ANTIOXIDANT ACTIVITY AND STABILITY OF NANOEMULSIONS CONTAINING SIAM WEED LEAF EXTRACT (*CHROMOLAENA ODORATA L.*) PREPARED BY THE SPONTANEOUS EMULSIFICATION METHOD

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Abstract

This study aimed to determine the optimum concentration of polysorbate 80 (Tween 80) in the formulation of nanoemulsions containing Siam weed (*Chromolaena odorata L.*) leaf extract, as well as to evaluate the antioxidant activity and stability of the encapsulated compounds. The methods employed included extract maceration, nanoemulsion formulation via spontaneous emulsification using polysorbate 80 at concentrations of 1–5%, and nanoemulsion characterization. Characterization parameters included organoleptic properties (color and odor), emulsion type, transmittance, particle size, antioxidant activity (IC_{50}) as determined by the DPPH assay, and antioxidant stability under varying ambient temperatures. The results showed that the optimum concentration of polysorbate 80 in the chitosan–alginate encapsulated nanoemulsion of Siam weed leaf extract was 5%, yielding a stable, clear brown nanoemulsion with an oil-in-water type and an average particle size of 49 nm. The antioxidant activity of the nanoemulsion, based on an IC_{50} value of 144.26 ppm, was categorized as moderate. Antioxidant stability was evaluated using a cycling test, where the DPPH inhibition activity of the nanoemulsion decreased from 11.89% before the test to 1.07% after the test. However, this decrease was smaller compared to that of the crude extract, indicating that the nanoemulsion of Siam weed (Chromolaena odorata L.) leaf extract exhibited improved antioxidant stability and greater resistance to environmental temperature changes. Further development of this nanoemulsion formulation is required to enhance antioxidant stability.

Keywords: Siam weed, nanoemulsion, polysorbate 80, spontaneous emulsification, antioxidant stability.

I. INTRODUCTION

Siam weed (*Chromolaena odorata* L.) is a non-cultivated plant that grows wild and is native to South and Central America. It is widely distributed in tropical regions and is considered an invasive species that can negatively affect crops and livestock. However, despite its invasive nature, this plant has been extensively utilized for various applications, including organic fertilizer, biopesticide, herbicide, and traditional medicine, due to its rich bioactive compounds (Paul et al., 2026). The utilization of plants in human life is also reflected in the Qur'an, Surah Luqman (31:10), which states:

"He created the heavens without pillars that you see and placed firmly set mountains upon the earth lest it should shift with you, and He dispersed therein every kind of moving creature. And We sent down rain from the sky and caused to grow therein [plants] of every noble kind."

This verse reflects the greatness and mercy of Allah SWT in providing various beneficial plants for all living beings. The term *karīm* refers to plants that are good, beneficial, and capable of providing value according to their nature.

The leaves are the most widely utilized part of the plant due to their high content of secondary metabolites, including flavonoids, tannins, alkaloids, and saponins (Chinyere et al., 2024). These phytochemicals contribute significantly to its biological activities, particularly antioxidant activity. Several recent studies have confirmed that *C. odorata* leaf extracts exhibit strong antioxidant potential due to their high phenolic and flavonoid content (Phetburom et al., 2025).

Antioxidants are compounds capable of inhibiting oxidation processes, thereby protecting cells from damage caused by free radicals. These compounds act by neutralizing reactive species and preventing oxidative stress, which is associated with cellular damage and degenerative diseases (Alwasel, 2023). The antioxidant activity of plant extracts can be evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical compound widely used to measure radical scavenging ability. The

DPPH assay is based on the reduction of the purple DPPH radical into a yellow-colored compound when reacting with antioxidant substances, indicating their scavenging activity (Rakhman, 2025).

The stability of antioxidant compounds in plant extracts is influenced by several factors, including pH, temperature, and storage duration. DPPH and antioxidant compounds are sensitive to environmental conditions such as light, temperature fluctuations, and storage time, which can lead to degradation and decreased antioxidant activity (Rakhman, 2025).
To enhance stability, antioxidant compounds can be protected through encapsulation, where bioactive compounds are entrapped within carrier systems at the nanoscale, such as nanoemulsions. Nanoemulsion systems have been shown to improve stability, solubility, and effectiveness of antioxidant compounds by protecting them from degradation and enabling controlled release (Ben et al., 2026). Furthermore, recent studies have demonstrated that nanoemulsion formulations exhibit improved antioxidant activity compared to crude extracts, as indicated by lower IC_{50} values and better physicochemical stability. For example, nanoemulsion systems prepared using plant extracts showed effective antioxidant activity using the DPPH method, confirming their potential as delivery systems for bioactive compounds.

