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Original Article

NANOENCAPSULATION OF ETHANOL EXTRACT OF PAPAYA LEAF (*CARICA PAPAYA* LINN.) USING CHITOSAN AND TESTING ITS EFFECTIVENESS AS AN ANTI-INFLAMMATORY

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ABSTRACT

Objective: Papaya is a plant typical of West Kalimantan which has many properties such as anti-inflammatory, analgesic, antimalarial, and antibacterial. This research aims to formulate ethanol extract of papaya leaves into a nanoencapsulated preparation and test its effectiveness as an anti-inflammatory.

Methods: Nanoparticle formulations made with the ionic gelation method use polymer chitosan (0.25%-1%) with crosslinker sodium tripolyphosphate (0.25%). Nanoencapsulation ethanol extract of papaya leaf was evaluated for characteristics including particle size distribution, index polydispersity, zeta potential, particle morphology, and entrapment efficiency. Furthermore, The efficacy of anti-inflammatory nanoencapsulation was then evaluated on male Wistar rats with carrageenan-induced inflammation using doses of 100 mg/kg and 200 mg/kg. The assessment of anti-inflammatory activity utilized the Rat hind paw edema method by observing the development of inflammation in the volume of the soles of the test animals' paws.

Results: The results of nanoencapsulation characterization showed that papaya ethanol extract in Formula 1 with a ratio of Chitosan: Papaya Leaf Extract Ethanol: NaTPP = 6:1:1 was the best formula, exhibiting an average particle size of 217.3±47.8 nm, a polydispersity index value of 0.271, a zeta potential value of+34.3 mV, an entrapment efficiency value of 65.54, and a particle morphology that is less spherical. The test for anti-inflammatory activity of papaya leaf ethanol extract nanoparticles, administered orally at a dosage of 200 mg/kgBW, demonstrated the highest percentage of anti-inflammatory efficacy at 61.538%. In comparison, the positive control group (diclofenac sodium) exhibited 54.325%, and the low-dose group (100 mg/kgBW) showed 51.585%. The results showed that the ethanol extract of papaya, when nanoencapsulated in chitosan nanoparticles, exhibits good characteristics and has significant potential for inhibiting inflammation in male Wistar rats induced by carrageenan.

Conclusion: The characterization results of the optimal chitosan-ethanol papaya leaf extract nanoparticles were obtained using Formula 1. Nanoparticles of chitosan-ethanol extract from papaya leaves at doses I and II exhibited anti-inflammatory activity that was not significantly different.

Keywords: Anti-inflammation, Ethanol extract of papaya leaves, Chitosan-tripolyphosphate, Nanoencapsulation

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INTRODUCTION

Inflammation is a normal response caused by the body's defenses to eliminate pathogens, prevent the spread of tissue damage, and repair tissue damaged due to pathological symptoms of a disease [1]. One of the plants that has been studied as having anti-inflammatory activity is papaya (*Carica papaya* L). The biochemical substances contained in papaya are flavonoids, alkaloids, tannins, saponins, polyphenols, and terpenoids [2-6].

Flavonoid compounds have been proven to show anti-inflammatory effects by inhibiting the oxidation of arachidonic acid to endoperoxide and reducing the activity of lipoxygenase and cyclooxygenase enzymes. This prevents the formation of leukotrienes and prostaglandins, which are mediators of inflammation [7]. In the body, the bioavailability of flavonoids is also very low due to enzymatic degradation and breakdown by intestinal microorganisms, making them less effective for oral use [8].

One way to overcome this problem is by formulating it into a nanoparticle preparation, specifically by encapsulating it using a polymer [9]. One of the drug carrier matrix materials used in nanoparticle technology is chitosan. Chitosan is selected to enhance penetration, extend contact duration, and boost effectiveness. The preference for chitosan in nanoparticle form is due to its biocompatibility, biodegradability, low toxicity, and mucoadhesive properties [10]. In its encapsulated form, the particles of the active ingredient can be easily absorbed by the walls of the small intestine, thereby enhancing their bioavailability [11, 12]. Nanoencapsulation is a reservoir system in which polymers surround the active drug ingredients. Nanoparticles are considered highly promising carriers for enhancing the bioavailability of biomolecules due to their improved diffusion and penetration abilities into the mucus layer [13].

The production of nanoparticles through plant-mediated methods is uncomplicated, readily accessible, cost-effective, and easily scalable. The metabolites and phytochemicals can be added, such as terpenoids, alkaloids, flavonoids, proteins, peptides, and tannins from plant leaf extracts to enhance the biosynthetic of nanoparticles [14].

Ionic gelation is one of the methods used in the preparation of nanoparticle formulations. The principle of this method involves the occurrence of ionic interactions between the positively charged amino groups in chitosan and the negatively charged polyanions. The most widely used polyanion crosslinker is sodium tripolyphosphate because it is non-toxic and multivalent [12].

