ORIGINAL ARTICLE

Nanoemulsion Formulation of *Andrographis paniculata* Extract: Comparative Evaluation of Eco-Friendly Surfactants for Sustainable Delivery Systems

Nurul Diyana Zainal[1], Fadzilah Adibah Majid[2], Nor Farahiyah Aman Nor[2], Harisun Yaakob[1]

[1] Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor
[2] Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu

ABSTRACT

Introduction: *Andrographis paniculata* (AP) is a medicinal plant widely recognized for its bioactive compound, andrographolide, which exhibits anti-inflammatory, antioxidant, and gastroprotective activities. However, poor aqueous solubility and limited oral bioavailability restrict its therapeutic effectiveness. Nanoemulsion systems offer a promising strategy to enhance solubility, stability, and delivery of plant-derived bioactives. This study aimed to comparatively evaluate the influence of eco-friendly, food-grade surfactants on the physicochemical characteristics of AP nanoemulsions. **Materials and Methods:** Nanoemulsions were formulated using coconut oil (10% w/w), freeze-dried AP extract (1% w/w), surfactant (5% w/w), and distilled water (84% w/w), with Tween 80, Tween 20, Span 80, polyethylene glycol 400 (PEG 400), and propylene glycol (PG) investigated as surfactants. Particle size and polydispersity index (PDI) were determined using dynamic light scattering, while entrapment efficiency (EE) was assessed by centrifugation followed by HPLC analysis. **Results:** Tween 80 and Span 80 produced nanoemulsions with the smallest droplet sizes (91.77 ± 2.14 nm and 87.73 ± 1.96 nm, respectively) and low PDI values (<0.13). Tween 80 exhibited the highest EE (95.34 ± 1.21%), followed by Span 80 (92.05 ± 1.48%). Other surfactants resulted in larger droplet sizes (>150 nm) and lower encapsulation efficiencies (<80%). **Conclusion:** These findings demonstrate that surfactant selection significantly affects nanoemulsion performance and highlight the potential of Tween 80 as an eco-friendly stabilizer for sustainable nanoemulsion-based delivery of AP extract in nutraceutical applications.
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Corresponding Author:
Harisun Yaakob, PhD
Email: harisun@utm.my
Tel: +60127830020

INTRODUCTION

Andrographis paniculata (AP), commonly referred to as “King of Bitters,” is traditionally used in Southeast Asian and Ayurvedic medicine for treating fever, inflammation, infections, and gastrointestinal disorders (1). Its primary active compound, andrographolide, has been extensively studied for its anti-inflammatory, antioxidant, hepatoprotective, and anti-ulcer activities. Despite its therapeutic potential, the clinical application of AP extract remains limited due to its poor aqueous solubility, instability in gastrointestinal fluids, and low oral bioavailability (2). These challenges necessitate the development of an effective delivery system that can enhance solubility and promote sustained release at the site of action.

Nanoemulsions, colloidal dispersions composed of oil, water, and surfactants, have emerged as a robust platform for encapsulating hydrophobic bioactives like andrographolide. Their small droplet size, typically below 200 nm, increases surface area and facilitates rapid absorption in the gastrointestinal tract (3,4). Moreover, nanoemulsions provide protection against degradation, control the release of active compounds, and potentially bypass first-pass metabolism. These advantages make them a suitable vehicle for enhancing the bioavailability of plant-based actives in oral and topical formulations (5).

The surfactant used in nanoemulsion formulation plays a pivotal role in determining the physical stability and biological performance of the final product. Beyond conventional chemical surfactants, there is increasing demand for eco-friendly, food-grade, and biodegradable alternatives to reduce environmental burden and support regulatory compliance, especially for nutraceutical and food-based applications (6). Surfactants derived from natural or non-toxic sources are preferred, particularly those recognized as safe for human consumption. Evaluating their emulsifying efficiency, compatibility with actives, and stability over time is essential for designing safe and sustainable delivery systems.

Several studies have individually explored the use of food-grade surfactants in herbal nanoemulsion systems, but few offer a comprehensive comparison using standardized formulation conditions (7). Moreover, while Tween series surfactants and Span 80 are frequently utilized, the roles of less conventional agents like polyethylene glycol (PEG 400) and propylene glycol (PG), especially as cosurfactants or stabilizers, remain underexplored in the context of AP nanoemulsions (8). A systematic evaluation comparing multiple surfactants under fixed formulation parameters will provide insights into their suitability, efficiency, and sustainability. This study seeks to address this research gap by evaluating the effect of various eco-friendly surfactants on the nanoemulsion characteristics of AP extract. By maintaining consistent oil and cosurfactant concentrations (9), the impact of surfactant type on key parameters such as droplet size, polydispersity index (PDI), and entrapment efficiency of andrographolide is assessed. The findings are expected to guide future formulation strategies for developing efficient, safe, and green delivery systems for plant-based therapeutic compounds.

MATERIALS AND METHODS

Plant Materials and Extraction

Fresh aerial parts of *Andrographis paniculata* were obtained from Naturemedics Laboratories Sdn. Bhd., Terengganu, Malaysia. The plant material was authenticated, and a voucher specimen number (to be inserted) was deposited for reference. The samples were washed thoroughly with distilled water, air-dried at room temperature (25 ± 2°C) for 5 days, and ground into fine powder (60 mesh particle size). Approximately 100 g of dried powder was subjected to aqueous extraction by boiling in 1 L of distilled water (solid-to-liquid ratio 1:10 w/v) at 100°C for 1 hour. The extract was allowed to cool to room temperature and filtered through Whatman No. 1 filter paper to remove plant debris. The filtrate was subsequently freeze-dried using a laboratory freeze dryer (Labconco FreeZone, USA) to obtain a dry powdered extract. The freeze-dried extract was stored in airtight containers at −20°C until further use in nanoemulsion formulation.

Chemicals and Reagents

Tween 20, Tween 80, Span 80, polyethylene glycol 400 (PEG 400), and propylene glycol (PG) were purchased from Sigma-Aldrich (USA). Coconut oil (food grade) was obtained from a local certified supplier in Malaysia. Methanol (HPLC grade) was purchased from Merck (Germany). All chemicals used were of food grade except for methanol.

Nanoemulsion Composition

The nanoemulsion formulations were prepared based on a previously optimized composition reported by Ibrahim (1) with minor modification. Each formulation consisted of 10% (w/w) coconut oil as the oil phase, 1% (w/w) freeze-dried *Andrographis paniculata* extract as the active component, 5% (w/w) surfactant, and 84% (w/w) distilled water as the aqueous phase. The total composition was maintained at 100% (w/w). To enable comparative evaluation of surfactant performance, only the type of surfactant was varied among formulations, while all other components and their respective concentrations were kept constant. The surfactants investigated in this study were Tween 20, Tween 80, Span 80, polyethylene glycol 400 (PEG 400), and propylene glycol (PG). This standardized formulation approach allowed direct assessment of the influence of surfactant type on droplet size, polydispersity index, and entrapment efficiency of the nanoemulsion systems.

Nanoemulsion Preparation

The AP extract (1% w/w) was dissolved in the oil phase (coconut oil). The surfactant (5% w/w) was added to the oil phase and mixed thoroughly. The aqueous phase (distilled water) was heated to 40°C. Both the oil phase and aqueous phase were heated to approximately 60 °C to reduce viscosity and facilitate emulsification. The aqueous phase was then gradually added to the oil phase under continuous stirring. The resulting mixture was subjected to high-speed homogenization using a rotor–stator homogenizer (Ultra-Turrax T25, IKA®, Germany) at 10,000–12,000 rpm for 3–5 min to obtain a coarse emulsion. The coarse emulsion was subsequently processed using a high-pressure homogenizer (IKA LAB 1000 High-Pressure Homogenizer, IKA®, Germany) operated at 800 bar for 5 cycles, as adapted from Ibrahim (1), to produce fine nanoemulsions with uniform droplet size distribution. All formulations were allowed to cool to room temperature prior to further characterization.
Particle Size and Polydispersity Index (PDI)

The droplet size and PDI were measured using dynamic light scattering (Malvern Zetasizer Nano ZS, Malvern Instruments, UK). Samples were diluted 1:100 with distilled water prior to measurement. All measurements were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD).