Nanoemulsions can be prepared using the spontaneous emulsification method, a low-energy technique that enables the formation of nanoscale droplets without requiring high mechanical energy input. This method is particularly advantageous for preserving sensitive bioactive compounds, such as antioxidants, during the nanoemulsion formation process (Walia et al., 2022). The physicochemical characteristics of nanoemulsions are strongly influenced by their formulation components, especially surfactants. Surfactants function by reducing the interfacial tension between oil and water phases, thereby facilitating the formation of a stable and homogeneous dispersion. Non-ionic surfactants are commonly used due to their stability and safety profile in pharmaceutical and food applications (Zhang & Li, 2022).
Polysorbate 80 (Tween 80) is one of the most widely used non-ionic surfactants because of its low toxicity, non-irritating nature, and excellent emulsifying properties. It has been shown to effectively stabilize nanoemulsions and improve their physicochemical stability (Qadir et al., 2026).

Several recent studies have demonstrated that the concentration of Tween 80 significantly affects nanoemulsion characteristics, including droplet size, stability, and antioxidant activity. For instance, optimization of Tween 80 concentration in nanoemulsion systems can lead to smaller particle sizes and improved stability. In addition, nanoemulsions stabilized with Tween 80 have shown enhanced antioxidant activity due to better protection and dispersion of bioactive compounds (Ananingsih et al., 2024). Furthermore, nanoemulsion systems have been reported to exhibit improved stability and antioxidant performance compared to non-encapsulated systems, particularly under varying environmental conditions such as temperature and pH (Xu et al., 2024).

METHODS

Extraction of Plant Material

Siam weed (*Chromolaena odorata L.)* leaves were collected, cleaned, and air-dried at room temperature. After drying, the leaves were weighed and ground into a fine powder. A total of 1 kg of dried leaf powder was placed into a maceration container. Subsequently, 96% ethanol was added until the sample was fully immersed and homogenized (Hatmayana et al., 2022).

The sample was macerated in a dark room for 3 × 24 hours. Every 24 hours, the filtrate was collected and replaced with fresh solvent. The filtrates obtained from the first, second, and third days were combined, and the solvent was evaporated using a rotary evaporator to obtain a semi-concentrated extract. The extract was further evaporated until a viscous extract was obtained. The final extract was weighed and stored in a tightly sealed sterile container (Hatmayana et al., 2022).

Preparation of Nanoemulsion

Aqueous Phase

The aqueous phase was prepared by mixing 4 mL of 0.3% (w/v) chitosan solution with 20 mL of 0.6% (w/v) sodium alginate solution using a magnetic stirrer until homogeneous. Then, 3 mL of 4% (v/v) sodium tripolyphosphate (TPP) was added while maintaining the pH at 5.0–5.5. Distilled water was added up to a total volume of 150 mL (Hatmayana et al., 2022).

Oil Phase

A total of 0.3 g of *Chromolaena odorata* leaf extract was dissolved in 7.2 mL of 96% ethanol. Polysorbate 80 (Tween 80) was then added as a surfactant (10 mL) with concentration variations of 1%, 2%, 3%, 4%, and 5% (Hatmayana et al., 2022)..

Emulsification Process

The nanoemulsion was prepared by adding the oil phase dropwise into the aqueous phase while

stirring continuously using a magnetic stirrer at 600 rpm for 4 hours (Hatmayana et al., 2022).

Emulsion Type Test

The emulsion type was determined by mixing 5 mL of nanoemulsion with one drop of methylene blue. If the dye dissolved, the nanoemulsion was classified as oil-in-water (O/W); otherwise, it was classified as water-in-oil (W/O) (Ma'arif et al., 2023).

Clarity Test

Nanoemulsion clarity was evaluated based on percent transmittance. A total of 1 mL of sample was diluted to 100 mL using distilled water. The transmittance was measured using a UV-Vis spectrophotometer at 650 nm. A good nanoemulsion shows 90–100% transmittance (Aprilya et al., 2021).

Particle Size Analysis

A total of 1 g of nanoemulsion sample was prepared and sonicated for 30 minutes. The sample was then placed in a cuvette, and particle size distribution was measured using a particle size analyzer.

Antioxidant Activity Test

DPPH Assay

A total of 1 mL of 0.5 mM DPPH solution was added into 10 mL ethanol and incubated in the dark for 30 minutes. The absorbance was measured at 515 nm. Standard ascorbic acid solutions (4–12 ppm) and sample solutions (10–50 ppm) were prepared and measured using the same procedure (Khotimah et al., 2023).

DPPH Inhibition Calculation

% Inhibition = (A0 − A1) / A0 × 100%
A0 = absorbance of control
A1 = absorbance of sample

IC50 Determination

The IC50 value was obtained from the linear regression of concentration versus percent inhibition, where 50% inhibition corresponds to the IC50 value (Khotimah et al., 2023).

Antioxidant Stability Test

The cycling test was conducted by storing samples at 4 ± 2°C for 24 hours and then at 40 ± 2°C for another 24 hours. This process was repeated for three cycles, followed by re-evaluation of antioxidant activity (Khotimah et al., 2023).

II. RESULT AND DISCUSSION

Nanoemulsion Characteristics

The characteristics of nanoemulsions obtained from five formulations with varying concentrations of polysorbate 80 (1%, 2%, 3%, 4%, and 5%) are presented in Table 1.

Table 1 Characteristics of Siam Weed (Chromolaena odorata L.) Leaf Extract Nanoemulsion

Polysorbate 80 Concentration (%)	Color	Odor	Reference* Color	Reference* Odor
1	yellow	Oily odor		
2	yellow	Oily odor		
3	brown	Oily odor	Clear brown	Leaf-like odor
4	brown	Oily odor		
5	brown	Oily odor		

Nanoemulsions of *Chromolaena odorata* leaf extract with polysorbate 80 concentrations of 1% and 2% produced clear yellow formulations, whereas concentrations of 3%, 4%, and 5% resulted in clear brown nanoemulsions. These results indicate that increasing the concentration of polysorbate 80 leads to a darker color in the nanoemulsion.

This phenomenon is related to changes in droplet size and light scattering behavior. Nanoemulsions with smaller droplet sizes tend to appear more transparent because light passes through with minimal scattering, while larger droplets increase light scattering and result in darker or more intense coloration.

In addition, surfactant concentration significantly influences the physicochemical properties of nanoemulsions. Increasing surfactant concentration reduces interfacial tension and can modify droplet size distribution, stability, and optical appearance (Chuacharoen et al., 2019). This improved solubilization and dispersion of bioactive compounds may contribute to increased color intensity in the system. Furthermore, droplet size in nanoemulsions is strongly affected by surfactant concentration, where higher surfactant levels generally promote the formation of smaller droplets and more stable systems, although excessive concentrations may lead to aggregation effects (Basumatary & Kumar, 2024).

Emulsion Type Test

The emulsion type of the *Chromolaena odorata* L. leaf extract nanoemulsion was determined using the methylene blue dye method, as presented in Table 2. The determination of emulsion type in this study

follows the dye solubility principle, where methylene blue, a water-soluble dye, dissolves in oil-in-water (O/W) emulsions but not in water-in-oil (W/O) systems. This method provides a clear visual distinction between emulsion types due to the affinity of the dye toward the aqueous phase (Iskandar et al., 2024).

Based on Table 2, methylene blue was soluble in all nanoemulsion formulations, indicating that the *Chromolaena odorata* leaf extract nanoemulsions were classified as oil-in-water (O/W) systems. This result is consistent with previous findings showing that chitosan–alginate-based nanoemulsions typically form O/W systems due to the hydrophilic nature of the continuous phase and stabilizing polymers.