This research aimed to formulate an ethanol extract of papaya leaves into a nanoparticle preparation using the ionic gelation method. The study involved both physical and chemical evaluations based on predetermined parameters. Additionally, its anti-inflammatory effectiveness was measured on male rats using the rat hind paw edema method.

MATERIALS AND METHODS

Materials

The materials used in this research include young papaya leaves (*Carica papaya* L.), diclofenac sodium 150 mg, CMC 0.5%, technical ethanol 70%, ethanol 96% p. a., magnesium powder, AlCl3 solution, distilled water (aquadest), aluminium foil, chitosan plates (Biotech Surindo), Na-TPP, and acetate buffer at pH 4.

Instruments

The equipment used includes glassware (Pyrex), blender (Linqi type FZ-10), rotary evaporator (Heodolph type Hei-VAP), spectrophotometer UV-Vis (Shimadzu type 2450), analytical balance

(Precisa type XB 4200C and BEL type M254Ai), centrifuge, PSA (Beckman colter delsaTM nano), scanning electron microscopy (SEM), and magnetic stirrer.

Plant determination

Papaya leaf collected from Pontianak, West Borneo. Papaya leaf (*Carica papaya*) was determined at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, West Kalimantan with number 076/A/lB/FMIPA/UNTAN/2015.

Collecting and extraction of papaya leaf

The samples used were papaya leaves in good condition, specifically undamaged, dark green, and not yellow. These leaves were collected during the dry season and when the photosynthesis process is at its peak, typically after 10 am, as they exhibit the highest secondary metabolite content [15]. After collection, the leaves were wet-sorted and cleaned of dirt by washing them with clean running water and draining them. Subsequently, the papaya leaves were air-dried in an open place, shielded from direct sunlight. Once almost dry, the leaves were chopped and further dried using an oven at a temperature of approximately 40 °C for 2 d. This drying process aims to reduce water content, preventing the growth of fungi, mold, and bacteria. Additionally, it deactivates enzymes that could decompose the metabolites and maintains sample stability for long-term storage. The dried papaya leaves were then crushed using a blender and stored in a dry container to prevent damage and contamination.

375 grams of papaya leaf simplicial were mixed with a 70% ethanol solvent until submerged and left for 24 h. The obtained filtrate was then collected, and the remaining residue from the filtration was soaked again with a new solvent [16]. The ethanol extract of papaya leaves was concentrated using an evaporator at a temperature of 60 °C with a speed of 100 rpm and water bath until a thick extract was obtained [17].

Drying shrinkage

One gram of extract was weighed and placed in a covered crucible that had been preheated to 105 °C for 30 min and previously tared. The used extract was then transferred to a crucible and heated in an oven at a temperature between 100 °C and 105 °C for an additional 30 min. After heating, it was weighed. This heating and weighing process was repeated until a constant weight of less than 0.50 mg was obtained for each gram of substance used [18]. The remaining solvent in the extract can be calculated using the following formula:

$$\text{Drying Shrinkage} = \frac{A-B}{A} x100$$

Information:

A = Weight before heating

B = Weight after heating

Phytochemical screening

The phytochemical screening consists of tests for alkaloids, flavonoids, saponins, steroids, triterpenoids, phenols, and tannins [19].

Determination of ethanol soluble essence content

1 g of extract (W0) was macerated with 20 ml of 96% ethanol for 24 h using a bottle or container while being shaken repeatedly for the first 6 h and left undisturbed for the remaining 18 h. It was quickly filtered to prevent ethanol evaporation. Subsequently, the crucible containing the residue was heated at a temperature of 105 °C until a constant weight was achieved (W1) [20].

$$\text{Ethanol soluble extract content} = \frac{W1}{W0} x\ 100\%$$

Description:

W0 = Initial weight of the extract

W1 = Constant weight of the crucible+sample after drying

Making chitosan nanoparticles-papaya leaf extract

The chitosan solution was prepared with a concentration ranging from 0.25% to 1%, dissolved in acetate buffer at pH 4. Simultaneously, a 0.25% Na-TPP solution was created by dissolving Na-TPP in distilled water. Subsequently, 0.16 grams of papaya leaf extract were combined with the 0.25% Na-TPP solution through dripping and stirring, employing a magnetic stirrer for one hour. Afterward, the mixture of papaya leaf extract and tripolyphosphate was gradually added drop by drop to the chitosan solution, which had a concentration varying from 0.25% to 1%. This addition took place at room temperature under the rotation of a magnetic stirrer, operating at a speed of 1500 rpm for three hours until a nanoparticle suspension was formed [21].

Table 1: Nanoencapsulation formula of chitosan-papaya leaf ethanol extract [18]

Material	Formula (% w/v)			
	F1	F2	F3	F4
EPLE*(grams)	0.16	0.16	0.16	0.16
Chitosan (%)	0.25	0.50	0.75	1.0
NaTPP (%)	0.25	0.25	0.25	025

Description: F1 = Chitosan (0.25%); F2 = Chitosan (0.50%); F3= Chitosan (0.75%); Chitosan (1.0%); EPLE =Ethanol Papaya Leaf Extract; Na-TPP = Natrium Tripolifosfat.