Entrapment Efficiency and HPLC Analysis

Entrapment efficiency (EE) was determined using a centrifugation method as described by Jamaludin (7). Briefly, 2 mL of nanoemulsion was centrifuged at 12,000 rpm for 30 minutes using a refrigerated centrifuge (Eppendorf 5810R, Germany) to separate free andrographolide from encapsulated drug. The supernatant containing free (unencapsulated) andrographolide was collected and analyzed using HPLC. The total andrographolide content in the nanoemulsion (before centrifugation) was also determined by dissolving a known quantity of nanoemulsion in methanol. The analysis was carried out using a C18 reverse-phase column, with a mobile phase of methanol:water (70:30, v/v) at a flow rate of 1.0 mL/min. Detection was done at a wavelength of 223 nm.

Entrapment efficiency was calculated using:

EE(%)=(Total andrographolide-Free andrographolide)/(Total andrographolide) x 100

Where:
Total andrographolide = amount present in nanoemulsion before centrifugation
Free andrographolide = amount detected in supernatant
All measurements were performed in triplicate (mean ± SD).

HPLC Analysis
Quantification of andrographolide was performed using an HPLC system (Agilent 1260 Infinity, USA) equipped with a UV detector.

Column: C18 reverse-phase column (4.6 x 250 mm, 5 μm)
Mobile phase: Methanol:Water (70:30 v/v)
Flow rate: 1.0 mL/min
Detection wavelength: 223 nm
Injection volume: 20 μL

The method was adapted from Ibrahim (1) with minor modifications.

Statistical Analysis
All experiments were performed in triplicate. Data were expressed as mean ± SD. Statistical comparisons were conducted using one-way ANOVA, with significance considered at $p < 0.05$.

RESULTS

Particle Size and Polydispersity Index (PDI)
The particle size and polydispersity index (PDI) of AP nanoemulsions prepared using different surfactants are summarized in **Table I**. Significant variations were observed depending on surfactant type. Nanoemulsions formulated with Tween 80 and Span 80 exhibited the smallest droplet sizes of 91.77 ± 2.14 nm and 87.73 ± 1.96 nm, respectively. These formulations also demonstrated low PDI values of 0.126 ± 0.008 and 0.117 ± 0.006, indicating narrow particle size distribution and good homogeneity. In contrast, formulations prepared with Tween 20, PEG 400, and PG produced significantly larger droplet sizes ranging from 156.45 ± 4.32 nm to 180.12 ± 6.27 nm. These systems also showed higher PDI values (>0.28), suggesting broader distribution and comparatively lower physical stability.

Entrapment Efficiency of Andrographolide
Entrapment efficiency (EE) values are presented in **Table I**. Tween 80-based nanoemulsions demonstrated the highest EE (95.34 ± 1.21%), followed by Span 80 (92.05 ± 1.48%). Nanoemulsions prepared using Tween 20, PEG 400, and PG exhibited lower EE values ranging from 70.40 ± 3.11% to 78.12 ± 2.36%. These results indicate that surfactant selection significantly influences andrographolide encapsulation performance.

Table I: Physicochemical properties of *Andrographis paniculata* nanoemulsions formulated with different surfactants. Particle size (nm), polydispersity index (PDI), and entrapment efficiency (EE, %) are expressed as mean ± standard deviation (SD) (n = 3).

Surfactant	Particle Size (nm)	PDI	EE (%)
Tween 80	91.77 ± 2.14	0.126 ± 0.008	95.34 ± 1.21
Span 80	87.73 ± 1.96	0.117 ± 0.006	92.05 ± 1.48
Tween 20	156.45 ± 4.32	0.289 ± 0.015	78.12 ± 2.36
PEG 400	172.30 ± 5.01	0.310 ± 0.018	74.50 ± 2.94
PG	180.12 ± 6.27	0.342 ± 0.021	70.40 ± 3.11

DISCUSSION

The present study demonstrates that surfactant type significantly influences the physicochemical characteristics of *Andrographis paniculata* nanoemulsions. As shown in **Fig. 1**, Tween 80 produced nanoemulsions with small droplet size and narrow size distribution. Its high hydrophilic–lipophilic balance (HLB ≈ 15) promotes effective stabilization of oil-in-water systems by reducing interfacial tension, thereby facilitating the formation of smaller droplets during homogenization (6). Smaller droplet size enhances surface area and contributes to improved kinetic stability (2). Span 80 also demonstrated favorable performance despite its lower HLB value (~4.3). This may be attributed to improved interfacial packing efficiency and strong interaction with the oil phase, which can enhance droplet stabilization under optimized conditions. Formulations containing Tween 20, PEG 400, and PG exhibited larger droplet sizes and higher PDI values, indicating less efficient emulsification under the fixed composition used in this study. PEG 400 and PG are generally more suitable as cosurfactants rather than primary emulsifiers, which may explain the lower stability observed.

