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Antioxidant activity and formulation evaluation of *Morinda citrifolia* L.leaf ethanol extract nanoparticles incorporated into moisturizing gel

Eriska Agustin[1*], Ranti Ayu Anjelina[1], Yola Yernianti[1]

[1] Faculty of Pharmacy, Kader Bangsa University, Palembang, Indonesia.

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Abstract: Skin is continuously exposed to environmental stressors such as ultraviolet radiation and pollution, which trigger the formation of reactive oxygen species (ROS) leading to oxidative stress, premature aging, and skin damage. Therefore, the development of topical antioxidant formulations from natural sources has become increasingly important. This study aimed to develop nanoparticles of *Morinda citrifolia* L. leaf ethanol extract and incorporate them into a moisturizing gel, followed by evaluation of their physicochemical properties and antioxidant activity. The extract was prepared by maceration using 96% ethanol, while nanoparticles were synthesized using the ionic gelation method with chitosan and sodium tripolyphosphate. Particle size, polydispersity index, morphology, and antioxidant activity (DPPH method) were evaluated. The nanoparticles exhibited sizes below 200 nm with a uniform distribution (PDI< 0.3). The optimized formulation showed the smallest particle size (FIII=77.5±6.36 nm) and good stability. Antioxidant activity increased after nanoparticle formulation, with IC_{50} values decreasing from 182.4±2.26 ppm (extract) to 145.4±6.89 ppm (FIII), indicating moderate activity. The gel formulations showed good physical stability and acceptable viscosity, although pH values were below the ideal skin range. The moisturizing test demonstrated significant improvement in skin hydration. In conclusion, nanoparticle incorporation enhanced antioxidant and moisturizing performance, indicating potential for topical applications.

Keywords: Antioxidant; Cosmetic; Gel; Moisturizer; Nanoparticle.

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Introduction

Skin is continuously exposed to environmental stressors such as ultraviolet (UV) radiation, pollution, and oxidative agents that can induce the formation of reactive oxygen species (ROS). Excessive ROS production leads to oxidative stress, which plays a critical in premature skin aging, inflammation, and various dermatological disorders. Therefore, the use of antioxidants in topical formulations has become an important strategy to protect the skin and maintain its physiological function (Putri and Sutoyo, 2025).

Natural antioxidant derived from medicinal plants have gained increasing attention due to their safety and biological activities. One of the widely used tropical plants is *Morinda citrifolia* L., which belongs to the Rubiaceae family. This plant contains various bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, which contribute significantly to its antioxidant and anti-inflammatory properties (Putri and Sutoyo, 2025; Vashti et.al, 2024). Previous studies have demonstrated that extract of *Morinda citrifolia* exhibit strong free radical scavenging activity, indicating its potential as a natural antioxidant source for

Email: eriska.agustin@gmail.com *(*Corresponding Author)*

pharmaceutical and cosmetic applications (Fitriana and Islami, 2025).

Morinda citrifolia leaves are rich in phenolic compounds, flavonoids, and anthraquinones that contribute to their pharmacological activities, particularly antioxidant effects. The antioxidant potency of Morinda citrifolia leaf extract has been demonstrated using DPPH assay, with reported IC_{50} values ranging from 200-350 ppm depending on the extraction solvent, indicating moderate to strong free radical scavenging activity (Thani et al., 2010).

Despite its promising bioactivity, the direct use of plant extracts in topical formulations is often limited by poor stability, low penetration through the skin barrier, and reduced bioavailability. To overcome the limitations, nanotechnology-based delivery systems have been widely developed. Nanoparticles offer several advantages, including increased surface area, enhanced solubility, improved stability of active compounds, and better skin penetration. Moreover, green synthesis of nanoparticles using plant extracts has been reported as an eco-friendly and efficient approach, where phytochemicals act as reducing and stabilizing agents (Sukumaran et.al, 2024; Tamilselvi et.al, 2025).

