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Original Article

OPTIMIZATION OF THE CONDITION TO PRODUCE ZEIN-*ACALYPHA INDICA*. l LEAF EXTRACT NANOPARTICLES AS ANTIOXIDANTS

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ABSTRACT

Objective: *Acalypha indica* L. (*A. indica* L.) is an herbal plant predominantly found in the wet tropics and known for its diverse activities. One way to increase its biological activities is by making it as nanoparticle form. This study aimed to optimize the condition to produce zein-leaf *A. indica* L. Nanoparticles (NPZA) as antioxidant.

Methods: The nanoparticles condition optimization was developed using a central composite design, employing two solvents for extraction: 50% ethanol ($NPZAE_{50\%}$) and methanol p. a (NPZAM). The optimized conditions include the amount of extract, the amount of zein, and the sonication time. The antioxidant capacity was evaluated using the Diphenyl Picryl Hydrazyl Method, while the nanoparticles were characterized using UV/Vis Spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), Particle Size Analyzer (PSA), and Transmission Electron Microscope (TEM).

Results: The optimum condition was found on 100 mg of extract ethanol 50%, 150 mg of zein, and a sonication time of 20 min, with antioxidant capacity of 0.935±0.15 mmol AAE/ml for $NPZAE_{50\%}$. The average particle size of $NPZAE_{50\%}$ was 27.5 nm with a spherical particle morphology.

Conclusion: In conclusion, the highest antioxidant capacity was achieved with 117 mg of extract, 175 mg of zein, and 23 min of sonication, with formula 20 showing the best results for $NPZAE_{50\%}$ and NPZAM.

Keywords: *Acalypha indica, Zein Nanoparticles, PSA, TEM, DPPH*

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INTRODUCTION

The utilization of nanoparticles in the medical field has become prevalent. Zein Nanoparticles (NPZ) can enhance drug stability oral bioavailability, control drug release, and improve drug targeting, effectively increasing the drug effectiveness [1, 2]. Typically, the synthesis of nanoparticles involves physical and chemical methods that employ toxic chemicals [3]. To mitigate the risk of toxicity, there is a shift towards utilizing environmentally friendly materials, such as plant extracts [3–6]. Numerous studies have been conducted on synthesizing nanoparticles using plant extracts, such as the production of Ag-conjugated nanoparticles utilizing rosemary leaf extract [7] and the synthesis of silver nanoparticles using *Senna siamea* flower extract [8]. However, plant extracts often have large particle sizes [9], leading to low solubility and reduced effectiveness. Modifications are required to address this issue, including reducing the particle size to the nanoscale, which can be achieved by incorporating a zein solution.

In recent years, proteins have been widely studied for the encapsulation of bioactive compounds. Proteins have the advantages of being metabolizable, biodegradable, biocompatible, and nontoxic. Several uses of proteins to produce nanoparticles have been reported, such as gluten, albumin, gliadin, and gelatin.

Zein, a prolamine found in corn, is soluble in ethanol at concentrations higher than 70% [10]. It forms aggregates via disulfide bonds within the corn structure, some of which can be disrupted during extraction or wet milling operations by reducing agents. Zein can bind to plant extracts, facilitating nanoparticles forming that serve as carriers [11, 12]. NPZ have also been utilized as encapsulation materials, such as incorporating curcumin to deliver bioactive compounds effectively [13, 14]. This protein has the ability to encapsulate both hydrophilic and hydrophobic molecules and is recognized by the FDA as Generally Recognized as Safe (GRAS) [15]. Recently, it has been utilized in various delivery system formulations, highlighting its adaptability as a nanocarrier. Zhang *et al.* focused on NPZ production by incorporating lecithin as antifungal agent. NPZ can also be employed as an antimicrobial agent by incorporating *Thymbra capitata* (L.) extract [16, 17].

Many plants, including *A. indica* L., are used in traditional medicine and contain secondary metabolites. *A. indica* L., commonly known as the Anting-anting plant, possesses active ingredients in its leaves, stems, and roots. Qualitative tests have indicated the presence of phenolics, flavonoids, alkaloids, essential oils, steroids, and triterpenoids in the root extract of *A. indica* L., which exhibits antioxidant properties [18]. Antioxidants are compounds that protect and repair cell damage caused by exposure to free radicals. Ravi *et al.* evaluated the antioxidant activity of *A. indica* L., revealing a high quantitative content of phenolic compounds and potent antioxidant properties [19]. In previous studies, *A. indica* has been reported to exhibit antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-diabetic, and wound-healing activities [20, 21].

