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ENCAPSULATION OF *Laurocerasus officinalis* AND ITS ANTIPROLIFERATIVE EFFECT ON COLORECTAL CANCER CELL LINE

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Aim. *Laurocerasus officinalis*, also known as cherry laurel, is frequently used in cancer treatment due to its high antioxidant capacity. Encapsulation technology aims to protect phenolic compounds from external factors, such as heat, light, and moisture, while preserving their bioactive properties. This study aimed to enhance the bioavailability of *Laurocerasus officinalis* fruit extract through starch-based encapsulation and to evaluate its cytotoxic, antiproliferative, and anti-migratory effects on the HCT-116 colorectal cancer cell line.

Methods. *Laurocerasus officinalis* was encapsulated and characterized by FT-IR and SEM analyses. Cytotoxicity of cherry laurel extract encapsulation in HCT-116 cells was assessed using CVDK-8, cell migration by wound healing assay, and cell death by acridine orange/ethidium bromide staining.

Results. The TPC value of cherry laurel fruit was determined as 13407.05 mg GAE/kg, while the TPC value of the starch-encapsulated extract was obtained as 1216.67 mg GAE/kg. On the other hand, encapsulation of cherry laurel fruit extract demonstrated an antiproliferative effect, reducing HCT-116 cell viability in a dose- and time-dependent manner. Further analyses supported the finding that encapsulated cherry laurel extract reduced cell migration and increased apoptotic cell death.

Conclusion. Overall, these findings suggest that the encapsulated Laurocerasus officinalis extract exhibits promising antiproliferative potential against HCT-116 cells; however, additional *in vitro* and *in vivo* studies are required to elucidate its mechanisms of action further and confirm its therapeutic efficacy.

Keywords: Cherry Laurel, Colorectal Cancer, Encapsulation, Cell Migration, Apoptosis.

Colorectal cancer (CRC) accounts for approximately 10% of all diagnosed tumors worldwide and remains a leading cause of cancer-related mortality [1]. Current treatment strategies include surgery, radiotherapy, chemotherapy, and immunotherapy, with agents such as 5-FU, topoisomerase I inhibitors, monoclonal antibodies, and immune-based therapies showing clinical benefit [2]. However, the development of chemoresistance and severe side effects limits the effectiveness of these approaches, highlighting the need for more selective and effective therapeutic strategies [3].

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Encapsulation technologies have gained attention in cancer therapy due to their ability to enhance stability, control release, and reduce toxicity by protecting bioactive compounds from environmental factors such as heat, light, and moisture [4, 5]. Plant extracts contain bioactive compounds with anticancer and antioxidant properties, but their instability and poor bioavailability restrict clinical application [6–8]. Encapsulation may overcome these limitations by improving targeted delivery and therapeutic efficacy in colorectal cancer [7]. Moreover, natural bioactive compounds such as curcumin, berberine, and resveratrol exhibit strong antitumor activity but are limited by low solubility and rapid systemic clearance [9]. Therefore, encapsulation-based delivery systems represent a promising strategy.

Laurocerasus officinalis (cherry laurel), a member of the Rosaceae family, is rich in phenolic acids, anthocyanins, and antioxidant compounds [10–15]. Previous studies have demonstrated its selective cytotoxic and apoptosis-inducing effects in various cancer cell lines, including gastric cancer cells [16, 15].

In this study, we aimed to enhance the bioavailability and cytotoxic efficacy of L. officinalis fruit extract in colorectal cancer cells through starch-based encapsulation and to compare the therapeutic effects of free and encapsulated extracts in HCT-116 cells.

Materials and Methods

Preparation of Laurocerasus officinalis Extract

Laurocerasus officinalis fruit was crushed after removing the seeds. The fruit (100 g) was dissolved in 1000 mL of ethanol in an ultrasonic water bath. A 1% formic acid solution was used for the acid extraction. Filtration was performed using a Whatman filter paper no. 1. The ethanol remaining in the filtrate was removed at 50–60 °C using a rotary evaporator [17].

Total Phenolic Content

The total phenolic content (TPC) of the extract and the starch-encapsulated extract was determined using the Folin-Ciocalteau method, as modified from Maurya and Singh (2010). After diluting the extract to the required concentration, 0.5 ml of the diluted sample was placed in a test tube, and 2.5 mL of Folin-Ciocalteau reagent (10%, w/w, in water) and 2 mL of sodium carbonate solution (7.5%, w/w, in water) were added sequentially. The test tube was left in the dark at room temperature for 60 min. At the end of the time, the absorbance was measured at 760 nm using a spectrophotometer. The TPC was calculated using a calibration curve prepared using a gallic acid standard, and the results were expressed as mg gallic acid equivalent/g (mg GAE/g) [18].

