-1}$ dose range. The number of *E. coli* bacteria colonies used as a control (treatment dose of 0 mg ml$^{-1}$) was 2.9x10$^{14}$, while for *S. enteridis* was 2.8x10$^{14}$.

ZnO nanoparticles reported has ability as high antibacterial activity against for both types of bacteria (Gram-positive and Gram-negative bacteria) [14]. Mechanism of inhibition of bacteria by nanoparticles is carried out through following mechanisms, i.e., cell penetration, adhesion to the bacterial surface through electrostatic forces, and the production of ROS (reactive oxygen species) which plays a role in inhibiting bacterial growth [15]. ZnO nanoparticles have both bactericidal and fungicide activity by destroying cell membranes [16]. Accordingly, ZnO nanoparticles are very potential and effective to be used for protection from bacterial infection. The lower size of the Zn nanoparticles makes it easier for them to penetrate into bacterial cells through the bacterial cell walls, thus limiting DNA replication [17]. There are several factors that influence of Zn nanoparticles antibacterial activity, i.e., particle size, surface area, concentration, and morphology of Zn nanoparticles [18].

The ability of NZF to kill *E. coli* and *S. enteridis* is also thought to be influenced by the activity of the phytogetic compounds contained therein. Several research results show that the phytogetic elements contained in guava leaves (flavonoids and gallocretechins) have antibacterial properties including against *E. coli* and *Salmonella* bacteria [19]. Antimicrobial activity of phytogetic compounds is largely controlled by the physico-chemical characteristics of plant compounds [20]. The phytogeticity of guava leaves (*P. guajava* L) was reported by Biswas et al. [19] as part of plants that contain antimicrobial compounds, i.e., tannins, essential oils (eugenol), fatty oils, resins, triterpenoids, flavonoids, and malic acid. The antimicrobial compounds in guava leaves have ability to suppress both of kinds bacteria (Gram-positive and Gram-negative bacteria) [19]. Guava leaves contain a wide spectrum of phytochemicals, i.e., alcohol sesquiterpenoids, triterpenoid acids, alkaloids, glycosides, steroids, flavonoids, tannins, and saponins [21].

### Table 4

| Dose (mg ml$^{-1}$) (B) | 0.00X | 0.00X |
|-------------------------|-------|-------|
| 2.5                     | 82.20Y| 99.84Y|
| 5                       | 98.83Z| 99.99Z|
| 10                      | 100.00Z| 100.00Z|
| Pooled SE               | 0.55  | 0.009 |
| p-value                 | <0.0001 | <0.0001 |

Interaction (A*B) NS NS

p-value 0.15 0.314

Similar letters in similar column indicate insignificant differences (p>0.05). NS = Unsignificant
3.3. Physical characteristics
Based on the evaluation of chemical and biological characteristics, and considering the application of NZF production when produced on a large scale, NZF produced using unheated water is better than that produced using heated water. Figure 2 shows the NZF product produced using guava leaf extract using unheated water. In the physical evaluation of NZF, two types of NZF were evaluated, which were oven-dried (60°C) and freeze-dried. Dried NZF is powdery, smooth like ash, and has a gray color (Figure 1). NZF dried by freeze-drying method did not go through the centrifugation process of separation between NZF sediment and liquid. After the NZF biosynthesis process resulted from the reaction between the Zn solution and plant extracts, the resulting liquid from the biosynthesis was then directly dried using the freeze-drying method.

3.3.1. Particle Size Analyzer (PSA). Analysis with the PSA (Particle Size Analyzer) is a test to analyze particle size [22]. The results of the analysis of the NZF size distribution dried by two types of drying, namely oven (60°C) and freeze-drying, are presented in Table 5. The size distribution of NZF oven-dried at 60°C has a particle size with a size distribution between 122.4-1484 nm with an average size of 645 nm. The percentage of particles with a size below 1000 nm is 88.9% and above 1000 nm is 11.1%. Meanwhile, NZF dried by the freeze-drying method has a particle size with a size distribution between 190.1-396.1 nm with an average size of 278 nm. The percentage of particles with a size below 1000 nm is 100%. In this experiment, the poly-dispersion index value for oven-dried NZF was 0.54, while those dried by the freeze-drying method were 0.88 (Table 5). This shows that the type of drying determines the homogeneity of the nanoparticles produced.

