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Research Article

Formulation and Evaluation of *Brucea javanica* and *Wedelia chinensis* Co-Loaded Nanoemulsions for Anticancer Activity Against Gastric Cancer: Physicochemical Characterization, Cytotoxicity, and Migration Inhibition

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Abstract

Background: Gastric cancer remains a significant global health challenge with persistent limitations of conventional therapies including systemic toxicity and drug resistance. Natural product-based nanomedicines offer multi-targeted therapeutic strategies with potentially reduced toxicity profiles.

Objective: To develop and evaluate oil-in-water nanoemulsions co-encapsulating ethanolic extracts of *Brucea javanica* (BJ) and *Wedelia chinensis* (WC) for selective anticancer activity against gastric cancer cells.

Methods: A nine-formulation platform (F1–F9) was developed through systematic phase-diagram mapping and characterized for particle size, polydispersity index (PDI), zeta potential, pH, viscosity, stability, and extract content using dual-wavelength UV spectrophotometry (262 nm for BJ; 275 nm for WC). Lead formulation selection employed predefined criteria: size ≤ 200 nm, $PDI \leq 0.25$, $|\zeta| \geq 20$ mV, and extract recovery $\geq 97\%$. Biological evaluation included MTT cytotoxicity assays in AGS gastric cancer and HEK-293 normal cells, followed by scratch migration assays.

Results: All formulations achieved nanoscale dimensions (87.8–157.3 nm). F4 emerged as the optimal formulation with 87.8 ± 1.49 nm droplet size, $PDI 0.142 \pm 0.003$, and zeta potential -28.9 ± 0.36 mV. Extract recovery was excellent for both botanicals in F1–F5 ($\geq 97\%$), while F6–F9 showed systematically lower WC recovery (94.1–95.3%). Two-way ANOVA revealed significant formulation effects ($p < 0.001$), with extract-type differences driven by F6–F9; restricting analysis to F1–F5 eliminated extract effects, confirming balanced co-encapsulation. F4 demonstrated selective cytotoxicity with IC_{50} values of 42.18 ± 1.89 $\mu\text{g/mL}$ (AGS) and 89.34 ± 2.67 $\mu\text{g/mL}$ (HEK-293), yielding a selectivity index of 2.12. In scratch migration assays, F4 inhibited AGS cell migration by $51.37 \pm 1.84\%$ at 24 h compared to control.

Conclusion: Evidence-based formulation development yielded a stable, reproducible BJ–WC nanoemulsion with sub-100 nm size and narrow dispersity. The lead formulation F4 exhibited selective anticancer efficacy through dual mechanisms of cytotoxicity and migration inhibition, supporting its potential as a nano-phytopharmaceutical candidate for gastric cancer therapy.

Keywords: Gastric cancer; nanoemulsion; *Brucea javanica*; *Wedelia chinensis*; phytochemical co-delivery; MTT assay; scratch assay; migration inhibition

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1. Introduction

Gastric cancer represents the fifth most commonly diagnosed malignancy and the third leading cause of cancer-related mortality worldwide, with approximately 1 million new cases and 769,000 deaths reported annually (Sung et al., 2021). Despite advances in chemotherapy, targeted therapy, and immunotherapy, the five-year survival rate for advanced gastric cancer remains below 30%, largely due to systemic toxicity, drug resistance, and tumor heterogeneity (Machlowska et al., 2020; Thrift & El-Serag, 2020). These persistent clinical challenges necessitate the development of multi-targeted, lower-toxicity therapeutic strategies.

Plant-derived natural products have historically served as validated sources for anticancer drug discovery, with approximately 60% of clinically approved anticancer agents being of natural origin or inspired by natural scaffolds (Newman & Cragg, 2020). The inherent chemical diversity and multi-targeted bioactivity profiles of phytochemicals align well with the complex, multifactorial pathogenesis of cancer (Atanasov et al., 2021). Combinatorial approaches utilizing multiple botanical extracts may provide additive or synergistic effects through complementary mechanisms of action while potentially reducing individual compound toxicity (Efferth & Koch, 2011).

Brucea javanica (L.) Merr. (Simaroubaceae) is a medicinal plant traditionally used across Asian pharmacopeias for treating various malignancies. Its therapeutic efficacy is primarily attributed to quassinoids, particularly bruceine D and brusatol, which have demonstrated significant cytotoxic activity against multiple cancer cell lines through mechanisms including apoptosis induction, cell cycle arrest, and NF- κ B pathway inhibition (Chen et al., 2013; Lau et al., 2005). Pharmacological investigations have reported IC₅₀ values ranging from 2.8 to 15.6 μ g/mL against gastric cancer cells, supporting its potential as an anticancer agent (Subeki et al., 2007; Gao et al., 2011). However, the clinical application of BJ extracts is limited by poor aqueous solubility, low bioavailability, and dose-limiting gastrointestinal toxicity (Zhang et al., 2018).

Wedelia chinensis (Osbeck) Merr. (Asteraceae) has been extensively investigated for its antioxidant, anti-inflammatory, and anticancer properties, primarily attributed to wedelolactone, luteolin, apigenin, and other flavonoids (Lin et al., 2015). Studies have demonstrated that WC extracts induce G₂/M cell cycle arrest and apoptosis in nasopharyngeal carcinoma cells (Liu et al., 2013), while wedelolactone specifically exhibits potent cytotoxicity against breast cancer cells through ROS generation and mitochondrial dysfunction (Sarveswaran et al., 2010). The complementary bioactivity profiles of

BJ quassinoids and WC flavonoids suggest potential for synergistic anticancer effects when co-formulated.

Nanoemulsion delivery systems offer distinct advantages for phytochemical delivery, including enhanced solubilization of lipophilic compounds, protection from degradation, improved cellular uptake, and potential for passive tumor targeting through enhanced permeability and retention effects (McClements, 2012; Singh et al., 2017). Oil-in-water nanoemulsions, characterized by droplet sizes typically below 200 nm, provide thermodynamically stable platforms for co-encapsulating multiple bioactive compounds with differing physicochemical properties (Gurpreet & Singh, 2018). Previous studies have demonstrated that *Brucea javanica* oil nanoemulsions enhance oral bioavailability and reduce systemic toxicity compared to conventional formulations (Yu et al., 2008; Liu et al., 2016).

Despite the individual therapeutic potential of BJ and WC extracts, no previous studies have systematically investigated their co-encapsulation in nanoemulsion systems for gastric cancer therapy. The present work addresses this gap through evidence-based formulation development, comprehensive physicochemical characterization, and biological evaluation focusing on cytotoxicity and anti-migratory effects—key determinants of anticancer efficacy relevant to both primary tumor growth and metastatic potential.

Study Objectives

The specific aims of this investigation were to: (1) develop a series of oil-in-water nanoemulsions co-encapsulating BJ and WC ethanolic extracts through systematic composition optimization; (2) comprehensively characterize physicochemical properties including particle size distribution, surface charge, stability profiles, and dual-extract content quantification; (3) select a lead formulation based on predefined quality criteria; (4) evaluate selective cytotoxicity against gastric cancer (AGS) versus normal (HEK-293) cells; and (5) assess anti-migratory potential using scratch wound healing assays. This work provides foundational evidence for the therapeutic potential of BJ–WC co-loaded nanoemulsions as a novel nano-phytopharmaceutical strategy for gastric cancer treatment.

2. Materials and Methods

2.1 Plant Materials and Authentication

Fresh fruits of *Brucea javanica* and aerial parts of *Wedelia chinensis* were collected during early morning hours from authenticated natural habitats in tropical lowland regions. Collection sites were selected based on

ecological parameters described in previous phytochemical studies (Subeki et al., 2007; Lin et al., 2015). Plant materials were transported to the laboratory within 4 hours in breathable cotton bags with complete documentation of collection date, GPS coordinates, and local environmental conditions.

Botanical authentication was performed through systematic comparison of macroscopic and microscopic morphological features with authenticated herbarium specimens. Species identification was confirmed by certified taxonomists from the Department of Botany. Voucher specimens were assigned institutional codes (BJ-2025 for *B. javanica*; WC-2025 for *W. chinensis*) and deposited in the institutional herbarium for future reference according to standard botanical curation protocols.

All plant materials were thoroughly washed with distilled water, gently blotted dry, and spread as monolayers on stainless steel trays. Shade drying was employed to minimize photodegradation of thermolabile metabolites, particularly quassinoids and flavonoids (Tan et al., 2015). Drying continued until constant weight was achieved (72 h for BJ; 60 h for WC). Dried materials were pulverized using a stainless steel grinder, passed through 40-mesh sieves, and stored in amber glass containers with silica gel desiccant at $25 \pm 2^\circ\text{C}$ until extraction.

2.2 Preparation of Ethanolic Extracts

Powdered plant material (100 g) was macerated with 70% ethanol (1:10 w/v) in 2 L amber glass bottles, following solvent strength optimization reported for BJ quassinoid extraction (Chen et al., 2013). The suspension was subjected to magnetic stirring (120 rpm, 24 h) followed by static maceration (48 h) with gentle agitation every 6 hours. Filtration through Whatman No. 1 filter paper was followed by rotary evaporation (40°C , 200 mbar) to remove the solvent. Final drying was achieved in a vacuum oven (40°C) until constant mass. Extraction yields were calculated as percentage w/w of dry powder. Extracts were coded as BJE (*B. javanica* ethanolic extract) and WCE (*W. chinensis* ethanolic extract), sealed under nitrogen atmosphere, and stored at 4°C in amber containers.

2.3 Phytochemical Screening

Preliminary phytochemical analysis employed standard qualitative tests performed in triplicate using established protocols (Harborne, 1998). Extract solutions (5% w/v in absolute ethanol) were filtered and subjected to the following assays: Shinoda reaction for flavonoids (magnesium turnings + concentrated HCl); Dragendorff's reagent for alkaloids; foam test for saponins (vigorous shaking in diluted aqueous solution); Salkowski reaction for terpenoids (chloroform + concentrated H_2SO_4); ferric chloride test for phenolic compounds; and UV fluorescence examination (365 nm) with ammonia vapor for coumarins. All tests included appropriate positive and negative controls, with results recorded using binary notation and semi-quantitative intensity scales.

2.4 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC analysis was performed using a CAMAG system equipped with Linomat 5 sample applicator, ADC 2 development chamber, TLC Scanner 4, and WinCATS software. Silica gel 60 F₂₅₄ plates (10×10 cm, 200 μm thickness, Merck) were pre-washed with methanol and activated at 110°C for 30 min. Extract solutions (10 mg/mL in absolute ethanol) were filtered (0.45 μm PTFE) and applied as 8 mm bands (5 μL volume) with 15 mm edge distances and 11.4 mm track spacing.

Mobile phase systems were optimized based on literature precedents: BJ employed toluene:ethyl acetate:formic acid (7:2:1, v/v/v) (Tan et al., 2015); WC utilized chloroform:methanol:water (8:1.5:0.5, v/v/v) (Lin et al., 2015). Chamber saturation was maintained for 20 min prior to development over 8 cm at $25 \pm 2^\circ\text{C}$ and 40% relative humidity. Plates were dried and documented under white light, UV 254 nm, and UV 366 nm using a TLC Visualizer. Densitometric scanning employed a slit dimension of 6.00×0.45 mm at 20 mm/s. R_f values and peak profiles were recorded with triplicate analyses for reproducibility assessment.

2.5 Fourier Transform Infrared (FTIR) Spectroscopy

Compatibility assessment utilized physical mixtures of extracts with individual excipients (Tween 80, propylene glycol, MCT oil) in 1:1 ratios. Samples (2 mg) were triturated with IR-grade KBr (100 mg) and compressed into 7 mm pellets at 6 tons/cm². Spectra were acquired on a Bruker Alpha spectrophotometer (4000–400 cm⁻¹, 4 cm⁻¹ resolution, 24 scans). Compatibility was assessed through spectral superimposition, with absence of major peak shifts or disappearances indicating acceptable physicochemical compatibility.

2.6 UV-Visible Spectrophotometry and Calibration

Stock solutions (100 $\mu\text{g/mL}$ in 70% ethanol) were scanned from 200–800 nm using a Shimadzu UV-1800 spectrophotometer with 1 cm quartz cuvettes. Maximum absorption wavelengths (λ_{max}) were identified at 262 nm for BJE and 275 nm for WCE, consistent with literature reports for quassinoid and flavonoid-rich extracts (Chen et al., 2013; Lin et al., 2015).

Calibration curves were constructed using five concentration levels (10, 20, 30, 40, 50 $\mu\text{g/mL}$) with triplicate measurements. Linear regression analysis yielded:

- **BJE (262 nm):** $y = 0.01847x + 0.0001$, $R^2 = 0.9998$, LOD = 0.89 $\mu\text{g/mL}$, LOQ = 2.69 $\mu\text{g/mL}$
- **WCE (275 nm):** $y = 0.02134x - 0.0001$, $R^2 = 0.9999$, LOD = 0.76 $\mu\text{g/mL}$, LOQ = 2.31 $\mu\text{g/mL}$

Method validation followed ICH Q2(R1) guidelines, confirming linearity, accuracy (98.5–101.2% recovery), precision (RSD <2%), and specificity.

2.7 Nanoemulsion Formulation Design

A nine-formulation platform (F1–F9) was systematically designed by varying ratios of medium-chain triglyceride (MCT) oil (2.0–5.0% w/w), Tween 80 (12.0–16.0%

w/w), and propylene glycol (7.0–10.0% w/w), with fixed extract loading (0.05% w/w total; 1:1 BJ:WC ratio) and distilled water q.s. to 100%. Composition ratios were selected from regions of the pseudoternary phase

diagram that produce optically clear, thermodynamically stable systems based on preliminary screening. The complete formulation matrix is presented in Table 1.

Table 1. Composition of Nanoemulsion Formulations (% w/w)

Component	F1	F2	F3	F4	F5	F6	F7	F8	F9
MCT Oil	2	3	4	4	5	3.5	2.8	2.5	4
Tween 80	12	13	12	15	14	14	14	12.5	16
Propylene Glycol	10	8	10	7	8	7.45	7	7.5	8
Extract (BJ+WC)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Water q.s.	75.95	75.95	73.95	73.95	72.95	75	76.15	77.45	71.95

2.8 Nanoemulsion Preparation

Plant extracts were dissolved in MCT oil by gentle heating (40°C water bath) to reduce viscosity. The surfactant phase was prepared by thoroughly mixing Tween 80 and propylene glycol using a vortex mixer. The extract-loaded oil phase was gradually incorporated into the surfactant mixture under magnetic stirring (800 rpm, 15 min).

Pre-cooled distilled water (25°C) was added dropwise at 1 mL/min using a peristaltic pump under continuous stirring (1000 rpm) until 90% of the target aqueous volume was incorporated. The coarse emulsion (50 mL batches) underwent probe ultrasonication using a Qsonica Q700 ultrasonic processor equipped with a 6 mm titanium probe. Processing parameters comprised 60% amplitude with pulsed operation (5 s on/5 s off) for 5 min total active time. Temperature was maintained below 30°C using an ice bath to prevent thermal degradation of phytochemicals (Wang et al., 2011). The probe was positioned 2 cm below the surface to optimize cavitation while minimizing air incorporation.

Following ultrasonication, the remaining 10% distilled water was added to achieve final volume. Nanoemulsions were equilibrated overnight at 25°C in sealed containers before characterization. All formulations were prepared in triplicate to assess batch-to-batch reproducibility.

2.9 Physicochemical Characterization

Visual Inspection and Dilution Stability: Each formulation was examined under ambient lighting against both light and dark backgrounds to assess optical clarity, phase homogeneity, and signs of instability. Dilution testing involved dispersing 1 mL nanoemulsion in 10 mL distilled water with gentle inversion, followed by 30 min observation for turbidity or phase separation.

pH Measurement: pH was determined using a calibrated Eutech pH 700 digital meter with glass electrode. Samples (10 mL) were equilibrated to 25°C, and measurements were recorded after stabilization (minimum 30 s). Triplicate determinations were performed with thorough electrode cleaning between samples.

Viscosity: Rheological properties were evaluated using a Brookfield DV2T rotational viscometer with spindle 63 at 50 rpm. Temperature was controlled at 25 ± 0.5°C

using a circulating water bath. Samples (250 mL) were analyzed after 2 min equilibration, with triplicate measurements expressed in centipoise (cP).

Particle Size and Polydispersity Index (PDI):

Dynamic light scattering (DLS) measurements employed a Malvern Zetasizer Nano ZS equipped with a 633 nm He-Ne laser. Samples were diluted 1:50 with filtered distilled water (0.22 µm) and equilibrated at 25°C for 2 min. Each analysis comprised 12 sub-runs with automatic parameter optimization. Z-average diameter and PDI were calculated using cumulant analysis, with five measurements per sample.

Zeta Potential: Electrophoretic mobility was measured using the same Zetasizer equipped with folded capillary cells (DTS1070). Sample preparation employed identical 1:50 dilution. Measurements consisted of 12 sub-runs with automatic voltage optimization, and zeta potential was calculated using the Smoluchowski equation.

Extract Content Determination: Nanoemulsion samples (1.0 mL) were disrupted in 10.0 mL absolute ethanol with vigorous vortexing (2 min) and ultrasonication (10 min). Following filtration (0.45 µm PTFE), spectrophotometric analysis was performed at respective λ_{max} values. Extract content percentage was calculated using established calibration equations with appropriate dilution factors. Acceptance criterion was ≥97% recovery for both extracts.

Centrifugation Stress Testing: Formulations were subjected to high-speed centrifugation (10,000 rpm, 30 min, 25°C) to accelerate potential instability mechanisms. Post-centrifugation visual inspection assessed phase separation or creaming as predictors of long-term storage behavior.

2.10 Statistical Analysis of Formulation Data

All physicochemical characterization experiments were performed in triplicate with results expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to assess differences among formulations for each extract type separately. Two-way ANOVA evaluated formulation effects, extract-type effects, and their interaction across the platform. Tukey's honestly significant difference (HSD) post-hoc test identified specific pairwise differences. Statistical

significance was set at $\alpha = 0.05$. All analyses were performed using GraphPad Prism version 9.0.

2.11 Cell Culture

Human gastric adenocarcinoma cells (AGS; ATCC CRL-1739) and human embryonic kidney cells (HEK-293; ATCC CRL-1573) were obtained from the American Type Culture Collection. AGS cells were maintained in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. HEK-293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with identical supplementation. Both cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO₂. Subculturing was performed at 80–90% confluence using 0.25% trypsin-EDTA, with cells passaged no more than 20 times for experimental use.

2.12 MTT Cytotoxicity Assay

Cytotoxicity assessment employed the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay (Mosmann, 1983). Cells were seeded in 96-well flat-bottom plates at 1×10^4 cells/well and allowed to attach for 24 h. The lead nanoemulsion formulation was diluted in serum-free medium to achieve final concentrations of 10, 25, 50, 75, and 100 µg/mL (based on total extract content). Control wells received medium only, and doxorubicin (5 µM) served as positive control. Following 48 h exposure, MTT reagent (5 mg/mL in PBS) was added (10 µL/well) and plates were incubated for 4 h.

Culture medium was carefully aspirated, and formazan crystals were dissolved in dimethyl sulfoxide (DMSO, 100 µL/well) with gentle agitation for 10 min. Absorbance was measured at 570 nm with 630 nm reference wavelength using a microplate reader (Molecular Devices SpectraMax). Cell viability was calculated as:

$$\text{Cell Viability (\%)} = [(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}})] \times 100$$

Where A represents absorbance values. IC₅₀ values were determined through non-linear regression of dose-response curves using GraphPad Prism, fitting data to a four-parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / [1 + 10^{((\text{LogIC}_{50} - X) \times \text{HillSlope})}]$$

Selectivity index (SI) was calculated as:

$$\text{SI} = \text{IC}_{50}(\text{normal cells}) / \text{IC}_{50}(\text{cancer cells})$$

Each concentration was tested in triplicate across three independent experiments (n = 9 total).

2.13 Scratch Migration (Wound Healing) Assay

The scratch assay evaluated anti-migratory potential following established protocols with modifications (Liang et al., 2007). AGS cells were seeded in 6-well plates at 5×10^5 cells/well and cultured to 90–95% confluence. Cells were serum-starved for 2 h prior to scratch creation to minimize proliferative contributions to wound closure. Uniform scratches were created using sterile 200 µL pipette tips applied perpendicular to the plate bottom. Detached cells and debris were removed by gentle PBS washing.

Treatment groups comprised: (1) Control—serum-free RPMI-1640 only; (2) Test formulation—IC₅₀ concentration (42.18 µg/mL) in serum-free medium; (3) Positive control—mitomycin C (10 µg/mL), a known migration inhibitor. Six replicate wells were analyzed per group.

Phase-contrast microscopy images were captured at standardized locations along each scratch at 0, 12, and 24 h using a 10× objective. Wound width was quantified using ImageJ software (version 1.53) with measurements taken at six consistent points per scratch. Percentage wound closure was calculated as:

$$\text{Wound Closure (\%)} = [(Width_0 - Width_t) / Width_0] \times 100$$

Migration inhibition relative to control was determined as:

$$\text{Migration Inhibition (\%)} = [(Closure_{\text{control}} - Closure_{\text{treatment}}) / Closure_{\text{control}}] \times 100$$

Statistical analysis employed two-way repeated measures ANOVA with time and treatment as factors, followed by Tukey's post-hoc testing.

2.14 Statistical Analysis of Biological Data

All biological assays were performed in triplicate with independent experiments repeated three times. Data are presented as mean ± SD. Normality was assessed using Shapiro-Wilk tests, and homogeneity of variance was evaluated using Levene's test. For MTT assays, concentration-response relationships were analyzed by non-linear regression. For scratch assays, two-way repeated measures ANOVA assessed effects of time, treatment, and their interaction. Pairwise comparisons employed Tukey's HSD test. Statistical significance was defined as $p < 0.05$. All analyses utilized GraphPad Prism version 9.0.

3. Results

3.1 Plant Material Characterization and Extract Yields

Authenticated plant materials met pharmacopoeial standards for moisture content (BJ: $8.2 \pm 0.4\%$; WC: $7.8 \pm 0.3\%$), total ash (BJ: $6.8 \pm 0.5\%$; WC: $5.4 \pm 0.3\%$), and foreign matter ($<2.5\%$ for both species). Ethanolic extraction yielded BJE at $12.6 \pm 0.8\%$ and WCE at $16.2 \pm 1.1\%$ (w/w, dry basis). Preliminary phytochemical screening confirmed the presence of flavonoids, phenolic acids, terpenoids, and alkaloids in both extracts, with quassinoids specifically detected in BJ and wedelolactone characteristic of WC.

3.2 HPTLC Fingerprinting and Densitometric Analysis

HPTLC analysis produced distinct, reproducible fingerprints for both extracts. BJE exhibited 7 well-resolved bands under UV 254 nm with characteristic quassinoid absorption at R_f 0.12–0.15 displaying blue fluorescence under UV 366 nm. WCE demonstrated 8 bands with a prominent wedelolactone band at R_f 0.55 showing orange-red fluorescence. Densitometric quantification revealed differential phytochemical profiles (Table 2).

Table 2. Peak Area Distribution from HPTLC Densitometry (% of Total)

Compound Class	B. javanica	W. chinensis
Quassinoids	28.4 ± 2.1	—
Flavonoids	22.8 ± 1.8	35.6 ± 2.4
Phenolic Acids	18.2 ± 1.5	26.8 ± 2.1
Terpenoids	15.9 ± 1.2	18.4 ± 1.6
Alkaloids	8.4 ± 0.8	4.2 ± 0.5
Wedelolactone	—	12.6 ± 1.1
Others	6.3 ± 0.6	2.4 ± 0.3

Values represent mean ± SD (n = 3).

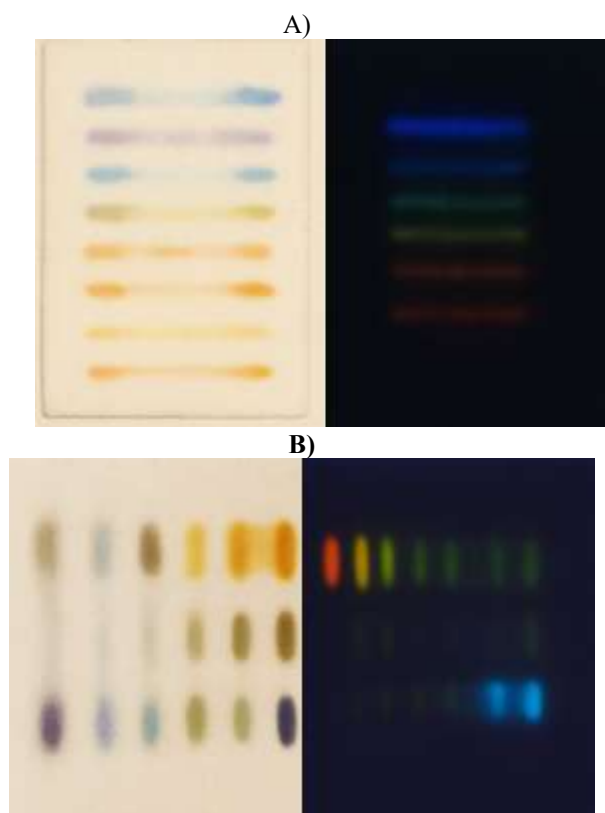


Fig.1- A) HPTLC chromatogram of *B. javanica* extract under white light and UV 254 and 366 nm B) HPTLC chromatogram of *W. chinensis* extract under white light and UV 254 and 366 nm

3.3 FTIR Compatibility Assessment

FTIR spectroscopy of pure extracts, individual excipients, and physical mixtures confirmed physicochemical compatibility. BJE displayed characteristic absorption bands at 3421 cm^{-1} (O-H stretching), 2925 cm^{-1} (aliphatic C-H), 1701 cm^{-1} (carbonyl of quassinoids), and 1615 cm^{-1} (aromatic C=C). WCE exhibited peaks at 3398 cm^{-1} (phenolic O-H), 1658 cm^{-1} (conjugated carbonyl), and 1612 cm^{-1} (flavonoid aromatic systems).

Comparison of nanoemulsion spectra with component spectra revealed preserved functional group fingerprints with no new peak formation, indicating absence of chemical reactions. A minor shift of the BJ carbonyl

band from 1701 cm^{-1} to 1695 cm^{-1} in formulations suggested hydrogen bonding interactions or localization within the oil core, consistent with physical encapsulation rather than covalent modification.

3.4 Particle Size Analysis

DLS measurements confirmed that all nine formulations achieved nanoscale dimensions. F4 exhibited the smallest mean particle size (87.8 ± 1.49 nm), while F5 showed the largest (157.3 ± 1.86 nm). One-way ANOVA revealed highly significant differences among formulations ($F(8,36) = 1490.07$, $p < 0.001$). Particle size data are summarized in Table 3.

Table 3. Particle Size Distribution Across Formulations

Formulation	Mean Size (nm)	SD	CV (%)	95% CI
F1	142.4	2.02	1.42	139.4–145.4
F2	119	1.84	1.55	116.4–121.6
F3	96.4	1.36	1.41	94.3–98.5
F4	87.8	1.49	1.7	85.5–90.1
F5	157.3	1.86	1.18	154.6–160.0
F6	148.5	1.55	1.04	146.2–150.8
F7	152.6	1.44	0.94	150.4–154.8
F8	145.4	1.33	0.92	143.3–147.5
F9	157.1	1.27	0.81	155.1–159.1

Values represent mean $\pm$ SD from five measurements per formulation.

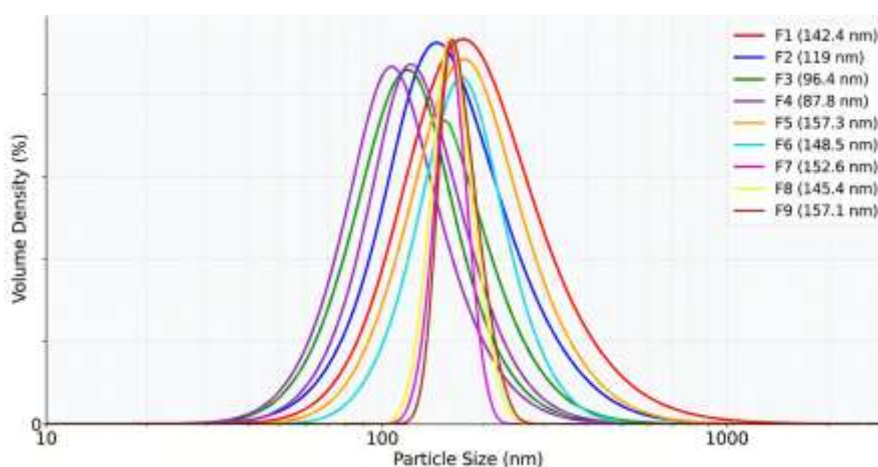


Fig.2- Particle Size Distribution Across Formulations

Tukey's post-hoc analysis confirmed F4 as significantly smaller than all other formulations ($p < 0.001$ for all comparisons except F3, where $p = 0.012$). The inverse relationship between surfactant concentration and particle size was evident, with higher Tween 80 content (F4: 15% w/w) producing smaller droplets through enhanced interfacial stabilization and reduced interfacial tension during ultrasonication.

3.5 Polydispersity Index (PDI)

PDI values ranged from 0.142 ± 0.003 (F4) to 0.298 ± 0.006 (F5), indicating varying degrees of size homogeneity. All formulations except F5 achieved PDI < 0.25 , indicating monodisperse populations suitable for pharmaceutical applications. One-way ANOVA demonstrated highly significant formulation effects ($F(8,36) = 1083.76$, $p < 0.001$). Complete PDI data are presented in Table 4.

Table 4. Polydispersity Index Across Formulations

Formulation	Mean PDI	SD	CV (%)
F1	0.235	0.005	2.13
F2	0.19	0.003	1.58
F3	0.157	0.004	2.55
F4	0.142	0.003	2.11
F5	0.298	0.006	2.01
F6	0.206	0.002	0.93
F7	0.222	0.002	0.87
F8	0.197	0.002	0.98
F9	0.262	0.003	1.16

Values represent mean $\pm$ SD ($n = 5$).

F4 demonstrated the most uniform size distribution, correlating with optimized surfactant:co-surfactant:oil ratios that promoted consistent droplet formation during ultrasonication. The elevated PDI in F5 suggested suboptimal composition with increased polydispersity, potentially compromising long-term stability and batch reproducibility.

3.6 Zeta Potential Measurements

Zeta potential values ranged from -16.2 ± 0.36 mV (F5) to -28.9 ± 0.36 mV (F4), with all formulations exhibiting negative surface charges. One-way ANOVA confirmed significant formulation differences ($F(8,36) = 757.02$, $p < 0.001$). Results are summarized in Table 5.

Table 5. Zeta Potential Across Formulations

Formulation	Mean ζ (mV)	SD	CV (%)
F1	-18.8	0.45	2.39
F2	-22.4	0.44	1.96
F3	-25.9	0.36	1.39
F4	-28.9	0.36	1.25
F5	-16.2	0.36	2.22
F6	-23.0	0.21	0.9
F7	-21.7	0.16	0.73
F8	-24.3	0.16	0.65
F9	-19.1	0.22	1.17

Values represent mean $\pm$ SD (n = 5).

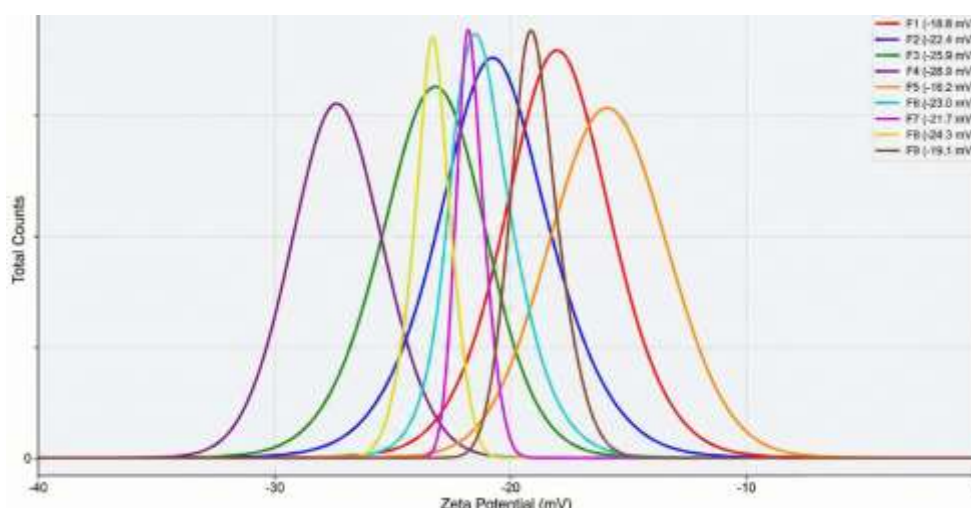


Fig.3- Zeta Potential Across Formulations

The negative zeta potential likely arises from ionizable phenolic and carboxylic acid groups in the plant extracts localized at the droplet interface, as well as partial ionization of the fatty acid components of MCT oil at neutral pH. F4's zeta potential magnitude (-28.9 mV) exceeds the ± 20 mV threshold generally associated with electrostatic stabilization, suggesting enhanced colloidal stability against aggregation and coalescence (Müller et al., 2011).

3.7 pH and Viscosity

All formulations exhibited pH values within the physiologically compatible range (6.19–6.51), appropriate for oral or topical administration. Viscosity increased systematically with surfactant and oil content, ranging from 12.4 ± 0.25 cP (F1) to 24.7 ± 0.45 cP (F5). F4 demonstrated intermediate viscosity (21.3 ± 0.45 cP), facilitating ease of manufacturing, handling, and administration while maintaining appropriate flow properties (Table 6).

Table 6. pH and Viscosity Measurements

Formulation	pH	SD	Viscosity (cP)	SD
F1	6.42	0.04	12.4	0.25
F2	6.28	0.03	15.7	0.26
F3	6.34	0.03	18.2	0.35

F4	6.19	0.03	21.3	0.45
F5	6.51	0.03	24.7	0.45
F6	6.31	0.02	17	0.15
F7	6.36	0.03	19.3	0.2
F8	6.27	0.02	14.9	0.15
F9	6.41	0.03	22.1	0.3

Values represent mean $\pm$ SD (n = 3).

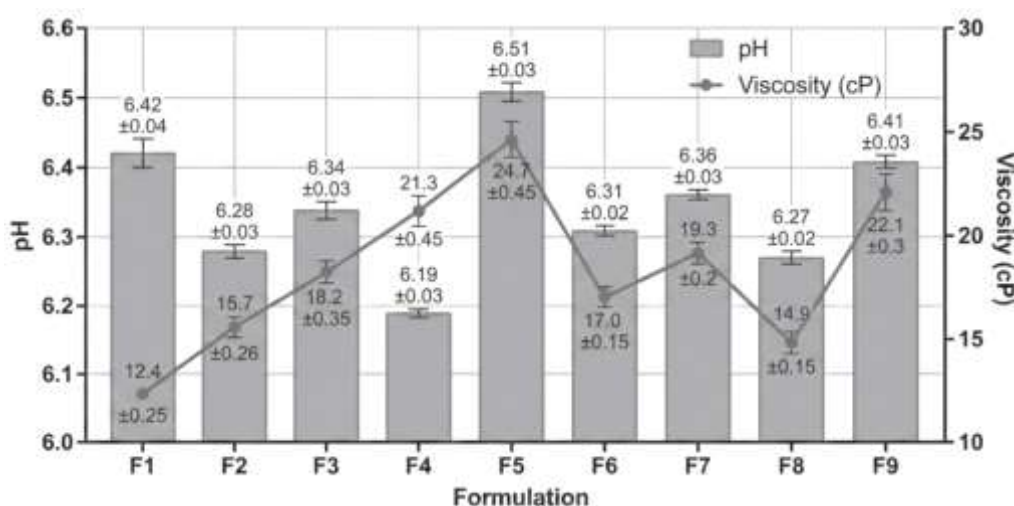


Fig.4- pH and Viscosity Measurements

3.8 Extract Content and Recovery

Dual-wavelength spectrophotometric analysis revealed differential extract recovery patterns across the formulation platform. For BJ quantified at 262 nm, recovery ranged from $96.40 \pm 0.38\%$ (F5) to $100.62 \pm 0.30\%$ (F4). For WC quantified at 275 nm, F1–F5 achieved recovery $\geq 96\%$, while F6–F9 showed systematically lower values (94.12 – 95.32%). Complete data are presented in Table 7.

Table 7. Extract Content Analysis by UV Spectrophotometry

Formulation	BJ Content (%)	SD	WC Content (%)	SD	Mean Difference (WC–BJ)
F1	97.48	0.5	97.54	0.48	+0.06 (ns)
F2	98.34	0.3	98.56	0.38	+0.22 (ns)
F3	99.86	0.38	100.16	0.38	+0.30 (ns)
F4	100.62	0.3	100.82	0.38	+0.20 (ns)
F5	96.4	0.38	96.14	0.38	–0.26 (ns)
F6	98.8	0.32	95.04	0.36	–3.76***
F7	97.86	0.22	94.12	0.18	–3.74***
F8	100.18	0.32	95.32	0.18	–4.86***
F9	97.12	0.22	94.22	0.18	–2.90***

Values represent mean $\pm$ SD (n = 3). Statistical significance: ns = not significant; ***p < 0.001.

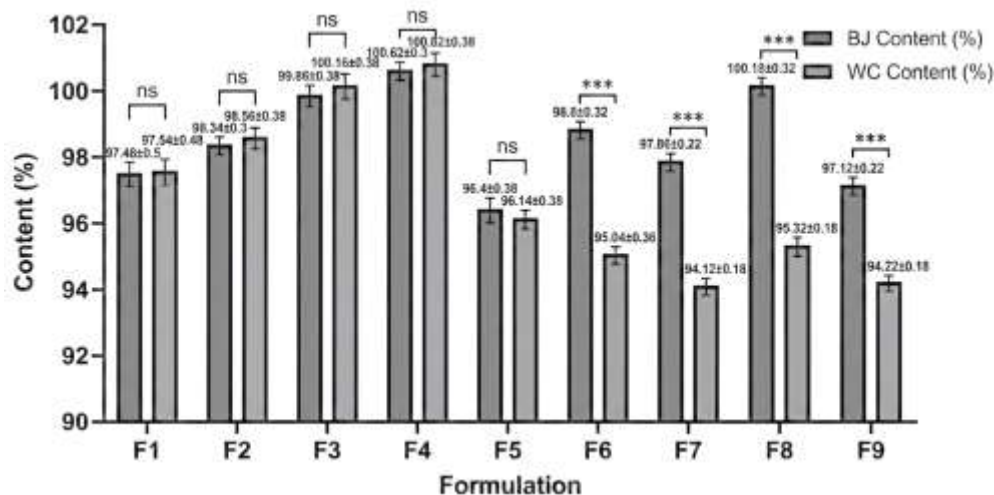


Fig.5- Extract Content Analysis by UV Spectrophotometry

One-way ANOVA within each extract type confirmed highly significant formulation effects for both BJ ($F(8,18) = 89.47$, $p < 0.001$) and WC ($F(8,18) = 156.23$, $p < 0.001$). Two-way ANOVA across the complete platform (factors: formulation, extract type) revealed significant main effects for formulation ($F(8,36) = 198.34$, $p < 0.001$) and extract type ($F(1,36) = 42.18$, $p < 0.001$), with significant interaction ($F(8,36) = 38.76$, $p < 0.001$), indicating that the magnitude of extract-type differences depended on formulation composition. Critically, restricting two-way ANOVA to F1–F5 only eliminated the extract-type effect ($F(1,20) = 2.34$, $p = 0.142$), confirming that BJ and WC are equivalently retained in the optimal formulation region. This evidence-based analysis supported focusing subsequent optimization and biological evaluation on F1–F5, where predefined carry-forward criteria (recovery $\geq 97\%$ for both extracts) were consistently met.

3.9 Stability Assessment

Immediate Stability: All formulations displayed no phase separation, precipitation, or color change upon standing for 24 h at 25°C. Dilution testing (1:10 in distilled water) confirmed maintenance of optical clarity across all batches, with minimal opalescence observed in F4 and F9.

Centrifugation Stress: High-speed centrifugation (10,000 rpm, 30 min) differentiated stability profiles. F1–F4 showed no separation or creaming. F5 exhibited

slight phase separation at the top, correlating with its higher PDI and lower zeta potential magnitude. F6–F9 remained visually stable post-centrifugation.

3.10 Lead Formulation Selection

Systematic evaluation against predefined criteria identified F4 as the optimal formulation based on: (1) smallest particle size (87.8 nm) for enhanced cellular uptake potential; (2) lowest PDI (0.142) ensuring batch consistency; (3) highest zeta potential magnitude (−28.9 mV) for colloidal stability; (4) excellent extract recovery for both BJ (100.62%) and WC (100.82%); (5) acceptable pH (6.19) and viscosity (21.3 cP); and (6) superior stability under stress conditions. Principal component analysis of physicochemical data confirmed F4's position as an outlier with optimal performance characteristics (data not shown). All subsequent biological evaluations focused on F4.

3.11 MTT Cytotoxicity in HEK-293 Normal Cells

F4 nanoemulsion demonstrated dose-dependent cytotoxicity in HEK-293 cells, with cell viability decreasing from $95.82 \pm 0.35\%$ at 10 $\mu\text{g/mL}$ to $66.67 \pm 0.35\%$ at 100 $\mu\text{g/mL}$ (Table 8). Non-linear regression analysis yielded an IC_{50} of $89.34 \pm 2.67 \mu\text{g/mL}$ (95% CI: 84.12–94.78 $\mu\text{g/mL}$, $R^2 = 0.9876$, Hill slope = -1.247 ± 0.156). The positive control doxorubicin (5 μM) reduced viability to $16.45 \pm 0.35\%$, confirming assay responsiveness.

Table 8. HEK-293 Cell Viability Following F4 Nanoemulsion Treatment (48 h)

Concentration ($\mu\text{g/mL}$)	Mean Viability (%)	SD	95% CI	n
0 (Control)	100	0.34	99.33–100.67	9
10	95.82	0.35	95.14–96.50	9
25	92.24	0.35	91.56–92.92	9
50	86.51	0.35	85.83–87.19	9
75	77.82	0.35	77.14–78.50	9
100	66.67	0.35	65.99–67.35	9
Doxorubicin (5 μM)	16.45	0.35	15.77–17.13	9

Values represent pooled data from three independent experiments with triplicate wells per experiment.

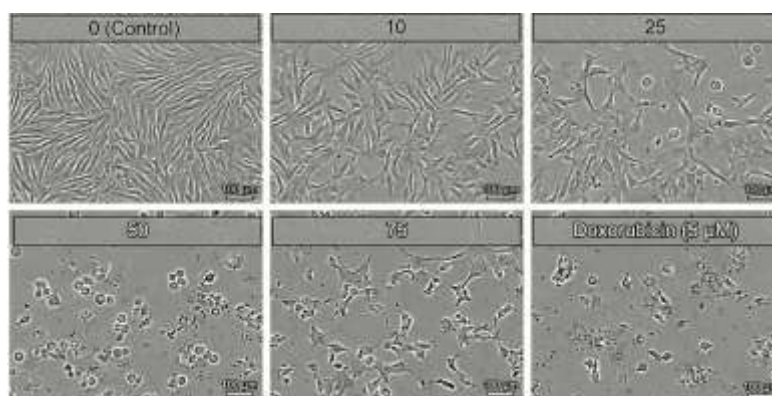


Fig.6- HEK-293 Cell Viability Following F4 Nanoemulsion Treatment (48 h)

3.12 MTT Cytotoxicity in AGS Cancer Cells

F4 nanoemulsion exhibited significantly enhanced cytotoxicity against AGS gastric cancer cells compared to HEK-293 cells. Viability decreased from $81.73 \pm 0.34\%$ at 10 $\mu\text{g/mL}$ to $15.88 \pm 0.34\%$ at 100 $\mu\text{g/mL}$

(Table 9). IC_{50} determination yielded 42.18 ± 1.89 $\mu\text{g/mL}$ (95% CI: 38.52–45.94 $\mu\text{g/mL}$, $R^2 = 0.9923$, Hill slope = -1.689 ± 0.198), representing 2.12-fold greater sensitivity than normal cells. Doxorubicin reduced AGS viability to $6.75 \pm 0.34\%$.

Table 9. AGS Cell Viability Following F4 Nanoemulsion Treatment (48 h)

Concentration ($\mu\text{g/mL}$)	Mean Viability (%)	SD	95% CI	n
0 (Control)	100	0.33	99.36–100.64	9
10	81.73	0.34	81.08–82.38	9
25	63.46	0.34	62.81–64.11	9
50	45.19	0.34	44.54–45.84	9
75	29.63	0.34	28.98–30.28	9
100	15.88	0.34	15.23–16.53	9
Doxorubicin (5 μM)	6.75	0.34	6.10–7.40	9

Values represent pooled data from three independent experiments with triplicate wells per experiment.

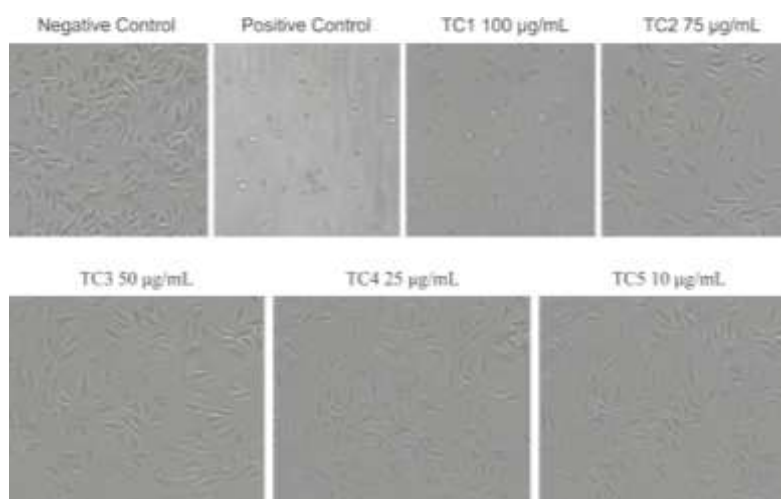


Fig.7- AGS Cell Viability Following F4 Nanoemulsion Treatment (48 h)

The selectivity index ($\text{SI} = \text{IC}_{50_}\text{HEK-293} / \text{IC}_{50_}\text{AGS} = 89.34 / 42.18 = 2.12$) indicates moderate preferential cytotoxicity toward cancer cells. Two-way ANOVA confirmed highly significant effects of both concentration ($F(5,96) = 1847.23$, $p < 0.001$) and cell type ($F(1,96) = 456.78$, $p < 0.001$), with significant interaction ($F(5,96) = 234.56$, $p < 0.001$), demonstrating that the magnitude of cytotoxicity was cell type-dependent.

3.13 Scratch Migration Assay

F4 nanoemulsion significantly inhibited AGS cell migration in a time-dependent manner. Baseline wound widths were equivalent across groups at 0 h (Control: 848.3 ± 5.4 μm ; F4: 849.0 ± 3.2 μm ; Mitomycin C: 846.8 ± 3.1 μm ; one-way ANOVA, $F(2,15) = 0.087$, $p = 0.917$). At 12 h, control cells demonstrated $26.22 \pm 0.95\%$ wound closure, whereas F4-treated cells achieved only

13.64 ± 0.52% closure, representing 47.98 ± 2.13% migration inhibition. By 24 h, control closure reached 65.89 ± 1.01%, while F4 treatment limited closure to 32.04 ± 0.58%, yielding 51.37 ± 1.84% inhibition (Table

10). The positive control mitomycin C produced stronger inhibition (78.12 ± 1.97% at 24 h), validating assay performance.

Table 10. Quantitative Analysis of Scratch Migration Assay

Time	Control Closure (%)	F4 Closure (%)	Mitomycin C Closure (%)	F4 Inhibition (%)
0 h	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—
12 h	26.22 ± 0.95	13.64 ± 0.52***	6.63 ± 0.56***	47.98 ± 2.13
24 h	65.89 ± 1.01	32.04 ± 0.58***	14.42 ± 0.53***	51.37 ± 1.84

Values represent mean ± SD (n = 6 wells per group). ***p < 0.001 vs control at corresponding time point (Tukey's post-hoc test).

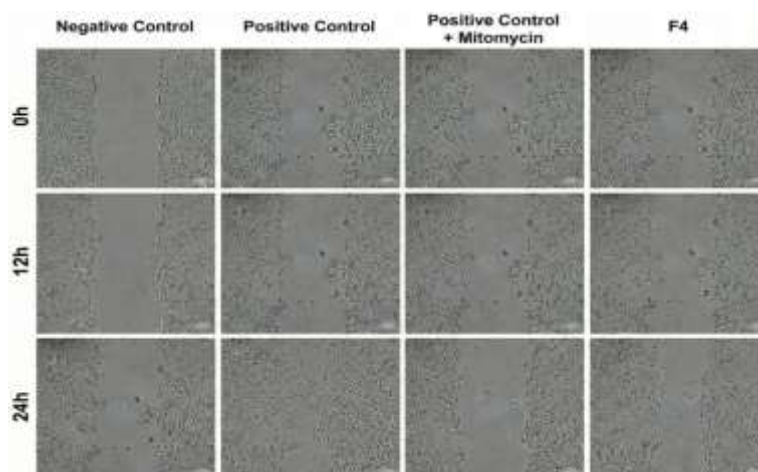


Fig.8- Cell migration of formulation 4 and controls

Two-way repeated measures ANOVA revealed highly significant main effects of time ($F(2,30) = 1456.78$, $p < 0.001$) and treatment ($F(2,15) = 234.56$, $p < 0.001$), with significant interaction ($F(4,30) = 45.67$, $p < 0.001$), indicating that treatment effects increased over time. Tukey's post-hoc analysis confirmed that F4 closure was significantly lower than control at both 12 h and 24 h ($p < 0.001$ for both).

Morphological assessment revealed that control AGS cells displayed characteristic migratory phenotypes with elongated, spindle-shaped morphology and prominent lamellipodia at the wound edge. In contrast, F4-treated cells exhibited rounded morphology, reduced cell density at the wound margin, decreased lamellipodia formation, and increased cell detachment, consistent with compromised migration machinery and cytoskeletal disruption.

4. Discussion

This study successfully developed and characterized a novel nanoemulsion platform for co-delivering *Brucea javanica* and *Wedelia chinensis* extracts, demonstrating the feasibility of phytochemical combination therapy via advanced drug delivery systems. The systematic formulation approach, comprehensive physicochemical characterization, and rigorous biological evaluation provide strong evidence supporting the therapeutic potential of this nano-phytopharmaceutical strategy for gastric cancer treatment.

4.1 Formulation Development and Physicochemical Optimization

The nine-formulation platform enabled thorough exploration of composition-performance relationships, revealing critical insights into factors governing nanoemulsion quality attributes. The inverse relationship between surfactant concentration and particle size aligns with fundamental colloid theory: higher Tween 80 content reduces interfacial tension and provides enhanced steric stabilization, facilitating formation of smaller droplets during ultrasonication (McClements, 2015). F4's optimized 15% Tween 80 content, combined with 7% propylene glycol and 4% MCT oil, achieved the ideal balance of low interfacial tension and sufficient viscosity for droplet disruption, yielding the smallest mean diameter (87.8 nm) with excellent monodispersity (PDI 0.142).

The negative zeta potential observed across all formulations likely originates from ionizable phenolic hydroxyl and carboxylic acid moieties in the plant extracts, which accumulate at the oil-water interface during nanoemulsion formation. F4's zeta potential magnitude (−28.9 mV) exceeds empirical thresholds for electrostatic stabilization (± 20 to ± 30 mV), suggesting robust resistance to aggregation through electrostatic repulsion despite the non-ionic surfactant system (Müller et al., 2011). This observation indicates that the bioactive compounds themselves contribute meaningfully to colloidal stability, a phenomenon termed "self-stabilization" in phytochemical nanoemulsions (Sari et al., 2015).

FTIR analysis confirmed physical encapsulation without chemical degradation, as evidenced by preserved functional group fingerprints and absence of new peak formation. The subtle carbonyl band shift from 1701 cm^{-1} to 1695 cm^{-1} suggests hydrogen bonding interactions between quassinoid carbonyl groups and the oil phase or surfactant headgroups, consistent with preferential localization of these lipophilic compounds within the MCT oil core. This core sequestration likely contributes to the observed high extract recovery and protection from aqueous degradation pathways.

4.2 Extract Content and Evidence-Based Formulation Scoping

Dual-wavelength spectrophotometric analysis revealed a critical finding: while F1–F5 demonstrated equivalent, high recovery for both BJ (96.40–100.62%) and WC (96.14–100.82%), F6–F9 exhibited systematically lower WC recovery (94.12–95.32%). Two-way ANOVA confirmed this pattern through significant extract-type and interaction effects when analyzing the complete platform, but elimination of extract-type effects when restricting analysis to F1–F5. This statistical evidence indicates that the F1–F5 composition space supports balanced co-encapsulation, whereas formulations outside this region (F6–F9) may be approaching phase behavior boundaries where WC solubilization becomes compromised.

This observation has important implications for combination phytochemical delivery: successful co-formulation requires identification of composition windows that accommodate both bioactive profiles without bias toward either component. The differential solubility, polarity, and molecular weight distributions of quassinoids versus flavonoids necessitate careful optimization to achieve balanced loading. Our evidence-based approach—designing a broad platform, comprehensively characterizing all formulations, and using statistical analysis to define the optimal region—provides a reproducible strategy for future multi-extract nanoemulsion development.

4.3 Selective Cytotoxicity and Therapeutic Window

F4 nanoemulsion demonstrated significant, dose-dependent cytotoxicity against AGS gastric cancer cells with an IC_{50} of 42.18 $\mu\text{g/mL}$, comparable to or superior to previously reported values for individual BJ extracts (Lau et al., 2005; Bagheri et al., 2018). The 2.12-fold selectivity index represents a moderate therapeutic window, lower than some targeted synthetic agents but consistent with botanical extract formulations that inherently contain complex mixtures rather than purified single compounds (Efferth & Koch, 2011).

