Trop J Nat Prod Res, February 2025; 9(2): 454 - 459 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Potential of Nanoencapsulated *Curcuma longa-Andrographis paniculata* as an Anticancer Agent

Sari E. Cahyaningrum[1], Andika P. Wardana[1]*, Fitriari I. Muhaimin[2], Amalia P. Purnamasari[1], Browi Nugroho[1], and Misni B. Misran[3]

[1] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia.*
[2] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, East Java, Indonesia.*
[3] *Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia.*

ARTICLE INFO

Article history:
Received 12 October 2024
Revised 01 November 2024
Accepted 21 November 2024
Published online 01 March 2025

ABSTRACT

Anticancer agents can inhibit and destroy cancer cells by various mechanisms. Some plants are reported to have bioactive components that can be developed as anticancer agents, such as *Curcuma longa* and *Andrographis paniculata*. Although herbal-based drugs have good potential, they have limitations when applied in vivo. Developing drug delivery systems is a potential solution by utilizing nano *C. longa-A. paniculata* as anticancer agents. The nanoencapsulation of *C. longa-A. paniculata* with carboxymethyl chitosan was performed using the ultrasonication method. The characterization of nano *C. longa-A. paniculata* (FB-NPs) included physicochemical properties, FTIR, AFM, stability, loading, and release of its bioactive components. FB-NPs had a particle size of 222.60 ± 0.87 nm. The loading amount and efficiency of FB-NPs were 17.83 ± 3.04% and 35.66 ± 6.08%, respectively. FB-NPs showed greater stability under changes in pH, temperature, and salt concentration compared to the free *C. longa-A. paniculata* formula (FB). The anticancer activity of FB-NPs against the MCF7 cell line showed stronger inhibition, with an EC_{50} value of 13.71 ± 2.66 µg/mL compared to FB. The nanoencapsulation process of *C. longa-A. paniculata* with carboxymethyl chitosan has potential as an herbal-based anticancer agent, which can significantly contribute to the development of breast cancer treatment.

Keywords: *Andrographis paniculata*, Anticancer, Carboxymethyl chitosan, *Curcuma longa*, Nanoencapsulation.

Introduction

Breast cancer is one of the most frequently diagnosed malignant in women, with an estimated 2.3 million cases worldwide.[1,2] Breast cancer is one of the most studied cancers, with a curable rate of 70.00-80.00% in patients with early-stage and non-metastatic disease. However, it still has high mortality rates in several countries, and a five-year estimation for mortality risk is still used in the recent diagnosis.[3] This cancer is a heterogeneous disease that has molecular features, including activation of human epidermal growth factor receptor 2 (HER2), activation of hormone receptors (estrogen receptor and progesterone receptor), and breast cancer gene (BRCA) mutation.[1] Several risk factors for breast cancer include sex, age, reproductive and hormonal status, molecular alterations, genetic predisposition, ionizing radiation, diet, lifestyle, and obesity.[1,4] Treatment options for breast cancer vary depending on the type and the stages of cancer, which include surgery, radiotherapy, immunotherapy, neo-adjuvant therapy, endocrine therapy, etc.[5,6] Most of these treatments intervene in various stages of cancer development, from the single cell to initiation stage promotion, migration, and progression.[7] Several medicinal herbs have been heavily researched for their anticancer potential due to their uniqueness compared to synthetic compounds or molecules, such as curcumin and green chiretta.[8,9]

*Corresponding author. E mail: andikawardana@unesa.ac.id
Tel: + 6231-8296427

Citation: Cahyaningrum SE, Wardana AP, Muhaimin FI, Purnamasari AP, Nugroho B, Misran MB. Potential of Nanoencapsulated *Curcuma longa-Andrographis paniculata* as an Anticancer Agent. Trop J Nat Prod Res. 2025; 9(3): 454 - 459 https://doi.org/10.26538/tjnpr/v9i2.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Curcumin is a bioactive component of turmeric obtained from the *Curcuma longa* plant, a member of the Zingiberaceae ginger family widely found in Indonesia. It is commonly used in food flavoring, coloring, and traditional medicine.[10,11] It is known to have antioxidant, antibacterial, anti-inflammatory, and anticancer properties. It is observed to have highly cytotoxic anticancer activity against breast and prostate cancer, inhibiting cell proliferation and triggering apoptosis.[12-14] Meanwhile, *Andrographis paniculata* is known to play a role in lowering fever, reducing inflammation, inhibiting the formation of atherosclerosis, and inhibiting the formation of reactive oxidative species (ROS).[15,16] In addition, previous studies have shown that the constituent of *A. paniculata* was found to have antitumor activity.[17] Encapsulating these anticancer agents into biocompatible and biodegradable nanoparticles (NPs) could improve the efficiency and effectiveness of their chemoprevention activity [18,19]. Furthermore, it may overcome their poor adsorption, low systemic bioavailability, gastrointestinal hydrolysis, and short half-life.[20] Chitosan as the shell for anticancer agents could be an option. The nanoencapsulation process can enhance anticancer activity, as seen in nanoencapsulated *Syzygium polycephalum* with carrageenan-folate, which showed increased inhibitory activity against T47D and HeLa cell lines.[21] Carboxymethyl chitosan (CMCS) is a derivative of chitosan that is more hydrophilic than chitosan. It consists of active hydroxyl (–OH), carboxyl (–COOH), and amine ($–NH_2$) groups that enhance its solubility in neutral pH.[22] CMC has potential as a carrier for various biological active agents due to its ability to control the drug release rate and high biocompatibility.[22,23] Previously, CMC has been observed to have anticancer and antitumor properties as a single molecule and conjugate of anticancer drugs.[22,24,25]This study synthesized and characterized the formulation of *C. Longa-A. Paniculat*e encapsulated with chitosan to investigate its anticancer potential against breast cancer. Physical and chemical characterization, as well as *in vitro* tests, were performed.

Materials and Methods

Material
C. longa rhizome, *A. paniculata* leave, HCl (Merck), NaOH (Merck), methanol, aquades, carboxymethyl chitosan, KCl (Merck), KH_2PO_4 (Merck), K_2HPO_4 (Merck), H_3BO_3 (Merck), curcumin (Sigma-Aldrich), quercetin (Sigma-Aldrich), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich), NaCl (Merck), ascorbic acid (Sigma-Aldrich).

Sample Preparation
Rhizome of *C. longa* L. (certificate No. 002/03.542.Jl.Genbinesia) and leaves of *A. paniculata* (Burm.f.) Wall. Ex Nees (certificate No. 002/03.543.Jl.Genbinesia) taken from Mount Lawu, Jogorogo, Ngawi, East Java, Indonesia (7º33'46"S 111º13'23"E). Samples of *C. longa* rhizomes and *A. paniculata* leaves were dried and grounded, respectively. A total of 1 kg each was extracted with methanol for 24 hours and repeated thrice. The extract of each sample was concentrated using a rotary vacuum evaporator (BUCHI R-100).

Herb formulation
The herb formula consists of *C. longa* and *A. paniculata* extract with ratio 3:1 and antioxidant bioactivity screening using the DPPH method.[26] The best results are selected to continue with the following process.

Nanoencapsulation of *C. longa-A. paniculat*e
The nanoencapsulation process of *C. longa-A. paniculata* herb formula refers to the previous research.[27] Nano herbs *C. longa-A. paniculata* were obtained from the nanoencapsulation process with carboxymethyl chitosan. Zeta potential, polydispersity index (PDI), and particle size were the three physicochemical characteristics of nano *C. longa-A. paniculata* that were determined by Dynamic Light Scattering (DLS, Zetasizer Nano ZS, Malvern). Using FTIR (Shimadzu IRTracer-100), the functional groups of the nano *C. longa-A. paniculata* were identified. Simultaneously, Scanning Electron Microscope (SEM, Zeiss EVO MA 10) analysis was conducted on the morphology of nano *C. longa-A. Paniculate.*

Loading and release of nano C. longa-A. paniculate
Nanoencapsulation is an application of nanotechnology that can improve stability, and bioavailability of bioactive compounds because nano-size can increase the surface area. Stability tests were performed based on the pH, salt concentration, and temperature. The test results were conducted by observing changes in the UV-Vis spectra (Shimadzu UV-1800) and turbidity levels using a turbidimeter.[27,28] The release study was conducted at pH 2, 7, and 8.5 (Eq. 1). The loading amount (%LA) and efficiency (%LE) calculations are determined using Eq. 2 and 3.

$$Ct^1 = Ct^0 + \frac{v}{V}\sum_0^{i-t} Ct \quad \ldots(1)$$

Where Ct^1: concentration correction at t time
Ct^0: measured concentration at t time
V : Total volume of buffer used
v : volume of aliquots

$$\%LA = \frac{Mass\ of\ samples\ on\ FB-NPs}{Mass\ of\ FB-NPs} x100\% \quad \ldots(2)$$

$$\%LE = \frac{Mass\ of\ samples\ on\ FB-NPs}{Mass\ of\ samples\ in\ feed} x100\% \quad \ldots(3)$$

Toxicity assay
MCF7 cell lines were cultured in a 96-well plate and then incubated at 37°C with 5.00% CO_2 until the cell growth percentage reached 70.00%. Cells were treated with each variation of formulation and incubated at 37°C with 5.00% CO_2 gas for 48 hours. A number of 100 µL of each sample and positive control of the microtube into each 96-well plate that already contains cells. About 9 mL of media in a tube to which 1 mL of "Resazurin Sodium Salt-Powder, BioReagent" (10 µL reagent for 90 µL medium), then 100 µL the mixture solution into each well of the microplate and then incubate for 1-2 hours until a color change is visible. Resazurin Sodium Salt reagent in a living cell will be reduced from the resazurin blue compound without intrinsic fluorescent value, being a red and very fluorescent resorufin compound. The conversion value is proportional to the number of active metabolic cells and can be measured quantitatively. The absorbance was measured at a wavelength of 570 nm using a multimode reader (TECAN Infinite M200 Pro).