Encapsulation of Laurocerasus officinalis Extract

Starch was used as the coating material for cherry laurel extracts. The encapsulation of the coated material was performed using a freeze dryer. Starch was mixed with distilled water at a 10% weight ratio. The extract was mixed with a starch solution at a 1:30 (w/w) ratio. The mixtures were homogenized at 4,000 rpm for 5 min, then exposed to an ultrasonicator for 20 min. The resulting mixtures were frozen at −18 °C and then dried in a lyophilizer at −55 °C and 50 Pa vacuum for 24 h. The powder mixtures were stored at 18 °C until use [19].

Characterization

The structural and morphological characteristics of the encapsulated cherry laurel extract were comprehensively evaluated using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). FTIR analysis was performed using a PerkinElmer Spectrum 100 spectrometer to identify functional groups and possible interactions between the cherry laurel extract and the starch-encapsulating matrix. Lyophilized samples were scanned over the spectral range of 4000–400 cm^{-1}, and each spectrum was averaged from 24 consecutive scans to enhance signal accuracy and reproducibility.

The surface morphology and particle structure of the encapsulated extract were examined using SEM (JEOL JSM-6510). Before imaging, the samples were mounted on aluminum stubs using double-sided adhesive carbon tape and sputter-coated with a thin layer of gold to improve electrical conductivity. Micrographs were recorded at different magnifications under high vacuum conditions to evaluate particle shape, surface texture, and the distribution of the encapsulated material within the starch matrix [20, 21].

Cell Line and Culture Conditions

The human colorectal cancer cell line (HCT-116) was provided by the Bingöl University Cancer Research Group (BUKAG). HCT-116 cells were cultured in DMEM supplemented with

10% FBS (Paisley, Scotland, United Kingdom) and 1% penicillin/streptomycin (v/v) (St. Louis, MO, USA). The cells were incubated in a sterile incubator containing 5% CO_2 at 37 °C.

Cell Viability (CVDK-8)

Cell viability was analyzed using a colorimetric assay (CVDK-8) according to a standard method. Briefly, 3×10^3 cells were seeded per well into 96-well plates, and treatments were applied for 24–48 hours. At the end of the treatment period, 20 µL of 5 mg/mL CVDK-8 was added to the plates, which were then incubated at 37 °C for 4 hours. Absorbance was read at 450 nm with a microplate reader (Bio-RadBenchmark, Hercules, CA, USA) [22].

Wound Healing-Scratch Assay

Cells (1×10^5) were seeded into 12-well plates and grown to a specific density. The cell layer was wounded using the tip of a sterile 200 µL pipette (width: ~1 mm). Cell debris was removed by washing twice with phosphate-buffered saline (PBS). Cells were treated and then allowed to migrate to bare areas. Images were taken under a microscope at 24 and 48 hour intervals. Wound closure was calculated using ImageJ software (Version 1.50) [23].

Acridine Orange and Ethidium Bromide Double Staining

Fluorescence-based determination of apoptosis in HCT-116 cells was performed using the acridine orange/ethidium bromide (AO/EtBr) staining method as described by Baskic et al. Colorectal cancer cells were incubated with encapsulated cherry laurel extract (10, 20, and 50 µg/mL) for 48 hours, after which cells were collected, washed with PBS, and stained with AO/EtBr (100 µL PBS, 10 µg/mL AO, and 10 µg/mL PI). The stained cells were photographed under a fluorescence microscope and compared to the control. The nuclei of viable cells stained green only because acridine orange is permeable, while apoptotic cells appeared red/orange due to combined staining with both fluorescent dyes. Using at least 20 cells per sample, the percentages of live, early apoptotic, late apoptotic, and necrotic cells were calculated in ImageJ (Version 1.50i). Results are expressed as the mean ± standard deviation of three independent experiments [24].