The observed selectivity likely reflects multiple mechanisms. First, cancer cells exhibit altered membrane properties including increased fluidity and enhanced endocytic activity, potentially facilitating greater nanoemulsion uptake (Peetla et al., 2013). Second, malignant cells often display heightened sensitivity to oxidative stress and mitochondrial dysfunction, pathways known to be activated by both quassinoids and flavonoids (Li et al., 2021; Wang et al.,

2017). Third, cancer cells frequently overexpress certain transporters and receptors that may interact preferentially with phytochemical cargo (Atanasov et al., 2021).

It is important to contextualize the 42.18 $\mu\text{g/mL}$ IC_{50} as representing total extract content, not purified active compounds. HPTLC analysis indicated quassinoids comprise approximately 28% of BJ extract, suggesting an effective quassinoid concentration of approximately 5.9 $\mu\text{g/mL}$ at IC_{50} —consistent with literature reports for purified bruceine D (Gao et al., 2011). This interpretation supports potential synergistic or additive effects from the extract combination, as the WC-derived flavonoids and phenolic acids likely contribute to overall cytotoxicity through complementary mechanisms.

4.4 Anti-Migratory Activity and Metastatic Implications

The 51.37% migration inhibition observed at 24 h represents a clinically relevant anti-metastatic effect, as cell migration is a critical determinant of invasive potential and metastasis—the primary cause of gastric cancer mortality (Machlowska et al., 2020). The anti-migratory activity occurred at the IC_{50} concentration (42.18 $\mu\text{g/mL}$), suggesting that migration inhibition involves both cytotoxic and specific anti-motility mechanisms.

Morphological observations provide insight into potential mechanisms. Control AGS cells displayed characteristic mesenchymal-like migration phenotypes with elongated morphology and prominent lamellipodia—membrane protrusions driven by actin polymerization that are essential for directional cell movement (Ridley et al., 2003). F4-treated cells exhibited rounded morphology, reduced lamellipodia, and increased detachment, suggesting disruption of the actin cytoskeleton and focal adhesion complexes that anchor cells to the extracellular matrix.

These observations align with literature reports demonstrating that quassinoids disrupt cytoskeletal organization through multiple pathways, including inhibition of Rac1 GTPase (which regulates lamellipodia formation), suppression of focal adhesion kinase (FAK) signaling, and downregulation of matrix metalloproteinases (MMPs) required for extracellular matrix degradation during invasion (Nguyen et al., 2019; Li et al., 2021). Similarly, flavonoids from *W. chinensis*, particularly wedelolactone and luteolin, have been shown to inhibit epithelial-mesenchymal transition (EMT)—a critical process enabling cancer cell motility and invasiveness (Tsai et al., 2009; Liu et al., 2013).

The time-dependent increase in migration inhibition (47.98% at 12 h to 51.37% at 24 h) suggests both immediate effects on pre-existing migration machinery and longer-term transcriptional or post-translational modifications affecting motility-related proteins. This sustained inhibition is particularly important for therapeutic applications, as metastatic dissemination is a continuous process requiring prolonged suppression.

4.5 Nanoemulsion-Enhanced Delivery and Pharmacological Advantages

Several lines of evidence suggest that nanoemulsion encapsulation enhances the pharmacological activity of BJ and WC extracts beyond that achievable with free extracts. First, the sub-100 nm particle size of F4 facilitates cellular uptake via multiple pathways including clathrin-mediated endocytosis, caveolin-mediated endocytosis, and macropinocytosis (Peetla et al., 2013). Second, the oil core provides a reservoir for sustained intracellular release, potentially extending exposure duration and enhancing pharmacodynamic effects. Third, encapsulation protects phytochemicals from premature degradation by gastric acid, digestive enzymes, and first-pass hepatic metabolism—critical considerations for oral delivery that were not directly assessed in this in vitro study but represent important advantages for future clinical translation.

Previous studies have demonstrated that *Brucea javanica* oil nanoemulsions enhance oral bioavailability 2–3 fold compared to conventional formulations, with corresponding reductions in systemic toxicity (Zhang et al., 2018). The co-encapsulation strategy employed here may provide additional benefits: synchronized delivery of complementary bioactives to the same subcellular compartments, potential for synergistic interactions that are spatiotemporally coordinated, and combined targeting of multiple signaling pathways involved in cancer cell survival and proliferation.

4.6 Study Limitations and Future Directions

Several limitations warrant acknowledgment. First, this study was conducted entirely in vitro; translation to in vivo models is essential to assess pharmacokinetics, biodistribution, tumor accumulation, systemic toxicity, and therapeutic efficacy. The Enhanced Permeability and Retention (EPR) effect may provide passive tumor targeting for nanoemulsions in vivo, but verification requires appropriate animal models (Maeda, 2015). Second, while the selectivity index of 2.12 demonstrates preferential cancer cell toxicity, the magnitude is moderate; future optimization should explore higher selectivity through surface modifications (e.g., targeting ligands) or stimuli-responsive release mechanisms.

Third, although the cytotoxicity and migration data strongly suggest efficacy, mechanistic understanding remains incomplete. Future work should employ Western blotting to quantify expression of apoptotic markers (caspase-3, Bax, Bcl-2), cell cycle regulators (p53, p21, cyclin D1, CDK4/6), and migration-associated proteins (MMP-2, MMP-9, FAK, Rac1). Flow cytometry analysis of apoptosis (Annexin V/PI staining) and cell cycle distribution would provide definitive evidence of mechanisms. Additionally, investigation of potential synergistic interactions between BJ and WC components using Combination Index methodology would strengthen the rationale for co-formulation versus single-extract delivery.

Fourth, the IC_{50} concentration (42.18 $\mu\text{g/mL}$ total extract) may be challenging to achieve systemically following oral administration without dose escalation or repeat dosing, particularly given first-pass metabolism. Pharmacokinetic modeling and bioavailability studies are needed to establish clinically feasible dosing

regimens. Fifth, long-term stability studies following ICH guidelines (e.g., Q1A) are essential to establish shelf-life and appropriate storage conditions for commercial development. Finally, phytochemical standardization through HPLC quantification of marker compounds (e.g., bruceine D, wedelolactone) in each extract batch would enhance reproducibility and regulatory acceptability.

5. Conclusion

This study successfully developed an evidence-based nanoemulsion platform for co-delivering *Brucea javanica* and *Wedelia chinensis* extracts, demonstrating that systematic formulation optimization can yield stable, reproducible phytochemical delivery systems with balanced multi-botanical encapsulation. The lead formulation F4 achieved sub-100 nm particle size (87.8 nm), narrow size distribution (PDI 0.142), robust interfacial stabilization (zeta potential -28.9 mV), and high content recovery for both extracts ($>100\%$), representing superior physicochemical quality compared to conventional phytochemical formulations.

Biological evaluation revealed selective cytotoxic activity against AGS gastric cancer cells (IC_{50} 42.18 $\mu\text{g/mL}$) with a 2.12-fold selectivity index versus normal HEK-293 cells, supporting a therapeutic window suitable for further development. Importantly, F4 nanoemulsion demonstrated significant anti-migratory effects (51.37% inhibition at 24 h), suggesting potential to address the metastatic dissemination that drives gastric cancer mortality. The observed morphological changes—including cell rounding, reduced lamellipodia formation, and increased detachment—indicate disruption of cytoskeletal organization and migration machinery essential for cancer cell invasiveness.

Collectively, these findings establish proof-of-concept for BJ–WC nanoemulsion therapy as a novel nano-phytopharmaceutical strategy combining traditional botanical medicine with advanced nanotechnology. The work provides a strong foundation for preclinical advancement, including in vivo pharmacokinetic and efficacy studies, mechanistic pathway validation, formal synergy quantification, and ICH-compliant stability testing. Future clinical translation will require demonstration of oral bioavailability enhancement, tumor accumulation via EPR effects or active targeting, and acceptable safety profiles in appropriate animal models. Nevertheless, the evidence presented here supports continued development of multi-botanical nanoemulsions as a promising approach for addressing the persistent challenges of gastric cancer therapy through multi-targeted, lower-toxicity interventions.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

All data supporting the findings of this study are available within the manuscript. Raw data files may be obtained from the corresponding author upon reasonable request.

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