Recent studies have shown that nanotechnology can further improve the pharmacological performance of phytochemicals. The reduction of particle size into the nanometer range increases the specific surface area, resulting in faster dissolution and enhanced interaction of bioactive compounds with biological membranes. In addition, nanoencapsulation protects phenolic compound against oxidation, hydrolysis, and photodegradation, thereby maintaining their biological activity during storage. These mechanisms may enhance antioxidant efficacy through improved stability and bioavailability rather than by altering the instrinsic antioxidant capacity of phytochemicals themselves (Priya et al., 2023).

Evidance supporting this approach has been reported in several nanoparticle-based studies, for example biosynthesized gold nanoparticles prepared using Morinda citrifolia leaf extract exhibited DPPH radical scavenging IC_{50} of 24,65 ppm, representing approximately an 8-to 14 fold improvement compared with the antioxidant activity reported for convensional Morinda citrifolia leaf extract (200-350 ppm) (Thani et al., 2010; Mekala et al., 2025). Similarly, fruit extracts of Morinda citrifolia showed marked differences in antioxidant potency depending on the extraction fraction, with the ethyl acetate fraction exhibiting an IC_{50} of 46,7 ppm, whereas methanol extracts showed an IC_{50} of 888,6 ppm, demonstrating that optimization of formulation and delivery systems substantially influences biological activity (Nagalingam et al., 2022).

Topical delivery systems, particularly gel formulations, are widely preferred in dermatological and cosmetic applications due to their non-greasy nature, easy of application, good spreadability, and patient acceptability. Gel based-moisturizers not only improve skin hydration but also serve as effective carriers for active compounds. Previous studies have shown that *Morinda citrifolia* extract can be successfully incorporated into gel formulations with acceptable physical stability and pH characteristics suitable for skin application (Handayani et.al, 2020; Eelager et.al, 2024).

However, there is still limited research focusing on the incorporation of nanoparticle-based *Morinda citrifolia* leaf ethanol extract into moisturizing gel systems, particularly in relation to its antioxidant activity and formulations characteristics. Therefore, the development of such a formulation is essential to enhance the stability, efficacy, and delivery of bioactive compounds (Yulizar et.al, 2021).

Based on these considerations, this study aims to formulate nanoparticle of *Morinda citrifolia* L. leaf ethanol extract, incorporate them into a moisturizing gel, and evaluate both the physicochemical properties of the formulation and its antioxidant activity. The results of this study are expected to contribute to the development of innovative natural-based topical antioxidant products with improved performance and therapeutic potential.

Materials and Methods

1. Materials

The instruments utilized in this study included an analytical balance (Mettler Toledo® XS205), magnetic stirrer (IKA® RW 20 Digital), rotary evaporator (Heldoph® RV 10), Brookfield viscometer (Brookfield DV2T Viscometer), pH meter (Mettler Toledo® S20), Particle Size Analyzer (Delsa™ Nano C Particle Analyzer), homogenizer (Ultra-Turrax® T25 Digital), spektrofotometer UV-Vis (Shimadzu UV-1800), along with other supporting laboratory equipment.

The materials employed in this research comprised *Morinda citrifolia* leaf collected from Surulangun Rawas Village (South Sumatra, Indonesia), chitosan (Sigma-Aldrich, USA), sodium tripolyphosphate (NaTPP) (Merck), glycerin (Brataco Chemical), Carbopol (Lubrizol Corporation), triethanolamine (TEA) (Merck), methyl paraben (Merck), propyl paraben (Merck), distilled water produced in-house, and deionized water (Amidis).

2. Methods

2.1 Preparation of Morinda citrifolia leaf Samples

Fresh *Morinda citrifolia* leaves collected from Surulangun Rawas Village, Rawas Ulu District, North Musi Rawas Regency, South Sumatra, Indonesia were thoroughly washed under running water and allowed to drain, yielding an initial mass of approximately 9 kg. The leaves were subsequently air-dried at ambient temperature until a slight brownish coloration was observed, resulting in a reduced weight of about 6 kg.