It was hypothesized that NPZ incorporating *A. indica* leaf extract improve antioxidant capacity, making them valuable as biomedical materials enriched with antioxidant agents for pharmaceutical use. This study aims to optimize the formulation of zein-leaf *A. indica* L. nanoparticles (NPZA) with maximum antioxidant capacity and to characterize the resulting nanoparticles. The NPZA is optimized using formula designed by Design Expert 7.0 software with varying *A. indica*. l ethanol 50% extract ($NPZAE_{50\%}$), *A. indica*. l methanol extract (NPZAM), amount of extract, zein, and sonication time. In addition to using extracts and zein, tween 20 is added to the formulation as a stabilizer/emulsifier for NPZA. Without a stabilizer, the solution would agglomerate, leading to unstable particle sizes that could increase over time. The antioxidant capacity of the formed nanoparticles will be evaluated using the DPPH method. The formula that demonstrates optimal antioxidant capacity will be characterized using UV/Vis Spectrophotometry to determine the maximum wavelength of the particles, FTIR to identify functional

groups, PSA to measure particle size, and TEM to examine the morphology of the resulting nanoparticles.

MATERIALS AND METHODS

Materials

The materials used in this research were methanol CH_3OH p. a (Merck, Germany), ascorbic acid $C_6H_8O_6$ (Merck, Germany), ethanol C_2H_5OH (Sigma Aldrich, USA), distilled water H_2O, tween 20 $C_{58}H_{114}O_{26}$ p. a (Merck, Germany), DPPH $C_{18}H_{12}N_5O_6$ p. a (Merck, Germany), zein (Sigma Aldrich, USA), Anting-anting (*Acalypha indica*. L) leaves.

Methods

Sample identification and preparation

Samples of *Acalypha indica* L. leaves, commonly known as the anting-anting plant, were collected from the yard and roadside area of Siteba Aia Pacah Padang, West Sumatra, Indonesia. The samples were identified, including the root, stem, leaf, and flower. The voucher specimen was deposited at the Andalas Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, West Sumatra, Indonesia No. ANDA00010072. To prepare the samples, they were first separated from impurities, washed with running water, and left to air-dry. The dried samples were ground into a fine powder for further analysis.

Extraction of *A. Indica*. L

The extraction was performed with two maceration processes using each different solvents, methanol p. a and ethanol 50%. Extraction was performed with a 1:20 ratio of sample to solvent for ±5 d, then filtered. The filtrate was then evaporated using rotary evaporator (YAMATO RE301, Japan) for±6 h, then dried in freeze drying (Christ, Germany). Extract standardization had been carried out in previous studies [22].

Preparation of zein-leaf *A. indica*. l nanoparticles (NPZA)

The preparation of NPZA involved the utilization of the Central Composite Design, employing two solvents: ethanol 50% ($NPZAE_{50\%}$) and methanol (NPZAM). NPZA was prepared by ultrasonication method. A total of 20 formulations were created for each extract, as outlined in table 1, varying extract amount, zein amount, and sonication time. Zein was dissolved in 100 ml ethanol, then *A. indica* L. leaf extract and 1 ml of Tween 20 were added. The mixture was homogenized using a sonicator [23].

Table 1: The composition of the NPZA formula of each EtOH50% and MeOH extract based on the central composite design using a design expert 7.0

Formula	Variable		
	A. indica. l leaf extract (mg)	Zein (mg)	Sonication time (min)
1	75	112.5	6
2	117	112.5	15
3	50	150.0	10
4	50	150.0	20
5	75	112.5	15
6	75	112.5	15
7	33	112.5	15
8	50	75.0	10
9	100	75.0	20
10	75	112.5	15
11	75	112.5	15
12	75	175.5	15
13	100	75.0	10
14	75	112.5	15
15	75	49.4	15
16	50	75.0	20
17	75	112.5	15
18	100	150.0	10
19	75	112.5	23
20	100	150.0	20