Nanoparticle characterization

Nanoparticle characterization included particle size, zeta potential, adsorption efficiency, and nanoparticle morphology [22]. Characterization to determine the particle size, zeta potential, and polydispersity index of nanoencapsulated powder was conducted using a particle size analyzer (PSA). Particle size was examined using the dynamic light scattering (DLS) technique, with parameters such as mean particle diameter (ZAve) and polydispersity index (PI). Zeta potential was measured using the laser Doppler electrophoresis (LDE) method.

Determination of total flavonoids

The nanoparticles were centrifuged at a speed of 10,000 rpm for 15 min. The filtrate was collected and soaked in 100 ml of 95% ethanol for 30 min. The absorption was measured using a UV-Vis Spectrophotometer with standard quercetin at a maximum wavelength of 256 nm. The flavonoid content in chitosan-papaya leaf ethanol extract nanoparticles can be determined using the equation derived from the quercetin calibration curve in ethanol [23]. The absorption efficiency of extracts by nanoparticles can be calculated using the following formula:

$$\text{Entrapment efficiency} = \frac{A-B}{A} x\ 100\%$$

Information:

A = Flavonoid content of the extract

B = Flavonoid content of nanoparticle

Dose papaya leaf ethanol extract

The dose of papaya leaf ethanol extract (*Carica papaya*) used for rats based on the literature is 100 mg/kgBW and 200 mg/kgBW. In the study, the doses of papaya leaf ethanol extract were used:

Extract dose 1 = 10 mg/100gBW of the rat

Extract dose 2 = 20 mg/100GBW of the rat

Dose chitosan nanoparticles-ethanol extract of papaya leaves

Doses of chitosan-papaya leaf extract nanoparticle

The doses of chitosan-papaya leaf extract nanoparticles used in this study are:

Nanoparticle dose 1 = 10 mg/100gBW of the rat

Nanoparticle dose 2 = 20 mg/100 gBW of the rat

Anti-inflammatory effect testing

The test of anti-inflammatory activity was conducted using the rat hind paw edema method, which involves inducing inflammation in the paw of the test animal using carrageenan as the inductor substance. This method was chosen because inflammation or edema is a symptom that can be used as a parameter to measure the anti-inflammatory potential of a compound [24]. This research has been approved by the ethical committee of the Medical University of Tanjungpura with No. 4598/UN22.9/TA/2015

The test animals used were male white Wistar rats. A total of 21 male Wistar white rats were divided into 7 groups, including a negative control group with the extract given as a 0.5% CMC suspension (K1), another negative control group with nanoencapsulation given as chitosan-NaTPP at 0.25% (K2), a positive control group with the extract given as a suspension of diclofenac sodium at 13.5 mg/kgBW (K3), an ethanol extract group of papaya leaves with dose I (K4), ethanol extract group of papaya leaves with dose II (K5), a nanoencapsulation group of ethanol extract of papaya leaves with dose I (K6), and nanoencapsulation group of ethanol extract of papaya leaves with dose II (K7). Before testing, the animals were acclimatized in the experimental room for around 1 w in individual cages [25]. The room used for testing should meet the requirements for temperature, humidity, light, and noise in accordance with the living needs of the test animals. Specifically, the room temperature should be set to 22 °±3 °C, with a relative humidity of 30–70%, and it should have 12 h of bright light and 12 h of darkness. Additionally, the room must always be kept clean, and the animals are to be provided with food and drink according to standards [26]

After 30 min of oral administration of the compound in both control and treatment groups, 0,1 ml of carrageenan suspension 1% was injected subplantar into the left hind of the rat. Inflammation in the left hind paw of rats was observed for 360 min at 30-minute intervals. The volume of inflammation is the difference between the volume of the rat's paw after being injected with carrageenan solution 1% and the volume of the rat's paw before being injected with the carrageenan solution. The percentage of inflammation at each time is determined using the following formula [27]:

$$\% \text{ inflammation} = \frac{Vt - Vo}{Vo} \text{ x } 100\%$$

Description:

Vt= volume of the rat's paw at time (t)

Vo= volume of rat's paws before carrageenin injection

After obtaining the inflammation percentage curve over time, the AUC (Area Under Curve) 0-360 for each individual is calculated using the formula:

$$AUC_{0\text{-}360} = \frac{V0+V30}{2}(t30 - t0) + \frac{V30+V60}{2}(t60 - t30) + \cdots + \frac{V330+V360}{2}(t360 - t330)$$

From the $AUC_{0\text{-}360}$ value in each group, the percentage of anti-inflammatory power can be calculated using the formula:

$$\% \text{ Anti-inflammatory power} = \frac{AUCk - AUCp}{AUCk} \text{ x } 100\%$$

Description:

AUC_P = Area under the curve of the inflammation percentage over time for the average treatment group [28].

Data analysis

Data analysis was performed statistically using the One-Way ANOVA (Analysis Of Variance) test. If there was a significant difference, it was followed by LSD (Least Significant Difference) testing ($p<0.05$). To assess the analgesic effectiveness, a comparison of the number of writhes between group dose I of extract and nanoparticle extract, as well as group dose II of extract and nanoparticle extract, was statistically analyzed using the T-test.

RESULTS AND DISCUSSION

Collecting and extraction of papaya leaf

The results of plant determination conducted in the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University on the plants indicated that the plant used is indeed the papaya plant (*Carica papaya* L.).

The initial stage carried out in this research was extracting papaya leaves using the maceration method. Maceration was chosen as the extraction method because it is used to extract heat-sensitive compounds. Additionally, the extract yield obtained is greater compared to other extraction methods, such as soxhlet extraction. [29]. The solvent used was 70% ethanol. Ethanol was chosen as the solvent for extraction because the compounds to be extracted are phenolic compounds, which are polar in nature. The extraction of phenolic compounds from plant tissues in the form of glycosides is carried out using methanol or ethanol as a solvent at room temperature through maceration. The yield of the extract resulting from the maceration process was 24.15% w/w.

Examination of papaya leaf extracts characteristics

Determination of drying loss aims to determine how many substances in the extract evaporate after heating to a temperature of 105^0C. The assessment of drying loss was employed to assess both the water content and solvent remaining in the extract, consequently identifying the extract group. According to the conducted test, the obtained drying loss percentage was 27.94%±0.2228, n=3. This percentage indicates that the extract used in this study is categorized as a thick extract, as the drying loss value is less than 30.00% [27].

Phytochemical screening is used to qualitatively detect the presence of secondary metabolite compounds in plants that exhibit biological activity. Identification of chemical compounds in the extract was carried out using the color reagent method with a tube test. The results of phytochemical screening showed that papaya leaf extract tested positive for alkaloids, flavonoids, steroids or terpenoids, and phenolics but tested negative for saponins and tannins [6].

Nanoparticle formulation results

The initial preparation of nanoparticles involved observing the presence of precipitates influenced by the duration of stirring to assess the stability of the nanoparticle suspension. Acetic acid with a pH of 4 was used as a solvent for chitosan because chitosan can only be fully protonated in solvents with an acidic pH range of less than 6.5 [30]. The formulation of extract nanoparticles was carried out with a sequential ratio of chitosan, Na-TPP, and extract, respectively, in a 6:1:1 ratio. The extract used had a concentration of 160 mg/ml. The formulation of nanoparticles is carried out by mixing the extract into a tripolyphosphate solution, where the extract is gradually dripped using a syringe with the assistance of a magnetic stirrer at a speed of 1500 rpm for 1 hour. Subsequently, the mixture of the extract and tripolyphosphate is added to a chitosan solution in a similar manner, using a magnetic stirrer at a speed of 1500 rpm for 3 h.

Nanoparticle characterization

Physicochemical characterization of nanoparticles is important because the physicochemical properties of nanoparticles not only influence the process of absorption and release of active substances but also determine how the particles interact with biological components such as proteins and membranes of the tissue where the nanoparticles work. Physicochemical characterization of nanoparticles, as the main drug carriers, includes assessing their shape, size, surface properties, and entrapment efficiency.

Table 2: Characterization results of chitosan-ethanol papaya leaf extract nanoparticle

Formulas	Chitosan: Na-TPP (6: 1)	Nanoparticle characterization results			
		Particle size (Nm)	PI	Zeta potential (Mv)	Entrapment efficiency (%)
1	0.25 %: 0.25%	217.3±47.8	0.271	+34.3	66,285%±1.477
2	0.5 %: 0.25%	357.0±54.5	0.293	+35.7	67,262%±0.362
3	0.75 %: 0.25%	599.7±103.6	0.229	+32.1	68,367%±0.648
4	1 %: 0.25%	648.2±170.8	0.287	+33.6	71,657%±1.122

Description: Na-TPP = Natrium Tripolifosfat; PI = Polydisperse Index. (Results are expressed as a mean±SD, n=3)

The particle size produced in Formula I is 217.3 nm. These results indicate that the particle size formed meets the criteria, as the size of nanoparticles suitable for drug delivery systems is<300 nm [31]. Additionally, the concentration of chitosan used influences the particle size produced; specifically, a higher concentration of chitosan leads to a larger particle size. This observation is consistent with Moura's research, which asserts that an increase in chitosan concentration will result in an increase in the size of the formed particles. The smaller particle size can enhance the solubility and absorption of the drug; thus, it is expected that its bioavailability will also increase.

The Polydispersity Index (IP) is a value that indicates the breadth of particle size distribution within a formulation. An Polydispersity Index (IP) value less than 0.4 indicates a narrow particle size distribution, while a value greater than 0.4 suggests a broad distribution [32]. The Polydispersity Index (IP) values generated from the four formulas show that the particle size distribution produced is monodisperse, signifying a uniform particle distribution in all four formulas, with IP values falling within the range of 0-0.3. Particle uniformity can influence the solubility and absorption of active substances.

The zeta potential value of nanoparticles is generally used to characterize the surface charge properties of nanoparticles. A particle is considered stable if it has a zeta potential value outside the range of±30 mV. The results of the zeta potential testing indicate that all four formulas carry a positive charge. This is linked to the type of mechanism involved in the ionic gelation nanoparticle formation, where the positive charge from the amino groups of chitosan is neutralized through interaction with the negative charge from the polyanion sodium tripolyphosphate. The residual positive-charged amino groups from chitosan result in a positive zeta potential value. The higher the zeta potential value, the more stable the formed nanoparticles. This effect is associated with the binding of anionic groups by the long amino groups of chitosan to maintain a high electric value, thus preventing aggregation [33]. The measurement results indicate that all four formulas exhibit good physical stability with zeta potential values>±30 mV. The concentration of chitosan can influence the stability of a particle, where a high concentration causes the -NH3 groups to neutralize on the surface, resulting in increased electrostatic forces between particles, making them more stable.

The entrapment efficiency was determined by comparing the total flavonoid content in the ethanol extract of papaya leaves with the free flavonoid content present in the nanoparticle formulation of chitosan-ethanol papaya leaf extract. Quercetin was used as the reference standard, chosen for its relevance to the flavonoid content found in papaya leaves, specifically flavonols.

The flavonoid content in the ethanol extract of papaya leaves was calculated using the equation Y = 0.0141x+0.1471, derived from the quercetin standard curve measured at a wavelength of 255.1 nm and subsequently verified. The results of the absorption efficiency for the chitosan-ethanol papaya leaf extract nanoparticle formulations, encompassing all four formulas, indicate that nanoparticles utilizing chitosan and NaTPP as polymers exhibit a strong capacity for absorbing active substances. The absorption rate exceeds 60% and approaches 100% [34].

The morphology of the formed nanoparticle preparations was examined using a scanning electron microscope (SEM). Based on the characterization results, F1 was identified as the optimal formula due to its particle size. Consequently, the morphology of the nanoparticle in F1 was observed. The results of the observation indicate that the morphology of the papaya leaf extract nanoparticle is irregular.

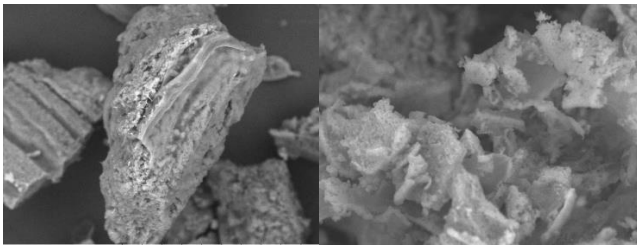

Fig. 1: Nanoparticle morphology results using SEM at 1000x and 1800x magnification

Anti-inflammatory activity test on extract

The testing results using the hind paw edema method in each treatment group revealed a correlation between the dose and the reduction in the area under the curve (AUC) value in rats. This correlation was observed in both the positive control group and the groups receiving extract doses I and II when compared to the negative control group. These findings indicate that the administration of ethanol extract from papaya leaves and sodium diclofenac (positive control) has anti-inflammatory activity. This activity is attributed to the inflammatory response induced by subplantar carrageenan injection in rats.

Table 3: Percentage of anti-inflammatory power in the 0.5% CMC group, diclofenac sodium and extract test dose group

Group treatment	Dosage (mg/kg BW)	AUC	% Anti-inflammatory power
CMC 0.5%	-	39500.000	-
Diclofenac sodium	13.5	18041.700	54.325
Papaya leaf ethanol extract	100	19125.015	51.582
Papaya leaf ethanol extract	200	17708.360	55.169

Description: AUC = Area Under Curve; CMC = Carboxymethylcellulose.

Based on the percentage of anti-inflammatory activity and the AUC values, the relationship between the average AUC and the percentage of anti-inflammatory activity can be determined. The smaller the percentage of rat inflammation produced, the smaller the AUC value, indicating a greater potential for the test material to reduce inflammation. The group receiving dose II of the extract (200 mg/kg body weight) exhibits the highest percentage of anti-inflammatory activity at 55.169%, followed by the positive control group (sodium diclofenac) at 54.325%, and the dose I group (100 mg/kg body weight) at 51.585%.

The data obtained were statistically analyzed using a one-way analysis of variance (ANOVA) method to observe significant differences among the groups. To determine whether the data is normally distributed or not, a normality test is conducted using the One-Sample Kolmogorov-Smirnov Test.

Table 4: Normality test

One-sample kolmogorov-smirnov test		**AUC**
N		**12**
Normal Parameters[a,,b]	Mean	1966.1474
	Std. Deviation	880.73175
Most Extreme Differences	Absolute	.264
	Positive	.264
	Negative	-.144
Kolmogorov-Smirnov Z		.913
Asymp. Sig. (2-tailed)		.375
a. Test distribution is Normal.		
b. Calculated from data.		

Statistical test results show that the data is normally distributed with a significance value of p = 0.375 (p>0.05). Subsequently, proceed with the homogeneity test using the Levene Test to determine whether the obtained data is homogeneous, considering a significance value above 0.05.

Table 5: Homogeneity test

Test of homogeneity of variances			
AUC			
Levene Statistic	df1	df2	Sig.
1.255	3	8	.353

Table 6: One-way ANOVA test

ANOVA					
AUC					
	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between Groups	7050863.485	3	2350287.828	12.690	.002
Within Groups	1481709.123	8	185213.640		
Total	8532572.608	11			

The analysis yielded a ρ value of 0.002 (ρ ≤ 0.05). Consequently, Ho was rejected, indicating a significant difference in the data. Subsequently, the LSD test was conducted to determine which group exhibited a value significantly different from the others.

Table 7: LSD test

AUC			
Group	**N**	**Subset for alpha = 0.05**	
		1	**2**
Extract papaya leaf dosage II	3	17708.3600	
Diclofenac sodium	3	18041.7000	
Extract papaya leaf dosage I	3	19125.0150	
CMC 0.5%	3		39500.0000
Sig.		.986	1.000
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

The results at table 7 revealed that the negative control group differed significantly from both the positive control group (diclofenac sodium 13.5 mg/kgBW) and the extract groups at doses I (100 mg/kgBW) and II (200 mg/kgBW). However, when comparing the positive control group with the extract group at dose II (200 mg/kgBW), no significant difference was observed (p>0.05). Therefore, it can be concluded that the extract at dose II exhibits anti-inflammatory activity equivalent to diclofenac sodium.

Anti-inflammatory activity test of extract nanoparticles

Based on the percentage of anti-inflammatory activity and the AUC values, the relationship between the average AUC is inversely proportional to the percentage of anti-inflammatory activity. The smaller the percentage of rat inflammation produced, the smaller the AUC value, indicating a greater potential for the test material to reduce inflammation. The group with the highest percentage of anti-inflammatory activity is the one with nanoencapsulated ethanol extract of papaya leaves at dose II (200 mg/kgBW), which is 61.538%. Following closely is the group with nanoencapsulated ethanol extracts of papaya leaves at dose I (100 mg/kgBW) with 54.201%.

Table 8: Percentage of anti-inflammatory power in the 0.25% chitosan-TPP group and nanoparticle test dose group

Treatment group	Dosage (mg/kg BW)	AUC	% Anti-inflammatory power
Chitosan-NaTPP 0.25%	-	2934.027	-
Nanoencapsulation of Papaya Leaf Ethanol Extract	100	1343.751	54.201
Nanoencapsulation of Papaya Leaf Ethanol Extract	200	1128.471	61.538

Description: NaTPP = Natrium Tripolifosfat; AUC = Area Under Curve. The obtained data were statistically analyzed using a one-way analysis of variance (ANOVA) method to observe significant differences among the groups.

Table 9: Normality test

One-sample kolmogorov-smirnov test		AUC
N		9
Normal Parameters[a,b]	Mean	21624.9933
	Std. Deviation	10727.25958
Most Extreme Differences	Absolute	.281
	Positive	.281
	Negative	-.164
Kolmogorov-Smirnov Z		.844
Asymp. Sig. (2-tailed)		.475
a. Test distribution is Normal.		
b. Calculated from data.		

Table 10: Homogeneity test

Test of homogeneity of variances			
AUC			
Levene Statistic	df1	df2	Sig.
.371	2	6	.705

Statistical test results show that the data is normally distributed and homogenous.

Table 11: One-way ANOVA test

ANOVA					
AUC	Sum of squares	df	Mean square	F	Sig.
Between Groups	8.403E8	2	4.201E8	31.393	.001
Within Groups	8.030E7	6	1.338E7		
Total	9.206E8	8			

The examination resulted in a ρ value of 0.002 (ρ ≤ 0.05). As a result, Ho was dismissed, signifying a noteworthy distinction in the data. Following this, the LSD test was performed to identify the group that displayed a value significantly distinct from the rest.

Table 12: LSD test

AUC			
Group	N	Subset for alpha = 0.05	
		1	2
Nanoencapsulation of Papaya Leaf Ethanol Extract Dosage II	3	13541.6500	
Nanoencapsulation of Papaya Leaf Ethanol Extract Dosage I	3	16125.0100	
Negative Control	3		35208.3200
Sig.		.680	1.000
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

It was found that the negative control group of nanoencapsulation significantly differed from the nanoextract group at dose I (100 mg/kgBW), and the nanoencapsulation group of the extract at dose II (200 mg/kgBW) showed a significant difference. Therefore, it can be concluded that both nanoencapsulation groups of the extract at dose I and dose II exhibit anti-inflammatory activity.

Table 13: Paired sample T-test papaya leaf extract dosage I and nanoparticle extract dosage I

Independent samples test										
		Levene's test for equality of variances		t-test for equality of means					95% confidence interval of the difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
AUC value	Equal variances assumed	1.565	.279	.643	4	.555	250.00033	388.54907	-828.78484	1328.78551
	Equal variances not assumed			.643	2.764	.569	250.00033	388.54907	-1048.6072	1548.60796

Anti-inflammatory effectiveness of chitosan-ethanol papaya leaf extract nanoparticles in anti-inflammatory activity

The anti-inflammatory effectiveness is evaluated by comparing the average inflammation percentage of nanoparticle extracts from ethanol papaya leaf dosage I with that of ethanol papaya leaf extract dosage I, and the average inflammation percentage of nanoparticle extracts from ethanol papaya leaf dosage II with that of ethanol papaya leaf extract dosage II. The anti-inflammatory effectiveness was statistically assessed using a T-Test.

Table 14: Paired sample T-test papaya leaf extract dosage II and nanoparticle extract dosage II

		Independent samples test								
		Levene's test for equality of variances		t-test for equality of means					95% confidence interval of the difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. Error difference	Lower	Upper
AUC value	Equal variances assumed	.673	.458	1.350	4	.248	347.22567	257.17956	-366.81928	1061.27061
	Equal variances not assumed			1.350	3.697	.254	347.22567	257.17956	-390.54465	1084.99599

The results of the statistical test indicate that the nano extract group at dose I does not show a significant difference ($p<0.05$) in anti-inflammatory effects compared to the extract group at dose I. Similarly, the nano extract group at dose II does not exhibit a significant difference ($p<0.05$) in anti-inflammatory effects compared to the extract group at dose II. Based on the results of this statistical test, we can conclude that formulating papaya leaf ethanol extract into a nanoparticle preparation using chitosan polymer and Na-TPP is no more effective than using the papaya leaf ethanol extract without nano encapsulation.

CONCLUSION

Based on the conducted research, the formulation of nanoparticles from the ethanol extract of papaya leaves can be achieved using chitosan polymer and sodium tripolyphosphate as a cross-linking agent with the ionic gelation method. The optimal chitosan-ethanol papaya leaf extract nanoparticle, produced by Formula 1, exhibits an average particle size of 217.3±47.8 nm, a polydispersity index value of 0.271, a zeta potential value of+34.4 mV, an absorption efficiency of 66.54%, and irregular particle morphology. The nanoparticles, administered at two different doses (dose I: 100 mg/kgBW of rats and dose II: 200 mg/kgBW of rats), demonstrate anti-inflammatory activity comparable to that of ethanol leaf extract of papaya at the corresponding doses. Statistical tests, including one-way analysis of variance (ANOVA) and T-test, indicate no significant differences ($p>0.05$) between the two formulations.

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CONFLICT OF INTERESTS

The author declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Each author contributed equally to the designing of the study, collection of data, and drafting of the manuscript.

REFERENCES

1. Kee JL, Hayes ER. Pharmacology: a nursing process approach. Jakarta: EGC Publisher; 1993.
2. Ezekwe AS, Ify E, Osuocha UK. Hypoglycemic, hypolipidemic and body weight effects of the unripe pulp of *Carica papaya L.* using diabetic Albino rat model. J Pharmacogn Phytochem. 2014;2(6):109-14.
3. Sukadana IM, Santi RS, Juliarti NK. Antibacterial activity of triterpenoid compounds from papaya seeds (*Carica papaya L.*). Jurusan Kimia FMIPA Univ Udayana. 2008;2(1):15-8.
4. Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V. Antioxidant and anti-inflammatory activities of extracts from *Cassia alata*, *Eleusine indica*, *Eremomastax speciosa*, *Carica papaya* and *Polyscias fulva* medicinal plants collected in cameroon. PLOS ONE. 2014;9(8):e103999. doi: 10.1371/journal.pone.0103999, PMID 25090613.
5. Rehena JF. Test the activity of papaya leaf extract (*Carica papaya. Linn*) as an *in vitro* antimalarial. Faculty of Teacher Training and Education, Pattimura University; 2010;11(1):96-100.
6. Alex A. Adedapo and Vivian Eguonor, Orherhe. Antinociceptive and anti-inflammatory studies of the aqueous leaf extract of in laboratory animals. Nigeria: Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. 2013;4(1):89-96.
7. Octavianus S, Fatimawali, Widya AL. Test the analgesic effect of ethanol extract of papaya leaves (*Carica papaya L.*) on male white mice (*Mus mucculus*). Manado: FMIPA UNSRAT Pharmacy Study Program. 2014;3(2):87-92.
8. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflamm Allergy Drug Targets. 2009;8(3):229-35. doi: 10.2174/187152809788681029, PMID 19601883.
9. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci. 2004;96(3):229-45. doi: 10.1254/jphs.crj04003x, PMID 15539763.
10. Rahmat D, Widowati W, Faried A, Nainggolan IM, Priyandoko D, Budiati A. Characterization of nanoparticles conditioned medium adipose tissue mesenchymal stem cell (CM-ATMSC). Int J App Pharm. 2022;14(3):104-6. doi: 10.22159/ijap.2022.v14s3.22.
11. Kartasasmita RH, Herowati R, Harmastuti N, Gusnidar T. Quercetin derivatives docking based on study of flavonoids interaction to cyclooxygenase-2. Bandung: Indo J Chem. 2009;9(2):297-302.
12. Rakesh B, Vijay M, Avinash D. New approaches in nanoparticulate drug delivery system-a review. Int J Curr Pharm Res. 2012;4(2):29-38.
13. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA. Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. J Phys Chem B. 2006;110(14):7238-48. doi: 10.1021/jp057170o, PMID 16599493.
14. Muhaimin M, Chaerunisaa AY, Rostinawati T, Amalia E, Hazrina A, Nurhasanah S. A review on nanoparticles of *Moringa oleifera* extract: preparation, characterization, and activity. Int J App Pharm. 2023;15(4):43-51. doi: 10.22159/ijap.2023v15i4.47709.
15. Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev. 2001;47(1):39-54. doi: 10.1016/s0169-409x(00)00120-4, PMID 11251244.
16. Andersen M, Markham KR. Flavonoids: chemistry, biochemistry, and applications. Taylor & Francis Group 2; 2006. p. 3-4.
17. Yulanda H. Extraction, fractionation, and characterization of Ginger rhizome starch. Bogor: Faculty of Mathematics and Natural Sciences, Bogor Agricultural Institute; 2007.
18. Mishra M, Kumar H, Singh RK, Tripathi K. Diabetes and nanomaterials. Digest Journey of Nanomaterials and Biostructures. 2008;3:109-13.
19. Setyawaty R, Aptuning BR, Dewanto D. Preliminary studies on the content of phytochemical compounds on the skin of salak