The dried leaves were then separated from the midribs to obtain the leaf blades, yielding approximately 2 kg of material. The obtained leaf blades were further processed by grinding using a blender to produce approximately 1 kg of fine powder. The powder was then sieved through an appropriate 40-mesh size to ensure uniform particle distribution and to obtain a homogeneous simplicia powder.

2.2 Preparation of *Morinda citrifolia* leaf Extracts (EDM)

The extraction of *Morinda citrifolia* leaf powder (1 kg of dried simplicia) was performed using the maceration method with 96% ethanol as the extraction solvent for 3 × 24 hours. The solvent was renewed every 24 hours to ensure maximum extraction efficiency. Following the maceration process, the mixture was filtered to separate the filtrate from the solid residue.

The collected filtrate was then concentrated using a rotary evaporator at 70°C to obtain a viscous extract (Wirasti et.al, 2020). The percentage yield of the extract was subsequently calculated using the following equation:

$$Yield\ (\%) = \frac{weight\ of\ extract}{weight\ of\ dried\ simplicia} x100$$

2.3 Preparation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM)

The Chitosan solution (0.2% w/v) was prepared by dissolving 0.2 g of chitosan powder in 100 mL of 1% acetic acid, followed by stirring with a magnetic stirrer at 1000 rpm for 30 minutes until a homogeneous solution was achieved.

Sodium tripolyphosphate (NaTPP) solution (0.1% w/v) was prepared by dissolving 0.1 g of NaTPP in 100 mL of distilled water and stirred at 1000 rpm for 30 minutes to ensure complete dissolution.

The preparation of *Morinda citrifolia* leaf extract nanoparticles (NEDM) was carried out using the ionic gelation method. All components were accurately weighed according to the formulation presented in Table 1. Initially, the *Morinda citrifolia* leaf extract (EDM), as the active compound, was mixed with the chitosan solution, which functioned as a stabilizing matrix and crosslinking medium. The mixture was stirred at 1500 rpm for 30 minutes using a magnetic stirrer until a uniform dispersion was obtained.

Table 1. Formulation Design of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM)

Ingredients	Function	Comparison of Consentrations			
		F0	FI	FII	FIII
Morinda citrifolia leaf Extract (EDM)	Active ingredient	0	1	1	1
Chitosan	Stabilizing and crosslinking agent	1	5	10	15
Sodium tripolyphosphate (NaTPP)	Crosslinking agent	1	1	1	1
Tween 80	Surfactant	0,5 %	0,5 %	0,5 %	0,5 %

Subsequently, the NaTPP solution was added dropwise as a crosslinking agent under continuous stirring at 1500 rpm for 30 minutes. Tween 80 was then incorporated as a surfactant, followed by further stirring at the same speed for an additional 30 minutes to enhance system stability. Finally, the resulting suspension was subjected to ultrasonication for 60 minutes to reduce particle size and improve nanoparticle stability (Agustin and Martavinus, 2025).

2.4 Evaluation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM)

Particle Size and Globule Distribution Analysis

Particle size an globule size distribution of the *Morinda citrifolia* leaf Extract Nanoparticle (NEDM) were determined using a Particle Size Analyzer (PSA). Approximately 1 mL of the NEDM sample was placed into a cuvette, which was then positioned in the sample holder for measurement over 15 minutes to obtain particle size values and distribution curves. After each measumerement, the cuvette was thoroughly cleaned to remove any residual lipid contaminants (Adheline, 2019).

