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ENCAPSULATİON OF Curcuma Longa L. EXTRACT: CYTOTOXİCİTY STUDY ON PROMYELOCYTİC LEUKEMİA (HL-60) CELLS

Curcuma Longa L. EKSTRESİNİN KAPSÜLLENMESİ: PROMİYELOSİTER LÖSEMİ (HL-60) HÜCRELERİ ÜZERİNDEKİ SİTOTOKSİSİTE ÇALIŞMASI

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ABSTRACT

Objective: There are great difficulties in the preparation and use of antitumor and therapeutic drugs. Preparation of highly efficient nano/micro devices to address these difficulties represents one of the new topics in the field of antitumor pharmaceuticals.

Methods: Biopolymers such as chitosan and alginate have been shown to be used as an absorption enhancing agent. In this study, the extracts of the turmeric (*Curcuma longa* L.) plant (TEX) was obtained by the Soxhlet method. Then, TEXs obtained in two different ways were encapsulated with alginate/chitosan. FT-IR, sem-edx and extract analyzes proved that encapsulation occurred and the presence of curcumin and phenolics. Drug loading efficiency (DL) and encapsulation efficiency (EE) of the obtained capsules were examined. EE and DL for TEX microcapsule 1 were 82.40% and 3.21%, respectively, and EE and DL for TEX microcapsule 2 were 88.16% and 4.68%, respectively. Additionally, the anti-proliferative activity and capsule release of the prepared capsules on the promyelocytic leukemia (HL-60) cell line were evaluated.

Results: According to the results of this analysis, it was determined that in the first 24 hours, TEX-1 Liposomes released 67.03% of their content into the environment at pH 5.5 and 58.24% of their existing content at pH 7.4. It was determined that TEX-2 liposome suspensions released 73.23% of their content at pH 5.5 and 66.89% of their content at pH 7.4 in the first 24 hours. In addition, the IC_{50} doses of TEX-1 and its liposomes after 48 hours were 242.3- 126.1µg/mL, respectively; The IC_{50} levels of TEX-2 and its liposomes were 81.2- 19.3µg/mL, respectively.

Conclusion: As a result of the study, it was observed that alginate/chitosan encapsulation strongly inhibited cellular proliferation in the cell line (HL-60).

Keywords: Cancer, Chitosan, Curcimin, *Curcuma longa*, HL-60.

ÖZET

Amaç: Antitümör ve terapötik ilaçların hazırlanması ve kullanımında önemli zorluklar bulunmaktadır. Bu zorlukların aşılmasına yönelik yüksek verimli nano/mikro cihazların hazırlanması, antitümör farmasötikler alanında yeni konulardan biri olarak öne çıkmaktadır.

Gereç ve Yöntem: Kitosan ve aljinat gibi biyopolimerlerin emilim artırıcı ajan olarak kullanılabildiği gösterilmiştir. Bu çalışmada, zerdeçal (*Curcuma longa L.*) bitkisinin ekstreleri (TEX) Soxhlet yöntemi ile elde edilmiştir. Daha sonra iki farklı şekilde elde edilen TEX'ler aljinat/kitosan ile enkapsüle edilmiştir. FT-IR, SEM-EDX ve ekstrakt analizleri, enkapsülasyonun gerçekleştiğini ve kurkumin ile fenolik bileşiklerin varlığını doğrulamıştır. Elde edilen kapsüllerin ilaç yükleme verimliliği (DL) ve enkapsülasyon verimliliği (EE) incelenmiştir. TEX mikrokapsül 1 için EE ve DL değerleri sırasıyla %82,40 ve %3,21 olarak bulunurken, TEX mikrokapsül 2 için EE ve DL değerleri sırasıyla %88,16 ve %4,68 olarak belirlenmiştir. Ayrıca, hazırlanan kapsüllerin promiyelositik lösemi (HL-60) hücre hattı üzerindeki antiproliferatif aktiviteleri ve kapsül salım davranışları değerlendirilmiştir.

Bulgular: Analiz sonuçlarına göre, ilk 24 saat içerisinde TEX-1 liposomlarının pH 5,5 ortamında içeriklerinin %67,03'ünü ve pH 7,4 ortamında mevcut içeriklerinin %58,24'ünü ortama saldığı belirlenmiştir. TEX-2 liposom süspansiyonlarının ise ilk 24 saat içerisinde pH 5,5 ortamında içeriklerinin %73,23'ünü ve pH 7,4 ortamında içeriklerinin %66,89'unu saldığı tespit edilmiştir. Ayrıca, 48 saat sonunda TEX-1 ve liposomlarının IC_{50} dozları sırasıyla 242,3 ve 126,1 µg/mL olarak bulunurken; TEX-2 ve liposomlarının IC_{50} değerleri sırasıyla 81,2 ve 19,3 µg/mL olarak belirlenmiştir.

Sonuç: Çalışma sonucunda, aljinat/kitosan ile enkapsülasyonun HL-60 hücre hattında hücresel proliferasyonu güçlü bir şekilde inhibe ettiği gözlemlenmiştir.

Anahtar Kelimeler: *Curcuma longa*, HL-60, