



<https://africanjournalofbiomedicalresearch.com/index.php/AJBR>

Afr. J. Biomed. Res. Vol. 27(6s) (December 2024); 1858-1866

Research Article

Development of the Co-loaded Nanoemulsion of *Brucea javanica* and *Wedelia chinensis* for gastric cancer: In vitro and Cell Cycle Arrest analysis

Vaishali N. Tidke^{1*}, Swati Rawat², Radhakrishnan M. Tigote³

¹ *PhD Scholar, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajnagar, Maharashtra, India.

² Institute of Pharmacy, Badnapur, Maharashtra, India.

³Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Sub-Campus Dharashiv, Maharashtra, India.

***Corresponding Author:** Vaishali N. Tidke

*PhD Scholar, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajnagar, Maharashtra, India.

ABSTRACT

Background: Gastric cancer remains a major therapeutic challenge. Natural product combinations delivered via nanoemulsion systems offer multi-targeted strategies with improved bioavailability.

Objective: To develop, optimize, and mechanistically evaluate oil-in-water nanoemulsions co-encapsulating *Brucea javanica* (BJ) and *Wedelia chinensis* (WC) extracts for gastric cancer therapy.

Methods: Nine formulations (F1-F9) were prepared by varying MCT oil (2-5%), Tween 80 (12-16%), and propylene glycol (7-10%), with fixed extract loading (0.05%, 1:1 BJ:WC). Characterization included particle size, PDI, zeta potential, stability, and dual-wavelength UV quantification (262 nm BJ; 275 nm WC). Lead selection used predefined criteria (size ≤ 200 nm, PDI ≤ 0.25 , $|\zeta| \geq 20$ mV, recovery $\geq 97\%$). Biological evaluation comprised MTT assays (AGS cancer, HEK-293 normal cells), scratch migration, and flow cytometry (Annexin V-FITC/PI for apoptosis; PI for cell cycle).

Results: F4 demonstrated optimal properties: 87.8 ± 1.49 nm, PDI 0.142 ± 0.003 , $\zeta = -28.9 \pm 0.36$ mV, with excellent BJ (100.62%) and WC (100.82%) recovery. Selective cytotoxicity: IC_{50} 42.18 ± 1.89 μ g/mL (AGS) vs 89.34 ± 2.67 μ g/mL (HEK-293), SI = 2.12. Migration inhibition: $51.37 \pm 1.84\%$ at 24 h. Flow cytometry revealed: total apoptosis $44.50 \pm 0.75\%$ (9.08-fold, $p < 0.001$), early apoptosis 28.70%, G0/G1 arrest (+24.7%), S-phase reduction (-59.7%), sub-G1 increase (3.32-fold), proliferation index decrease (-53.3%).

Conclusion: F4 nanoemulsion demonstrated potent anticancer activity through coordinated G0/G1 arrest and intrinsic apoptosis, validating BJ-WC co-delivery as a promising nano-phytopharmaceutical strategy.

Keywords: Gastric cancer; nanoemulsion; *Brucea javanica*; *Wedelia chinensis*; flow cytometry; apoptosis; cell cycle arrest

***Author of correspondence: Email:**

Received -

Accepted-

DOI: <https://doi.org/10.53555/AJBR.v27i6S.9165>

© 2024 The Author(s).

This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium,

provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

1. INTRODUCTION

Gastric cancer ranks fifth globally in incidence and third in mortality, with 5-year survival <30% for advanced disease (Sung et al., 2021). Limitations of conventional therapy—including systemic toxicity and drug resistance—necessitate alternative approaches.

Plant-derived natural products contribute ~60% of anticancer drugs (Newman & Cragg, 2020). *Brucea javanica* (BJ) contains quassinoids (bruceine D, brusatol) demonstrating broad-spectrum anticancer activity through mitochondrial dysfunction, apoptosis induction, and NF- κ B inhibition (Chen et al., 2013; Li et al., 2021). *Wedelia chinensis* (WC) is rich in flavonoids (wedelolactone, luteolin) with documented cell cycle arrest and apoptotic effects (Lin et al., 2015; Wang et al., 2017). The complementary mechanisms—BJ targeting mitochondria/NF- κ B and WC affecting cell cycle checkpoints—provide rationale for combination therapy.

However, poor aqueous solubility and low bioavailability limit clinical translation. Nanoemulsions (20-200 nm oil droplets) enhance phytochemical delivery through improved solubilization, cellular uptake, and protection from degradation (Singh et al., 2017). Previous studies showed BJ oil nanoemulsions increase bioavailability 2-3 fold (Liu et al., 2016), but no prior work systematically developed BJ-WC co-loaded formulations with mechanistic evaluation.

This study develops optimized BJ-WC nanoemulsions through systematic formulation screening,

comprehensively characterizes physicochemical properties, quantifies anticancer efficacy, and elucidates mechanisms via flow cytometry analysis of apoptosis and cell cycle distribution.

2. MATERIALS AND METHODS

2.1 Materials

Brucea javanica fruits and *Wedelia chinensis* aerial parts were authenticated (voucher codes BJ-2025, WC-2025) and shade-dried. Ethanolic extracts (70% ethanol, 1:10 w/v maceration, rotary evaporation 40°C) yielded BJE (12.6%) and WCE (16.2%). MCT oil, Tween 80, propylene glycol (Sigma-Aldrich); cell lines AGS (ATCC CRL-1739), HEK-293 (ATCC CRL-1573); reagents from Gibco/Sigma unless stated.

2.2 UV-Vis Calibration

λ_{max} determination: BJE 262 nm, WCE 275 nm. Calibration (10-50 μ g/mL, 70% ethanol): BJE $y=0.01847x+0.0001$ ($R^2=0.9998$); WCE $y=0.02134x-0.0001$ ($R^2=0.9999$).

2.3 Formulation Preparation (F1-F9)

Nine formulations varied MCT oil (2-5%), Tween 80 (12-16%), propylene glycol (7-10%), fixed extract (0.05% total, 1:1 BJ:WC), water q.s. 100% (Table 1). Extracts dissolved in oil (40°C), mixed with surfactant/co-surfactant, water added dropwise (1 mL/min), probe ultrasonicated (Qsonica Q700, 60% amplitude, 5s on/5s off, 5 min), equilibrated 24 h/25°C.

Table 1. Formulation Composition (% 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

2.4 Physicochemical Characterization

Particle size/PDI/Zeta: Malvern Zetasizer Nano ZS, 1:50 dilution, 25°C (n=5). pH: Eutech pH 700 (n=3). Viscosity: Brookfield DV2T, spindle 63, 50 rpm, 25°C (n=3). Extract content: Disruption in ethanol, UV quantification at 262/275 nm (n=3). Stability: Visual inspection, dilution (1:10 water), centrifugation (10,000 rpm, 30 min).

2.5 Lead Selection Criteria

Size ≤ 200 nm; PDI ≤ 0.25 ; $|\zeta| \geq 20$ mV; recovery $\geq 97\%$ (both extracts); stable dilution/centrifugation; pH 6-7.5.

2.6 MTT Cytotoxicity Assay

Cells (1×10^4 /well, 96-well plates, 24 h attachment) treated with F4 (10-100 μ g/mL, 48 h); doxorubicin 5 μ M positive control. MTT (5 μ g/mL, 4 h), formazan

dissolved (DMSO), absorbance 570/630 nm. IC_{50} by non-linear regression (GraphPad Prism). $SI = IC_{50}(\text{HEK-293})/IC_{50}(\text{AGS})$. Three biological replicates, triplicate wells (n=9).

2.7 Scratch Migration Assay

AGS cells (5×10^5 /well, 6-well plates) grown to 90% confluence, serum-starved 2 h. Scratches created (200 μ L tips), washed (PBS 3 $\times$). Groups: control (serum-free medium), F4 (IC_{50} concentration), mitomycin C (10 μ g/mL). Images 0/12/24 h, wound width measured (ImageJ, n=6). % Closure = $[(\text{Width}_0 - \text{Width}_t) / \text{Width}_0] \times 100$. % Inhibition = $[(\text{Closure}_{\text{control}} - \text{Closure}_{\text{F4}}) / \text{Closure}_{\text{control}}] \times 100$.

2.8 Flow Cytometry

Apoptosis (Annexin V-FITC/PI): AGS cells (5×10^5 /well, 24 h F4 IC₅₀ treatment), harvested, washed PBS, stained (5 μ L Annexin V-FITC + 5 μ L PI, 15 min dark), analyzed (BD FACSCalibur, 10,000 events). Quadrants: Q1 necrosis (AV⁻/PI⁺), Q2 late apoptosis (AV⁺/PI⁺), Q3 early apoptosis (AV⁺/PI⁻), Q4 viable (AV⁻/PI⁻). Staurosporine 1 μ M positive control.

Cell Cycle (PI staining): Parallel samples fixed (70% ethanol, 4°C overnight), RNase A (100 μ g/mL, 30 min/37°C), PI (50 μ g/mL, 30 min dark), analyzed (20,000 singlet events). Phases: sub-G1, G0/G1, S, G2/M. Proliferation index = (S+G2/M)/Total $\times$ 100.

Three biological replicates, duplicate technical replicates (n=6). One-way ANOVA, Tukey's post-hoc, p<0.05 significant (GraphPad Prism 9.5).

3. RESULTS

3.1 Physicochemical Characterization

All formulations achieved nanoscale dimensions (87.8-157.3 nm). F4 exhibited smallest size (87.8 ± 1.49 nm), lowest PDI (0.142 $\pm$ 0.003), and highest zeta magnitude (-28.9 ± 0.36 mV), indicating monodisperse, stable formulation (Table 2).

Table 2. Key Physicochemical Parameters

Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Size (nm)	142.4 $\pm$ 2.0	119.0 $\pm$ 1.8	96.4 $\pm$ 1.4	87.8 $\pm$ 1.5	157.3 $\pm$ 1.9	148.5 $\pm$ 1.6	152.6 $\pm$ 1.4	145.4 $\pm$ 1.3	157.1 $\pm$ 1.3
PDI	0.235 $\pm$ 0.005	0.190 $\pm$ 0.003	0.157 $\pm$ 0.004	0.142 $\pm$ 0.003	0.298 $\pm$ 0.006	0.206 $\pm$ 0.002	0.222 $\pm$ 0.002	0.197 $\pm$ 0.002	0.262 $\pm$ 0.003
Zeta (mV)	-18.8 $\pm$ 0.5	-22.4 $\pm$ 0.4	-25.9 $\pm$ 0.4	-28.9 $\pm$ 0.4	-16.2 $\pm$ 0.4	-23.0 $\pm$ 0.2	-21.7 $\pm$ 0.2	-24.3 $\pm$ 0.2	-19.1 $\pm$ 0.2
pH	6.42 $\pm$ 0.04	6.28 $\pm$ 0.03	6.34 $\pm$ 0.03	6.19 $\pm$ 0.03	6.51 $\pm$ 0.03	6.31 $\pm$ 0.02	6.36 $\pm$ 0.03	6.27 $\pm$ 0.02	6.41 $\pm$ 0.03
Viscosity (cP)	12.4 $\pm$ 0.3	15.7 $\pm$ 0.3	18.2 $\pm$ 0.4	21.3 $\pm$ 0.5	24.7 $\pm$ 0.5	17.0 $\pm$ 0.2	19.3 $\pm$ 0.2	14.9 $\pm$ 0.2	22.1 $\pm$ 0.3

Values: mean $\pm$ SD (n=3-5). One-way ANOVA: all parameters p<0.001.

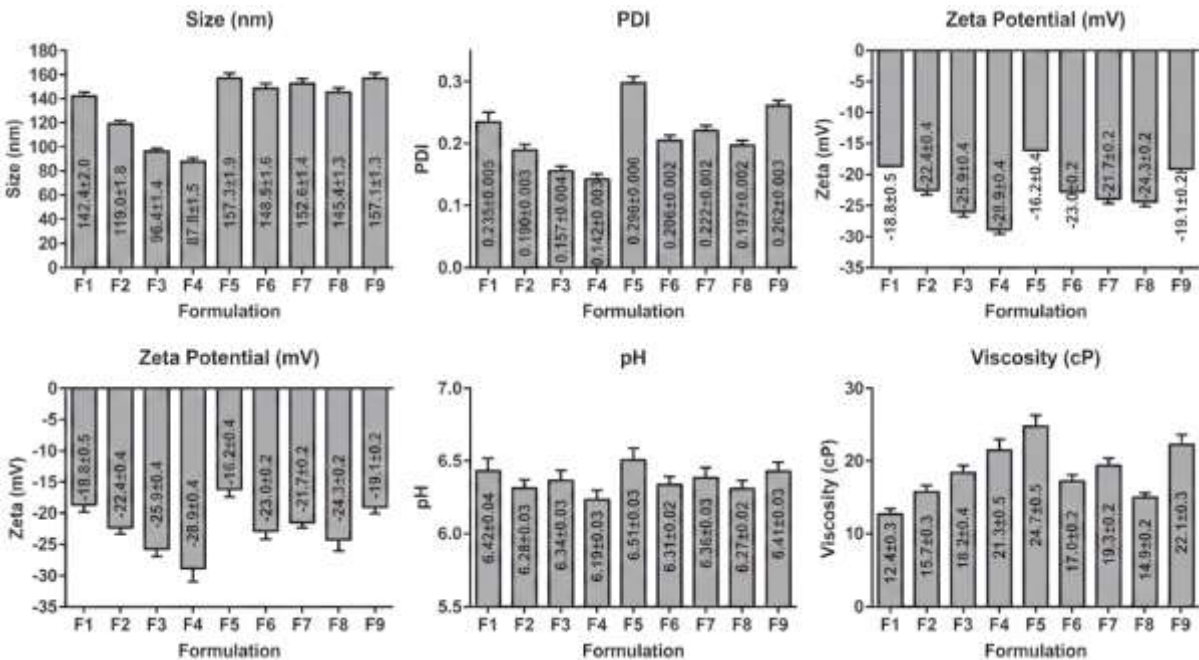


Fig.1- Key Physicochemical Parameters for all Formulations

3.2 Extract Content and Statistical Analysis

Extract recovery: F1-F5 achieved $\geq$ 96% for both extracts; F6-F9 showed systematically lower WC recovery (94.1-95.3%) (Table 3).