Antioxidant Activity Assay of Morinda citrifolia leaf Extract Nanoparticle (NEDM)

a. Preparation of 50 ppm DPPH Solution

A total of 50 mg of DPPH was dissolved in ethanol p.a and transferred into a 50 mL volumetric flask. The volume was adjusted to the mark with ethanol p.a to obtain a 1000 ppm stock solution. This stock solution was subsequently diluted to 50 ppm for sample test (Agustin et.al, 2023).

b. Determination of Maximum Wavelength of DPPH Solution

The maximum absorption wavelength of DPPH was determined by mixing 2 mL of 50 ppm DPPH solution with 2 mL of ethanol p.a in a test tube. The absorbance was scanned within the wavelength range of 400–600 nm using a UV-Visible spectrophotometer to identify the λmax (Agustin et.al, 2023).

c. Preparation of Vitamin C Stock Solution

A total of 50 mg of vitamin C was dissolved in ethanol p.a and diluted to 50 mL in a volumetric flask to obtain a 1000 ppm stock solution. Serial dilutions were then prepared to obtain concentrations of 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 ppm (Agustin et.al, 2023).

d. Preparation of *Morinda citrifolia* leaf Extract (EDM) Solution

A total of 100 mg of *Morinda citrifolia* leaf extract (EDM) was dissolved in ethanol and diluted to 100 mL to produce a 1000 ppm stock solution. Serial dilutions were prepared to obtain concentrations of 60, 90, 120, 140, and 160 ppm (Agustin et.al, 2023).

e. Preparation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM)

A total of 100 mg of nanoparticle formulation (NEDM) from each formulation batch was dissolved in ethanol and diluted to 100 mL to obtain a 1000 ppm stock solution. Serial dilutions were subsequently prepared to achieve concentrations of 60, 90, 120, 140, and 160 ppm (Agustin et.al, 2023).

f. Determination of Sample Absorbance

Each test solution at 2 mL specific concentration was mixed with 2 mL of DPPH solution (1:1) and vortexed until homogeneous. The mixture was then incubated in the dark for 30 minutes. After incubation, the absorbance was measured using a UV-Visible spectrophotometer at a wavelength of 517 nm (Agustin et.al, 2023). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

g. Data Analysis

The measured absorbance values were used to calculate the percentage of radical scavenging activity (% inhibition) for each concentration of extract and nanoparticle samples. The formula is :

$$Inhibition(\%) : \frac{abs\ control - abs\ sample}{abs\ control} x100$$

The IC_{50} value was determined using linear regression analysis based on the equation $y = bx + a$, where x represents the sample concentration and y represents the percentage of inhibition. Statistical analysis of the formulation characteristics was performed using analysis of variance (ANOVA).

2.5 Preparation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM) Gel

The formulation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM) Gel is presented in Table 2.

Carbopol (1.5%) was first dispersed in distilled water as the initial step in the preparation of the gel moisturizer formulation. Subsequently, triethanolamine (TEA) (0.5%) was added to neutralize the Carbopol dispersion and facilitate gel formation. The mixture was continuously stirred until a homogeneous gel base was obtained. Glycerin (15%), methyl paraben (0.2%), propyl paraben (0.18%), and *Vernonia amygdalina* leaf extract nanoparticles (NEDA) at concentrations of 0%, 0.5%, 1%, and 2% were then incorporated into the gel base. Distilled water was added to adjust the final volume to 100%, and the entire formulation was thoroughly mixed and homogenized to produce a stable gel moisturizer (Agustin and Yanti, 2024).

Table 2. Formulation of *Morinda citrifolia* leaf Extract Nanoparticle Gel (NEDM Gel)

Ingredients	Functions	Concentration (%)			
		F0	F1	F2	F3
Morinda citrifolia leaf Extract Nanoparticle (formula FIII)	Active ingredient	0	0,5	1	2
Glycerin	Humectant	15	15	15	15
Carbopol	Gelling agent	1,5	1,5	1,5	1,5
Triethanolamine (TEA)	Alkalizing agent	0,5	0,5	0,5	0,5
Methyl paraben	Antibacterial preservative	0,2	0,2	0,2	0,2
Propyl paraben	Antifungal preservative	0,18	0,18	0,18	0,18
Distilled water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