Determination of antioxidant capacity

Validation of the analysis method was carried out, including linearity, accuracy, precision, LOD, and LOQ parameters using ascorbic acid as a standard [24]. To determine antioxidant capacity, 2 ml of each ascorbic acid standard solution with concentrations ranging from 0.001 to 0.04 mmol were pipetted, followed by the addition of 2 ml of $NPZAE_{50\%}$ and NPZAM. To the vial, 0.5 ml of a 0.3 mmol DPPH solution was added. The vial was then shaken thoroughly and left to stand for 15 min. After incubation, the absorbance was measured using UV-Vis Spectrophotometer (Thermo Scientific GENESYS 10S, USA) at 517 nm [25]. The antioxidant capacity is reported as mmol Equivalent of Ascorbic Acid per milliliter (mM AAE/ml) [26].

Optimization condition of zein-leaf *A. indica*. l nanoparticles (NPZA)

To determine the optimal conditions, the relationship between antioxidant capacity and the amount of *A. indica* leaf extract (A), the amount of zein (B), and sonication time (C) was investigated using Design Expert 7.0. The aim of this study is to identify the ideal combination of these factors to yield optimal antioxidant capacity. This parameter is used as a response to assess the bioactivity of compounds. With optimal antioxidant capacity, it is expected that the size will be at the nanoscale, then the nanoparticles were characterized to assess their properties [23].

Characterization of Zein-*A. indica*. l leaf extract nanoparticles

The nanoparticles derived from the optimal formula were characterized using several methods, including UV/Vis Spectrophotometer, FTIR (Thermo Scientific Nicolet 380), PSA (Horiba SZ-100, Japan), and TEM (HT7700 TEM, Hitachi, Japan). The confirmation of NPZ from *A. indica* L. was performed using UV-Vis spectrophotometer. The sample was diluted with ethanol 50%, and the UV/Vis spectra were recorded using a quartz cuvette, with ethanol 50% as the blank. Spectrometric readings were collected in the range of 200-800 nm [27].

To determine the functional groups present in the ethanol 50% extract and $NPZAE_{50\%}$, a FTIR spectrophotometry was done. The extract solution and nanoparticles were pipetted onto an infrared cell with KBr crystal windows to identify any changes in the functional groups between the ethanol 50% extract and $NPZAE_{50\%}$ [28].

The particle size of $NPZAE_{50\%}$ was determined using Particle Analyzer Horiba SZ-100 (PSA). Furthermore, the morphological characteristics of $NPZAE_{50\%}$ were examined using high-resolution transmission electron microscopy (HRTEM) with a JEOL JEM 2100 instrument to visualize the particle morphology at a higher resolution [6].

RESULTS

Extraction of *A. indica*. L

The yield of the extracts obtained was 14.83% for the methanol extract, which exhibited a dark green color, and 5.94% for the ethanol 50% extract, which had a dark brown color.

Formation of NPZAE$_{50\%}$ and NPZAM

The NPZA formula consists of a mixture of zein and plant extracts using two solvents, methanol and ethanol 50%. The effect of these two solvents on the sample was compared among the 20 formulas tested, with variations in the amount of zein, *A. indica* leaf extract, and sonication time. Formulas with more extract than zein exhibited a more intense color, while formulas with less extract appeared more faded. This observation aligns with the laser beam transmission through NPZAE$_{50\%}$.

Antioxidant capacity of NPZAE$_{50\%}$ and NPZAM

The validation parameters determined included linearity with r value was 0.999, accuracy 98.37%, precision with RSD value 1.34%, LOD value of 0.001 mmol and LOQ of 0.004 mmol. The antioxidant capacity of the ethanol extract of *A. indica* L. was found to be 0.014±0.000 mmol AAE/ml, while the methanol extract exhibited lower antioxidant capacity of 0.008±0.001 mmol AAE/ml. Table 2 demonstrates the variations in the antioxidant capacity among different NPZA formulas.