Table 2 Emulsion Type of Siam Weed (Leaf Extract Nanoemulsion Using Methylene Blue

Polysorbate 80 Concentration (%)	Methylene Blue Solubility	Emulsion Type
1	Soluble	O/W
2	Soluble	O/W
3	Soluble	O/W
4	Soluble	O/W
5	Soluble	O/W

Note: O/W = oil-in-water emulsion

Clarity Test

The clarity of the *Chromolaena odorata* leaf extract nanoemulsion was evaluated using a UV-Vis spectrophotometer at a wavelength of 650 nm. The results are presented in Table 3.

Table 3 Clarity of Siam Weed Leaf Extract Nanoemulsion

Polysorbate 80 Concentration (%)	Transmittance (%)
1	99.364
2	99.304
3	98.927
4	98.285
5	98.403

Nanoemulsions are generally characterized by high optical clarity, with transmittance values above 90%, indicating small droplet size, uniform dispersion, and system stability (Xu et al., 2024). High transmittance occurs because nanoscale droplets scatter light minimally, resulting in a transparent or clear appearance. Based on Table 3, all nanoemulsion formulations exhibited transmittance values greater than 90%, indicating that the systems formed were transparent, homogeneous, and stable nanoemulsions. These results confirm that the formulated nanoemulsions possess desirable physicochemical properties, particularly in terms of clarity and dispersion quality.

Particle Size Analysis

The particle size of the *Chromolaena odorata* L. leaf extract nanoemulsion was measured using a particle size analyzer (PSA), and the results are presented in Table 4.

Table 4 Particle Size of Siam Weed (Chromolaena odorata L.) Leaf Extract Nanoemulsion

Parameter	Standard	Polysorbate 80 Concentration (%)				
		1	2	3	4	5
D4,3 (nm)	<100*	254	46	47	55	49
D50/Dmode Ratio	≈1**	1.506	0.922	0.941	0.875	0.98
Absorbance	–	0.031	0.028	0.063	0.079	0.111

The measurement was conducted using the laser diffraction (LD) method, which is widely used for analyzing particle size distribution across nano- to microscale ranges due to its accuracy and rapid analysis capability (Kim & Jin, 2020). Based on Table 4, the average particle size (D4,3), which represents the particle size of the nanoemulsion system, shows that formulations containing 2%, 3%, 4%, and 5% polysorbate 80 fall within the nanoscale range (<100 nm), indicating successful nanoemulsion formation. In contrast, the formulation with 1% polysorbate 80 produced larger particles (254 nm), suggesting insufficient surfactant concentration to stabilize the emulsion system.

The decrease in particle size with increasing surfactant concentration is consistent with previous studies, where higher surfactant levels reduce interfacial tension and facilitate the formation of smaller droplets (Chuacharoen et al., 2019).

Furthermore, particle size distribution uniformity can be evaluated using the ratio of D50 (median diameter) to Dmode (most frequent diameter). A ratio approaching 1 indicates a more homogeneous particle size distribution. As shown in Table 4, the nanoemulsion with 5% polysorbate 80 exhibited a D50/Dmode ratio closest to 1 (0.98), indicating the most uniform distribution. Uniform particle size distribution contributes to improved nanoemulsion stability, as systems with homogeneous droplet sizes are less prone to aggregation and phase separation (Xu et al., 2024). Based on these results, the nanoemulsion formulated with 5% polysorbate

80 demonstrated the most optimal characteristics, with a particle size of 49 nm and a D50/Dmode ratio of 0.98. This was further supported by visual observations after storage, where no turbidity, sedimentation, or phase separation occurred, indicating good stability of the nanoemulsion system.

Antioxidant Activity Test

The antioxidant activity of ascorbic acid, *Chromolaena odorata* L. leaf extract, and its nanoemulsion was evaluated using the DPPH method with a UV-Vis spectrophotometer at a wavelength of 515 nm. The results are presented in Tables 5–7.

