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Phytochemical Screening, GC–MS Profiling, and Colloidal Characterization of *Toona sinensis* and *Carica papaya* Leaf-Extract Nanoemulsions for Potential Plant-Extract Nanoemulsion Development

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ABSTRACT

Environmentally friendly insecticides are increasingly needed to replace synthetic pesticides that drive pest resistance and contribute to environmental pollution. This study explores the potential of combining *Toona sinensis* and *Carica papaya* (Ts/Cp) leaf extracts in a nanoemulsion formulation to develop an effective and sustainable botanical insecticide. The formulation consisted of a 20% oil phase (OP, 1:1 v/v extract in 70% ethanol) and an 80% aqueous phase (AP, 97% water and 3% Tween 80), prepared using low-energy magnetic stirring (2500 rpm, 25 °C, 35–45 minutes). Phytochemical screening revealed the presence of dominant secondary metabolites, including flavonoids, tannins, saponins, and steroids. Gas chromatography–mass spectrometry (GC-MS) analysis indicated that T. sinensis is rich in phenolics and polar glycosides with strong antioxidant activity, while *C. papaya* contains more lipophilic compounds such as fatty acid esters, terpenoids, and phytosterols, resulting in a complementary bioactive spectrum. Stability analysis showed minimal pH changes (5.9–6.9), indicating good chemical stability. Among formulations, the 25:75 (Ts: Cp) ratio yielded optimal characteristics, including a small particle size (17.3 nm), a low PDI (0.32), and homogeneous dispersion. However, a low zeta potential suggests reliance on steric stabilization. This formulation is recommended for further bioassay and optimization studies.

1. INTRODUCTION

Pest attacks remain one of the main obstacles to increasing agricultural productivity, both on small-scale and industrial-scale farms. Dependence on synthetic pesticides often has negative impacts, including pest resistance, harmful residues, and environmental damage. Therefore, botanical insecticides derived from plants are gaining attention for their environmentally friendly properties, biodegradability, and relative safety to humans and non-target organisms [1, 2]. One plant with potential as a source of botanical insecticide is *Toona sinensis*, which is widely known in Asian ethnopharmacology. *T. sinensis* plant contains various secondary metabolites such as flavonoids, terpenoids, alkaloids, and polyphenols that have been proven to have biological activity, including antibacterial, antioxidant, and insecticidal properties [3]. In addition, *Carica papaya* leaves are rich in bioactive compounds, including fatty acids, flavonoids, tannins, and phytosterols. *C. papaya* leaf extract has insecticidal and larvicidal activity against insect pests and disease vectors, making it a potential raw material for botanical insecticide [4, 5].

The combination of two different types of plant extracts can produce synergistic, additive, or even antagonistic effects. Synergistic effects can broaden the spectrum of pest control, reduce application doses, and increase the duration of effectiveness. The combination of *T. sinensis* and *C. papaya* was selected for the development of botanical insecticides because both possess complementary spectra of bioactive compounds and modes of action, thereby potentially producing synergistic effects against pests. *T. sinensis* is known to be rich in phenolic compounds and flavonoids that act as antifeedants, inhibiting feeding activity and disrupting insect metabolism [6]. Meanwhile, *C. papaya* contains lipophilic fractions, such as terpenoids, fatty acids, and phytosterols, that contribute to direct toxic effects, including disruption of the nervous system and cellular physiology [7]. Additionally, the phytosterol content in both plants has the

potential to disrupt the regulation of insect growth hormones, particularly the molting process, thereby acting similarly to insect growth regulators (IGRs). This indicates that the combination of these two extracts enables multiple modes of action—namely, antifeedant and growth-inhibiting effects—which simultaneously suppress pest populations across various developmental stages. This multi-target approach not only enhances the effectiveness of the resulting botanical insecticides but also serves as a crucial strategy for slowing the development of pest resistance while supporting the development of more environmentally friendly and sustainable pesticides. However, the combination of *T. sinensis* and *C. papaya* leaf extracts as a botanical insecticide formulation has not been comprehensively studied.

Phytochemical analysis is an important step in identifying the active components in plant extract formulations. Meanwhile, gas chromatography–mass spectrometry (GC–MS) is an analytical technique widely used to detect volatile and semi-volatile compounds, making it highly relevant for its potential as a botanical insecticide based on *T. sinensis* and *C. papaya* formulations. *T. sinensis* extract contains sesquiterpenes and flavonoids that can act as deterrents against pests, while *C. Papaya* contains various fatty acids that contribute to its larvicidal properties. However, most studies remain partial, focusing on only one plant or one group of compounds and failing to demonstrate the formulation's potential as a botanical insecticide. The development of botanical insecticides focuses not only on the identification of compounds, but also on formulations using nanoemulsion, microencapsulation, and controlled release systems, which have been proven to increase the stability of active ingredients, prolong the duration of action, and improve the effectiveness of insecticides in the field [8-10]. Modern formulations still focus on essential oils or single extracts, and this technology has not been applied to combinations of *T. sinensis* and *C. papaya* leaf extracts.