fruit (*Salacca zalacca*). Pharm J Indones. 2020;6(1):1-6. doi: 10.21776/ub.pji.2020.006.01.1.
20. Anam S, Yusran M, Trisakti A, Ibrahim N, Khumaidi A, Ramadanil ZMS. Standardization of ethyl acetate extract of sanrego wood (Lunasia amara Blanco). Online Journal of Natural science. 2013;2(3):1-8.
21. Mardliyati E, Muttaqien SE, Setyawati DR. Synthesis of chitosan-tripolyphosphate nanoparticles using ionic gelation method: influence of concentration and volume ratio on particle characteristics; 2012.
22. Abdullah M, Khairurrijal. Review: nanomaterial characterization. J Nanosci Nanotechnol. 2009;1(2):1-9.
23. Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharm. 2005;295(1-2):235-45. doi: 10.1016/j.ijpharm.2005.01.042, PMID 15848008.
24. Ab Cong HH, Khaziakhmetova VN, Zigashina LE. Rat paw oedema modeling and NSAIDs: timing of effects. Int J Risk Saf Med. 2015;27(s1):S76-7.
25. Arts JW, Kramer K, Arndt SS, Ohl F. The impact of transportation on physiological and behavioral parameters in wistar rats: implications for acclimatization periods. ILAR J. 2012;53(1):E82-98. doi: 10.1093/ilar.53.1.82, PMID 23382273.
26. Indonesian Food and Drug Authority. BPOM Regulation Number 18 of 2021 concerning Guidelines for Preclinical Pharmacodynamic Testing of Traditional Medicines. Jakarta: Indonesian Food and Drug Authority; 2021.
27. Mansjoer S. Anti-inflammatory effect of white ginger essential oil (*Curcuma zedoaria Rosc*.) on artificial edema in male white Wistar rats. Indonesian Pharm Mag. 1997;8:35-41.
28. A'yunin Q. Anti-inflammatory effect of tapak liman (*Elephantopus scaber L.*) leaf infusion on male white rats [thesis]. Surakarta: Faculty of Pharmacy, Muhammadiyah University of Surakarta; 2004.
29. Waree T. Chitosan nanoparticles: a promising system for drug delivery. Naresuan University Journal. 2003;11(3):51-66.
30. Voigt R. Pharmaceutical technology textbook. 5th ed. Yogyakarta: UGM-press; 1995.
31. Gupta RB, Kompella UB. Nanoparticle drug delivery for technology. Vol. 159. New York: Taylor & Francis Group; 2006.
32. Moura MJ, Faneca H, Lima MP, Gil MH, Figueiredo MM. In situ forming chitosan hydrogels prepared via ionic/covalent co-cross-linking. Biomacromolecules. 2011;12(9):3275-84. doi: 10.1021/bm200731x, PMID 21774479.
33. Mohanraj VJ, Nanopartikel CY. A review Nigeria. Trop J Pharm Res. 2006;5(1):561-73.
34. Lin Y, Sonaje K, Lin KM, Juang J, Mi F, Yang H. Multi-ion-crosslinked nanoparticles with pH-responsive characteristics for oral delivery of protein drugs. J Control Rel. 2008;132(2):141-9. doi: 10.1016/j.jconrel.2008.08.020.