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Original Article

Influence of ginger essential oil nanoemulsion delivery system on antioxidant activity and postharvest *Davallia* frond vase life

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Abstract

Plant essential oils have limited water solubility, for which problem oil-in-water emulsions provide a good solution. The aim of this work was to prepare a ginger essential oil (*Zingiber officinale* Rosc.; GEO) coarse emulsion, microemulsion, and nanoemulsion all with the same formula, determine their characteristics, and compare their antioxidant activities and utility as holding solutions for *Davallia* fronds. A coarse emulsion was firstly formed by a magnetic stirrer of GEO with a 1:2 by weight mixture of Tween 20 and Span 20 at weight ratio of 1:8, respectively. The coarse emulsion droplets (810.0 nm) were then broken by sonication and high-pressure homogenization to create the microemulsion (426.1 nm) and nanoemulsion (76.4 nm), respectively. The three emulsions exhibited ς-potential values more negative than -30 mV, indicating them to be stable. All three emulsions demonstrated DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ free radical scavenging capacities significantly higher than those of GEO in ethanol. Meanwhile, the nanoemulsion significantly improved the Fe^{2+} chelating effect Finally, in a bioefficacy experiment with *Davallia* fronds, both the microemulsion at concentrations of 5 and 10 mg/mL and the coarse emulsion at 10 mg/mL were found to extend frond vase life. The nanoemulsion demonstrated superior frond longevity at low concentration (5 mg/mL), but at higher concentration (10 mg/mL), vase life and leaf chlorophyll were not improved and malondialdehyde formation increased. Based on the current investigation, emulsification significantly enhances the antioxidant activities of GEO. Vase solutions containing high concentrations of coarse emulsion (10 μg/mL), microemulsion (5 and 10 μg/mL), and low concentration of nanoemulsion (5 μg/mL) increased *Davallia* frond longevity from 6.3 days to up to 11.9 days. Our findings suggest that there is an optimal concentration range for using essential oil emulsions in different delivery systems as a preservation solution in cut fronds.

Keywords: Cut leaf, Delivery vehicle, Fern, Oil-in-water, Holding solution

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Introduction

Essential oils are complex mixtures of typically lipophilic and water-soluble compounds that are primarily classified into alkaloids, monoterpenes, phenolic acids, flavonoids, isoflavones, carotenoids, and aldehydes (Seow et al., 2014). Ginger (*Zingiber officinale* Rosc.) is a *unique* aromatic herb possessing both non-volatile and volatile oils and is an important horticultural crop in tropical Southeast Asia. Ginger *essential oil (GEO)* extracted from the *rhizome* contains numerous naturally occurring aromatic compounds that account for the oil's various bioactivities (Kumar et al., 2014). In particular, compounds such as α-zingiberene, geranyl acetate, β-myrcene, β-bisabolene, α-curcumene, α-farnesene, D-limonene, α-sabinene, and β-sesquiphellandrene have been identified as contributing to its antioxidant properties (Wahyuni et al., 2024). Despite the useful properties of essential oils, their application into aqueous-based matrices is somewhat restricted by their limited solubility in water (Yammine et al., 2024). Water-immiscible essential oils are commonly diluted with organic solvents including dimethyl sulfoxide (Cao et al., 2009), ethanol (Li et al., 2010), and methanol (Torres-Martínez et al., 2017; Zouari et al., 2012); however, such water-miscible organic solvents can alter the polarity of the aqueous phase when mixed with a liquid medium (Chen et al., 2014). Nano- and micromaterials have been developed in a number of delivery systems, including biomimetic nanoparticles (Hasan et al., 2021), solid lipid particles (Dang et al., 2014), liposomes (Hasan et al., 2014; Hasan et al., 2019), and emulsions (Bhargava et al., 2015; Zhang et al., 2014), to improve solubility, stability, controlled release, antioxidant and antibacterial activity, and demonstrate targeted delivery properties. An alternative approach for solubilizing an essential oil is to create an oil-in-water emulsion which have been showed to be a promising delivery system to offer several beneficial properties in terms of enhanced solubility, high encapsulation efficiency, high physical stability, and high bioavailability and overcome the different limitations of essential oils (Yammine et al., 2024). Polysorbate has been preferred as the surfactant for essential oil delivery systems (Cortés et al., 2021), and may be used to improve emulsion stability (Anton et al., 2008). This and other emulsifiers are surface-active materials that have the ability to adsorb at the oil-water interface, decrease interfacial tension and prevent droplet coalescence (Ozturk and McClements, 2016). After mixing oil and surfactant, the initially large emulsion droplets are broken into smaller droplets using a high energy method, ultimately yielding a fine oil emulsion (Håkansson and Rayner, 2018). Nanoemulsions, characterized as finely dispersed emulsions, have proven to be an effective delivery system for the encapsulation, protection, and transport of lipophilic bioactive compounds (Choi and McClements, 2020). The smaller droplet size of a fine emulsion may not only facilitate active molecule transport across biological membranes, but also raise the surface-area-to-volume ratio, increasing functionality (Christaki et al., 2022; Zulfiqar et al., 2019). Enhanced antioxidant functionality of nanoemulsions has been demonstrated in comparison with similar non-nanoformulated systems and with organic solvents (Lou et al., 2017). In terms of applications, essential oil nanoemulsions have been used as postharvest antimicrobial agents and have demonstrated efficient decay control and quality maintenance of fresh lettuce (Bhargava et al., 2015), citrus (Yang et al., 2021), and papaya (Yu et al., 2024).

Davallia fern is marketed as cut foliage and functions as filler in floral arrangements or complement colorful floral bouquets, owing to the beautiful symmetry of its fronds and lush green foliage (Safeena et al., 2019). In a previous study, we showed GEO emulsified with sodium alkyl benzene sulfonate at a weight ratio of 4:1 to successfully extend the vase life of *Davallia* frond. After pulsing with 25 mg/L essential oil for 6 h, fronds were placed in a holding solution with 5 mg/L essential oil (Teerarak and Laosinwattana, 2019). However, little has been done to compare GEO nanoemulsions with similar non-nanoformulated delivery systems in terms of antioxidant activities and *Davallia* frond vase life. This study addresses that gap by producing matched emulsions with different oil droplet sizes using a magnetic stirrer, sonicator, and high-pressure homogenizer, then comparing their antioxidant activities and efficacy as a holding solution for vase life extension of *Davallia* fronds.

Material and Methods

The schematic diagram of the methodology is illustrated in Figure 1. The first experiment involves the formation of GEO emulsion using a low or high energy method to generate coarse emulsion,

microemulsion, and nanoemulsion, and the second experiment is conducted to compare their antioxidant activities. Then, the third experiment involves utilizing all three emulsions as floral preservative solutions for *Davallia* fern.

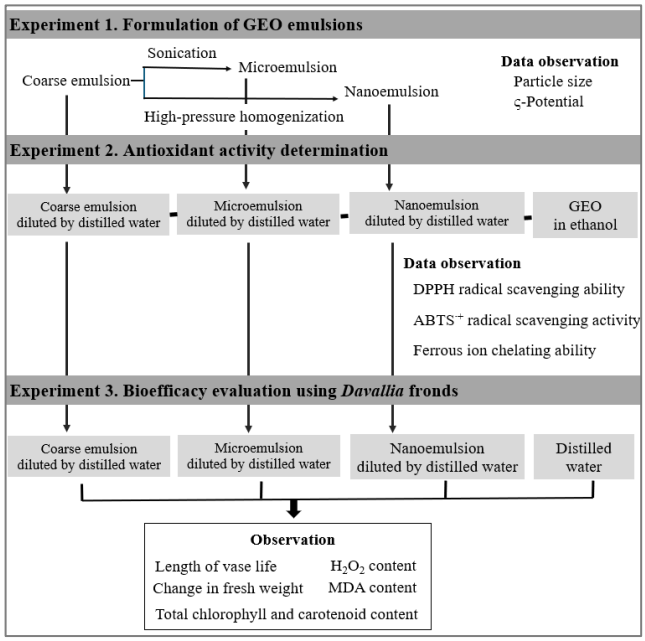

Figure-1. Schematic diagram of this study.

Essential oil

Ginger essential oil (*Zingiber officinale* Rosc.) was purchased from the Thai-China Flavours and Fragrances Industry Co., Ltd. (Nonthaburi, Thailand), CAS No.: 8007-08-7 which is HAACP-certified.

Plant material

Davallia fronds were obtained from a commercial garden in Ratchaburi Province, Thailand. They were harvested early in the morning, placed upright in a bucket partially filled with tap water and covered with a plastic film to prevent moisture loss, and brought to the laboratory within 3 h.

Formulation of GEO emulsions

To produce oil-in-water emulsions, a pre-concentrate was first prepared by magnetic stirring of GEO with a 1: 2 by weight mixture of Tween 20 (surfactant) and Span 20 (co-surfactant) at a weight ratio of 1:8, respectively. This yielded a 10 mg/mL stock solution in the form of a coarse emulsion. To produce fine emulsions, 50 mL of the coarse emulsion was dispersed by sonication at 37 kHz for 30 min or by high-pressure homogenization (M-110P Microfluidizer Processor, Newton, MA, USA) at 15000 psi for two cycles.

Particle size and ς-potential analysis

Measurements were collected at least three times, and the average size is reported. The ς-potential of the oil/water emulsion was measured using a Nano plus Zeta/Nano Particle Analyzer. Electrophoretic mobility values of colloidal particles were automatically calculated from ς- potential using the Smolochowski equation (Shaw, 1970), and the results are expressed in mV.

Antioxidant activity

Preparation of GEO delivery systems

Organic solvent formulation. A stock solution of 10 mg/mL GEO in absolute ethanol was prepared, then used to produce a range of dilutions (1-10 mg/mL). All dilutions were equilibrated for 2 h at room temperature before use in antioxidant activity evaluations.

Oil-in-water formulation. Oil-in-water emulsions of GEO were performed as previously described. Each emulsion stock solution (10 mg/mL) was then used to produce a range of dilutions (0.1-10 mg/mL). All dilutions were equilibrated for 2 h at room temperature before use in antioxidant activity evaluations.

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability

The method of Miliauskas et al. (2004) was adopted for the determination of DPPH radical-scavenging activity. Briefly, 4 mL of ethanol-DPPH solution (0.1 mM) was added in each of test tubes containing 2 mL of each GEO formulation at the optimum concentration for maximum antioxidant activity. The resulting solution was kept standing for 30 min before the absorbance was read at 517 nm. A positive control with ascorbic acid was prepared in the same manner. The percentage scavenging activity of GEO delivery system was calculated and the GEO concentration (mg/mL) that provided 50% scavenging ability (EC_{50}) was determined by plotting activity against concentration.

$ABTS^{\cdot+}$ radical scavenging activity

The ability of the various GEO delivery systems to scavenge the $ABTS^{\cdot+}$ was assessed according to Re et al. (1999). First, the radical was generated by reacting with 7 mM ABTS and 2.45 mM potassium persulfate (final concentration) and held in the dark at room

temperature for 16 h. After incubation, the radical solution was further diluted with ethanol to an absorbance of 0.7 ± 0.2 units at 750 nm. To determine the scavenging activity, 180 µL of diluted solution and a 20 µL aliquot of GEO sample were mixed and the absorbance was taken after 5 min at 750 nm. Ascorbic acid was used as a positive control. $ABTS^{\cdot+}$ radical-scavenging activity was determined as a percentage and the EC_{50} (mg/mL) was calculated from a plotted graph scavenging activity against GEO concentration.

Ferrous ion chelating ability

The capacity of the various GEO delivery systems to chelate ferrous ions was determined according to the method of Dinis et al. (1994) with EDTA as a reference standard. Each GEO sample (2.5 mL) was mixed with 0.2 mL of 2 mM $FeCl_2$ and then 0.4 mL of 5 mM ferrozine to initiate the reaction. The resultant mixture was kept standing for 10 min before the absorbance was measured at 562 nm. The percentage of ferrous ion chelating ability of GEO delivery system was calculated.

Bioefficacy evaluation using *Davallia* fronds

GEO emulsion treatment

Only undamaged fronds were used. The fronds were graded for uniformity of appearance and size (25-30 cm in length) and recut to a uniform length of 12 cm. These cut fronds were placed into holding solutions comprised of the various GEO formulations (coarse emulsion, microemulsion, and nanoemulsion) diluted by distilled water to concentrations of 5 and 10 ug/mL, where they were maintained until the end of the experiment.

Length of vase life

Vase life of cut fronds was evaluated daily. Fronds were considered terminated when they showed yellowing or symptoms of desiccation.

Change in fresh weight

Individually numbered fronds were weighed at the beginning of the experiment and re-weighed daily. Relative fresh weight was expressed as percentages of change from the initial weight.

Determination of total chlorophyll and carotenoid content

Total chlorophyll and carotenoid contents of leaf samples were assessed according to Lichtenthaler and Wellburn (1983). Briefly, frond samples (0.1 g) were ground in 80% aqueous acetone and extracted at 4 °C for 48 h in the dark. The pigment extract was filtered, and the final volume of extract was increased to 10 mL with 80% aqueous acetone. The quantification was performed with a spectrophotometer (GENESYS 20, Thermo Fisher Scientific, USA). The amount of these pigments was expressed as mg per g of dry weight (mg/g DW) using the equation reported by Lichtenthaler and Wellburn (1983).

Determination of malondialdehyde (MDA) content

For determination of MDA content, *Davallia* fronds (0.1 g) were ground in cold 20% trichloroacetic acid (TCA). After centrifugation, aliquot 1 mL of supernatant was mixed with 4 mL of 0.5% thiobarbituric acid in 20% TCA and 0.1 mL of 4% butylhydroxytoluene solution. This mixture was incubated at 95 °C for 30 min and then immediately cooled in ice bath. The MDA concentration was calculated from the absorbance of the thiobarbituric acid reactive substances measured at 532 nm and corrected for nonspecific absorbance at 600 nm and assuming an extinction coefficient of 155 mM^{-1} cm^{-1}. The MDA content was expressed as µmol/g on the basis of fresh weight (Heath and Packer, 1968).

Determination of hydrogen peroxide (H_2O_2) content

The determination of H_2O_2 levels in *Davallia* fronds was assessed according to (Velikova et al., 2000). Frond samples (0.5 g) were extracted with 5 mL of 0.1% (w/v) TCA and the extract was centrifuged at 10,000×*g* for 20 min. The reaction mixture of 1 mL extract, 1 mL of phosphate buffer (pH 7.0) and 2 mL of 1000 mM potassium iodide was mixed. The absorbance of the mixture was measured at 390 nm. A calibration curve plotted using authentic H_2O_2 was used to determine the H_2O_2 concentration. The results were expressed as nmol/g fresh weight.

Statistical analysis

A completely randomized experimental design was used, with ten replications per treatment for determination of vase life and relative fresh weight. Three replicates were used for measuring antioxidant activities and content of chlorophyll, carotenoid, MDA, and H_2O_2. Statistical significance was tested by one-way analysis of variance (ANOVA). Tukey's test was applied to compare the mean values when ANOVA showed significant differences.

Results

Characterization of GEO emulsion formulations

The average droplet size, ς-potential, and polydispersity index (PDI) of GEO coarse and fine emulsions are presented in Table 1, and droplet size distributions in Figure 2. As expected, the method of mixture preparation significantly influenced emulsion droplet size. All three emulsions exhibited a strong surface charge with values less negative than -30 mV, reflecting stable emulsions. The fine emulsions had smaller PDI values, indicating the two-step emulsification process to produce a relatively narrow size distribution; as illustrated in Figure 2, distributions were also monomodal. Visually, both the coarse emulsion and the sonicated microemulsion were milky white, while the nanoemulsion was transparent.

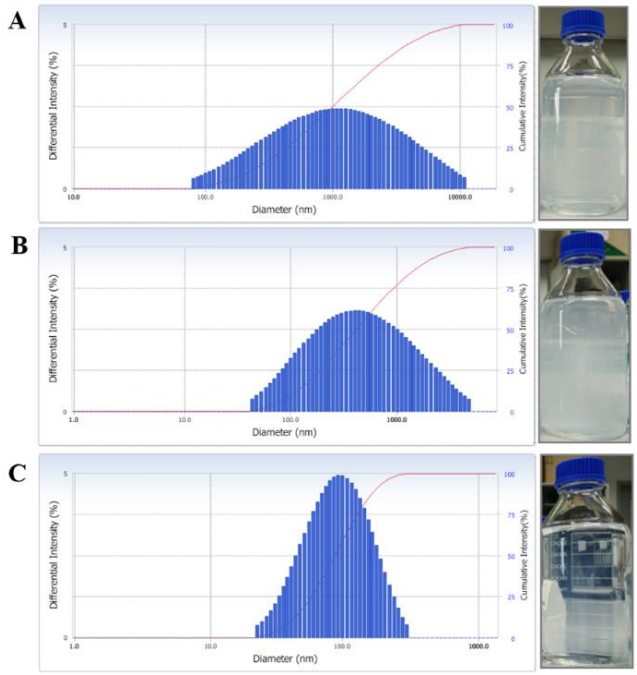

Figure-2. Particle size distribution and visual appearance of ginger essential oil emulsions produced by magnetic stirring (A), sonication (B), and high-pressure homogenization (C).

Comparative study of antioxidant activity

The antioxidant activity of GEO formulated in four delivery systems (organic solvent, coarse emulsion, microemulsion, and nanoemulsion) was evaluated *in vitro* in terms of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging ability and ferrous ion chelating ability, with ascorbic acid and EDTA as respective reference compounds.

Table-1. Average droplet size (nm) ς-potential (mV), and polydispersity index (PDI) of coarse and fine ginger essential oil emulsions.

Homogenization method	Oil droplet size (mm)	ς-Potential (mV)	Polydispersity index
Magnetic stirring	.8100±13.5	-33.63±1.89	0.409±0.006
Sonication	±426.111.7	-37.68±1.75	0.±2880.004
High-pressure homogenization	76.4±8.6	-35.61±1.81	0.2±370.004

Figure 3A plots the results of the DPPH free radical scavenging assay. At high concentrations of 1–10 mg/mL, GEO diluted in absolute ethanol scavenged 10.55-54.94% of DPPH$^{\bullet}$. Emulsions exhibited enhanced scavenging capacity, with values ranging from 18.85 to 74.12% for the coarse emulsion (0.1-1 mg/mL), 20.24 to 88.51% for the microemulsion (0.1-1 mg/mL), and 34.91 to 100% for the nanoemulsion (1-5 mg/mL). For comparison, the synthetic antioxidant ascorbic acid (0.001 to 0.1 mg/mL) scavenged 2.04 to 100% of DPPH$^{\bullet}$. The EC_{50} values obtained for each emulsion are listed in Table 2; a low value indicates high free radical scavenging capacity. All emulsions demonstrated EC_{50} values significantly lower ($p \leq 0.05$) than that of GEO in ethanol; these values were not as low as, but also not significantly different from ($p > 0.05$), the ascorbic acid control.

Figure 3B similarly illustrates the results of the ABTS assay. As with the DPPH assay, ascorbic acid demonstrated highly effective scavenging capacities at low concentrations of 0.1-1 mg/mL, while GEO in all delivery systems was effective at concentrations of 1-10 mg/mL. EC_{50} values among the GEO emulsions did not differ significantly ($p > 0.05$), but were significantly lower than that of GEO in ethanol ($p \leq 0.05$) and significantly greater ($p > 0.05$) than that of ascorbic acid.

The results of the ferrous ion chelating assay are shown in Figure 3C. The reference standard (EDTA) again demonstrated potent ability at low concentrations (0.05-1 mg/mL). GEO in ethanol (0.05-10 mg/mL) exhibited no detectable chelating activity. The coarse emulsion and microemulsion exhibited low levels of chelation at the highest tested concentration of 10 mg/mL, while the nanoemulsion exhibited modest chelation ability at all concentrations tested (1-10 mg/mL).

Bioefficacy assay using postharvest *Davallia* fronds

To further evaluate the GEO emulsion delivery systems as potential carriers of bioactive substances, we applied them as holding solutions for *Davallia* fronds and evaluated their effects on vase life. Vase solutions containing 10 µg/mL coarse emulsion, 5 and 10 µg/mL microemulsion, or 5 µg/mL nanoemulsion effectively extended frond longevity, from 6.3 days in untreated fronds to as much as 11.9 days in treated fronds. Notably, when using the nanoemulsion at 10 µg/mL, vase life was not significantly different from untreated fronds (Figure 4A). As illustrated in Figure 5, frond senescence during vase time (evaluated based on symptoms of desiccation or signs of yellowing) was dependent on the preservative solution and correlated with the length of vase life.

Figure 4B presents the change in frond fresh weight during vase time, expressed as a percentage of initial fresh weight. In control leaves, fresh weight continuously decreased during vase time, dropping to 89% on day four and then declining sharply through the end of the experiment. Conversely, fronds held in 10 µg/mL microemulsion exhibited increased fresh weight during the first five days of vase time, and maintained greater values than controls throughout the experiment. For all other treatments, fresh weight remained close to 100% during the first four days of vase life and did not drop below 90% until days 7-9.

The effect of holding solutions on frond chlorophyll and carotenoid contents is illustrated in Figures 6A and B. Both total chlorophyll and carotenoid content decreased continuously as vase time increased. All emulsion formulas generally showed values similar to the control treatment, though somewhat lower total chlorophyll and carotenoid contents were observed in the control and the 10 µg/mL nanoemulsion.

Similarly, the effects of holding solutions on frond lipid peroxidation and hydrogen peroxide content are illustrated in Figures 6C and D. Fronds held in 5 µg/mL coarse emulsion and 10 µg/mL nanoemulsion showed lipid peroxidation levels similar to control throughout the experiment. Meanwhile, the other emulsions demonstrated significantly lower lipid oxidation on day 12. Regarding H_2O_2 content, no differences were evident in the first nine days. On day 12, all emulsion samples exhibited significantly lower H_2O_2 content relative to the control.

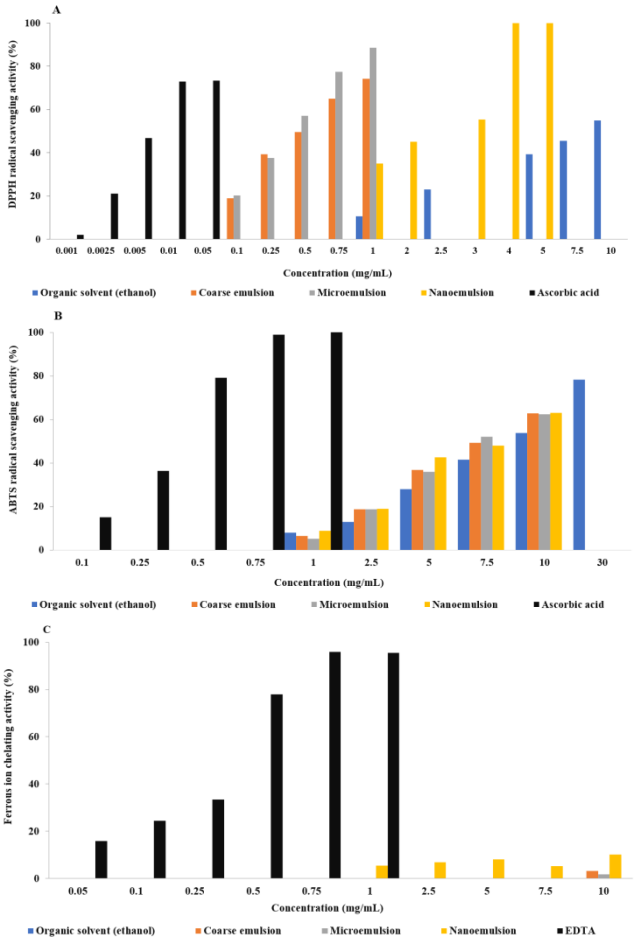

Figure-3. Antioxidant activity of ginger essential oil according to delivery system, measured as DPPH (A) and ABTS (B) scavenging activities and ferrous ion chelating activity (C).

Table-2. DPPH and ABTS scavenging activities (as EC_{50}) of ginger essential oil according to delivery system.

Delivery system	DPPH EC_{50} (mg/mL)	ABTS EC_{50} (mg/mL)
Ethanol solution	8.42±0.10a	8.42±0.39a
Coarse emulsion	0.41±0.02b	7.42±0.32b
Microemulsion	0.32±0.05b	7.24±0.14b
Nanoemulsion	1.76±0.06b	7.17±0.36b
Ascorbic acid	0.02±0.00b	0.25 ± 0.01c

Means with different letters in the same column indicate significant differences ($P < 0.05$) among treatment according to Tukey's studentized range test.

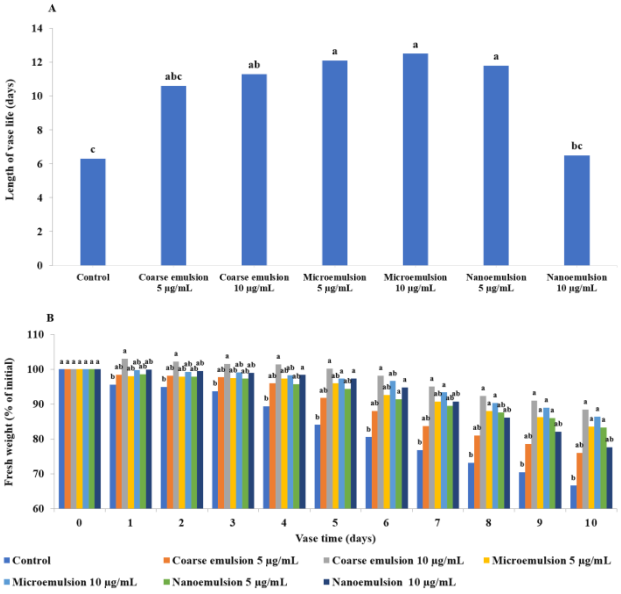

Figure-4. Length of vase life (A) and relative fresh weight (B) of *Davallia* fern fronds treated with ginger essential oil emulsions. Different letters above bars indicate significant differences (P < 0.05) among treatment according to Tukey's studentized range test.

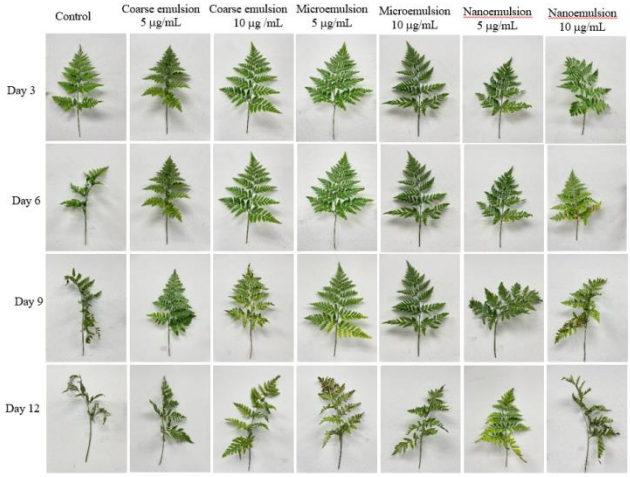

Figure-5. Photographs of *Davallia* fern fronds maintained in ginger essential oil emulsions for 12 days.

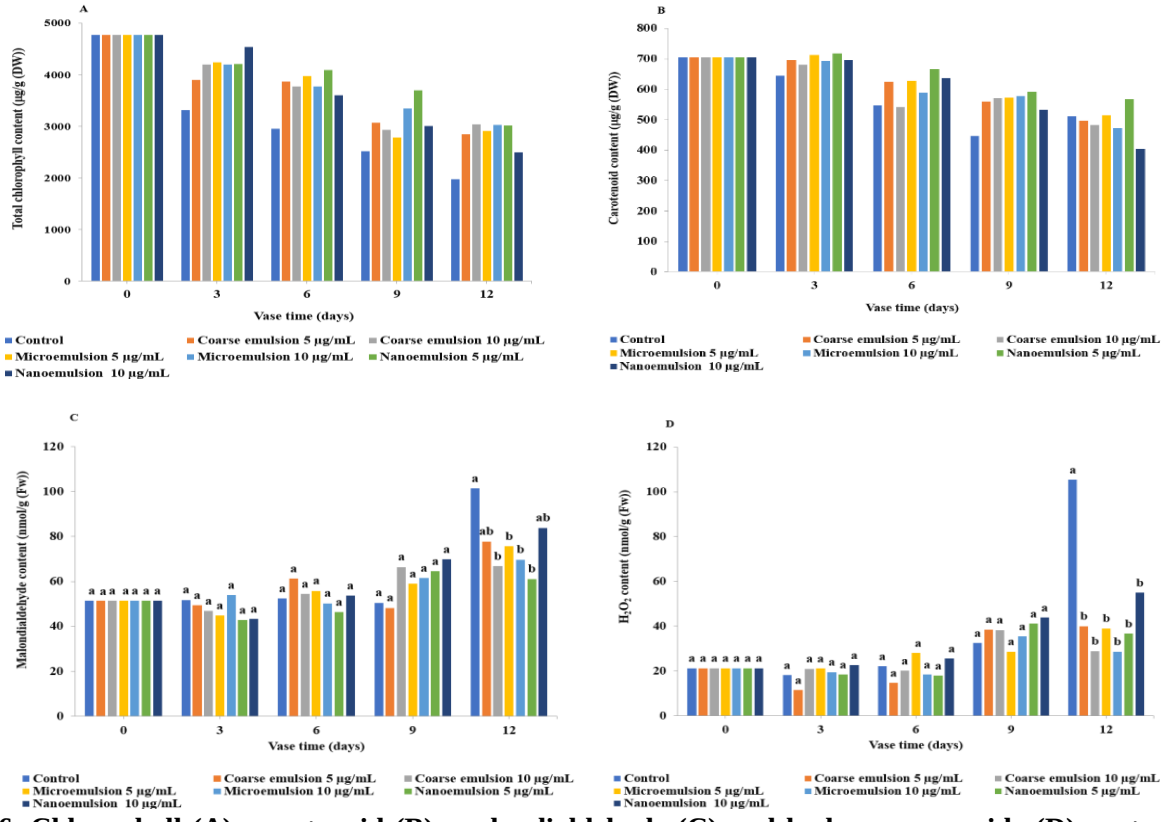

Figure-6. Chlorophyll (A), carotenoid (B), malondialdehyde (C) and hydrogen peroxide (D) contents of *Davallia* fern fronds treated with ginger essential oil emulsions. Different letters above bars indicate significant differences (P < 0.05) among treatment according to Tukey's studentized range test.

Discussion

This study investigated whether incorporation of GEO in a water-in-oil emulsion and refinement of that emulsion enhances its dispersibility in water, promotes its antioxidant activity, and prolongs the vase life of *Davallia* fern fronds.

An oil-in-water coarse emulsion was firstly formed as an aqueous solution containing GEO and a mixture of Tween 20 (surfactant) and Span 20 (co-surfactant) at weight the ratio of 1:8, respectively, all combined under magnetic stirring. Then, 30 min of sonication at 37 kHz and two cycles of high-pressure homogenization at 15000 psi were performed to obtain a microemulsion and nanoemulsion, respectively. External energy in the form of stirring was provided to facilitate microemulsion; this energy aids in overcoming kinetic energy barriers or mass transport limitations that retard spontaneous formation of an emulsion (McClements, 2012). Ultrasonication is acoustic cavitation that promotes the disruption of oil droplets, thereby minimizing droplet size (Leong et al., 2017). High-pressure homogenization is a high-energy emulsification method characterized by strong *shearing force (Yadav and Kale, 2020)* that efficiently facilitates formation of very fine emulsions or nanoemulsions. Decreasing droplet size can promote physical stability, bioavailability, and optical transparency (McClements, 2012). In this study, the emulsions all exhibited ς-potential values more negative than -30 mV, which indicate the droplet electrical charge is strong enough for repulsive or electrostatic effects to oppose droplet coalescence (Sabbah et al., 2016). The obtained PDI values were 0.409±0.006 for the coarse emulsion, 0.288±0.004 for the microemulsion, and 0.237±0.004 for the nanoemulsion, and all emulsions showed a monomodal size distribution. Uniformly small particle size with a monomodal distribution is highly desired as it promotes a more physically stable dispersion; PDI < 0.3 indicates a population of particles with homogeneity (Hasan et al., 2016).

The potential of an essential oil to inhibit free radicals may depend on the specific compounds involved in eliminating and preventing free radical formation (Ma et al., 2018). That is to say, EO antioxidant activity is related to its chemical composition (Peng et al., 2016). Typically, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ are used to evaluate the ability of plant extracts to act as free radical scavengers by single electron transfer (Chaves et al., 2020). In this study, all emulsions demonstrated significantly improved $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging capacities compared to GEO in ethanol. These results are consistent with previous observations in which surfactants assist dissolution of an essential oil in an aqueous phase, resulting in higher antioxidant activity (Lou et al., 2017). In addition, all GEO emulsions demonstrated comparable scavenging activities, suggesting that an emulsion's antioxidant properties correspond to its active ingredients and not droplet size. This work also assayed ferrous ion chelating ability, another common measure of antioxidant activity. Ferrous ions are known to trigger lipid peroxidation through the Fenton reaction, as well as to accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxy radicals (Juan et al., 2021). Here, the GEO nanoemulsion demonstrated greater chelating capability than the coarse emulsion, the microemulsion, and GEO in ethanol. Essential oils are composed of dozens to hundreds of compounds, and it is probable that the constituents capable of chelating metal ions have very low aqueous solubility and/or are present at only low levels, resulting in low overall chelating activity. Nanoemulsions are capable of dissolving a number of hydrophobic compounds, and hence hold immense promise for enhancing essential oil chelating capacities.