Table 2: Antioxidant capacity of NPZAE$_{50\%}$ and NPZAM

Formula	Factor			Response [mM AAE/ml]	
	A. Indica. l Leaf Extract (mg)	Zein (mg)	Sonication time (min)	Antioxidant capacity NPZAE$_{50\%}$	Antioxidant capacity NPZAM
1	75	112.5	6	0.480±0.03	0.365±0.04
2	117.0	112.5	15	0.885±0.07	0.790±0.05
3	50	150.0	10	0.610±0.07	0.345±0.07
4	50	150.0	20	0.650±0.01	0.375±0.05
5	75	112.5	15	0.665±0.05	0.410±0.04
6	75	112.5	15	0.665±0.29	0.405±0.01
7	33	112.5	15	0.535±0.04	0.345±0.02
8	50	75.0	10	0.515±0.08	0.290±0.04
9	100	75.0	20	0.805±0.01	0.710±0.03
10	75	112.5	15	0.675±0.09	0.400±0.03
11	75	112.5	15	0.655±0.06	0.400±0.02
12	75	175.5	15	0.500±0.05	0.530±0.08
13	100	75.0	10	0.680±0.06	0.660±0.03
14	75	112.5	15	0.680±0.11	0.410±0.03
15	75	49.4	15	0.570±0.05	0.540±0.08
16	50	75.0	20	0.575±0.05	0.560±0.08
17	75	112.5	15	0.660±0.06	0.420±0.05
18	100	150.0	10	0.815±0.04	0.575±0.04
19	75	112.5	23	0.710±0.04	0.515±0.05
20	100	150.0	20	0.935±0.15	0.865±0.03

*Response data represent mean±SD (n=3)

The highest antioxidant capacity was obtained in NPZA formula 20, with values 0.935±0.15 mmol AAE/ml for NPZAE$_{50\%}$ and 0.865±0.03 mmol AAE/ml for NPZAM. It was observed that the average antioxidant capacity of NPZAE$_{50\%}$ formulas was significantly higher compared to NPZAM ($p<0.05$). This indicates a statistically significant difference in the antioxidant activities between the two formulations.

The antioxidant capacity test results were analyzed using Design Expert 7.0.0 software. Fig. 1 shows the optimum composition of NPZA obtained in extract amount of 117 mg, zein amount of 175 mg, and sonication time of 23 min.

In this study, data analysis was performed using a quadratic model with ANOVA to determine the effects of A (*A. indica* leaf extract), B (zein), and C (sonication time) on the antioxidant capacity. The ANOVA results indicate that the quadratic model used is significant, with p-value<0.05. This suggests that the model effectively explains the data variability.

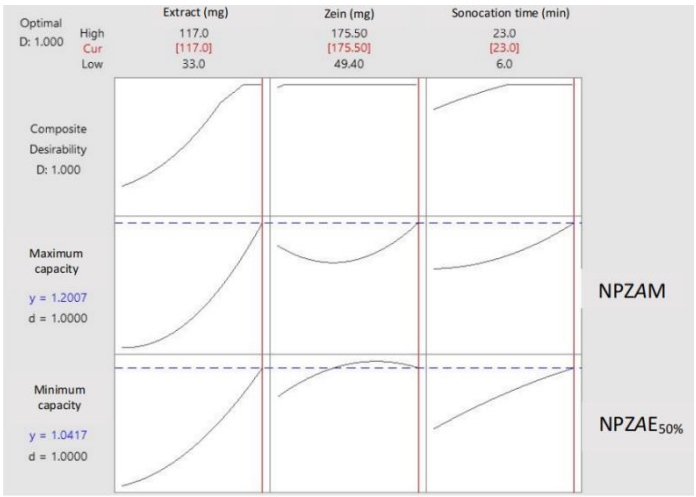

Fig. 1: Optimum response of NPZAM and NPZAE$_{50\%}$

The analysis reveals that *A. indica* leaf extract had a highly significant effect on the response ($p<0.001$), followed by sonication time, which also shows significance ($p<0.01$). On the other hand, zein amount did not significantly affect the response ($p>0.05$).

This statistical model yields an R^2 = 0.9239, meaning that the model explains 92.39% of the total data variability. The adjusted R^2 = 0.8553 suggests that the model remains reliable after adjusting for the number of variables. Additionally, the adequate precision = 12.3437 suggests that the model has an acceptable signal-to-noise ratio, making it suitable for design space exploration.

Characterization of Zein *A. indica*. l nanoparticles ethanol extract 50% (NPZAE$_{50\%}$)

The characterization of NPZAE$_{50\%}$ were carried out using UV/Vis Spectrophotometer, FTIR, Transmission Electron Microscopy (TEM), and Particle Size Analysis (PSA). In this characterization, formula 20 of NPZAE$_{50\%}$ was utilized instead of NPZAM due to its higher antioxidant capacity.

Absorption spectrum NPZAE$_{50\%}$

The UV/Vis spectrophotometer was used to determine the specific wavelength of the formed nanoparticles (NPZAE$_{50\%}$). The UV/Visible spectrum characteristics of NPZAE$_{50\%}$ revealed the presence of two distinct peaks at 230 nm and 240 nm. These peaks indicate the occurrence of chemical reactions during the formation of NPZAE$_{50\%}$.

Particle size and morphology of NPZAE$_{50\%}$

Particle size analysis using a Particle Size Analyzer (PSA) revealed that the average particle size of NPZAE$_{50\%}$ was 27.5 nm. This size indicates that the NPZAE$_{50\%}$ formula has successfully formed colloidal nanoparticles in the nanoscale range.

The morphology of the nanoparticles was further analyzed using transmission electron microscopy, as shown in fig. 3.

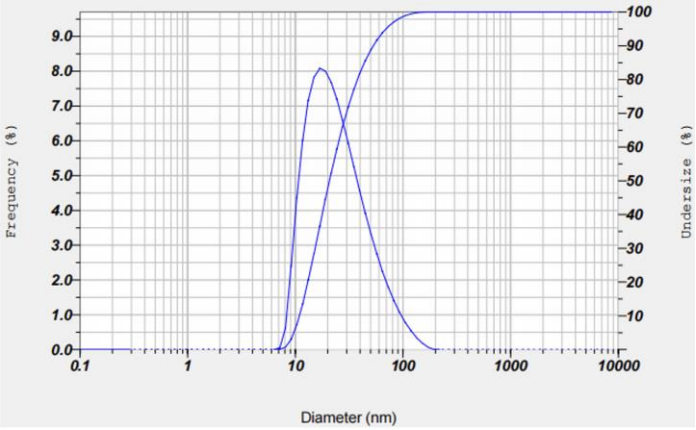

Fig. 2: Particle size distribution shows average size of NPZAE$_{50\%}$ was 27.5 nm (n = 3)

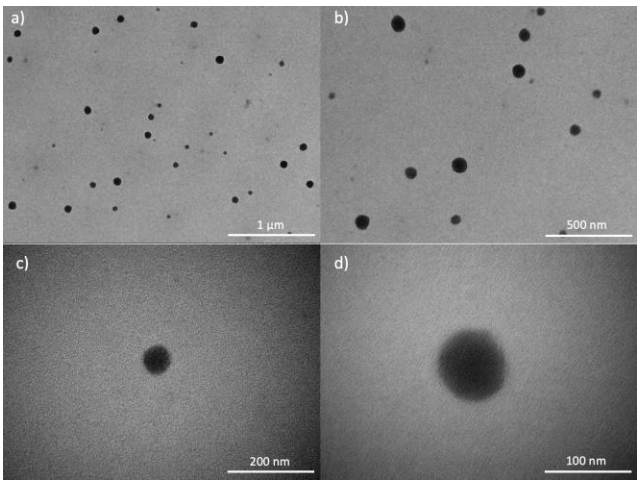

Fig. 3: TEM micrographs of NPZAE$_{50\%}$ at magnifications of (a) 10,000 (b) 20,000 (c) 50,000 and (d) 100,000 times shows the particle shape of NPZAE$_{50\%}$ were spherical

In fig. 3. A and 3. B, the particle sizes appear to be different. This variation could be attributed to the non-uniform distribution of particles, which is supported by the PSA characteristics with a Polydispersity Index (PI) of 0.372. However, when observed on a scale of 1 µm and 500 nm, it is evident that the particle shape of NPZAE$_{50\%}$ were spherical.

NPZAE$_{50\%}$ and extract of A. indica. l functional group

FTIR characterization was carried out to determine the functional group of NPZAE$_{50\%}$ and extract of *A. indica*. L. NPZAE$_{50\%}$ FTIR spectrum and *A. indica*. l extract can be seen in fig. 2.