Results and Discussion

Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu's method. Table 1 shows the TPC values for the plain and starch-encapsulated cherry laurel extracts. Based on the TPC results, the plain cherry laurel extract exhibited a markedly higher total phenolic content (13407.05 mg GAE/kg) compared to the starch-encapsulated extract (1216.67 mg GAE/kg), indicating that the encapsulation process led to a substantial reduction in measurable phenolic compounds. However, it may improve stability and controlled release of bioactive constituents.

SEM and FT-IR Analyses

The SEM analysis revealed that the starch-encapsulated cherry laurel extract exhibited irregularly shaped, compact, and aggregated particle structures at 500× magnification. The particles appeared to be embedded within a continuous starch matrix, suggesting effective coating and encapsulation of the extract (Fig. 1, *A*). The FT-IR spectra of the starch-encapsulated cherry laurel extract exhibited a broad band at ~3287 cm^{-1} corresponding to O–H stretching vibrations, indicating the presence of hydroxyl groups from both phenolic compounds and starch. The peaks observed at ~2932 and 2830 cm^{-1} were attributed to C–H stretching vibrations of aliphatic chains. Additionally, characteristic polysaccharide bands at ~1149 and 1076 cm^{-1} confirmed the presence of the starch matrix. These findings demonstrate successful encapsulation and suggest possible hydrogen-bond interactions between the bioactive compounds and the starch carrier (Fig. 1, *B*).

Encapsulated cherry laurel extract reduced cell viability in HCT-116

In this set of experiments of our study, the HCT-116 cell line was exposed to increasing concentrations of encapsulated cherry laurel extract (0, 10, 25, 50, 100, 250, 500, 1000 µg/mL) for 24 and 48 hours. The CVDK-8 cell viability assay results shown in Figure 2, *A* and 2, *B* revealed a dose-dependent decrease in cell viability in the HCT-116 cell line at both time points. This cytotoxic effect increased at concentrations of 50 µg/mL and above. The IC50 values for encapsulated cherry laurel extract in the HCT-116 cell line were calculated as 79.74 µg/mL and 56.82 µg/mL at 24 and 48 hours, respectively. In addition, dose-dependent changes in morphological shapes and cell densities were observed in the HCT-116 cell line after the application of encapsulated cherry laurel extract (Fig. 2, *C*).