In the bioefficacy assay using *Davallia* fern fronds, most emulsion treatments increased vase life. Notably, both limited amount of the coarse emulsion (5 µg/mL) and excessive amount of the nanoemulsion (10 µg/mL) resulted in shorter vase life periods not significantly different from control. Nanoemulsion positively influenced the quality and extended the vase life of *Davallia* fern at optimal concentrations but was ineffective at high concentration. The possible toxic effects of nanoemulsions are linked to their ability to markedly increase the bioavailability of bioactive compounds and the excessive translocation of active substances across a semipermeable membrane (Wani et al., 2018), which has a detrimental impact on the vase life of cut fern. Thus, the ability of GEO emulsion to prolong *Davallia* fern vase life appears related to droplet size: both a small droplet size at low concentration and a large droplet size at high concentration tend to extend vase life. Nanoemulsions with small particles of <100 nm exhibit high differential uptake efficiency when used in a vase solution and hence promote transportation

of essential oil components to the leaf. Meanwhile, the general effect of GEO emulsions on vase life extension could be explained by essential oil antimicrobial activities allowing water uptake by the cut fern stems to be maintained, thereby resulting in better maintenance of fresh weight relative to untreated fern. The GEO constituents borneol and linalool are known to have strong antimicrobial properties (Nychas et al., 2003), and preservative solutions containing these components inhibit microbial growth and prevent bacterial plugging of water-conducting tissues. Addition of essential oils to vase solutions was also reported to have positive effects on leaf chlorophyll in sunflower (Othman and Esmail, 2020); however, in this study, total chlorophyll and carotenoid contents did not significantly differ between untreated and treated fern fronds. In contrast, lower MDA content was observed on day 12 in fronds treated with coarse emulsion at 10 µg/mL, microemulsion at 5 and 10 µg/mL, and nanoemulsion at 5 µg/mL. MDA content is a measure of lipid peroxidation and hence reactive oxygen species (ROS) activity, as such chemical species cause damage to biomolecules such as lipids, proteins, and DNA. Polyunsaturated fatty acids in the plasma membrane are susceptible to oxidative ROS, particularly •OH and $O_2^{\bullet-}$, reaction with which yields conjugated dienes, lipid peroxyl radicals and hydroperoxides (Ayala et al., 2014). We also assayed H_2O_2 concentration directly, which is indicative of oxidative stress and produced in plant cells in response to drought, chilling, intense light, UV radiation, wounding, and pathogen infection (Sharma et al., 2012); it is also involved in regulation of the senescence process (Peng et al., 2005). This assay revealed reduced H_2O_2 concentrations for all GEO emulsion treatments. Overall, untreated cut fern fronds showed high levels of accumulated MDA and H_2O_2, which were indicative of severe oxidative stress and corresponded to shortened vase life. These results are consistent with the report of Zulfiqar et al. (2024) that borage (*Borago officinalis* L.) leaf extract possesses antioxidant properties, evidenced by decreased oxidative stress and reduced MDA and H_2O_2 concentrations. Through its antioxidant capabilities, GEO may help retain membrane integrity against oxidation either directly by interacting with lipid peroxyl radicals or indirectly by suppressing lipoxygenase activity (Beligni et al., 2002). Finally, the GEO nanoemulsion at 10 µg/mL (higher concentration) demonstrated no improvement in *Davallia* frond vase life and leaf chlorophyll content relative to the control alongside slightly increased malondialdehyde formation relative to other emulsion treatments, indicating acceptable phytotoxicity.

Conclusion

In this study, a GEO coarse emulsion (droplet diameter .8100 nm, PDI 0.409) was firstly formed by magnetic stirring of GEO with a 1: 2 by weight mixture of Tween 20 (surfactant) and Span 20 (co-surfactant) at weight ratio of 1:8, respectively. This solution was then sonicated at 37 kHz for 30 min or subjected to a high-pressure homogenizer at 15000 psi for two cycles to obtain a microemulsion (diameter 426.1 nm, PDI 0.288) and a nanoemulsion (diameter 76.4 nm, PDI 0.237), respectively. All formulations exhibited high negative zeta potentials indicative of emulsion stability (−33.63, −37.68, and −35.61 respectively), along with monomodal size distributions. DPPH and ABTS assays revealed all emulsions to have superior free radical scavenging activities compared to GEO in ethanol, while ferrous ion chelating assays showed the GEO nanoemulsion specifically to have enhanced chelating efficiency. Bioefficacy assays revealed the high concentration of coarse emulsion (10 µg/mL), both concentrations of microemulsion (5 and 10 µg/mL), and the low concentration of nanoemulsion (5 µg/mL) to extend the vase life of *Davallia* fern fronds. Moreover, treatment with emulsions restricted chlorophyll and carotenoid degradation, reduced malondialdehyde content, and reduced hydrogen peroxide content over time compared to the control. However, the high concentration of nanoemulsion (10 µg/mL) failed to increase frond vase life and leaf chlorophyll content over the control, and increased malondialdehyde formation relative to other treatments.

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Contribution of Authors

Kowwilaisang P: Collected and analyzed data, wrote the manuscript.
Teerarak M: Designed research methodology, analyzed and interpreted data, data presentation literature review and manuscript writing
Laosinwattana C: Project administration, provided laboratory facility, read and approved the manuscript

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