The spectra of the nanoparticles and extracts exhibited peaks at 3330 cm^{-1} and 3343 cm^{-1}, corresponding to C-H and O-H bonds with strong intensity and N-H amines with moderate intensity. A slightly shift in these peaks was observed in both spectra. The FTIR spectrum also revealed peaks at 2978.14 cm^{-1} to 2900 cm^{-1}, indicating O-H stretching of carboxylic acids and vibrations of C-H bonds. No shift in the IR wave was observed at 2978.14 cm^{-1}. Peaks associated with aromatic rings were observed at 1464.96 cm^{-1} and 1453.39 cm^{-1}, representing aromatic C-H bonds.

At 2331.98 cm^{-1}, a peak corresponding to C≡N bonds was observed in NPZAE$_{50\%}$, indicating the presence of less stable bonds. This peak

is specific to NPZAE$_{50\%}$ and suggests protein binding in the colloid. Another peak at 1085.94 cm^{-1} indicated the presence of C-N aliphatic amines with varying bonds and C-O bonds in esters, ethers, alcohols, and carboxylic acids.

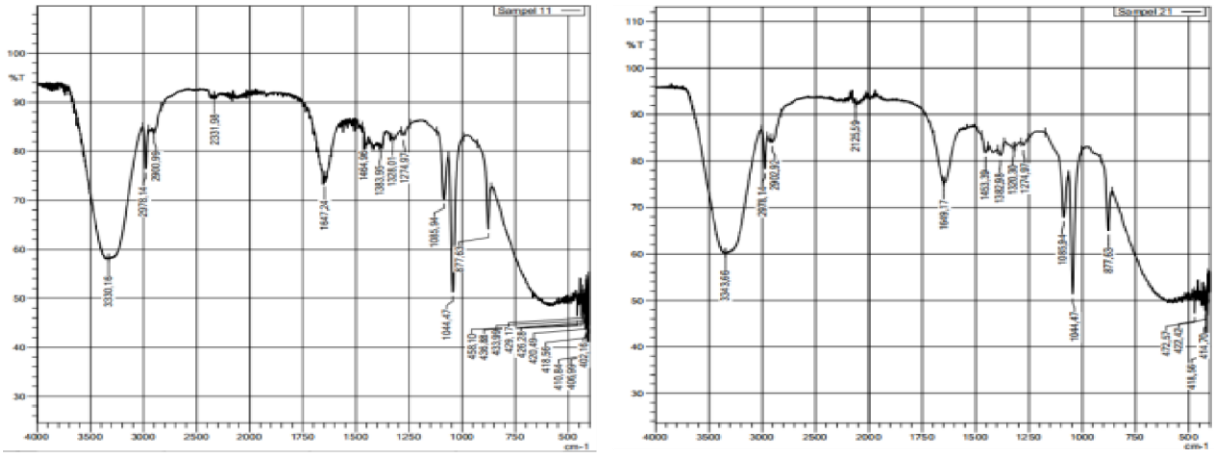

Fig. 2: FTIR spectrum of (a) NPZAE$_{50\%}$ and (b) extract of *A. indica*. L

DISCUSSION

In this study, the yield of the methanol extracts obtained was higher than ethanol 50% extract. Ethanol 50% has ability to extract both polar and semi-polar compounds. Methanol is more affordable and being more polar due to its smaller molecular size, can extract a broader range of highly polar compounds, such as low molecular weight phenolics. Although methanol is a toxic solvent, subsequent separation or evaporation of methanol can reduce the potential hazard. Methanol is generally used as the initial solvent, and the resulting extract is then further processed to reduce the remaining methanol content. Maceration method was performed at room temperature to prevent the degradation of bioactive compounds caused by exposure to high temperatures [29].

An increase in colloids formed leads to a higher intensity of transmitted light. NPZA is a colloidal solution with nano-sized particles. When a light beam interacts with colloidal particles, some light is transmitted, while some is scattered randomly in all directions by the particles. Thus, the intensity of scattered light can indicate the particle amount in the medium [30, 31].