2.6 Evaluation of *Morinda citrifolia* leaf Extract Nanoparticle (NEDM) Gel

a. Organoleptic Test

The organoleptic properties of the NEDM gel moisturizer were evaluated under various temperature conditions. Room temperature (25–28°C) was used for general observation, elevated temperatures (30–40°C) were applied to simulate hot environmental conditions, and lower temperatures (15–20°C) were used to represent cold conditions. Any changes in color and odor were carefully observed and recorded (Wirasti et.al, 2020). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

b. Viscosity Test

Viscosity measurements were carried out using a Brookfield viscometer. The gel sample was placed onto a cone with a diameter of 5 cm, followed by the application of a plate to spread the sample into a thin layer. During measurement, care was taken to avoid friction between the cone and the plate to ensure accurate readings (Wirasti et.al, 2020). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

c. pH Test

Prior to analysis, the pH meter was calibrated using standard buffer solutions. Approximately 0.1 g of the gel sample was dispersed in 10 mL of distilled water, and the pH was measured. The acceptable pH range for topical formulations is between 4.5 and 6.5 (Daswi et.al, 2022). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

d. Spreadability Test

A total of 0.5 g of the gel sample was placed at the center of a graduated circular glass plate. A second transparent glass plate was then placed on top, followed by the application of an additional load to reach a total weight of 150 g. After 1 minute, the diameter of the spread gel was measured. An acceptable spreadability range for gel formulations is typically between 5 and 7 cm (Salima and Nurwaini, 2023). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

e. Moisturizing Effect Test

Environmental factors such as temperature and humidity can significantly affect the stability and performance of gel formulations. Elevated temperatures may lead to degradation of active components, while high humidity levels may promote microbial growth or induce physical instability. Therefore, the moisturizing efficacy test was conducted at room temperature (25–28°C) with a relative humidity of approximately 46–55% (Tutik et.al, 2021).

The evaluation was performed on five female volunteers aged 19–25 years with dry skin conditions using a digital skin analyzer. The test area was located on the forearm with a diameter of 4.5 cm. Prior to application, the skin was cleansed and dried thoroughly. Baseline skin hydration levels were measured and recorded. The gel formulation was then applied to the designated area and allowed to absorb. Subsequent measurements were taken after 10 minute, and the percentage of skin hydration was recorded (Tutik et.al, 2021).

Skin moisture levels were categorized as dehydrated (0–29%), normal (30–45%), and well-hydrated (50–100%). Water content plays a crucial role in determining the physical characteristics of gel formulations; higher water content generally produces softer and more flowable gels, whereas lower water content results in firmer and more rigid systems. The optimal water content for NEDM gel moisturizers typically ranges from 70% to 80% (Tutik et.al, 2021). Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

Result and Discussion

The plant material used in this study was taxonomically identified to ensure its authenticity and accuracy. The determination was conducted at the Herbarium Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung (ITB). Based on the plant identification report (No. 6738/ITI.C11.2/TA.00/2025), the specimen was confirmed as *Morinda citrifolia* L., belonging to the family Rubiaceae.

The identification was carried out based on morphological characteristics, including leaf shape, venation pattern, leaf arrangement, and other distinctive features, and further verified through comparison with standard herbarium specimens and taxonomic references.

The extraction of *Morinda citrifolia* leaves was carried out using the maceration method with 96% ethanol as the solvent. This method is widely applied in phytochemical studies due to its simplicity and effectiveness in extracting thermolabile bioactive compounds. Ethanol is considered an appropriate solvent because it can dissolve a wide range of polar and semi-polar secondary metabolites, including flavonoids and phenolic compounds, which are known to contribute significantly to antioxidant activity (Zhu et.al, 2020).