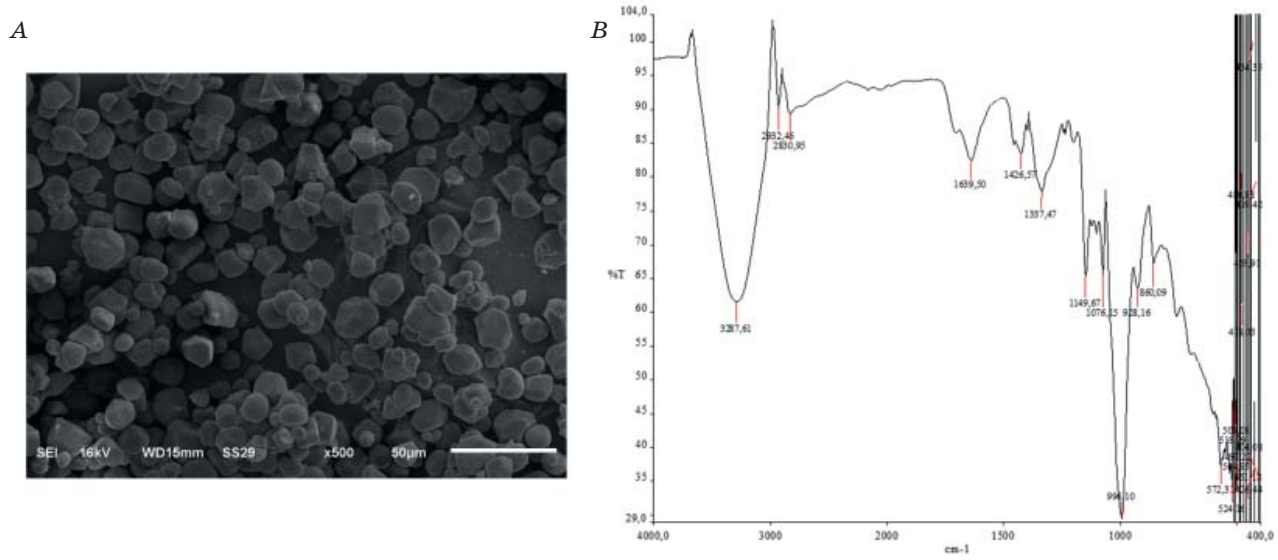

Fig. 1. **SEM and FT-IR images of encapsulated cherry laurel extract**

Encapsulated cherry laurel extract Inhibits In Vitro Cell Migration in HCT-116

To determine the effects of encapsulated cherry laurel extract on cell migration, a wound healing assay was performed in HCT-116 cell lines (Fig. 3, *A*). Encapsulated cherry laurel extract was applied to HCT-116 cells at the indicated concentrations (10, 25, and 50 µg/mL) for 48 hours. As shown in Fig. 3, *B* and the histograms reporting the percentage wound closure values, application of 25 and 50 µg/mL of encapsulated cherry laurel extract resulted in a significant inhibition of migration. At the same time. the control group exhibited significant cell migration to close the scratch.

Encapsulated-cherry laurel extract Induced Apoptosis of HCT-116 Cells

Encapsulated cherry laurel extract at 10, 25, and 50 µg/mL levels resulted in a consistent increase in orange and red staining and a decrease in green staining in nuclei (Fig. 4, *A*), indicating cell damage and apoptosis. The apoptotic rate was determined by analyzing the fluorescence intensities of red, orange, and green cells (Fig. 4, *B*). After 48 hours of treatment with encapsulated cherry laurel extract, the 50 µg/mL treatment group showed a significant increase in apoptotic cell death in the HCT-116 cell line compared to the control group.

Discussion

Colorectal cancer remains a major health problem due to its high mortality rate and the development of chemoresistance, highlighting the need for novel and complementary therapeutic strategies [25]. Phytochemicals such as polyphenols, flavonoids, and anthocyanins in cherry laurel extract have attracted attention for their potential anticancer activity. However, despite promising *in vitro* findings, natural compounds often suffer from low water solubility, chemical instability, and limited cellular uptake [26]. Encapsulation technologies, particularly nanocarrier systems, have been shown to enhance bioavailability and facilitate intracellular delivery of such compounds [27], while also improving therapeutic efficacy and reducing side effects in colorectal cancer treatment [28]. Similarly, anthocyanin-containing microcapsules from blueberry fruit significantly reduced colorectal cancer cell viability and migration [29].

In our study, the TPC value decreased after encapsulation (13407.05 vs. 1216.67 mg GAE/kg), likely due to the masking of phenolic compounds by the starch matrix. Comparable findings were reported in cagaita fruit, where encapsulated samples showed lower TPC values than pure extracts [30]. SEM analysis demonstrated angular, amorphous particles with smooth, non-porous surfaces and slight agglomeration, indicating successful encapsulation. Similar glassy and irregular morphologies have been observed in freeze-dried, anthocyanin-rich encapsulated systems [31]. FT-IR spectra reveal characteristic bands corresponding to hydroxyl (–OH), aliphatic C–H, and polysaccharide-related functional groups, suggesting that cherry laurel extract can be successfully incorporated into the starch matrix. The presence of a broad O–H stretching

band and glycosidic C–O–C vibrations suggests hydrogen-bond formation between phenolic compounds and starch, indicating that encapsulation stabilizes bioactive components within the carrier system while maintaining their structural integrity [32, 33]. In the study by Ivana and colleagues, the biochemical characterization of bay leaf and fruit extracts was determined. The quality of the extract was indicated by its total phenolic content. The results showed that the TPC and TFC values changed significantly ($P < 0.005$) and that cherry bay leaves were richer in methanol-soluble phenolic compounds compared to the fruit. Many studies have confirmed that leaf extracts contain higher concentrations of phenolic compounds compared to other parts of the same plant [37].

On the other hand, Yiğit and his team investigated the antifungal activity and optimization of silver nanoparticles synthesized from cherry laurel leaf extraction. FT-IR spectroscopy was used to characterize the molecules and functional groups in the newly synthesized AgNPs. At the same time, SEM characterization

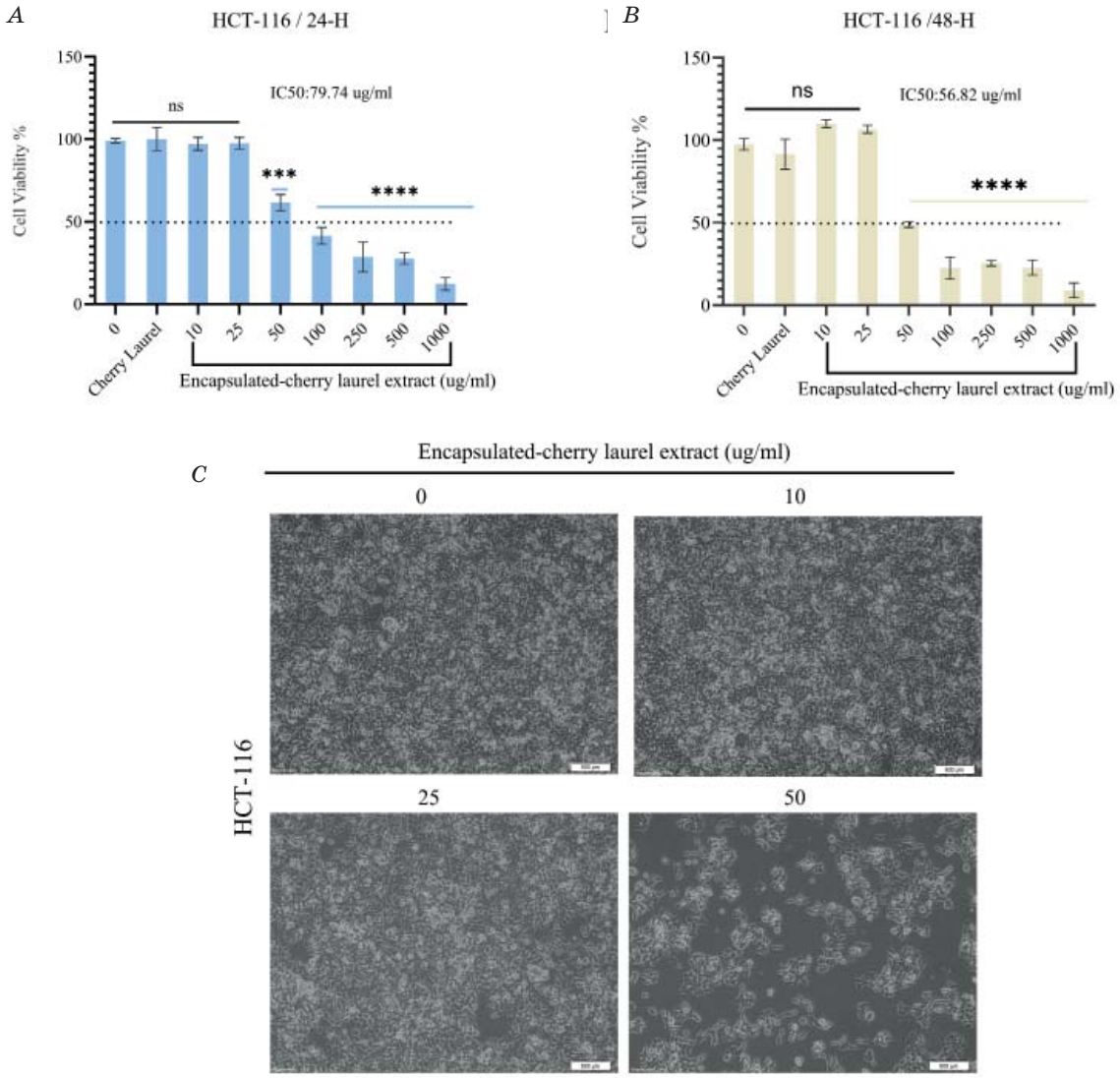

Fig. 2. **Effects of encapsulated cherry laurel extract on the viability of the HCT-116 colorectal cancer cell line**

HCT-116 cells were treated with increasing concentrations of encapsulated cherry laurel extract (0, 10, 25, 50, 100, 250, 500, and 1000 μg/mL) for 24 and 48 h (*A, B*). Cell viability was evaluated using the CVDK-8 assay, demonstrating a dose-dependent reduction following treatment. Morphological alterations in HCT-116 cells after 48 h exposure were observed using an Olympus CKX41 inverted phase-contrast fluorescence microscope at 10× magnification (*C*). Data are presented as mean ± SD ($n = 3$ per group). Statistical significance was determined by one-way ANOVA (***$P < 0.001$; **** $P < 0.0001$).

was performed for the size, shape, and morphology of the synthesized AgNPs. The results showed that the AgNPs synthesized from laurel leaves were highly effective and exhibited antifungal activity against many fungal pathogens [38].

Biologically, encapsulated cherry laurel extract significantly reduced HCT-116 cell viability in a dose- and time-dependent manner, consistent with previous reports of starch-based nanocarriers loaded with plant extracts suppressing colon cancer cell proliferation [34]. The extract also inhibited cell migration, particularly at 25 and 50 µg/mL, paralleling findings that starch nanoparticle-based systems can suppress migration in colon cancer cells [35]. Furthermore, treatment with 50 µg/mL induced apoptotic cell death, supporting previous evidence that encapsulated natural compounds can promote apoptosis in cancer cell lines [36].

Overall, our findings indicate that starch-based encapsulation effectively stabilizes cherry laurel extract and enhances its antiproliferative, anti-migratory, and

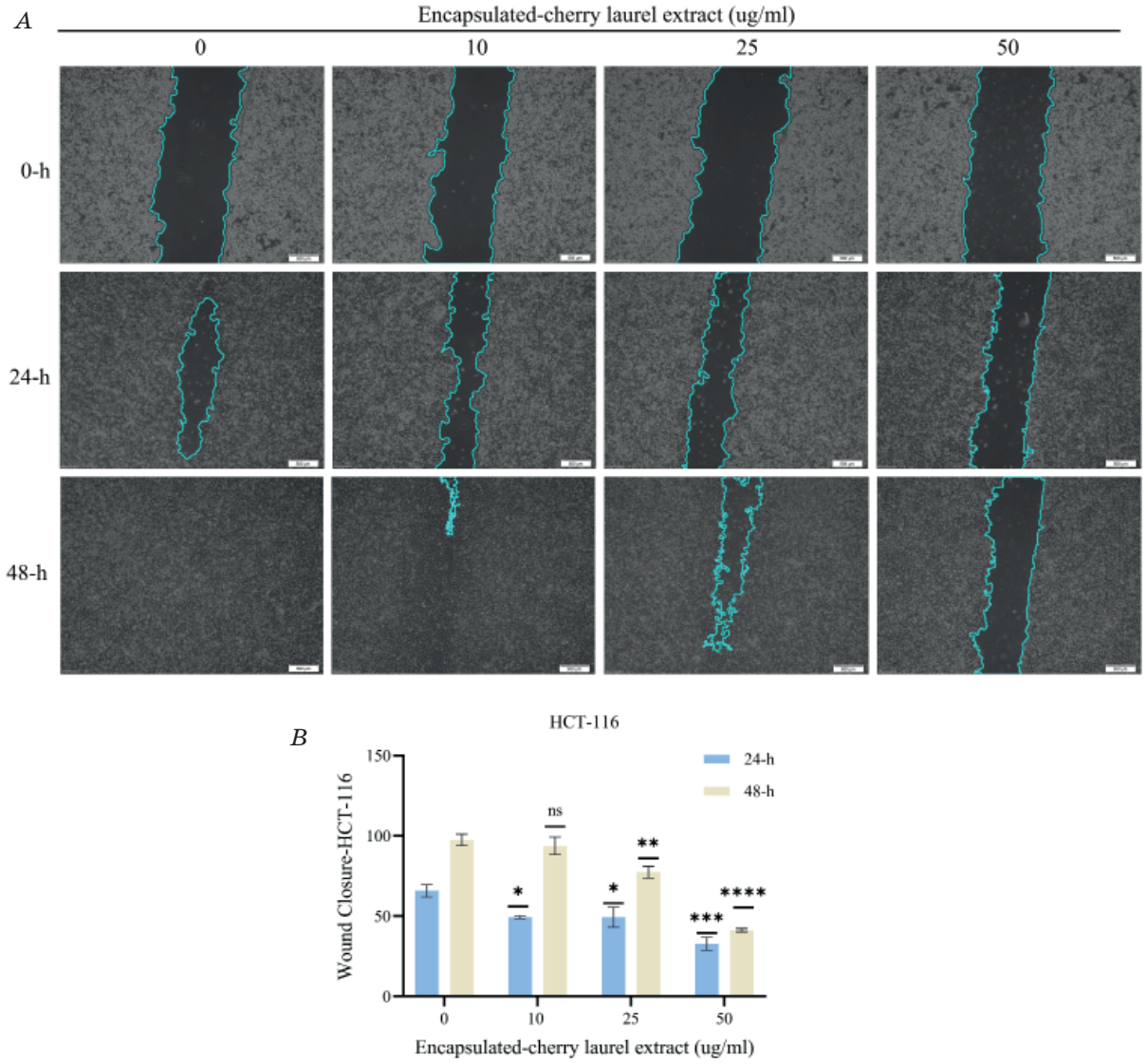

Fig. 3. **Inhibitory effect of encapsulated cherry laurel extract on the migratory capacity of HCT-116 cells (*A*) Cell migration was evaluated using a wound-healing assay at 0, 24, and 48 h after treatment with different concentrations of encapsulated cherry laurel extract. A linear scratch was created across the center of the cell monolayer, and wound closure was monitored microscopically over 48 h to assess migration (original magnification 4×). (*B*) Representative images (scale bar: 500 µm) illustrate wound closure at 0 h (blue bars) and 24 h and 48 h after treatment (yellow bars).**
Data are presented as mean ± SD ($n = 3$ per group). Statistical analysis was performed using one-way ANOVA (*$P < 0.05$; **$P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant).

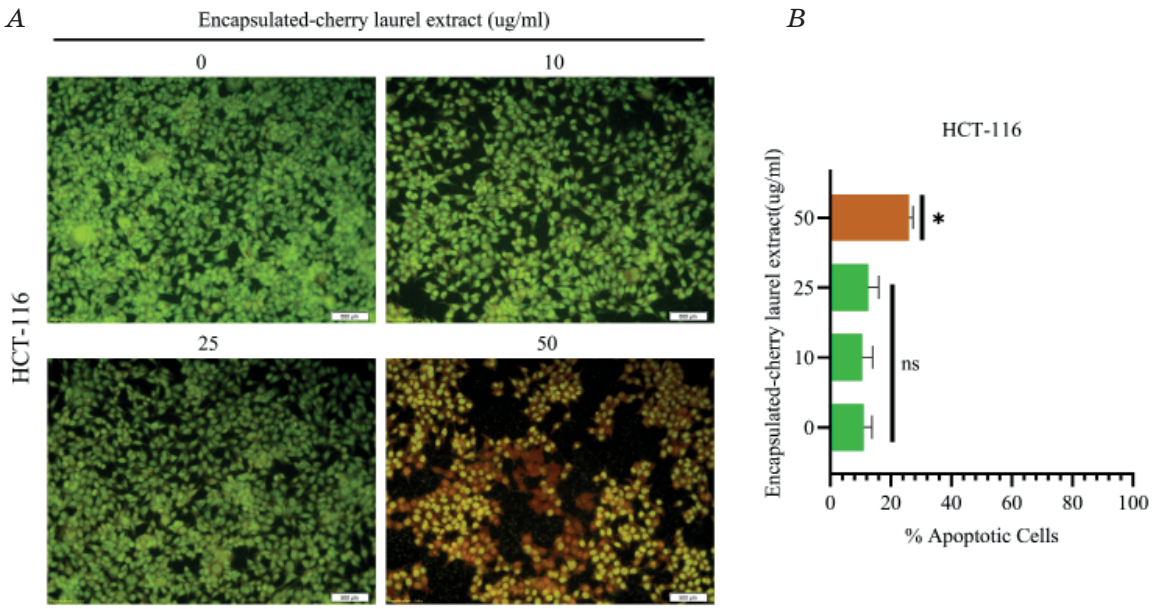

Fig. 4. **Effects of Encapsulated cherry laurel extract on cell death in HCT-116 cells (*A*) Apoptosis analysis by acridine or ange/ethidium bromide (AO/EB) staining. In cells treated with Encapsulated cherry laurel extract 10 and 20 µg/mL, no apparent color change was observed, indicating limited apoptotic activity. Treatment with Encapsulated cherry laurel extract 50 µg/mL further enhanced apoptosis. (*B*) Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test (GraphPad Prism 10.0, GraphPad Software Inc.)** (* $P < 0.05$; *ns* = not significant).

pro-apoptotic effects in HCT-116 cells. Nevertheless, further mechanistic and in vivo studies are required to clarify the molecular pathways involved.