Nanoemulsions are two-phase dispersion systems consisting of an oil phase (OP), an aqueous phase (AP), and surfactants, with droplet sizes on the nanometer scale that provide high kinetic stability and a large surface area [11]. In oil-in-water (O/W) systems, the OP serves as a carrier for lipophilic compounds, while the AP provides a continuous medium for more polar components. The stability of nanoemulsions is determined by mechanisms that inhibit droplet coalescence, which can occur through electrostatic repulsion or steric hindrance. In formulations using nonionic surfactants such as Tween 80, stabilization is dominated by steric mechanisms, namely the formation of a protective layer around the droplets that prevents aggregation even when the zeta potential is relatively low. This approach is particularly important in systems containing mixtures of multicomponent compounds. Ethanol extracts, especially at a 70% concentration, are known to be capable of extracting compounds with a wide range of polarities, ranging from phenolics and flavonoids (polar) to terpenoids, fatty acids, and phytosterols (semi-polar to non-polar). Therefore, nanoemulsions serve as a compatible system because they can accommodate the lipophilic fraction in the OP while retaining the polar components in the AP, resulting in a homogeneous and stable system. In addition to enhancing apparent solubility and particle distribution, nanoemulsions also help protect active compounds from degradation and improve their stability.

The use of nanoemulsions in ethanol extract formulations is a rational approach. This is due to the characteristics of ethanol extracts, particularly 70% ethanol, which can extract compounds with a wide range of polarities, from polar components (phenolics and flavonoids) to semi-polar and non-polar compounds (terpenoids, fatty acids, and phytosterols). Consequently, the resulting extract is complex and not fully stable in an aqueous system. O/W nanoemulsions allow the lipophilic fraction of the extract to be incorporated into the OP. At the same time, the more polar components remain dispersed in the continuous phase, thereby bridging the polarity gap within a single homogeneous system.

Furthermore, the extremely small droplet size in nanoemulsions increases the surface area and apparent solubility of active compounds, thereby enhancing bioavailability and biological efficacy. The use of nonionic surfactants, such as Tween 80, also provides adequate steric stabilization, even in a multicomponent system. Nanoemulsions are not only suitable for oil-based compounds but also hold great potential for ethanol extract formulations as they can improve stability, delivery efficiency, and the overall performance of botanical insecticides.

Environmental factors, leaf age, extraction methods, and processing conditions greatly influence variations in the chemical composition of extracts. These variations can lead to differences in bioactivity, requiring standardization of extraction methods and phytochemical analysis to ensure consistent results [12, 13]. The formulation of botanical insecticides is expected to correlate directly with their chemical profile and bioassay activity, thereby identifying the main compounds responsible for insecticidal activity [14, 15]. A good botanical insecticide is not only effective in killing pests, but also safe for natural enemies, pollinators, and the environment. Formulations based on *T. sinensis* and *C. papaya* are still very limited, suggesting great potential for the sustainable use of local plants. This is because these plants are easily found in various regions of Indonesia, especially in West Sumatra, particularly in Solok. Therefore, their use can support farmers' independence in reducing their dependence on synthetics [16-18]. Thus, an integrative approach combining phytochemistry, formulation, and bioassay is urgently needed to develop innovative, locally based botanical insecticides that are value-added, effective, environmentally friendly, accessible, safe, and sustainable. This study aims to characterize the phytochemical constituents, GC–MS profiles, and particle size analysis (PSA) results, as well as the zeta potential, of nanoemulsion formulations containing *T. sinensis* and *C. papaya* leaf extracts as botanical insecticides. However, it should be noted that bioactivity testing is beyond the scope of this study.

2. MATERIAL AND METHODS

2.1 Materials

Leaves of *T. sinensis* and *C. papaya* (Ts/Cp) were collected from several areas in Solok Regency, West Sumatra province, Indonesia, using random sampling. All leaves were identified at the Central Laboratory, Andalas University, Indonesia, and the Center for Tropical Biopharmaceutical Studies, Jl. Taman Kencana No. 3, Science Techno Park Area, CRC Building, 2nd Floor, Bogor City, Indonesia. Simplicia processing consists of collecting raw materials, wet sorting, washing, counting, drying, dry sorting, milling, packaging, and storage.

In this study, the samples used were green Ts/Cp leaves harvested from shoots, free of defects and pests. Tween 80 (polyoxyethylene (20) sorbitan monooleate) with catalog number 8.22187 and a hydrophilic-lipophilic balance (HLB) value of approximately 15 is a nonionic surfactant with relatively hydrophilic properties. Therefore, Tween 80 is commonly used as an emulsifier to form and stabilize O/W emulsions, as surfactants with high HLB values are more compatible with the AP.

2.2 Extraction method

Dried sample preparations from Ts/Cp leaves were sun-dried for 7 days, blended, and sieved to obtain a powder. The powder from each leaf was formulated in a percentage ratio (100% *T. sinensis*; 75% *T. sinensis* + 25% *C. papaya*; 50% *T. sinensis* + 50% *C. papaya*; 25% *T. sinensis* + 75% *C. papaya*, and 100% *C. papaya*) of 50 g of dry weight basis for 100% and mixed with 70% ethanol at a ratio of 1:10, with a solvent volume of 500 mL in a dark glass reagent bottle for each treatment. The maceration was carried out in a sterile, sealed container at room temperature (± 25 °C) for 7 days, until complete. The pH values of the ethanol extracts from Ts/Cp leaves before and after maceration were measured using a Hanna HI2211 pH meter (Hanna Instruments, USA). The solution was filtered using membrane filtration (MF) to separate the pulp from the filtrate. The resulting maceration, a thick liquid extract, was then concentrated using a rotary evaporator to obtain an ethanol extract from the Ts/Cp leaf formulation.

2.3 Phytochemical screening

Phytochemical screening was conducted to determine the presence of various secondary metabolites in the leaf formulations of Ts/Cp. Secondary metabolites from the flavonoid group were examined using cyanidine reagent (magnesium powder and concentrated hydrochloric acid); phenolic groups using iron (III) chloride reagent; saponins using the foam and water test; and steroids, alkaloids, and triterpenoids using Lieberman-Burchard reagent (acetic anhydride and concentrated sulfuric acid).

2.4 Gas chromatography–mass spectrometry analysis

GC–MS analysis of *Toona sinensis* and *Carica papaya* leaf extracts was performed at the Central Laboratory of Andalas University, Padang, Indonesia, using an Agilent 7890 GC System coupled to an Agilent 5975C MS detector (Agilent Technologies, USA) and an autosampler. Separation was performed using an HP Ultra 2 capillary column (5% phenyl methylpolysiloxane, length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm). Helium carrier gas (purity ≥ 99.999%) was used at a constant flow rate of 1.2 mL/min. A 5 µL (microliter) sample was injected in split mode with a split ratio of 1:10, and the injector temperature was set to 250 °C. The oven temperature program starts at 80 °C (held for 2 minutes), then increases by 10 °C/minute to 280 °C, and is held for 10 minutes, resulting in a total analysis time of approximately 32 minutes. Detection is performed using electron impact ionization (EI) at 70 eV. The ion source temperature is set to 230 °C, and the quadrupole temperature to 150 °C. A solvent delay of 3 minutes is applied to prevent detector saturation. Data acquisition is performed in scan mode with a mass range of m/z 50–600. Compound identification was performed by comparing mass spectra with databases such as the National Institute of Standards and Technology (NIST) and selecting compounds with a similarity level ≥ 80%. The analysis was performed without derivatization, limiting compound identification to volatile and semi-volatile components. Polar compounds such as sugars and glycosides may not have been optimally detected; therefore, the GC–MS results were interpreted with caution as profiles of dominant volatile compounds.

2.5 Nanoemulsion botanical insecticide

The spontaneous emulsification mechanism has modified the method described by Toor et al. [19], where the OP is an extraction solution from the formulation/Cp with a 1:1 v/v ratio of Ts/Cp, and extracts soluble bioactive compounds from a 70% ethanol solution. Meanwhile, the AP consists of 97% water and 3% Tween 80. Furthermore, the solution of both phases is emulsified using a low-energy magnetic stirrer at 2500 rpm for 35-45 minutes at 25 °C, via a dripping process.

Tween 80 was selected for its ability to effectively reduce the interfacial tension between the OP (containing hydrophobic phytochemical compounds) and the air phase, thereby facilitating the formation of nano-sized droplets with a homogeneous distribution. Its hydrophilic nature also supports steric stabilization via the polyoxyethylene chains, thereby increasing colloidal stability and preventing coalescence in O/W systems. The rational use of Tween 80 in this formulation aims to produce stable O/W nanoemulsions with optimal physicochemical characteristics.

2.6 Particle size analysis and zeta potential

The particle size and polydispersity index (PDI) of nanoemulsions of *Toona sinensis* and *Carica papaya* leaf extracts were analyzed using a dynamic light scattering (DLS)-based particle size analyzer. At the same time, the zeta potential was measured using electrophoretic light scattering (ELS) on instruments such as the Malvern Zetasizer Nano ZS (Malvern Instruments, UK) or equivalent devices. Before measurement, the instrument was stabilized for approximately 30 minutes, and all analyses were conducted at 25 °C. The nanoemulsion samples were diluted in deionized water, the dispersion medium, at a 1:100 (v/v) ratio to avoid multiple scattering and ensure optimal particle distribution. Solvent parameters such as viscosity (0.8872 cP), refractive index (1.333), and dielectric constant (78.5) were adjusted to match the aqueous dispersion medium at 25 °C. To ensure homogeneity, the diluted samples were sonicated in an ultrasonic bath at 40 kHz and 100–150 W for 10 minutes (optimal range: 5–15 minutes) to reduce particle agglomeration without damaging the nanoemulsion structure. After sonication, the samples were allowed to stand for 1–2 minutes to remove air bubbles. Particle size measurements were performed using a clean DLS cuvette with a sample volume of 1–2 mL, and each sample was analyzed in triplicate to obtain the hydrodynamic diameter (Z-average) and PDI value. Zeta potential measurements were performed using a folded capillary cell filled with a bubble-free sample. Analysis was performed using the Smoluchowski equation, assuming a low ionic strength in the dispersion medium. Each sample was measured in triplicate, and the replicates were combined into a single composite sample for analysis.

2.7 Data analysis

All experiments were conducted three times using three independent groups (n = 3), except for the GC-MS and PSA-Zeta potential tests, for which the samples were pooled. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics. Differences between groups were analyzed using one-way ANOVA followed by Duncan's test. A p-value < 0.05 was considered statistically significant.

3. RESULT AND DISCUSSION

3.1 The pH

The pH before and after the maceration process in the *T. sinensis* and *C. papaya* leaf extract formulations showed very small changes, but increased with pH values ranging from slightly acidic to near neutral (5.9 – 6.9), and no significant differences before and after treatment (p > 0.05), indicating good chemical stability of the system (Table 1). However, significant differences (p < 0.05) were observed among the formulations, suggesting that the composition of the *T. sinensis* and *C. papaya* leaf extracts significantly influenced pH. The 100% *Toona sinensis* formulation experienced a slight increase from pH 5.92 to 5.94, while the 75% *Toona sinensis* + 25% *Carica papaya* mixture increased from 6.92 to 6.96. The 50:50 composition, the pH was relatively stable (6.10 to 6.11), and in the 25% *Toona sinensis* + 75% *Carica papaya* formulation, there was no change (6.33 remained 6.33).

Table 1. The pH before and after the maceration process of formulation from *Toona sinensis* and *Carica papaya* leaf extract

Formulation	Before	After
100% *Toona sinensis*	5.92 ± 0.03 e	5.94 ± 0.03 e
75% *Toona sinensis* + 25% Carica papaya	6.92 ± 0.02 a	6.96 ± 0.03 a
50% *Toona sinensis* + 50% Carica papaya	6.10 ± 0.04 d	6.11 ± 0.02 d
25% *Toona sinensis* + 75% Carica papaya	6.33 ± 0.02 c	6.33 ± 0.02 c
100% Carica papaya	6.57 ± 0.04 b	6.60 ± 0.03 b
CV (%) - Duncan's Test	0.58**	0.50**
SE	0.03	0.03

Note: CV: Coefficient of variation, SE = Standard error, n = 3 samples.

The 100% *Carica papaya* extract increased slightly from 6.57 to 6.60. This increase indicates that the maceration process did not cause significant chemical degradation or the formation of large amounts of new acidic/basic compounds, so that the natural buffer system of phenolic compounds, flavonoids, and sugar components in both leaves likely maintained pH stability. Chemically, leaf extracts generally contain secondary metabolites, such as polyphenols and weak organic acids, which tend to maintain pH within a stable range during solvent extraction at low temperatures, such as maceration. The pH stability is important because it affects the stability of bioactive compounds and formulation safety, and indicates that the combination of the two ingredients does not cause strong acid-base interactions. A pH value close to neutral is also consistent with the characteristics of leaf extracts, which are rich in antioxidants and glycosides that are generally not very acidic or very alkaline [20, 21].

3.2 Phytochemical analysis

Phytochemical screening of *Toona sinensis* and *Carica papaya* leaf extract formulations showed that the main secondary metabolites detected were flavonoids, tannins, saponins, and steroids. In contrast, alkaloids, quinones, and triterpenoids were not detected in all or most formulations (Table 2). Flavonoids were positively identified in formulations A, D, and E, but negatively in B and C, indicating that the proportion of ingredients in the formulation affects the presence or concentration of soluble flavonoids beyond the detection limit of the qualitative test. Tannins showed positive results in formulations A, B, and C, and negative results in D and E, indicating that tannin components are more dominant in certain compositions, possibly influenced by the contribution of *Toona sinensis*, which is known to be rich in phenolic compounds. Saponins were detected in A, B, D, and E, but not in C, indicating a relatively broad distribution of active surface glycoside compounds in both leaf types, but sensitive to the mixing ratio. Steroid compounds were detected positively in all formulations (A–E), indicating that this group is a consistent and stable component despite variations in extract composition. The absence of alkaloids in all specific reagents reinforces that these two materials are not major sources of alkaloids, or their levels are very low. The synergistic properties of *Toona sinensis* and *Carica papaya* leaf extracts are due to their rich flavonoid, tannin, and saponin content, which act as antioxidants, antimicrobials, and other bioactive compounds. At the same time, alkaloid and quinone content is generally more limited. Qualitative phytochemical screening is strongly influenced by concentration, solvent, and interactions within the extract matrix [12, 22].

Table 2. Phytochemical formulation from *Toona sinensis* and *Carica papaya* leaf extract

Phytochemical		A	B	C	D	E
Flavonoid		+	-	-	+	+
Alkaloid	Wagner	-	-	-	-	-
	Mayer	-	-	-	-	-
	Dragendorf	-	-	-	-	-
Tannin		+	+	+	-	-
Saponin		+	+	-	+	+
Quinone		-	-	-	-	-
Steroid		+	+	+	+	+
Triterpenoid		-	-	-	-	-

Note: A = 100% *Toona sinensis*, B = 75% *Toona sinensis* + 25% *Carica papaya,* C = 50% *Toona sinensis* + 50% *Carica papaya*, D = 25% *Toona sinensis* + 75% *Carica papaya,* and E = 100% *Carica papaya*.

The undetection of flavonoids in the mixed formulation (75% *Toona sinensis* + 25% *Carica papaya* and 50:50) may be due to the concentration-dependent detection limit in the qualitative phytochemical assay. Although both extracts yielded positive results individually, their combination likely reduced the flavonoid concentration below the detection threshold. Additionally, matrix interactions between bioactive compounds such as tannins and saponins can interfere with the colorimetric response, leading to false-negative results. Therefore, the absence of flavonoids does not necessarily indicate their absence; rather, it reflects the limitations of the qualitative screening method. The presence of tannins in

formulations A, B, and C and their absence in formulations D and E indicate that the tannins primarily originate from *Toona sinensis*. The positive result observed at the 50% concentration (C) but not at the 25% concentration (D) suggests that the qualitative test's detection behavior is threshold-dependent. At lower concentrations, the tannin concentration may be below the detection limit. Additionally, matrix interactions with other phytochemicals, particularly saponins from *Carica papaya*, may interfere with the colorimetric reaction, resulting in false-negative results. Therefore, the absence of tannins in formulation D does not necessarily indicate the total absence of tannins, but rather the limitations of the qualitative detection method.

3.3 Gas chromatography–mass spectrometry analysis

The composition of active compounds analyzed in 100% *Toona sinensis* leaf extract shows a predominance of polyols, sugars and their derivatives, phenolics, fatty acids, and terpenoids (Table 3). The presence of glycerin (glycerol), maltol, pyrogallol (1,2,3-benzenetriol), various glycosides such as methyl-α/β-glucopyranoside, ethyl-α-D-glucopyranoside, and arabinitol indicates a strong polar fraction rich in antioxidant compounds. Simple phenolic compounds such as pyrogallol and ethyl gallate are known to exhibit free radical-scavenging and antimicrobial activities. The discovery of fatty acid derivatives such as linoleic and linolenic monoglycerides, monostearin, phytol, and squalene indicates the contribution of bioactive lipophilic components that act as antioxidants, anti-inflammatories, and precursors to other bioactive compounds. *Toona sinensis* is rich in polyphenols, flavonoids, and terpenoid components that contribute to its antioxidant and pharmacological activities [6, 23].

Table 3. Active compound of 100% *Toona sinensis* leaf extract

Compound	Molecular Formula
Glycerin (Glycerol)	$C_3H_8O_3$
Maltol	$C_6H_6O_3$
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$
1,2,3-Benzenetriol (Pyrogallol)	$C_6H_6O_3$
α-D-Lyxofuranoside, methyl	$C_6H_{12}O_5$
DL-Arabinitol (Arabinitol)	$C_5H_{12}O_5$
Quinic acid	$C_7H_{12}O_6$
Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
Ethyl gallate	$C_9H_{10}O_5$
9,12-Octadecadienoic acid glycerol ester (Linoleic monoglyceride)	$C_{21}H_{38}O_4$
9,12,15-Octadecatrienoic acid glycerol ester (Linolenic monoglyceride)	$C_{21}H_{36}O_4$
Octadecanoic acid glycerol ester (Monostearin)	$C_{21}H_{42}O_4$
Ethyl tetracosanoate	$C_{26}H_{52}O_2$
Phytol acetate (2,6,10,14-Hexadecatetraen-1-ol, acetate)	$C_{22}H_{36}O_2$
Squalene (2,6,10,14,18-Pentamethyl-eicosapentaene)	$C_{30}H_{50}$

Meanwhile, 100% *Carica papaya* leaf extract shows a spectrum of compounds that is also dominated by polyols and sugars (glycerin, L-arabinitol, methyl β-D-glucopyranoside), disaccharides/glycosides (methyl α-D-galactopyranoside and glucopyranose derivatives), as well as lipid and sterol components (Table 4). The presence of fatty acids and their esters (e.g., pentadecanoate, hexadecanoate ethyl ester, and linolenate derivatives), phytol, cholesterol, ergosterol, and campestenone derivatives indicates a strong non-polar fraction with potential biological activity as antioxidants, anti-inflammatories, and membrane stabilizers. The combination of sugar alcohols and glycosides supports humectant properties and antioxidant bioactivity, while sterols and terpenoids contribute to the widely reported pharmacological activity of papaya leaves. Comparatively, both extracts are rich in polar and semi-polar compounds, but T. sinensis appears to be more prominent in simple aromatic phenolics (e.g., pyrogallol and ethyl gallate), while *C. Papaya* is more prominent in sterol and fatty acid ester components. *Toona sinensis* and *Carica papaya* leaves contain a mixture of polyphenols, terpenoids, fatty acids, and phytosterols that play a role in antioxidant and other bioactive activities [24, 25].

Table 4. Active compound of 100% *Carica papaya* leaf extract

Compound	Molecular Formula
Glycerin (Glycerol)	$C_3H_8O_3$
L-Arabinitol	$C_5H_{12}O_5$
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
α-D-Galactopyranoside, methyl	$C_7H_{14}O_6$
β-D-Glucopyranose, 4-O-β-D-galactopyranosyl	$C_{12}H_{22}O_{11}$
Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$
Pentadecanoic acid	$C_{15}H_{30}O_2$
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
Phytol	$C_{20}H_{40}O$
9,12,15-Octadecatrienoic acid (Z,Z,Z)	$C_{18}H_{30}O_2$
trans,trans-9,12-Octadecadienoic acid, propyl ester	$C_{21}H_{38}O_2$
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{36}O_4$
Cholesterol	$C_{27}H_{46}O$
Ergosta-5,24(28)-dien-3-ol (3β)	$C_{28}H_{44}O$
4-Campestene-3-one	$C_{28}H_{46}O$

Table 5. Active compound of formulation 75% *Toona sinensis* + 25% *Carica papaya* leaf extract

Compound	Molecular Formula
Glycerin (Glycerol)	$C_3H_8O_3$
1,3,5-Triazine-2,4,6-triamine (Melamine)	$C_3H_6N_6$
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$
1,2,3-Benzenetriol (Pyrogallol)	$C_6H_6O_3$
α-D-Lyxofuranoside, methyl	$C_6H_{12}O_5$
L-Arabinitol	$C_5H_{12}O_5$
Quinic acid	$C_7H_{12}O_6$
Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$
Ethyl gallate	$C_9H_{10}O_5$
4H-1-Benzopyran-4-one, 2,3-dihydro-7-hydroxy-2,2-dimethyl-	$C_{11}H_{12}O_3$
2-Cyanoethyl-6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-	$\pm C_{14}H_{19}NO_2$

The active compounds in the formulation of *Toona sinensis* and *Carica papaya* leaf extracts indicate that changes in the composition ratio affect the dominance of the detected metabolite groups. In the 75% *T. sinensis* + 25% *C. papaya* formulation, the prominent components were dominated by polar compounds such as glycerin, various sugar derivatives and glycosides (methyl glucopyranoside, xylopyranoside derivatives, arabinitol), and simple phenolics such as pyrogallol and maltol derivatives (Table 5). The presence of ethyl gallate and other phenolic derivatives indicates strong antioxidant and antimicrobial activity potential, as gallic acid esters and polyphenols are known to be major contributors to the free radical scavenging capacity of leaf extracts. The discovery of quinic acid and oxygenated aromatic compounds further reinforces the extract's character as a phenolic-based antioxidant source, as reported in *T. sinensis* leaves.

In the 50%:50% formulation, the composition of compounds appears to be the most diverse, including polyols (glycerin, xylitol), sugar glycosides, oxygenated phenolics, and an increasing number of lipophilic components, such as steroid/sterol derivatives (e.g., cholestadienol derivatives) and aromatic heterocyclic compounds (Table 6). This diversity indicates a relatively balanced contribution from both materials, so that the polar fraction (sugar, polyol, phenolic) and the semi-nonpolar fraction (terpenoids and sterols) are extracted in equal amounts. Chemically, the combination of polyphenols and sterols/terpenoids is often associated with multi-role bioactive activities, including antioxidant, anti-inflammatory, and cell membrane protection, as they work through complementary mechanisms.

Table 6. Active compound of formulation 50% *Toona sinensis* + 50% *Carica papaya* leaf extract

Compound	Molecular Formula
Glycerin (Glycerol)	$C_3H_8O_3$
2-Oxazolidinone, 4,4-dimethyl-	$C_5H_9NO_2$
Diethanolamine	$C_4H_{11}NO_2$
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
α-D-Lyxofuranoside, methyl	$C_6H_{12}O_5$
Oxazolidine, 2-butyl-2-ethyl-	$C_9H_{19}NO$
Xylitol	$C_5H_{12}O_5$
Quinic acid	$C_7H_{12}O_6$
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$
3β-Hydroxy-5-cholen-24-oic acid	$C_{24}H_{40}O_3$
2-Cyanoethyl-6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-	-
6,27-Dinorergosta-5,23-dien-3-ol (3β)	$C_{26}H_{42}O$
3-(4-Hydroxy-5,7-dimethyl-3-methylene-2-oxo-3,3a,4,5,8,8a	-

Meanwhile, the formulation of 25% *T. sinensis* + 75% *C. papaya* showed a stronger shift towards non-polar and semi-polar compounds, characterized by the presence of fatty acid esters (e.g., palmitic acid ethyl ester and linolenic acid ester), phytol, various sterols (cholesta-, ergosta-derivatives), as well as terpenoid and lactone derivatives (Table 7). Although polar compounds such as glycerin and glycosides were still detected, the dominance of lipid and sterol components indicated a major contribution from the chemical matrix of *C. papaya* leaves. Compounds such as phytol and phytosterols have been reported to have antioxidant and anti-inflammatory activities and to help stabilize membrane structure. Overall, the three formulations showed a pattern in which an increase in the proportion of *T. sinensis* enriched the phenolic-glycoside fraction, a strong antioxidant. In contrast, an increase in the proportion of *C. papaya* enriched the fatty acid ester, terpenoid, and sterol fractions. Both leaves are rich in polyphenols, sugar alcohols, terpenoids, and phytosterols that contribute to various biological activities.

Table 7. Active compound of formulation 25% *Toona sinensis* + 75% *Carica papaya* leaf extract

Compound	Molecular Formula
1,2-Cyclopentanedione	$C_5H_6O_2$
Glycerin (Glycerol)	$C_3H_8O_3$
1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$
DL-Arabinitol	$C_5H_{12}O_5$
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
Ethyl α-D-glucopyranoside	$C_8H_{16}O_6$
Pentadecanoic acid	$C_{15}H_{30}O_2$
Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$
Phytol	$C_{20}H_{40}O$
trans,trans-9,12-Octadecadienoic acid, propyl ester	$C_{21}H_{38}O_2$
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z)	$C_{21}H_{36}O_4$
4-[(4-Methoxyphenyl)methyl]-1,2-oxazole-3,5-diamine	$C_{11}H_{13}N_3O_2$
5-Pregnen-3β-ol-20-one, methyl ether	$C_{22}H_{34}O_2$
5-Androstene-3β,16β,17α-triol	$C_{19}H_{32}O_3$
Murolan-3,9(11)-diene-10-peroxy	—
Boscartol F	—
Ergosta-5,24(28)-diene-3-ol (3β)	$C_{28}H_{46}O$
N-acetyl-4-tert-butylamphetamine	$C_{15}H_{23}NO$
Cholesta-5,22-diene-3-ol (3β)	$C_{27}H_{44}O$
Pregnan-3β-ol	$C_{21}H_{36}O$

3.4 Particle size analysis and zeta potential

Particle size and zeta potential in nanoemulsions of *Toona sinensis* and *Carica papaya* leaf extracts varied significantly across mixed formulations. The 100% *T. sinensis* formulation had a very small particle size (14.8 nm) with a zeta potential of -3.4 mV, while the 100% *C. papaya* formulation was also in the small nano range (13.6 nm) with a zeta potential of −7.7 mV (Figure 1). The 25% *T. sinensis* + 75% *C. papaya* mixture still produced small particle sizes (17.3 nm) with a charge of -10.3 mV. These formulations met the criteria for nanoemulsions (< 200 nm) and exhibited fine droplet sizes, which are generally correlated with high surface area and the potential for increased kinetic stability and bioavailability of the active compounds. In contrast, the 75% *T. sinensis* + 25% *C. papaya* formulation (160.2 nm; +8.2 mV) and especially the 50%:50% formulation (287.2 nm; −13.1 mV) exhibited significantly larger particle sizes, with the 50:50 formulation even exceeding the general nanoemulsion limit and approaching coarse microemulsion. The ratio of extract composition affects the droplet formation process, possibly through interactions between phytochemical components (e.g., saponins, polyphenols, and natural surface-active compounds) and surfactants, thereby altering interfacial tension and emulsification efficiency [26].

The zeta potential values for all formulations ranged from −13.1 to +8.2 mV, indicating low charge (Figure 1). According to colloid theory, dispersion systems with zeta potential ≥ 30 mV are generally considered to have good

electrostatic stability. At the same time, values below this range indicate stability that is more dependent on steric stabilization from nonionic surfactants or stabilizing polymers. The formulations have very small nanoparticle sizes, and their long-term stability is likely determined more by the type and concentration of surfactants than by electrostatic repulsion alone. The difference in charge sign (positive in the 75:25 formulation and negative in the others) also indicates a difference in the dominance of ionized functional groups from the extract components at the droplet interface. Overall, single-based formulations (100% *T. sinensis* or 100% *C. papaya*) and 25:75 mixtures appear to be the most promising in terms of particle size, but further optimization is needed to increase zeta potential and improve dispersion stability.

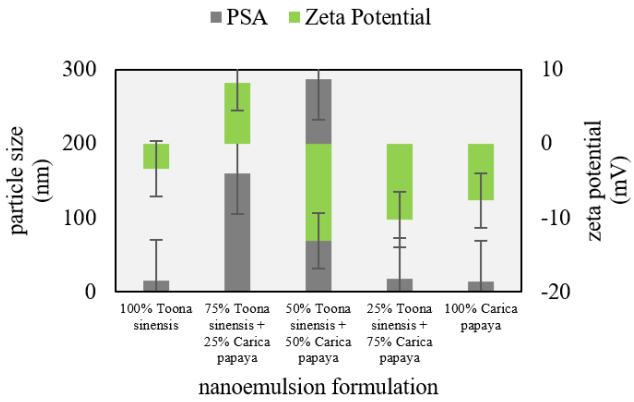

Figure 1. Particle size and zeta potential of nanoemulsion formulations from *T. sinensis* and *C. papaya* leaf extract
PSA: particle size analysis

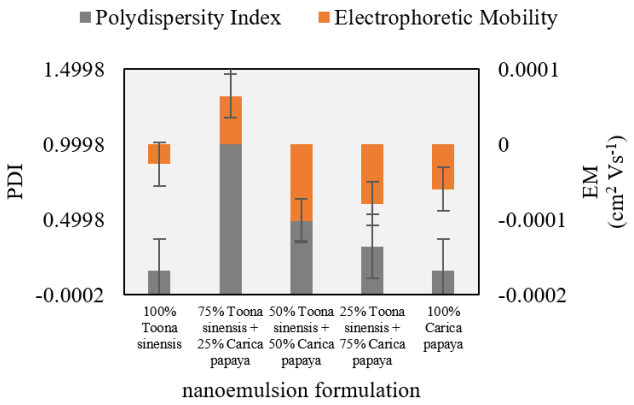

Figure 2. Polydispersity index (PDI) and electrophoretic mobility of nanoemulsion formulations from *T. sinensis* and *C. papaya* leaf extract

The PDI and electrophoretic mobility of nanoemulsions extracted from *Toona sinensis* and *Carica papaya* leaves indicate differences in droplet size uniformity and surface charge characteristics between the formulations. The lowest PDI values were found in the 100% *T. sinensis* and 100% *C. papaya* formulations (both 0.16), indicating a very narrow particle size distribution and a relatively homogeneous dispersion system (Figure 2). The 25% *T. sinensis* + 75% *C. papaya* formulation was also considered good (PDI 0.32), as nanoemulsions with PDI < 0.3–0.4 are generally considered to have a sufficiently uniform size distribution and physical stability. The 50:50 formulation showed a moderate PDI (0.57), suggesting increased droplet-size heterogeneity. Meanwhile, the 75% *T. sinensis* + 25% *C. papaya* formulation had a very high PDI (1.30), indicating a highly polydisperse and less stable system, possibly due to an imbalance in the composition of bioactive components and surfactants, which triggered droplet coalescence or aggregation during emulsion formation. The smaller the PDI value, the more uniform the particle size and the better the quality of the resulting nanoemulsion [27].

The electrophoretic mobility values of all formulations were in the small range, both negatively and slightly positively charged (approximately −0.000102 to +0.000064), indicating that the nanoemulsion droplets carried a weak surface charge (Figure 2). The negative mobility values in most formulations indicate the dominance of negatively charged groups from phenolic compounds, organic acids, or surfactant components at the droplet interface. In contrast, the small positive values in the 75:25 formulation suggest a possible contribution from cationic groups or the adsorption of certain components from the extract. The magnitude of electrophoretic mobility is directly proportional to the zeta potential; low values indicate weak electrostatic stability, so the system's stability is likely to depend more on steric stabilization by nonionic surfactants or polymer layers on the droplet surface. Thus, formulations with low PDI (100% *T. sinensis*, 100% *C. papaya*, and 25:75) are the most promising for homogeneity. Further increasing the surface charge or optimizing the surfactant is still necessary to enhance long-term colloidal stability [28].

4. CONCLUSION

Phytochemical screening revealed the presence of dominant secondary metabolites, including flavonoids, tannins, saponins, and steroids. GC-MS analysis indicated that *T. sinensis* is rich in phenolics and polar glycosides with strong antioxidant activity [1,2,3-Benzenetriol (Pyrogallol) and Ethyl gallate], while *C. papaya* contains more lipophilic compounds such as fatty acid esters, terpenoids, and phytosterols, resulting in a complementary bioactive spectrum. Stability analysis showed minimal pH changes (5.9–6.9), indicating good chemical stability. Among formulations, the 25:75 (Ts: Cp) ratio produced optimal characteristics, including small particle size (17.3 nm), low PDI (0.32), and homogeneous dispersion. However, a low zeta potential suggests reliance on steric stabilization. This formulation is recommended for further bioassay and optimization studies.

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