Table 5. Antioxidant Activity of Ascorbic Acid

Concentration (ppm)	Absorbance	DPPH Inhibition (%)	IC_{50} (ppm)
4	0.2511	44.80	
6	0.1376	69.75	
8	0.1011	77.77	3.04
10	0.0883	80.59	
12	0.0841	81.51	

Table 6. Antioxidant Activity of *Chromolaena odorata* Leaf Extract

Concentration (ppm)	Absorbance	DPPH Inhibition (%)	IC_{50} (ppm)
10	0.4695	-3.21	
20	0.4277	5.98	
30	0.4074	10.44	99.31
40	0.3728	18.05	
50	0.3662	19.50	

Table 7. Antioxidant Activity of Nanoemulsion

Concentration (ppm)	Absorbance	DPPH Inhibition (%)	IC_{50} (ppm)
10	0.4678	-2.84	
20	0.4397	3.34	
30	0.4114	9.58	144.26
40	0.4015	11.74	
50	0.4008	11.89	

The main parameter for evaluating antioxidant activity using the DPPH method is the inhibitory concentration (IC_{50}). A lower IC_{50} value indicates stronger antioxidant activity, whereas a higher IC_{50} value indicates weaker activity. The IC_{50} value is obtained by plotting sample concentration (x-axis) against percentage inhibition (y-axis) to generate a linear regression equation, where y = 50 corresponds to the IC_{50} value (Oncogenic et al., 2021). Based on the regression analysis, the IC_{50} value of *Chromolaena odorata* leaf extract was 99.31 ppm, indicating strong antioxidant activity. Meanwhile, the nanoemulsion exhibited an IC_{50} value of 144.26 ppm, which falls into the moderate antioxidant category. Both samples showed lower antioxidant activity compared to ascorbic acid (IC_{50} = 3.04 ppm), which is classified as a very strong antioxidant.

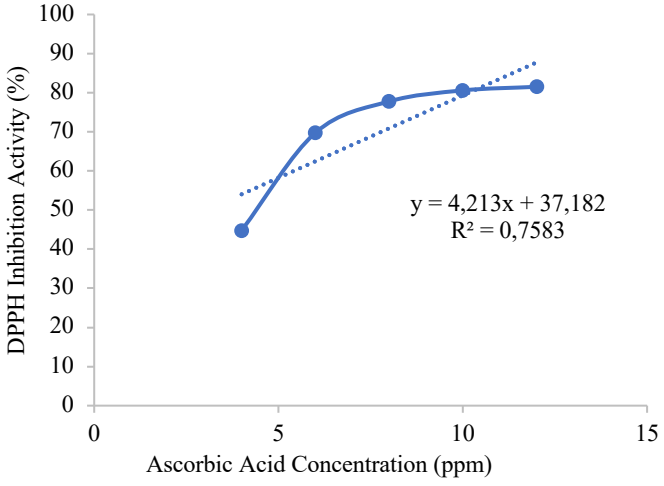


Figure 1 Linear Regression Equation of Ascorbic Acid ($C_6H_8O_6$)

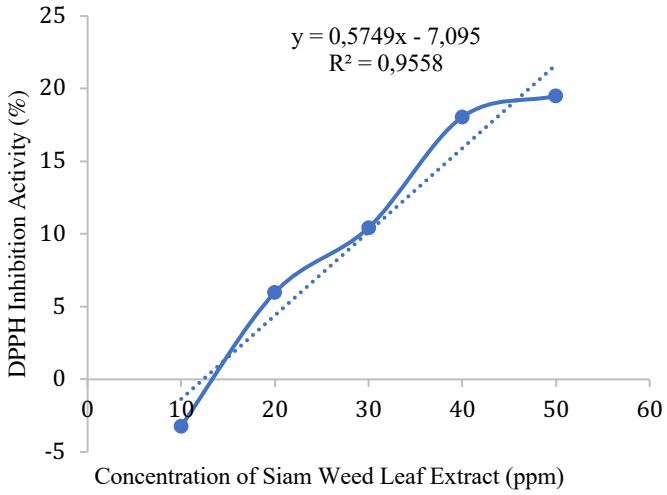


Figure 2 Linear Regression Equation of Siam Weed (*Chromolaena odorata* L.) Leaf Extract