Limitations of the Study

While our findings demonstrate that starch-encapsulated Laurocerasus officinalis extract exerts antiproliferative, anti-migratory, and pro-apoptotic effects in HCT-116 colorectal cancer cells, several limitations must be acknowledged. First, the study was conducted exclusively in a single cancer cell line. Reliance on one model introduces the risk of cell line-specific artifacts, including mutations acquired during long-term culture or laboratory-specific adaptations, which may not reflect the broader biology of colorectal cancer. Second, although the extract reduced viability and induced apoptosis in HCT-116 cells, we did not assess its effects on normal colon epithelial cells or primary cultures of the same tissue origin. Without such comparisons, the therapeutic window (the balance between anticancer efficacy and potential toxicity to healthy cells) remains undefined. This is a critical consideration, as many compounds can indiscriminately kill both malignant and normal cells. Therefore, the selectivity of encapsulated cherry laurel extract must be rigorously evaluated in future studies using pseudonormal cell lines (e.g., CCD 841 CoN) or primary colon epithelial cells. Finally, our work is limited to in vitro assays. Thus, in vivo validation will be essential to determine pharmacokinetics, bioavailability, and systemic safety. These limitations highlight that our current results should be regarded as preliminary, and further mechanistic and translational studies are required before therapeutic applications can be considered.

Conclusion

The data from this study show that cherry laurel extract was successfully encapsulated. The study demonstrates that treating colorectal cancer cells (HCT-116) with encapsulated cherry laurel extract reduces cell viability in a dose- and time-dependent manner. It also shows that encapsulated cherry laurel extract reduces the migratory ability of HCT-116 cells, leading to apoptotic cell death. Although effective results were obtained with encapsulated cherry laurel extract in HCT-116 cells, further studies are needed to determine its efficiency and understand the underlying mechanisms. Furthermore, it is recommended that in vivo studies be conducted across

different colorectal cancer cell lines and with different drug combinations. It is predicted that more effective and reliable results can be obtained through these approaches.

Author Contributions

Deniz Ozdemir, Esma Nur Bulut, Seher Saruhan, Can Ali Ağca — data supply and analysis, carrying out some observations and investigations, manuscript article writing, editing, translation, and paper preparation, provided the new literature data for review. All authors contributed to the manuscript's revision and read and approved the submitted version.

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Conflict of Interest

The authors declare no conflict of interests.

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ІНКАПСУЛЯЦІЯ ЛАВРОВИШНІ ЛІКУВАЛЬНОЇ ТА ЇЇ АНТИПРОЛІФЕРАТИВНИЙ ВПЛИВ НА КЛІТИННУ ЛІНІЮ КОЛОРЕКТАЛЬНОГО РАКУ

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Мета. *Laurocerasus officinalis*, також відомий як лавр вишневий, часто використовується в лікуванні раку завдяки своїй високій антиоксидантній здатності. Технологія інкапсуляції спрямована на захист фенольних сполук від зовнішніх факторів, таких як тепло, світло та волога, зберігаючи при цьому їхні біоактивні властивості. Метою цього дослідження було підвищення біодоступності екстракту плодів *Laurocerasus officinalis* шляхом інкапсуляції на основі крохмалю та оцінка його цитотоксичної, антипроліферативної та антиміграційної дії на клітинну лінію колоректального раку HCT-116.

Методи. *Laurocerasus officinalis* був інкапсульований та охарактеризований за допомогою FT-IR та SEM аналізів. Цитотоксичність інкапсульованого екстракту лаврового соку в клітинах HCT-116 оцінювали за допомогою CVDK-8, міграцію клітин за допомогою аналізу загоєння ран та загибель клітин за допомогою фарбування акридиновим оранжевим/бромідом етидію.

Результати. Значення TPC плодів лаврового соку було визначено як 13407,05 мг GAE/кг, тоді як значення TPC екстракту, інкапсульованого крохмалем, було отримано як 1216,67 мг GAE/кг. З іншого боку, інкапсуляція екстракту плодів лаврового соку продемонструвала антипроліферативний ефект, знижуючи життєздатність клітин HCT-116 залежно від дози та часу. Подальші аналізи підтвердили висновок про те, що інкапсульований екстракт лаврового соку зменшує міграцію клітин та збільшує апоптотичну загибель клітин.

Висновок. Загалом, ці результати свідчать про те, що інкапсульований екстракт *Laurocerasus officinalis* проявляє багатообіцяючий антипроліферативний потенціал проти клітин HCT-116; однак, необхідні додаткові дослідження *in vitro* та *in vivo*, щоб далі з'ясувати механізми його дії та підтвердити його терапевтичну ефективність.

Keywords: Вишневий лавр, колоректальний рак, інкапсуляція, міграція клітин, апоптоз.

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