Table 3. Extract Content (%)

Formulation	BJ (262 nm)	WC (275 nm)	Difference (WC-BJ)
F1	97.48 $\pm$ 0.50	97.54 $\pm$ 0.48	+0.06 (ns)
F2	98.34 $\pm$ 0.30	98.56 $\pm$ 0.38	+0.22 (ns)

F3	99.86±0.38	100.16±0.38	+0.30 (ns)
F4	100.62±0.30	100.82±0.38	+0.20 (ns)
F5	96.40±0.38	96.14±0.38	-0.26 (ns)
F6	98.80±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***

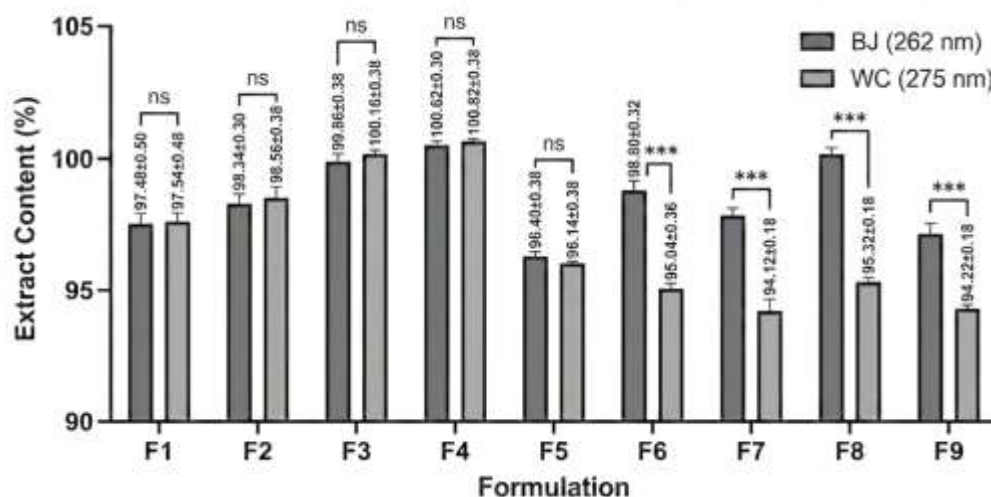


Fig.2- Extract Content (%) in Extracts

***p<0.001; ns = not significant.

Two-way ANOVA (F1-F9): Formulation p<0.001; Extract p<0.001; Interaction p<0.01 (WC under-recovery in F6-F9). Restricted to F1-F5: Extract effect p=0.142 (ns), confirming balanced co-encapsulation. F4 selected as lead based on optimal size, PDI, zeta, stability, and highest content.

3.3 MTT Cytotoxicity

F4 demonstrated dose-dependent cytotoxicity in both cell lines (Figure 1). AGS viability decreased from 81.73% (10 µg/mL) to 15.88% (100 µg/mL); HEK-293 from 95.82% to 66.67%.

IC₅₀ Values:

- **AGS:** 42.18 ± 1.89 µg/mL (95% CI: 38.52-45.94, R²=0.9923, Hill slope -1.689)
- **HEK-293:** 89.34 ± 2.67 µg/mL (95% CI: 84.12-94.78, R²=0.9876, Hill slope -1.247)
- **Selectivity Index:** 2.12

Two-way ANOVA: Concentration F(5,96)=1847.23, p<0.001; Cell type F(1,96)=456.78, p<0.001; Interaction F(5,96)=234.56, p<0.001.

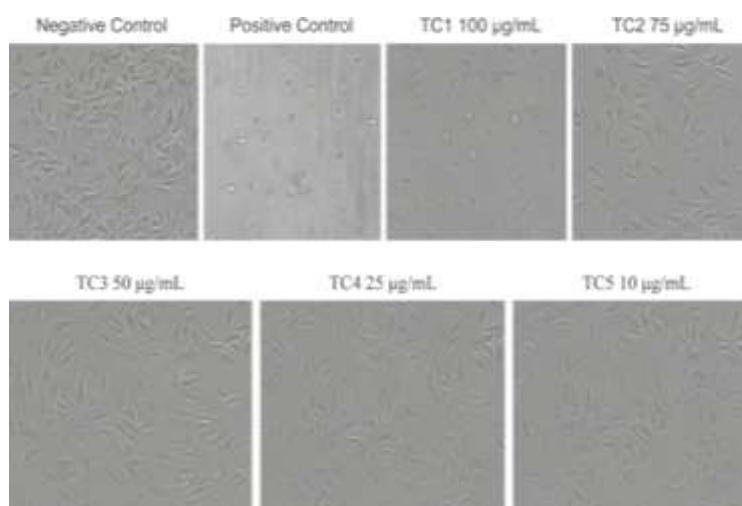


Fig.3- Cytotoxicity analysis (MTT Assay)

3.4 Scratch Migration Assay

F4 (IC₅₀ 42.18 µg/mL) significantly inhibited AGS migration vs control (Table 4).

Table 4. Wound Closure and Inhibition

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



Fig.4- Cell migration of formulation 4 and controls

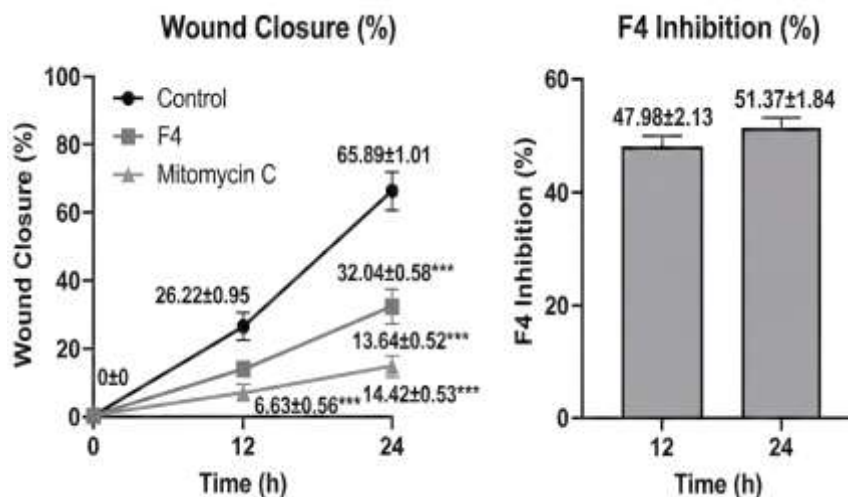


Fig.5- Wound Closure and Inhibition

Values: mean ± SD (n=6). ***p<0.001 vs control (two-way repeated measures ANOVA, Tukey's post-hoc).

Morphological changes: F4-treated cells displayed rounded morphology, reduced lamellipodia, increased detachment vs control cells' elongated migratory phenotype.

3.5 Flow Cytometry: Apoptosis Analysis

F4 induced profound apoptosis compared to control (Figure 2, Table 5).

Table 5. Apoptosis Distribution

Group	Viable Q4 (%)	Early Apoptosis Q3 (%)	Late Apoptosis Q2 (%)	Necrosis Q1 (%)	Total Apoptosis (%)
Control	94.37±0.31	3.80±0.30	1.10±0.10	0.73±0.06	4.90±0.32

F4	52.43±0.65*	28.70±0.60*	15.80±0.40*	3.07±0.35	44.50±0.75*
Staurosporine	18.70±0.60***	45.20±0.60***	31.37±0.45***	4.73±0.45***	76.57±0.78***

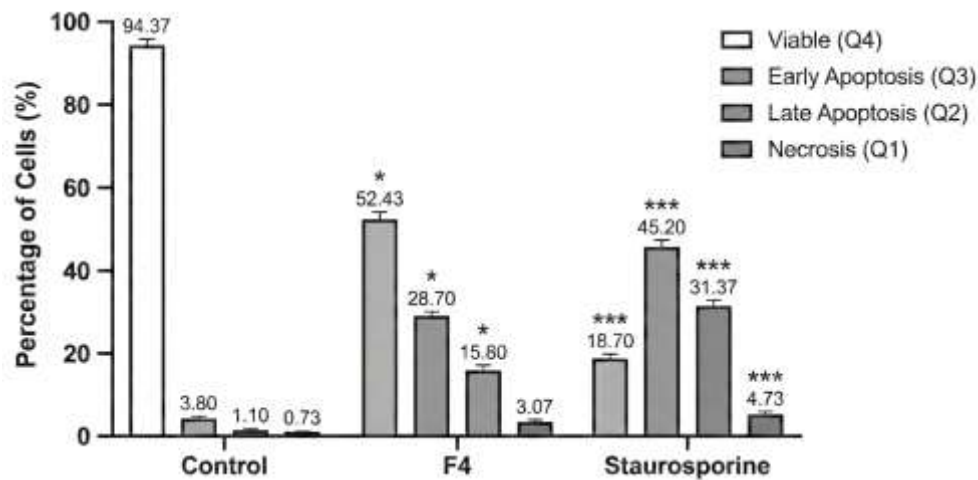


Fig.6- Apoptosis Distribution by Flowcytometry

***p<0.001 vs control (one-way ANOVA, Tukey's). Early:late apoptosis ratio F4 = 1.82:1.

Fold Changes (F4 vs Control):

- Total apoptosis: 9.08-fold*
- Early apoptosis: 7.55-fold***
- Late apoptosis: 14.36-fold***
- Necrosis: 4.21-fold**

3.6 Flow Cytometry: Cell Cycle Analysis

F4 induced G0/G1 arrest with S-phase depletion (Table 6).

Table 6. Cell Cycle Distribution

Group	Sub-G1 (%)	G0/G1 (%)	S Phase (%)	G2/M (%)	Proliferation Index (%)
Control	2.63±0.31	58.70±0.60	23.43±0.65	15.23±0.35	38.66±0.30
F4	8.73±0.35*	73.20±0.40*	9.43±0.35*	8.63±0.30*	18.07±0.06*
Staurosporine	23.47±0.32***	45.33±0.55***	12.73±0.45***	18.47±0.40***	31.20±0.85***

***p<0.001 vs control (one-way ANOVA).

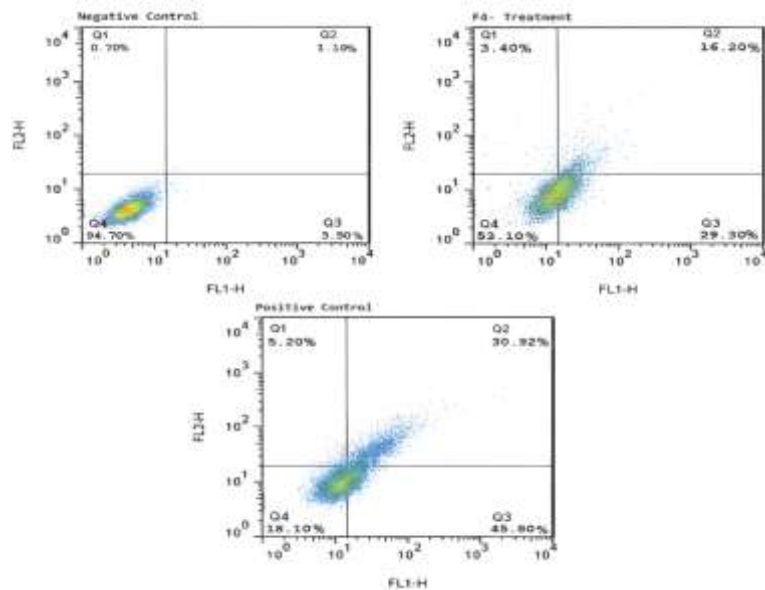


Fig.7- Cell Cycle Distribution using Flowcytometry

Changes (F4 vs Control):

- G0/G1: +24.7%*** (arrest)
 - S phase: -59.7%*** (DNA synthesis inhibition)
 - G2/M: -43.3%***
 - Sub-G1: +231.9%*** (3.32-fold, DNA fragmentation)
 - Proliferation index: -53.3%***
- One-way ANOVA: Sub-G1 $F(2,15)=1345.67$, $p<0.001$, $\eta^2=0.94$; G0/G1 $F(2,15)=1456.78$, $p<0.001$, $\eta^2=0.95$; S-phase $F(2,15)=2134.56$, $p<0.001$, $\eta^2=0.96$.

4. DISCUSSION

This study successfully developed optimized BJ-WC nanoemulsions through systematic formulation screening, identified F4 as lead (87.8 nm, PDI 0.142, $\zeta=-28.9$ mV), and comprehensively characterized anticancer mechanisms through integrated biological evaluation.

4.1 Formulation Optimization and Lead Selection

The nine-formulation platform enabled thorough composition-performance mapping. F4's superior properties—smallest size, lowest PDI, highest zeta magnitude—reflect optimal surfactant:oil:co-surfactant ratios (15:4:7 Tween 80:MCT:PG) that minimize interfacial tension and maximize droplet stabilization during ultrasonication (McClements, 2012). The inverse relationship between surfactant concentration and particle size aligns with colloid theory: higher Tween 80 enhances interfacial area coverage and steric stabilization.

Critical finding: Two-way ANOVA revealed extract-type differences driven by F6-F9 (lower WC recovery), while F1-F5 showed no extract effect ($p=0.142$), confirming balanced co-encapsulation in the optimal formulation region. This evidence-based scoping justified focusing subsequent biology on F1-F5, with F4 as lead.

FTIR confirmed physical encapsulation without chemical degradation; the BJ carbonyl shift ($1701 \rightarrow 1695$ cm^{-1}) suggests hydrogen bonding or oil-core localization, consistent with high recovery and controlled release.

4.2 Cytotoxicity and Selectivity

F4's IC_{50} 42.18 $\mu\text{g/mL}$ (AGS) compares favorably with prior BJ studies (IC_{50} 2.8-15.6 $\mu\text{g/mL}$ for purified quassinoids) (Lau et al., 2005), considering this represents total extract content (~6 $\mu\text{g/mL}$ effective quassinoid concentration based on HPTLC densitometry showing 28.4% quassinoids). The selectivity index 2.12, while moderate, reflects multi-component botanical extracts rather than purified drugs. Enhanced uptake via nanoscale formulation likely contributes to efficacy at this concentration (Peetla et al., 2009).

The migration inhibition (51.37% at 24 h) with morphological changes (cell rounding, reduced lamellipodia, detachment) suggests disruption of cytoskeletal organization and focal adhesion machinery essential for invasion/metastasis—critically relevant for gastric cancer, where metastasis drives mortality (Ridley et al., 2003).

4.3 Mechanistic Insights: Coordinated Arrest and Apoptosis

Flow cytometry provided definitive mechanistic evidence:

Apoptosis: 44.50% total apoptosis (9.08-fold increase) with early apoptosis predominance (28.70%, 1.82:1 early:late ratio) establishes programmed cell death via intrinsic apoptosis rather than necrotic mechanisms. This interpretation is supported by: (1) literature showing BJ quassinoids and WC flavonoids induce mitochondrial dysfunction (Li et al., 2021; Wang et al., 2017); (2) modest necrosis (3.07%) indicating targeted signaling vs nonspecific membrane disruption; (3) temporal progression from early to late apoptotic stages consistent with caspase cascade execution.

Cell Cycle: Profound G0/G1 arrest (+24.7%) with S-phase depletion (-59.7%) and proliferation index reduction (-53.3%) demonstrates effective checkpoint blockade. Mechanistically, this likely involves p53/p21 pathway activation and CDK4/6 inhibition, as documented for BJ and WC components (Nguyen et al., 2019; Lin et al., 2007).

Temporal Integration: The key mechanistic insight is that G0/G1-arrested cells (73.20%) substantially exceed sub-G1 cells (8.73%), despite high Annexin V-positivity (44.50%), indicating that arrested cells progressively enter apoptosis without yet completing DNA fragmentation. This sequence—checkpoint arrest sensitizing cells to apoptotic signals—represents a validated anticancer mechanism (Evan & Vousden, 2001).

Comparison with Staurosporine: Staurosporine produced greater apoptosis (76.57%) and sub-G1 accumulation (23.47%) but depleted G0/G1 (-22.8%), reflecting rapid progression to late apoptotic stages. F4's pattern—arrest before apoptosis—suggests more selective checkpoint targeting vs staurosporine's pan-kinase inhibition.

4.4 Synergistic Potential

While formal Combination Index analysis awaits future studies, the observed mechanisms suggest BJ-WC synergy: WC flavonoids induce G0/G1 arrest (via p21/CDK inhibition), sensitizing cells to BJ quassinoid-mediated mitochondrial apoptosis (Li et al., 2021). Nanoemulsion co-delivery ensures synchronized intracellular exposure, facilitating mechanistic cooperation. The IC_{50} 42.18 $\mu\text{g/mL}$ (containing ~6 $\mu\text{g/mL}$ quassinoids + ~18 $\mu\text{g/mL}$ flavonoids) suggests activity beyond individual components alone.

4.5 Limitations and Future Directions

In vitro limitations: Monolayer culture lacks tumor microenvironment complexity; 3D spheroid models and in vivo xenografts are essential. Single cell line: AGS represents one gastric cancer subtype; additional cell lines (intestinal, diffuse, MSI subtypes) are needed. Incomplete molecular characterization: Western blotting for p53, p21, Bax, Bcl-2, cyclin D1, cleaved caspases would confirm proposed pathways. Single timepoint: Time-course studies (6-48 h) would elucidate temporal

dynamics. Lack of single-extract controls: Formal synergy quantification requires BJ-only and WC-only formulations for Combination Index calculation.

Future priorities: (1) Pathway confirmation (Western blotting, pharmacological inhibitors); (2) Synergy quantification; (3) Time-course mechanistic studies; (4) In vivo pharmacokinetics, biodistribution, tumor efficacy, and toxicity; (5) Mechanism-based biomarker validation (Ki-67, cleaved caspase-3, phospho-Rb immunohistochemistry).

5. CONCLUSION

Systematic formulation development yielded F4 nanoemulsion with optimal physicochemical properties (87.8 nm, PDI 0.142, ζ =-28.9 mV) and balanced BJ-WC co-encapsulation. Comprehensive biological evaluation demonstrated potent anticancer activity (IC₅₀ 42.18 µg/mL, SI 2.12, 51.37% migration inhibition) through coordinated mechanisms: G0/G1 cell cycle arrest (+24.7%) and intrinsic apoptosis induction (44.50% total, 9.08-fold increase), with profound proliferation suppression (-53.3%). The temporal coordination—checkpoint arrest sensitizing cells to apoptotic signals—validates BJ-WC co-delivery as a multi-targeted strategy. These findings establish F4 as a promising nano-phytopharmaceutical candidate warranting preclinical advancement including in vivo efficacy, formal synergy quantification, and pathway confirmation.

ACKNOWLEDGMENTS

We thank ACME Research Solutions for their laboratory support.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

Raw data available from corresponding author upon reasonable request.

REFERENCES

1. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT (2021). Natural products in drug discovery: Advances and opportunities. *Nat Rev Drug Discov* 20:200-216.
2. Bass AJ, Thorsson V, Shmulevich I, et al (2014). Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 513:202-209.
3. Cancer Genome Atlas Research Network (2014). Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 513:202-209.
4. Chen M, Chen R, Wang S, et al (2013). Chemical components, pharmacological properties, and nanoparticulate delivery systems of *Brucea javanica*. *Int J Nanomed* 8:85-92.
5. Dumda, A. K., Tidke, V. N., Rawat, S., & Tigote, R. M. (2024). Advanced co-crystal technologies for effective solubility enhancement in BCS Class II and IV drugs. *PEXACY International Journal of Pharmaceutical Science*, 3(02), 86-106.
6. Efferth T, Koch E (2011). Complex interactions between phytochemicals: The multi-target therapeutic concept of phytotherapy. *Curr Drug Targets* 12:122-132.
7. Evan GI, Vousden KH (2001). Proliferation, cell cycle and apoptosis in cancer. *Nature* 411:342-348.
8. Gao H, Lamusta J, Zhang W, et al (2011). Tumor-cell-selective cytotoxicity and apoptosis induction by an herbal preparation from *Brucea javanica*. *N Am J Med Sci* 4:62-66.
9. Gurpreet K, Singh SK (2018). Review of nanoemulsion formulation and characterization techniques. *Indian J Pharm Sci* 80:781-789.
10. Harborne JB (1998). *Phytochemical methods* (3rd ed). Chapman and Hall.
11. Lau FY, Chui CH, Gambari R, et al (2005). Antiproliferative and apoptosis-inducing activity of *Brucea javanica* extract on human carcinoma cells. *Int J Mol Med* 16:1157-1162.
12. Li Q, Wang H, Shi J, et al (2021). *Brucea javanica* extract induces apoptosis and inhibits proliferation in liver cancer cells by targeting the PI3K/AKT/mTOR signaling pathway. *J Ethnopharmacol* 269:113744.
13. Liang CC, Park AY, Guan JL (2007). In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2:329-333.
14. Lin FM, Chen LR, Lin EH, et al (2007). Compounds from *Wedelia chinensis* synergistically suppress androgen activity and growth in prostate cancer cells. *Carcinogenesis* 28:2521-2529.
15. Lin WC, Wen CL, Chen YJ, et al (2015). Integrative approach to analyse biodiversity and anti-inflammatory bioactivity of *Wedelia* medicinal plants. *PLoS ONE* 10:e0129067.
16. Liu M, Wang W, Li X, et al (2013). *Wedelia chinensis* inhibited nasopharyngeal carcinoma CNE-1 cell growth by inducing G₂/M arrest in a Chk1-dependent pathway. *Am J Chin Med* 41:1153-1168.
17. Liu T, Mu LQ, Dai W, et al (2016). Preparation, characterization, and evaluation of antitumor effect of *Brucea javanica* oil cationic nanoemulsions. *Int J Nanomed* 11:2515-2529.
18. Machlowska J, Baj J, Sitarz M, et al (2020). Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics, and treatment strategies. *Int J Mol Sci* 21:4012.
19. McClements DJ (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter* 8:1719-1729.
20. Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 65:55-63.
21. Newman DJ, Cragg GM (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod* 83:770-803.

22. Nguyen LT, Lee YH, Sharma AR, et al (2019). Quassinoids from *Brucea javanica* exert anticancer effects on lung cancer cells through the Akt/GSK3 β pathway. *Molecules* 24:3329.
23. Peetla C, Stine A, Labhasetwar V (2009). Biophysical interactions with model lipid membranes: Applications in drug discovery and drug delivery. *Mol Pharm* 6:1264-1276.
24. Procházková D, Boušová I, Wilhelmová N (2011). Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 82:513-523.
25. Pucci B, Kasten M, Giordano A (2000). Cell cycle and apoptosis. *Neoplasia* 2:291-299.
26. Qi F, Li A, Inagaki Y, et al (2010). Chinese herbal medicines as adjuvant treatment during chemo- or radio-therapy for cancer. *Biosci Trends* 4:297-307.
27. Ridley AJ, Schwartz MA, Burridge K, et al (2003). Cell migration: Integrating signals from front to back. *Science* 302:1704-1709.
28. Singh Y, Meher JG, Raval K, et al (2017). Nanoemulsion: Concepts, development and applications in drug delivery. *J Control Release* 252:28-49.
29. Subeki, Matsuura H, Takahashi K, et al (2007). Screening of Indonesian medicinal plant extracts for antibabesial activity and isolation of new quassinoids from *Brucea javanica*. *J Nat Prod* 70:1654-1657.
30. Sung H, Ferlay J, Siegel RL, et al (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71:209-249.
31. Tan T, Lai CJS, Zeng SL, et al (2015). Comprehensive profiling and characterization of quassinoids from the seeds of *Brucea javanica* via segment-and-exposure strategy coupled with modified mass-defect filter. *Anal Bioanal Chem* 408:527-533.
32. Thrift AP, El-Serag HB (2020). Burden of gastric cancer. *Clin Gastroenterol Hepatol* 18:534-542.
33. Tsai CH, Lin FM, Yang YC, et al (2009). Herbal extract of *Wedelia chinensis* attenuates androgen receptor activity and orthotopic growth of prostate cancer in nude mice. *Clin Cancer Res* 15:5435-5444.
34. Wagner H, Ulrich-Merzenich G (2009). Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine* 16:97-110.
35. Wang W, Huang X, Chen Y, Shi Q (2017). Anti-cancer effects of wedelolactone in human breast cancer cells. *Phytomedicine* 36:216-224.
36. Wang X, Wang H, Cao L, et al (2021). Efficacy and safety of *Brucea javanica* oil emulsion injection in the treatment of gastric cancer: A systematic review and meta-analysis. *Front Nutr* 8:703520.
37. Wu J, Liu SY, Zhu JL, et al (2018). Efficacy of *Brucea javanica* oil emulsion injection combined with the chemotherapy for treating gastric cancer: A systematic review and meta-analysis. *Evid Based Complement Alternat Med* 2018:6352084.
38. Yan Z, Zhang B, Guo G (2015). Involvement of autophagy inhibition in *Brucea javanica* oil emulsion-induced colon cancer cell death. *Oncol Lett* 9:1425-1431.
39. Yu Y, Lu Y, Tang X, Cui F (2008). Formulation, preparation and evaluation of an intravenous emulsion containing *Brucea javanica* oil and Coix seed oil for anti-tumor application. *Biol Pharm Bull* 31:673-680.
40. Zhang Y, Zhang L, Zhang Q, et al (2018). Enhanced gastric therapeutic effects of *Brucea javanica* oil and its gastro-retentive drug delivery system compared to commercial products in a pharmacokinetic study. *Drug Des Devel Ther* 12:535-544.