Statistical analysis
Statistical analyses were performed using GraphPad Prism 10. p-value of the data were analysed using paired Student's t-test with two-tailed distributions. A p-value < 0.05 was considered statistically significant.

Results and Discussion

Phytochemical screening for secondary metabolites
C. longa-A. paniculata Herbal Formula
Herb formula of *C. longa-A. paniculata* was synthesized with various compositions, and its antioxidant activity formula was measured. Overall results show FA has the best antioxidant activity compared to FB and FC. The IC_{50} values of each formula were 308.02 ± 16.17 µg/mL, 400.53 ± 49.21 µg/mL, and 1699.14 ± 152.19 µg/mL (Figure 1). However, during the nanoencapsulation process, FA and FC were observed to be unstable compared to FB. Therefore, FB was used for nanoencapsulation and anticancer activity testing, while *C. longa-A. paniculata* was formulated and its nano properties evaluated (FB and FB-NPs) containing 2.16 mg curcumin/g *C. longa* extract and 0.06 mg quercetin/g *A. paniculata* extract. The bioactive component level is used as the base of loading-release data formulation.

Characterization of nano C. longa-A. paniculata
C. longa and *A. paniculata* were formulated as nano herbs encapsulated with carboxymethyl chitosan. The characterization of FB-NPs consisted of particle size and polydispersity index (PDI) (Table 1). Particle size ensures that each sample is classified as a nanoparticle. Nanoparticles are colloid-sized particles with a maximum diameter of 500 nm.[29] The particle sizes of FB and FB-NPs are 571.90 ± 65.09 nm and 222.6 ± 0.87 nm, respectively. The polydispersity index (PDI) measures sample heterogeneity based on particle size caused by size distribution, aggregation, and agglomeration in the sample. The PDI of FB and FB-NPs are observed at 0.56 ± 0.10 and 0.24 ± 0.018, respectively (Table 1). These results indicate that the size distribution of FB and FB-NPs tends to be homogeneous. PDI values less than 0.05 (mono dispersion sample) indicate a narrow particle size distribution; hence, the particle size is uniform (homogeneous). Meanwhile, a PDI value of more than 0.7 indicates an extensive particle size distribution, making the particle size not uniform.[27]

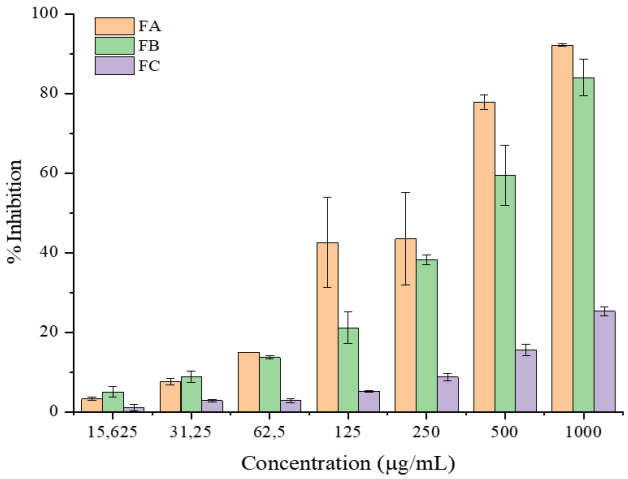
Table 1. Physicochemical Properties of FB-NPs

Parameters	FB	FB-NPs
Size ± SD (d.nm)	571.9 ± 65.09	222.6 ± 0.87
PDI ± SD	0.56 ± 0.10	0.24 ± 0.02

Note:
PDI : Polydispersity index
SD : Standard Deviation
FB : *C. longa-A. paniculate* formula
FB-NPs : Nano *C. longa-A. paniculata*

The characterization of FTIR spectra of FB-NPs showed an absorption at 3358 cm^{-1}, corresponding to the OH stretching group of the FB formula, which overlapped with the -NH stretching of the amine group in carboxymethyl chitosan. The absorption at 2931 cm^{-1} indicates the presence of the C-H sp^3 group from FB. The characteristics of the carboxymethyl chitosan groups were observed at wave numbers 1602 cm^{-1} (N-H deformation of the primary amine), 1511 cm^{-1} (asymmetric stretching vibration of COO^-), and 1030 cm^{-1} (C-O stretching in -CH_2-OH) (Figure 2). The surface morphology with SEM analysis for FB-NPs. The result of the SEM analysis with magnification 50 X shows that the particle size of FB-NPs is around 344.5 nm and 453.1 nm (Figure 3). The morphology of FB-NPs consists of granules with regular small nanocapsules.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



Formula	Composition (%)	
	C. longa	*A. paniculata*
FA	100	0
FB	75	25
FC	0	100

Figure 1: Antioxidant activity of *C. longa-A. paniculata* formulas

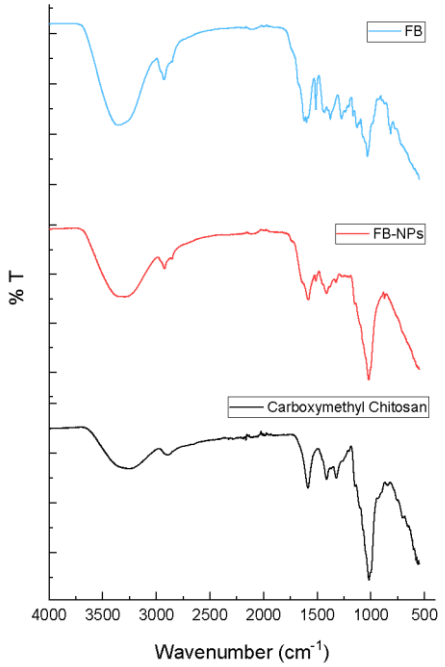


Figure 2: FTIR Spectrum of Carboxymethyl Chitosan, FB-NPs, and FB

Loading and release of nano C. longa-A. paniculate

The loading of FB-NP bioactive components is determined based on the loading efficiency (%LE) and loading amount (%LA). The loading efficiency of FB-NPs is 35.66 ± 6.08%, which shows the percentage of FB formula components trapped in the nanocapsule micelles. The loading amount of FB-NPs is 17.83 ± 3.04%, indicating the number of FB formula components trapped in the total nanocapsules. The release percentage of FB-NP bioactive components was tested in the human digestive system at pH 2, 7, and 8.5 for 8 hours (Figure 4). The release is expressed in % release curcumin equivalent. The release of FB-NPs at pH two is observed at 2.89 ± 0.92%, pH seven at 5.78 ± 1.21%, and pH 8.50 at 7.29 ± 0.95%. Poor release at pH two might be due to the aggregation of carboxymethyl chitosan polymers, resulting in bioactive components of FB-NPs being unable to be released. Meanwhile, the best release has been observed in pH 7 and 8.5, indicating that release at pH > 7 is the most optimum pH for release. This condition causes the solubility of carboxymethyl chitosan to increase, which triggers the release of the bioactive components in FB-NPs.

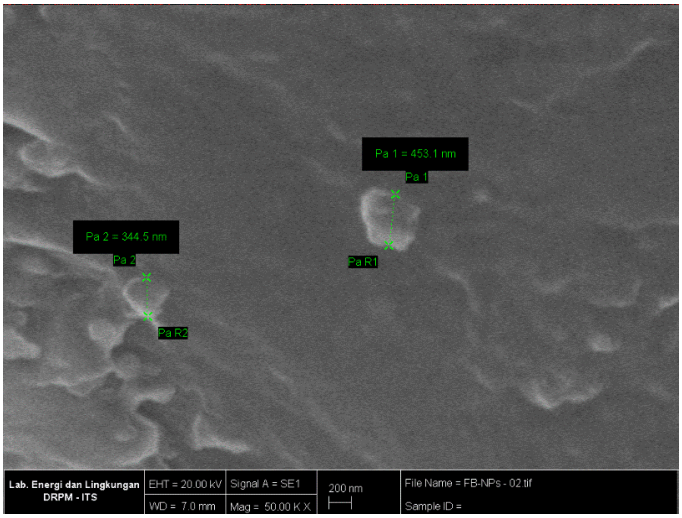


Figure 3: SEM analysis of FB-NPs magnification 50 x

pH Stability of nano C. longa-A. paniculata

Changes in pH affect the stability of the bioactive components in the FB formula and FB-NPs. Stability is observed based on changes in UV-Vis spectra and the turbidity level of the FB and FB-NPs formulas at pH 2-10. Generally, λmax FB at pH 6 (basic pH) is around 345 nm; a hypochromic shift occurs at pH conditions 7-10. Meanwhile, there was a hyperchromic and batochromic shift at pH 5 to 424 nm. In acidic conditions at pH 2-4, FB undergoes hypochromic and batochromic shifts (Figure 5a-b). It indicates that the bioactive component of FB is unstable due to changes in pH. The level of FB turbidity also shows that acidic pH tends to increase, with a decrease in turbidity at pH 2-3 due to the formation of coagulants at the bottom of the bottle. It is suspected that the bioactive components of FB undergo degradation or decomposition into species with lower solubility at acidic pH (Figure 5c). The stability of bioactive components FB-NPs tends to be stable at pH 2-5. While at pH 6-10, there is a hypochromic shift at λ_{max}. The turbidity level of FB-NPs affected by changes in pH shows that pH 2 has the highest turbidity level and decreases with a decrease in pH. The reduction in turbidity level is influenced by the solubility of carboxymethyl chitosan, where changes in pH do not significantly affect the level of tolerance of FB-NPs (Figure 5c). Below pH 5, free –COO− ions will form protonated –COOH. As a result, electrostatic repulsion between chains decreases, allowing the formation of hydrogen bonds, which further increases viscosity. However, deceleration of depolymerization occurs in an alkaline environment, decreasing viscosity.[30] This shows that FB-NPs are more able to maintain the stability of bioactive components than FB at pH changes.

Temperature Stability of nano C. longa-A. paniculata

The stability of FB and FB-NPs against temperature changes is observed at 30-100ºC. In general, temperature changes do not significantly affect the stability of FB bioactive components. In FB-NPs

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

heating at 40-70ºC, UV-Vis spectra tend to experience hyperchromic shifts, while at 80-100ºC, hypochromic shifts occur.

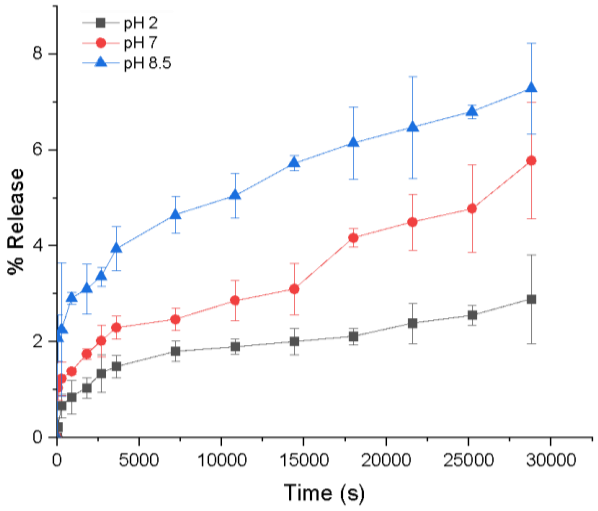


Figure 4: Release of bioactive components from FB-NPs

It is suspected that heating results in the decomposition of carboxymethyl chitosan, thus affecting the UV-Vis absorption band FB-NPs (Figure 6). Observation of the turbidity level on FB shows that the higher the temperature, the turbidity level decreases, but there is a coagulant (crust) at the bottom of the bottle. It suggests that heating results in the degradation of bioactive components in FB. Conversely, FB-NPs with temperature changes tend to be more stable from the constant turbidity. It aligns with several studies where herbal nanocapsule preparations are relatively stable to temperature changes compared to unencapsulated herbal formulas.[27,28]

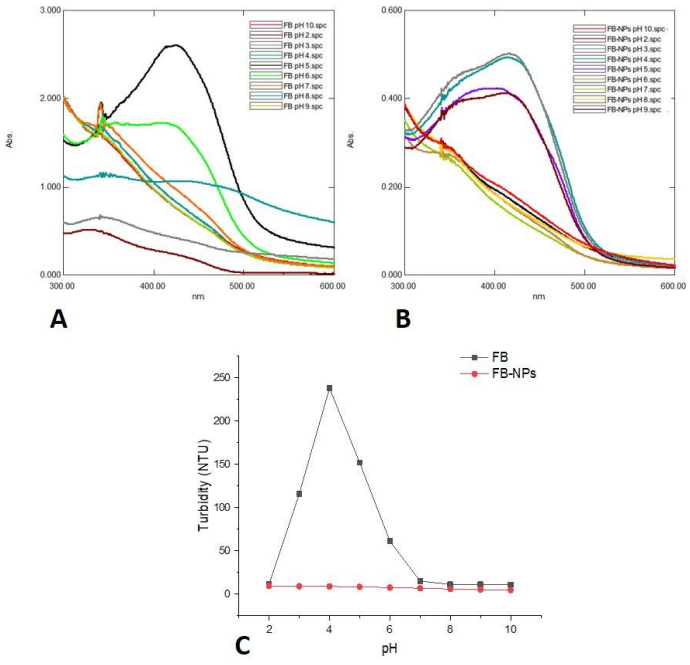


Figure 5: Effect of pH on UV-Vis spectral stability of (a) FB and (b) FB-NPs; (c) Effect of pH on turbidity level FB and FB-NPs

Salt Stability of nano C. longa-A. paniculata

Adding salt concentration (NaCl) to FB results in a significant hypochromic shift in the UV-Vis absorption band. In FB-NPs, adding salt concentration tends not to affect bad shifts absorption of UV-Vis (Figure 7). Adding 0.1 M NaCl increases the turbidity level of FB while adding NaCl causes the turbidity level to decrease (Figure 7). However, coagulants (crusts) were formed at the bottom of the bottle. It is

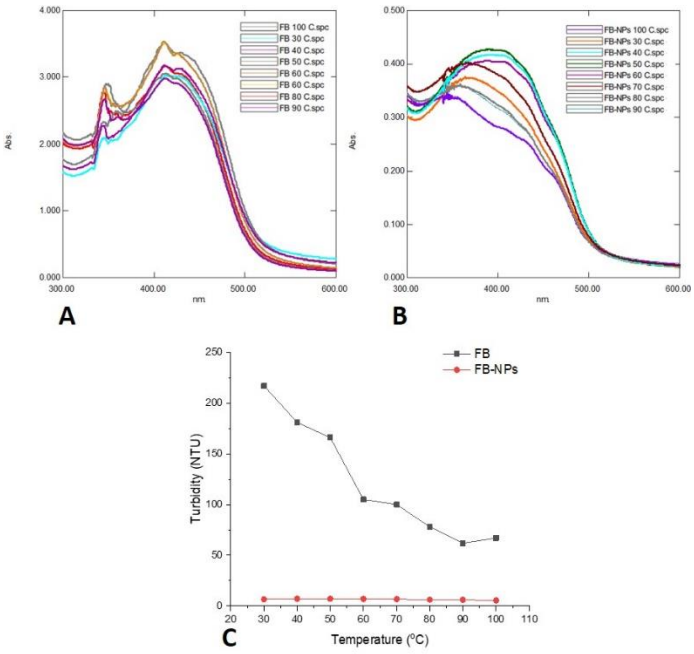


Figure 6: Effect of Temperature on UV-Vis spectral stability of (a) FB and (b) FB-NPs; (c) Effect of Temperature on turbidity level FB and FB-NPs

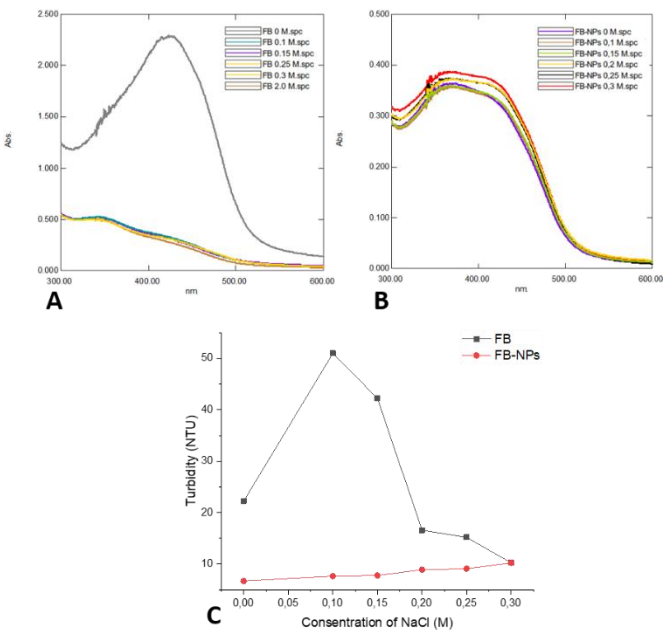


Figure 7: Effect of Salt concentration on UV-Vis spectral stability of (a) FB and (b) FB-NPs; (c) Effect of Salt concentration on turbidity level FB and FB-NPs

suspected to be caused by the salting-out effect, which results in ionic solubility that decreases the solubility in FB components.[31] Conversely, in FB-NPs, the addition of salt concentration is directly proportional to the increase in turbidity, although not significant. This suggests that FB

nanoencapsulation with carboxymethyl chitosan can retain the bioactive components of FB.

Toxicity of nano C. longa-A. paniculate

The anticancer activity of the herbal nano formula *C. longa-A. paniculata* (FB-NPs) was carried out using the prestoblue method against breast cancer cells (MCF7 cell line) (Figure 8). The test results showed that FB-NPs had a lower EC_{50} value compared to FB (free), respectively 13.71 ± 2.66 µg/mL and 18.43 ± 6.44 µg/mL. This lower dosage in nanoformulation indicates the efficiency of dosage induced by nano encapsulation. Nanoencapsulation improves drug stability, solubility, bioefficacy, bioavailability, and biodistribution. It can potentially deliver active molecules effectively targeted to specific organs.[32,33]. Lower dosage also gives benefits of decreasing the accumulation of the drugs during the treatment regimen while still achieving the desired effect.[33] This observation suggests encapsulation of the nanoherbal of *C. longa-A. paniculata* has better dosage efficacy than unencapsulated formula and has potential as an anticancer agent.

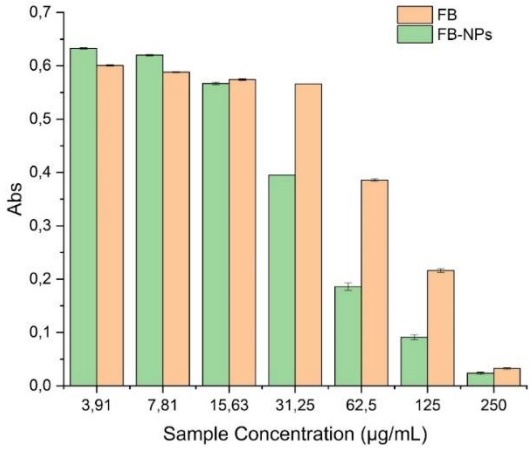


Figure 8: Anticancer activity of FB and FB-NPs on MCF-7 cell line

Conclusion

The combination of *Curcuma longa* and *Andrographis paniculata* was successfully nano-encapsulated using carboxymethyl chitosan as the shell material. The encapsulation efficiency and release profile were thoroughly evaluated, revealing that the nano-encapsulated formulation exhibited an optimal pH of 7 for the effective release of its bioactive compounds. Additionally, the encapsulated formulation demonstrated enhanced stability under conditions of high pH, elevated salt concentrations, and increased temperatures. Notably, the nano-encapsulated formulation showed significantly improved anticancer activity, achieving an EC_{50} value of 13.71 ± 2.66 µg/mL, in comparison to the non-encapsulated formulation. These findings underscore the potential of developing nano-based herbal therapies for cancer treatment in the future.

Conflict of interest

The author reports no conflicts of interest in this work.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was sponsored by the 2023 Non-APBN Funding of Universitas Negeri Surabaya under the Equity Collaboration scheme, contract number B/87631/UN38.11.1/LK.04.00/2023.

References

1. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, Ruddy KJ, Tsang J, Cardoso F. Breast Cancer. Nat Rev Dis Primers. 2019; 5(1): 66.
2. Smolarz B, Nowak AZ, Romanowicz H. Breast Cancer-Epidemiology, Classification, Pathogenesis and Treatment (review of literature). Cancers. 2022; 14(10): 2569.
3. Taylor C, McGale P, Probert J, Broggio J, Charman J, Darby SC, Kerr AJ, Whelan T, Cutter DJ, Mannu G, Dodwell D. Breast Cancer Mortality in 500 000 Women with Early Invasive Breast Cancer Diagnosed in England, 1993-2015: Population Based Observational Cohort Study. BMJ. 2023; 381: e074684
4. Britt KL, Cuzick J, Phillips K-A. Key Steps for Effective Breast Cancer Prevention. Nat Rev Cancer. 2020; 20(8): 417-436.
5. Hong R, Xu B. Breast Cancer: An Up-to-Date Review and Future Perspectives. Cancer Commun. 2022; 42(10): 913-936.
6. Lukasiewicz M, Kowalski S, Makarewicz M. Antimicrobial and Antioxidant Activity of Selected Polish Herbhoneys. *LWT-Food Sci Technol*. 2015; 64(2): 547-553.
7. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, Falco VD, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J. Lastra JMP. Cancer Chemotherapy and Beyond: Current Status, Drug Candidates, Associated Risks and Progress in Targeted Therapeutics. Genes & Diseases. 2023; 10(4): 1367-1401.
8. Samuel SM, Kubatka P, Büsselberg D. Treating Cancers using Nature's Medicine: Significance and Challenges. Biomolecules; 2021; 11(11): 1698
9. Naeem A, Hu P, Yang M, Zhang J, Liu Y, Zhu W, Zheng Q. Natural Products as Anticancer Agents: Current Status and Future Perspectives. Molecules. 2022; 27(23): 8367.
10. Sutaria D, Grandhi BK, Thakkar A, Wang J, Prabhu S. Chemoprevention of Pancreatic Cancer using Solid-Lipid Nanoparticulate Delivery of A Novel Aspirin, Curcumin and Sulforaphane Drug Combination Regimen. Int J Oncol. 2012; 41(6): 2260-2268.
11. Wardana AP, Aminah NS, Kristanti AN, Fahmi MZ, Abdjan MI, Sucipto TH. Antioxidant, Anti-inflammatory, Antiviral and Anticancer Potentials of Zingiberaceae Species Used as Herbal Medicine in Indonesia. Trop J Nat Prod Res. 2024; 8(9)
12. Wang K, Zhang C, Bao J, Jia X, Liang Y, Wang X, Chen M, Su H, Li P, Wan JB, He C. Synergistic Chemopreventive Effects of Curcumin and Berberine on Human Breast Cancer Cells Through Induction of Apoptosis and Autophagic Cell Death. Sci Rep. 2016; 6(1): 26064.
13. Qiao E-Q, Yang H-J, Yu X-F, Gong L-J, Zhang X-P, Chen D-B. *Curcuma zedoaria* Petroleum Ether Extract Reverses The Resistance of Triple-Negative Breast Cancer to Docetaxel Via Pregnane X Receptor. Annals Transl Med. 2021; 9(17)
14. Hu S, Xu Y, Meng L, Huang L, Sun H. Curcumin Inhibits Proliferation and Promotes Apoptosis of Breast Cancer Cells. Exp Ther Med. 2018; 16(2): 1266-1272.
15. Rajanna M, Bharathi B, Shivakumar B, Deepak M, Prashanth DS, Prabakaran D, Vijayabhaskar T, Arun B. Immunomodulatory Effects of *Andrographis paniculata* Extract in Healthy Adults–An Open-Label Study. J Ayurveda Integr Med. 2021; 12(3): 529-534.
16. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P. Harnessing The Medicinal Properties of *Andrographis paniculata* for Diseases and Beyond: A Review