The formation of NPZA occurs due to the ability of zein to absorb extracts facilitated by the cavities on the zein surface [23]. Zein has a wide range of surface functional groups, allowing the protein to interact with a variety of substances. It also allows for the encapsulation of hydrophobic and hydrophilic bioactive compounds into micro and nanostructures [32]. As the material is scaled down to the nanosized, there is a significant increase in the surface area-to-volume ratio [33]. According to Li, the interaction between active ingredient and NPZ, as observed through FTIR measurements, involves molecules binding to the surface of NPZ through hydrogen bonds and electrostatic interactions [34]. Zein's hollow surface acts as a host for the *A. indica* L. extract, with the extract adhering and adjusting to the zein surface. The formation of nanoparticles involves pore interaction, and to stabilize the interfacial bond between zein and the extract, tween 20 was added [23]. The formation of NPZA colloids is expected to enhance the effectiveness of *A. indica* L. leaf extract, as demonstrated by comparing the antioxidant capacity of NPZA with that of the *A. indica* L. leaf extract alone.

The DPPH assay is commonly used to determine the ability to scavenge free radicals and total antioxidant capacity. The antioxidant capacity of the *A. indica* L. methanol extract was lower compared to the ethanol extract. This difference can be attributed to the polarity of the two solvents used. Methanol is more polar than ethanol 50%. Similar findings have also been reported in several previous studies [35, 36]. The differences between the results of this study and other studies may attributed to variations in the plant matrix, solvent choice affecting extract composition and antioxidant activity, the presence of phenolic and flavonoid compounds with more hydroxyl groups, extraction methods and conditions like temperature and duration [37]. The significantly higher antioxidant capacity also observed in NPZAE$_{50\%}$ formulas compared to NPZAM ($p<0.05$). This may be attributed to the type of extract used in the formulations. NPZAE$_{50\%}$ utilizes an ethanol extract, which, based on the results of this study, contains higher antioxidant capacity compared to the methanol extract used in NPZAM.

In this study, the highest antioxidant capacity was found in NPZA formula 20, which was measured at 0.935±0.15 mmol AAE/ml for NPZAE$_{50\%}$ and 0.865±0.03 mmol AAE/ml for NPZAM, were found to be higher compared to *A. indica* extract alone. Similarly, previous research reported an increase 110% in the antioxidant activity of Jambolan extract nanoencapsulated with whey proteins and pectin. This enhancement in antioxidant activity could be attributed to the increased surface area resulting from the smaller particle sizes achieved through nanoencapsulation. This process facilitates greater chemical interactions, stabilizes active compounds, and improves their dispersibility, bioaccessibility, and bioavailability [38].

Upon analyzing the results using the Design Expert software, the optimum value for antioxidant capacity has not been achieved. This can be observed in fig. 1, where the antioxidant capacity for both types of nanoparticles show a potential for optimization with an extract amount of 117 mg, zein amount of 175 mg, and sonication time of 23 min. However, it is noteworthy that in the laboratory setting, the highest observed antioxidant capacity was obtained from formula 20, which had a total extract amount of 100 mg, zein amount of 150 mg, and a sonication time of 20 min. High antioxidant capacity was obtained because of the greater amount of zein and extract compared to other formulas. With a large amount of zein, the extract will be absorbed more on the surface of the zein. Molecular interactions between zein and bioactive compounds in the extract can increase the stability and potential antioxidant capacity of NPZA [32]. A similar result has been observed in thymol-loaded NPZ, where the inhibition of DPPH radicals increased along with increasing the concentration of thymol within the nanoparticles [39].

The quadratic model used in this study explains the data variability, as evidenced by the high R^2 value (0.9239). The value of Adjusted R^2, which closely matches R^2, further indicates the stability of the model. The adequate precision was 12.3437 confirms that the model has an

appropriate signal-to-noise ratio for optimization purposes. The analysis showed that *A. indica* leaf extract is the most significant variable ($p<0.001$), followed by sonication time ($p<0.01$), and the zein amount did not significantly affect the response ($p>0.05$). These results indicate that controlling the *A. indica* leaf extract and sonication time plays a crucial role in enhancing the antioxidant capacity.