Entrapment efficiency results further support the importance of surfactant molecular structure. As shown in **Fig. 2**, the high EE observed with Tween 80 may be attributed to its long hydrophobic chain, which enhances andrographolide incorporation into the oil core of the nanoemulsion droplets (7) Efficient encapsulation is essential for protecting lipophilic bioactives from

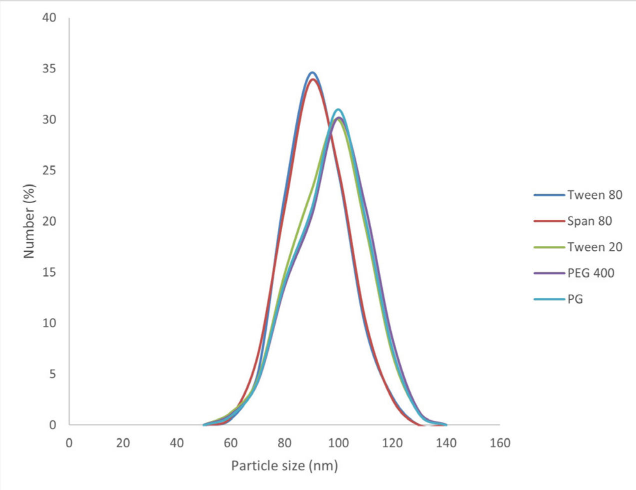

Fig. 1: Particle size and polydispersity index (PDI) of *Andrographis paniculata* nanoemulsions formulated with different surfactants. Tween 80 and Span 80 produced the smallest droplet sizes (91.77 ± 2.14 nm and 87.73 ± 1.96 nm, respectively) with low PDI values (0.126 ± 0.008 and 0.117 ± 0.006), indicating uniform and stable systems. In contrast, Tween 20, polyethylene glycol 400 (PEG 400), and propylene glycol (PG) resulted in larger droplet sizes and broader size distributions. Data are presented as mean ± SD (n = 3).

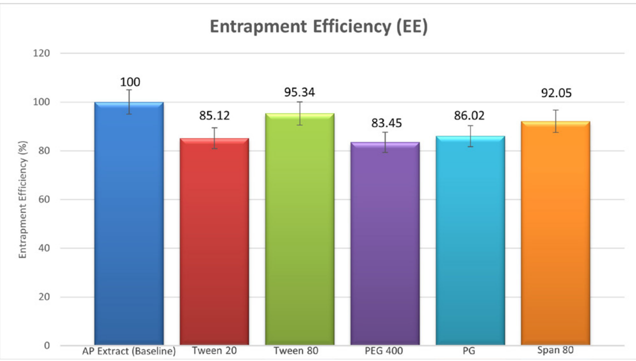

Fig. 2: Entrapment efficiency (EE) of andrographolide in *Andrographis paniculata* nanoemulsions prepared using different surfactants. Tween 80 achieved the highest EE (95.34 ± 1.21%), followed by Span 80 (92.05 ± 1.48%), whereas Tween 20, polyethylene glycol 400 (PEG 400), and propylene glycol (PG) showed lower encapsulation efficiencies. Data are presented as mean ± SD (n = 3).

degradation and improving bioavailability. Overall, the findings emphasize that proper surfactant selection is critical for achieving stable, high-performance nanoemulsion systems. The successful use of food-grade surfactants supports the development of sustainable delivery systems suitable for nutraceutical and functional food applications.

CONCLUSION

The study demonstrated that surfactant type significantly affects the physicochemical properties of *Andrographis paniculata* nanoemulsions. Tween 80 produced nanoemulsions with the smallest droplet size, lowest PDI, and highest entrapment efficiency. These findings highlight the importance of selecting appropriate eco-friendly surfactants to develop stable and sustainable nanoemulsion-based delivery systems. The results provide a basis for further biological evaluation and potential nutraceutical applications.

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