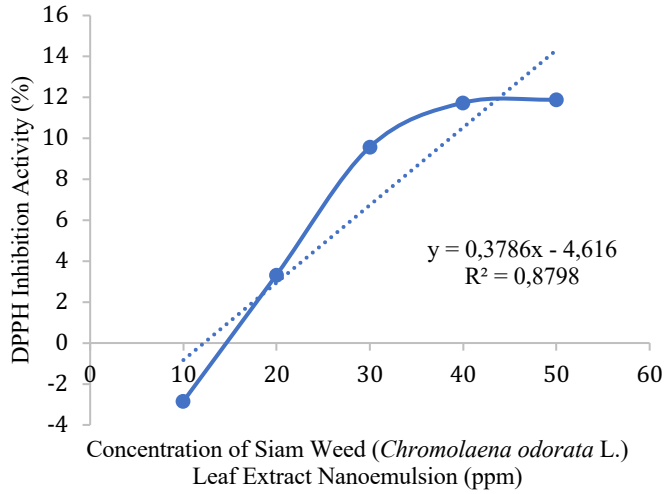


Figure 3 Linear Regression Equation of Siam Weed (*Chromolaena odorata* L.) Leaf Extract Nanoemulsion

These results indicate a decrease in antioxidant activity after the extract was formulated into a nanoemulsion. A similar trend has been reported in previous studies, where nanoemulsion systems showed reduced apparent antioxidant activity due to encapsulation effects (Xu et al., 2024). This decrease may be attributed to the encapsulation matrix, which can hinder the interaction between active compounds and DPPH radicals during the assay. In addition, insufficient incubation time may prevent the complete release of active compounds from the nanoemulsion system. Encapsulation systems are known to protect bioactive compounds, but may also delay their reactivity in in vitro assays (Xu et al., 2024). Furthermore, the concentration of active compounds within the nanoemulsion system also influences antioxidant activity. Lower effective availability of the extract in the dispersed system may contribute to the reduced radical scavenging activity observed.

Antioxidant Stability Test

The antioxidant stability of *Chromolaena odorata* L. leaf extract and its nanoemulsion was evaluated using the cycling test method. The results are presented in Table 8.

Table 8. Antioxidant Stability of Siam Weed (*Chromolaena odorata* L.) Leaf Extract and Nanoemulsion Using Cycling Test

Sample	DPPH Inhibition Activity (%)		Decrease (%)
	Before (C1)	After (C2)	C
Nanoemulsion	11.89	1.07	10.82
Extract	19.50	7.27	12.23

Notes:

C = decrease in DPPH inhibition activity (%)
C1 = before cycling test
C2 = after cycling test

This study employed the cycling test method because it simulates temperature fluctuations that may affect the stability of chemical compounds. Temperature variation is known to accelerate degradation of antioxidant compounds through oxidation and structural changes (Xu et al., 2024). Based on Table 8, the percentage of DPPH inhibition before the cycling test was 19.50% for the extract and 11.89% for the nanoemulsion. After the cycling test, both samples showed a decrease in antioxidant activity, with values dropping to 7.27% for the extract and 1.07% for the nanoemulsion. However, the decrease in antioxidant activity in the nanoemulsion (10.82%) was lower than that of the extract (12.23%). This indicates that the nanoemulsion system provided better protection of antioxidant compounds against temperature-induced degradation. Encapsulation within nanoemulsion systems is known to improve stability by protecting bioactive compounds from environmental stress such as heat, light, and oxidation (Chuacharoen et al., 2019). Therefore, the *Chromolaena odorata* leaf extract nanoemulsion encapsulated in a chitosan–alginate system and prepared using spontaneous emulsification exhibited greater stability and resistance to temperature changes compared to the crude extract. This improved stability is attributed to the protective effect of the encapsulation matrix, which helps maintain the integrity of antioxidant compounds during storage and environmental exposure.

III. Conclusion

Based on the results of this study, it can be concluded that:

1. The optimum concentration of polysorbate 80 in the nanoemulsion formulation of *Chromolaena odorata* L. leaf extract using the spontaneous emulsification method was 5%, producing a clear brown nanoemulsion with an oil-in-water (O/W) type and an average particle size of 49 nm.
2. The inhibitory concentration (IC_{50}) value of the nanoemulsion of *Chromolaena odorata* L. leaf extract was 144.26 ppm, indicating moderate antioxidant activity within the range of 100–150 ppm.
3. The nanoemulsion of *Chromolaena odorata* L. leaf extract exhibited a decrease in antioxidant activity of 10.82%, but demonstrated greater stability compared to the crude extract.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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