Based on UV/Vis spectrophotometry results, it can be inferred that the absorption characteristics of each compound influence the UV/Visible spectrum of NPZ. Aswathy *et al.* reported the absorbance spectrum of 5-fluorouracil (5-FU) loaded biocompatible fluorescent NPZ in the range of 270 nm and particle size of 800 nm, larger than the $NPZAE_{50\%}$ size [40]. The wavelength of the spectrum is directly proportional to the particle size, with larger wavelength values corresponding to larger particle sizes. For instance, the UV-Visible absorption spectrum of *A. indica*-mediated copper oxide nanoparticles exhibited an optical absorption range around 220 nm, with a particle size of 26-30 nm [41].

According to Zhao *et al.*, nanoparticles typically range from 1 to 100 nm [42]. Spasojevic *et al.* investigated zein and zein/rosin nanoparticles interacting with polyanion gum arabic, resulting in particle sizes of 200 nm [43]. Luo *et al.* reported zein particles with diameters up to 364 nm, which are larger than observed in the present study. This difference can be attributed to the higher concentration of zein used in previous study (5 mg/ml) compared to that utilized in this research (1 mg/ml) [39]. This finding aligns with the research conducted by Zhang *et al.*, that characterized zein-sodium caseinate nanoparticles and observed spherical particle shapes with size of 247 nm. Similarly, lecithins-NPZ obtained were spherical, with particle sizes ranging from 160-180 nm [44].

The increase in particle size can be influenced by the increasing concentration of zein in the solvent phase. Higher solute concentrations lead to an increased nucleation rate; however, excessively high concentrations can result in core aggregation, subsequently forming larger particles [45]. In this study, the particles showed well-defined spherical morphology. The particle size distribution, as observed in fig. 3, aligns with the polydispersity index (PI) data obtained through Dynamic Light Scattering (DLS). The nanoparticles produced showed no evidence of particle aggregation, which is a common occurrence during the lyophilization process to produce dry powder. However, the stability of the nanoparticles over time was not evaluated in this study. Future studies should include zeta potential analysis and long-term stability testing to assess the colloidal stability of the formulation under various conditions. These analyses are essential for confirming the long-term stability and ensuring the effectiveness of NPZA for practical applications.

The shift in the wavenumber 3330 cm^{-1} of the O-H and 1647 cm^{-1} of the amide bonds in NPZA observed in the FTIR analysis suggests the occurrence of hydrogen bond interactions during nanoparticle formation. Zhao *et al.* reported similar findings in NPZ-stabilized Pickering emulsions (ZPE) containing gallic acid [42]. Additionally, according to Padua *et al.* zein fractions are amphiphilic, containing both hydrophobic (leucine, alanine, proline) and hydrophilic (glutamine) amino acids, which may contribute to the formation of NPZA through hydrophobic interactions [46].

CONCLUSION

Based on the obtained research results, it can be concluded that NPZA can be successfully produced using *A. indica* L. extract and zein, resulting in colloidal compounds capable of transmitting laser light. The formation of NPZA is facilitated by zein, which acts as a carrier agent, and tween 20, which serves as a colloid stabilizer. The highest antioxidant capacity values were observed in formula 20 for $NPZAE_{50\%}$ (0.935±0.15 mmol AAE/ml) and NPZAM (0.865±0.03 mmol AAE/ml). The optimization results from Design Expert 7.0.0 software indicated that the optimal antioxidant capacity could be achieved with an extract amount of 117 mg, zein amount of 175 mg, and sonication time of 23 min. Characterization of NPZA using UV/Vis spectrophotometry revealed absorption peaks at wavelengths of 230 nm and 240 nm for $NPZAE_{50\%}$. FTIR analysis indicated a shift in wave number, indicating the occurrence of chemical reactions during NPZA formation. The particle size distribution, as determined by PSA, was found to be 27.5 nm, and the particle morphology observed through TEM exhibited a spherical shape. Further research needs to be conducted to evaluate their stability over extended periods, along with more *in vitro* studies to evaluate other bioactivities for therapeutic applications.

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AUTHORS CONTRIBUTIONS

R. R., Y. Y., and I. B. conceptualized and designed the study. F. I. and D. H. conducted the experiments and collected the data. F. I., D. H., and U. D. S performed data analysis and interpretation. D. H. and R. R. were responsible for manuscript writing and editing. R. R., Y. Y., and I. B. provided supervision of the manuscript.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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