of Its Phytochemistry and Pharmacology. Asian Pac J Trop Dis. 2014; 4(3): 213-222.
17. Harikrishnan A, Veena V, Kancharla R, Chavan S, Ramalingam RJ, Al-Lohedan HA, Pandiaraj S, Karrupiah P, Selvaraj A. Anti-Breast Cancer Activity of Bioactive Metabolites from *Andrographis paniculata* Through Inhibition of PI3K Activity in Triple Negative Breast Cancer (MDA-MB-231) Cells. J Mol Struct. 2023; 1294: 136506.
18. Swetha M, Keerthana C, Rayginia TP, Anto RJ. Cancer Chemoprevention: A Strategic Approach using Phytochemicals. Front Pharmacol. 2022; 12: 809308.
19. Seshadri VD, Oyouni AAA, Hawsawi YM, Aljohani SA, Al-Amer O, AlZamzami W, Mufti AH. Chemopreventive Role of Tin Oxide-Chitosan-Polyethylene Glycol-Crocin Nanocomposites Against Lung Cancer: An In Vitro and In Vivo Approach. Process Biochem. 2022; 120: 186-194.
20. Shirode AB, Bharali DJ, Nallanthighal S, Coon JK, Mousa SA, Reliene R. Nanoencapsulation of Pomegranate Bioactive Compounds for Breast Cancer Chemoprevention. Inter J Nanomed. 2015; 10: 475-484.
21. Wardana AP, Aminah NS, Fahmi MZ, Kristanti AN, Zahrah HI, Takaya Y, Choudhary MI. Nanoencapsulation of *Syzygium polycephalum* Extract Using Folate Modified κ-Carrageenan as Vehicles for Pronounced Anticancer Activity. Trop J Nat Prod Res. 2020; 4(11): 945-952
22. Shariatinia Z. Carboxymethyl Chitosan: Properties and Biomedical Applications. Inter J Biol Macromol. 2018; 120: 1406-1419.
23. Gopi S, Thomas S, Pius A. Handbook of Chitin and Chitosan. Elsevier; 2020.
24. Inamuddin A, Mohammad A. *Applications of nanocomposite materials in drug delivery*. Elsevier; 2018.
25. Siahaan P, Mentari NC, Wiedyanto UO, Hudiyanti D, Hildayani SZ, Laksitorini MD. The Optimum Conditions of Carboxymethyl Chitosan Synthesis on Drug Delivery Application and Its Release of Kinetics Study. Indones J Chem. 2017; 17(2): 291-300.
26. Tukiran T, Wardana AP, Hidayati N, Shimizu K. An Ellagic Acid Derivative and Its Antioxidant Activity of Stem Bark Extracts of *Syzygium polycephalum* Miq.(Myrtaceae). Indones J Chem. 2018; 18(1): 26-34.
27. Wardana AP, Aminah NS, Kristanti AN, Fahmi MZ, Zahrah HI, Widiastuti W, Ajiz HA, Zubaidah U, Wiratama PA, Takaya Y. Nano *Uncaria gambir* as Chemopreventive Agent Against Breast Cancer. Inter J Nanomed. 2023: 4471-4484.
28. Wardana AP, Aminah NS, Kristanti NA, Manuhara YSW, Fahmi MZ, Sucipto TH, Abdjan MI, Indriani. *Gynura procumbens* Nanoencapsulation: A Novel Promising Approach to Combat Dengue Infection. Rasayan J Chem. 2023; 16(2)
29. Perera G, Zipser M, Bonengel S, Salvenmoser W, Bernkop-Schnürch A. Development of Phosphorylated Nanoparticles as Zeta Potential Inverting Systems. European J Pharm Biopharm. 2015; 97: 250-256
30. Qin Y. Seaweed Hydrocolloids as Thickening, Gelling, and Emulsifying Agents in Functional Food Products. Bioactive Seaweeds for Food Applications. Elsevier; 2018: 135-152.
31. Noubigh A, Mgaidi A, Abderrabba M, Provost E, Fürst W. Effect of Salts on The Solubility of Phenolic Compounds: Experimental Measurements and Modelling. J Sci Food Agric. 2007; 87(5): 783-788.
32. Bhandari M, Nguyen S, Yazdani M, Utheim TP, Hagesaether E. The Therapeutic Benefits of Nanoencapsulation in Drug Delivery to The Anterior Segment of The Eye: A Systematic Review. Front Pharmacol. 2022; 13: 903519.
33. Ngwuluka NC, Abu-Thabit NY, Uwaezuoke OJ, Erebor J, Ilomuanya M, Mohamed RR, Soliman M, Elella MA, Elella NAA, Ebrahim. Natural Polymers in Micro-and Nanoencapsulation for Therapeutic and Diagnostic Applications: Part I: Lipids and Fabrication Techniques. Nano Microencapsul Tech Appl. 2021: 3-54.