| Drying type     | Size distribution (nm) | Average size (nm) | Polydispersity index |
|-----------------|------------------------|-------------------|---------------------|
| Oven dry        | 122.4-1484             | 645               | 0.54                |
| Freeze Dry      | 190.1-396.1            | 278               | 0.88                |

The drying method really determines the size and distribution of the nanoparticles produced. If we look at the average NZF particle size for the two types of drying, it can be ascertained that NZF has met the nanoparticle standards issued by the British Standards Institution, where the size scale for nanomaterials for the benefit of scientific terms is the size range of 1-1000 nm [23]. Size of particle and distribution of particle size reported can influence drug mobilization in the body. Poly-dispersion index value indicated the stability of the nanoparticle, where the increased polydispersity index value indicated more aggregated particles [24]. Poly-dispersities close to 0 indicates a homogeneous dispersion. Meanwhile, a poly-dispersion index with a value of more than 0.5 indicates high heterogeneity.

The biosynthesis of nanoparticles using extracts from natural materials is strongly influenced by the concentration of the extracts used. The results of this study generally show that the particle size distribution of the NZF nanoparticles produced is still diverse. The factor causing the variability of NZF particle size is probably the result of the technical implementation of the NZF biosynthesis process, especially when the pH of the solution increases during the NZF biosynthetic reaction. During the NZF biosynthesis process, the pH was increased by adding 5M NaOH. There is still a difference in the solution stirring speed when increasing the pH, which will cause a variation in the size of the resulting NZF particles. In connection with the process of increasing the pH, there is a reaction to form a complex between Zn and phytogenic where the technical speed of solution stirring will contribute to the particle size of the complex compound resulted from the reaction between Zn and phytogenic. Despite that, particles with a diameter of <1000 nm is acceptable as nano-sized carriers that can be used by the pharmaceutical industry [25].

In [26], the mechanism of metal nanoparticle synthesis through the green synthesis method. It can be explained that the reducing agent used in this study is guava leaf extract which acts as a catcher for Zn sulfate precursors. After the Zn$^{2+}$ cation is reduced to a metal with a zero charge, the guava leaf extract compound is around the surface of the nanoparticles formed. When the Zn metal is still positively charged or has not been reduced, the particle size is on the angstrom scale, and the guava leaf extract
compound as a trapping agent is more dominant than the cation so that the resulting particle size follows the size of the guava leaf extract compound. More and more reduction reactions occur with the passage of reaction time so that the particles undergo merging and produce a larger particle size. The tendency of particles to aggregate is due to the effect of the continuous motion of the particles occurring in solution. This tendency causes the particle diameter to be non-uniform. Nanoparticle aggregation occurs in two stages. In the first stage, the particles approach and collide with each other, and in the second stage, the colliding particles stick together [27].

3.3.2. Analysis by Scanning Electron Microscopy (SEM). SEM analysis used to determine structure of reaction products formed. The test results by means of SEM on NZF products dried by freeze-drying and oven-drying methods are shown in Figure 3 and Figure 4.

![Figure 1](image1.png)  
**Figure 1.** SEM results of freeze-dried NZF with a magnification of 20000x (left) and 50000x (right).

![Figure 2](image2.png)  
**Figure 2.** SEM results of oven-dried NZF with a magnification of 20000x (left) and 50000x (right).

Based on the results of tests using SEM, in general, NZF is known to have the form of nanoparticles, some of which are nanometer-sized, while others are still micrometer-sized agglomerations. The shape of the clots is a display of the carbon element, where NZF has a carbon element sourced from phytogenic compounds of guava leaf extract. The clumps of carbon particles show an imperfect spherical shape [26]. Nano Zn-Fitogenik dried using the freeze-drying method look more even, both in shape and particle size. Meanwhile, NZF which was oven-dried at 60° C show more variations in the shape of particles. The non-uniform particle morphology in nanoparticle synthesis occurs due to the influence of polarity, electrostatic Zn energy, and large energy on the surface of the sample, which is common when the synthesis process takes place [9]. Agglomeration (collection and/or accumulation of particles or substances into one) occurs allegedly because there are still many chemical compounds contained in guava leaf extract that play a role as traps or templates for ZnSO4 precursors. The size of the nano Zn produced is highly dependent on the size of the template surrounding the surface of the nanoparticles [28].
3.4. Fourier Transform Infrared Spectroscopy (FTIR) test

In this experiment, Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to identify functional groups contained in NZF. The working principle of FTIR is based on the absorption or transmittance of infrared rays by the molecules that make up a compound in the sample being tested [29]. If the frequency of a functional group vibration is the same as the frequency of infrared radiation, the molecules will absorb the light. This means some infrared rays are absorbed by the molecule, while some are transmitted. Referring to the database of organic components of FTIR spectroscopy reported by Segneanu et al. [30], we can find out the content of specific functional groups found in guava leaf extract, freeze-dried NZF and oven-dried NZF, as shown in Table 6. Typical functional groups found in guava leaf extract were measured as a comparison. The functional groups present in NF were derived from guava leaf extracts which were used in the NZF biosynthesis process.

**Table 6. Functional groups of guava leaf extract, freeze-dried and NZF oven-dried.**

| No | Guava leaf extract (freeze dried) | NZF (freeze dried) | NZF (Oven-dried) | Functional groups |
|----|----------------------------------|--------------------|------------------|------------------|
| 1  | -                                | -                  | 3236             | O-H stretching   |
| 2  | 3225                             | 3151               | -                | C-H aromatic     |
| 3  | 2922                             | -                  | -                | C-H alkane       |
| 4  | -                                | -                  | 2181             | N=C=O; N=C=S; N=C=N; N3; C=C=O Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes |
| 5  | -                                | -                  | 2069             |                  |
| 6  | -                                | -                  | 1643             |                  |
| 7  | 1601                             | -                  | 1618             | NH2 Amine        |
| 8  | -                                | 1504               | -                | C=C Aromatic, lignin |
| 9  | 1442                             | -                  | 1432             | S=O Sulfat       |
| 10 | 1347                             | -                  | -                | S=O Sulfone      |
| 11 | 1205                             | -                  | -                | N-O Amine oxide aromatic |
| 12 | -                                | -                  | 1145             | P=O Fosfat       |
| 13 | -                                | -                  | 1098             | Si=OR Silane     |
| 14 | -                                | 1090               | -                | O-C polysach     |
| 15 | 1035                             | -                  | -                | C=N Amines       |
| 16 | -                                | 1018               | -                | P-OR Ester       |
| 17 | -                                | -                  | 983              | P-H Phosphine    |
| 18 | 869                              | -                  | -                |                  |
| 19 | -                                | -                  | 867              | NH2 dan N-H Amines |
| 20 | 818                              | 861                | -                | S-OR Ester       |
| 21 | 765                              | -                  | 752              | S-OR Ester       |
| 22 | 728                              | -                  | -                | S-OR Ester       |
| 23 | 697                              | -                  | -                |                  |
| 24 | 658                              | -                  | -                |                  |
| 25 | -                                | 654                | -                |                  |
| 26 | 640                              | -                  | -                |                  |
| 27 | -                                | -                  | 630              | Zn               |
| 28 | -                                | -                  | 572              | Zn               |
| 29 | -                                | -                  | 459              | Zn               |
| 30 | -                                | -                  | 435              | Zn               |
Table 6 shows the functional group comparison between guava leaf extract, namely freeze-dried NZF and oven-dried NZF. The functional group found in the same guava leaf extract, also contained in freeze-dried NZF, is the C-H aromatic group, S-OR Ester. Meanwhile, the functional groups found in the guava leaf extract which are also contained in oven-dried NZF are NH$_2$ Amine, S=O Sulfate. Based on Table 6, it can be seen that there is a shift in the functional groups that occur from the functional groups found in the guava leaf extract to those contained in NZF. The shift in wave numbers shows that there is an interaction between functional groups and Zn nanoparticles due to the oxidation process as a result of the reduction of Zn nanoparticles [26]. The –OH group of terpenoids and flavonoids in leaf extract is responsible for the reduction of metal ions, and the -COO group participates in the stabilization of the nanoparticles [31]. The –OH group participates in the oxidation-reduction process, while the carbonyl and carboxylate groups are involved in particle stabilization. From Table 6 it can be seen that the type of NZF drying appears to affect the NZF functional groups.

Tiwari and Declan, [32] revealed the mechanism for the formation of metal nanoparticles, which can explain the mechanism of NZF formation in this experiment. NZF involves a complexation process between Zn$^{2+}$ and guava leaf extract. Complex compounds are formed by coordinating covalent bonds between ligands and metals. The ligand will donate the lone pair to the metal ion providing the empty orbital. Metal ions act as acids while ligands act as bases. The complex compounds formed have a more stable chelating effect [32]. Based on FTIR data, guava leaf extract contains compounds that have hydroxy and carbonyl functional groups. This functional group acts as a ligand that donates the lone pair to the Zn$^{2+}$ orbital, then Zn$^{2+}$ and the polar group form complex compounds in a nano-sized template. The binding of metal cations to the extracted compounds involves various kinds of physico-chemical interactions that can play a role alone or together [33]. These processes include the occurrence of coordination/chelating bonds, ion-exchange, electrostatic interactions, acid-base interactions, hydrogen bonding, and physical adsorption.

### 3.5. X-Ray Diffraction Analysis (X-RD)

The X-RD test aimed to determine the structure and quality of the NZF crystals produced. The results of the X-RD analysis of NZF products presented in Table 7.

| Parameters                      | Freeze dryer | Oven dryer   |
|---------------------------------|--------------|--------------|
| Crystal size distribution (nm)  | 0-495        | 0-1194       |
| Average crystal size (nm)       | 121          | 240          |
| Degree of Crystallinity (%)     | 56.33        | 56.44        |
| Crystal Form                    | Trigonal     | Tetragonal   |

In general, the XRD peaks detected in NZF samples by the drying process with the freeze-drying method are the same as those with NZF samples that were oven-dried (60° C). NZF dried by freeze-drying method appears to have a higher intensity, is narrower and sharper, which indicates that the crystal quality is better. The NZF diffraction pattern for both types of drying is not the same as the XRD diffraction pattern of pure crystal ZnSO$_4.7$H$_2$O reported by Saha and Podder [34]. This indicates that the reaction process between Zn and phytogenic compounds causes the formation of a new element which is different from ZnSO$_4.7$H$_2$O, which is used as an ingredient in the manufacture of NZF. In Table 7, it can be seen that the NZF crystal size range of those dried by the freeze-drying method is 0-495 nm with an average crystal size of 121 nm. Meanwhile, oven-dried NZF has a range of NZF crystal sizes of 0-1194 nm with an average crystal size of 240 nm.

This study shows that the NZF drying method affects the distribution and average size of the resulting NZF crystals. The degree of crystallinity of the NZF for those dried by freeze-drying method is 56.33%, while those for oven-drying is 56.44%. The degree of crystallinity is a quantity that states the amount of crystal content in a material by comparing the area of the crystal curve with the total area of amorphous and crystalline [35]. Solid dosage forms can be classified into two groups, namely crystalline
solids whose constituent particles are regularly arranged, and amorphous solids whose constituent particles do not have perfect order [36]. The shape of crystalline forms of oven-dried NZF and freeze-dried NZF seem to be different. The Trigonal shape (hexagonal axes) for NZF is dried using the freeze-drying method, while the oven-dried results in Tetragonal shape. The crystal structure is related to physicochemical properties, namely melting point of chemical reactivity, dissolution rate, and bioavailability of the drug which will affect the absorption rate in the digestive tract [37].

4. Conclusion
Green synthesis of Zn nanoparticles using guava leaves (*Psidium guajava*) has succeeded in forming a Nano Zn-Fitogenik (NZF) product. Nano Zn-Fitogenik contains phenolic and Zn compounds, and functions as an antioxidant. NZF also has a strong ability as an antibacterial on the pathogenic bacteria *Escherichia coli* and *Salmonella enteridis*. Therefore, NZF has the potential to be used as feed additives. NZF production is recommended to use guava leaf (*Psidium guajava*) extracted with unheated water. The average particle size of the oven-dried NZF (60° C) is 645 nm, while those dried by freeze-drying method has a size of 278 nm. NZF particle sizes are still within the standard range of nanomaterial sizes of 1-1000 nm.

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