The maceration process was conducted for 3 × 24 hours with periodic replacement of the solvent every 24 hours to maintain the concentration gradient between the plant matrix and the solvent. This condition enhances the diffusion of bioactive compounds into the solvent and improves extraction efficiency. Previous studies have shown that extraction conditions, including solvent type and extraction duration, significantly influence the yield and phytochemical content of *Morinda citrifolia* leaves (Ramayani et.al, 2021).

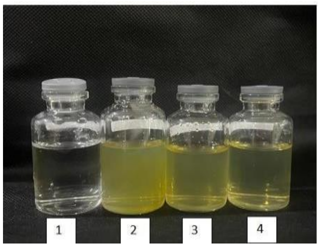

Figure 1. NEDM preparation (1) F0, (2) FI, (3) FII, (4) FIII

Particle size and polydispersity are critical parameters in evaluating nanoparticle systems, as they influence stability, drug release, and penetration ability through the skin. Based on results obtained, all formulations (F0-FIII) exhibited particle sizes within the nanometer range (<200 nm), indicating succesfull formation of nanoparticles using the ionic gelation method (Khan et.al, 2020). Among the formulations, FIII showed the smallest particle size with value of 77.5±6.36 nm on day 0 and 91.5±4.94 nm on day 14. This suggests that increasing the concentration of active components of formulation optimization contributes to reduced particle size, possibly due to enhanced crosslinking interactions between chitosan and NaTPP. Smaller particle sizes are advantageous as they provide a larger surface area, leading to improved bioavailability and enhanced skin penetration (Dash et.al, 2021)

Table 3. The results of particle size and polydispersity index analysis NEDM

Formula	**Particle Size (nm)**		**Polydispersity Index**	
	Day 0	**Day 14th**	**Day 0**	**Day 14th**
0	114±4,24	119±2,12	0.0013	0.0002
I	108±9,89	118±2,12	0.0082	0.0002
II	94,5±3,53	105±12,7	0.0013	0,014
III	77,5±6,36	91,5±4,94	0,0067	0.0028

The PDI values obtained for all formulations ranged from 0,0002 to 0,014, which indicates a highly uniform particle size distribution. The PDI below 0,3 generally reflects a homogeneous systems, while value approaching zero indicate a highly monodisperse system. Therefore, the results suggest that the prepared nanoparticles possess excellent uniformity and dispersion stability (Mohammed et.al, 2017).

The antioxidant activity of *Morinda citrifolia* leaf extract (EDM), its nanoparticle formulations (NEDM), and vitamin C as a positive control was evaluated using the DPPH radical scavenging method. The results are expressed in terms of IC_{50} values, where lower IC_{50} values indicate stronger antioxidant activity. The ethanol extract of *Morinda citrifolia* leaves (EDM) showed an IC_{50} value of 182.4±2.26 ppm, which is categorized as a weak antioxidant.

Table 4. The Result of Antioxidant Activity Test

Sample Test	The IC_{50} value (ppm)
Vitamin C (+ Control)	0.718±0.0078
Nanoparticle FI	166.3±7.29
Nanoparticle FII	146.1±1.97
Nanoparticle FIII	145.4±6.89
Extract Konvensional (extract of *Morinda citrifolia* leaf)	182.4±2.26

The nanoparticle formulations (FI–FIII) demonstrated improved antioxidant activity compared to the crude extract (EDM). Specifically, formulation FI showed an IC_{50} of 166.3±7.29 ppm (weak category), while formulations FII and FIII exhibited IC_{50} values of 146.1±1.97 ppm and 145.4±6.89 ppm, respectively, which fall into the moderate antioxidant category. This improvement indicates that nanoparticle formulation enhances the antioxidant performance of the extract (Duraisamy et.al, 2022).

Statistically analysis confirmed that these differences were significant. The normality test demonstrated that the IC_{50} values of all formulations were normally distributed ($p>0,05$), whereas the homogeneity test indicated unequal variances among the groups ($p<0,05$). Therefore, one-way ANOVA followed by the Games-Howell post hoc test was performed. The analysis revealed a statistically significant difference in IC_{50} values among the crude extract and the nanoparticle formulation ($p<0,05$), confirming that nanoparticle incorporation significantly enhanced antioxidant activity of Morinda citrifolia leaf extract.

The enhance antioxidant activity may be attributed to the physicochemical characteristics of the nanoparticle system. Reducing the particle size to the nanometer range increases the specific surface area, thereby improving the dissolution and dispersion of bioactive compounds and facilitating their interaction with free radicals. Furthermore, nanoparticle encapsulation protects phenolic and flavonoid compounds from degradation caused by oxidation and their bioavailability (Duraisamy et al., 2022; Patra et al., 2018; Danaei et al., 2018). The absence of significant difference between formulations FII and FIII would suggest that increasing the polymer concentration beyond the level used in FII did not provide additional improvement in antioxidant activity, indicating that the antioxidant enhancement may have reached an optimal level under the formulation conditions employed.

Furthermore, the ionic gelation method using chitosan and sodium tripolyphosphate (NaTPP) contributes to the formation of stable nanostructures that protect active compounds from degradation. Previous studies have demonstrated that plant extract-loaded chitosan nanoparticles significantly improve antioxidant activity compared to crude extracts, as reflected by lower IC_{50} values (Raza, 2021).

Organoleptic evaluation is a fundamental parameter in assessing the physical quality and acceptability of topical formulations. This evaluation includes observations of color, odor, and overall appearance of the gel under different storage conditions. In this study, all formulations (F0–F3) exhibited relatively stable organoleptic characteristics during storage, with no significant changes in color or odor observed under varying temperature conditions. The gel formulations generally showed a semi-solid consistency with a homogeneous appearance. The incorporation of *Morinda citrifolia* leaf extract nanoparticles (NEDM) contributed to a slightly darker coloration in higher concentrations (F2 and F3), which is attributed to the presence of phytochemical compounds such as phenolics and flavonoids. These results are consistent with previous studies reporting that Carbopol-based gels exhibit good physical stability and acceptable sensory characteristics when properly formulated (Kaur et al., 2021). The pH of topical formulations is a critical parameter that influences skin compatibility, stability of active compounds, and overall product safety (Kaur et.al, 2021; Rowe et.al, 2019).

The results indicate that all formulations exhibited acidic pH values ranging from 3.31 to 3.51. A slight decrease in pH was observed with increasing concentration of NEDM, which may be attributed to the presence of acidic phytochemical constituents such as phenolic compounds. However, the obtained pH values are below the acceptable physiological skin pH range (4.5–6.5), suggesting that the formulations may have the potential to cause skin irritation if applied directly without further adjustment. The acidic nature of the formulation is likely influenced by several factors, including the use of Carbopol, residual acetic acid from chitosan preparation, and the intrinsic acidity of plant extracts (Sahu et.al, 2020).

Previous studies have reported that Carbopol-based gel formulations typically require careful pH adjustment to achieve optimal viscosity and skin compatibility, as the polymer exhibits pH-dependent swelling behavior (Rowe et al., 2019). Additionally, plant extract-based formulations often show acidic pH due to the presence of organic acids and phenolic compounds, which contribute to antioxidant activity but may affect formulation stability (Sahu et al., 2020).

Viscosity is critical rheological parameter in topical gel formulations because it affects spreadability, adhesion, stability, and drug release. In this study, the incorporation of Morinda citrifolia leaf extract nanoparticle increased the viscosity of the gel, with F0 showing the lowest viscosity (25000 cPs) and F2 the highest (72000 cPs). All formulation fell within the acceptable viscosity range for topical gels (20000-100000 cPs), while F1-F3 were within the optimal range of 20000-80000 cPs, providing a suitable balance between spreadability and skin retentions (Kaur et al., 2021). The increased viscosity from F0-F2 is likely due to enhanced physical interactions between the nanoparticle system and the three dimensional Carbopol gel network, resulting in greates resistance to flow (Ozon et al., 2025). From a formulation perspective, the higher viscosity of F2 is expected to improve skin adhesion and prolong the residence time of the active compounds without exceeding the recommended viscosity range, indicating that nanoparticle incorporation successfully optimized the rheological properties of the moisturizing gel.

The moisturizing effect of the gel formulations was evaluated by measuring skin hydration levels before and after application on volunteers. The results showed a clear increase in skin moisture content after 10 minutes of application for all formulations, including the base and nanoparticle-containing formulations.

Based on the data, the baseline (base gel) showed an increase in hydration values from approximately 32–36% to 67–86%. This indicates that the gel base itself already possesses moisturizing properties, which can be attributed to the presence of humectants such as glycerin and the water-retaining capacity of Carbopol. Moisturizers in gel form are known to enhance skin hydration by forming a thin film on the skin surface and reducing transepidermal water loss (TEWL), thereby improving moisture retention (Goh et.al, 2019; Teng et.al, 2019). For the nanoparticle-containing formulations, an improvement in hydration was also observed. The F1 showed hydration values ranging from 60.8% to 88%, which is comparable to the base formulation but slightly lower in some repetitions. This may indicate that the concentration of active ingredients in this formulation has not yet reached an optimal level for enhancing hydration. The F2 demonstrated higher hydration values (69.6–82.7%) compared to F1, indicating improved moisturizing performance. This suggests that increasing the concentration of *Morinda citrifolia* extract nanoparticles enhances the hydrating effect, likely due to improved interaction between active compounds and the skin surface.

The highest moisturizing effect was observed in F3, with hydration values ranging from 72.9% to 95.4%. This indicates a significant improvement in skin hydration compared to both the base and other formulations. The enhanced performance of this formulation can be attributed to the nanoparticle system, which increases surface area, improves penetration, and enhances the retention of bioactive compounds on the skin. Nanoparticles are known to facilitate better delivery and prolonged residence time of active ingredients, thereby improving their moisturizing and protective effects (Kim et.al, 2023; Kim et.al, 2019).

In general, skin hydration values above 50% are categorized as well-hydrated, indicating that all formulations successfully improved skin moisture status from normal or dehydrated conditions to a hydrated state. This is consistent with previous studies reporting that topical gel formulations can significantly increase skin hydration within a short time after application, with effects observed as early as 10–30 minutes.

One limitation of the present study is that nanoparticle characterization was limited particle size analysis. Although particle size is a key parameter influencing the performance of nanoparticle-based delivery systems, additional characterization techniques, including Transmission Electron Microscopy (TEM), FTIR, and zeta potention measurement, would provide more comprehensive information regarding particle morphology, chemical interactions between the extract and polymer matrix, and colloidal stability. These analysis were beyond the scope of the present study because of limited access to specialized analytical instruments. Therefore, future studies should incorporate these characterization methods to provide a more comprehensive understanding of the physicochemical properties and stability of the developed nanoparticle systems.

Conclusion

The present study successfully prepared Morinda citrifolia leaf ethanol extract (EDM) nanoparticles using the ionic gelation method, producing nanosized particles with a uniform size distribution. Compared with the crude extract, the nanoparticle formulations exhibited lower IC_{50} values, and statistical analysis demonstrated significant differences in antioxidant activity among the tested formulation ($p<0,05$). The gel formulations also showed stable organoleptic characteristics and acceptable viscosity for topical application, although further optimization is required to adjust the pH to the physiological skin range. In addition, all formutions significantly improved skin hydration ($p<0,05$), with the formulation F3 producing the highest moisturizing effect. These findings suggest that incorporating Morinda citrifolia leaf extract into a nanoparticle-based gel formulation has the potential to improve its antioxidant activity and moisturizing performance. However, further studies involving comprehensive nanoparticle characterization and long-term stability evaluation are required to confirm these findings and support future product development

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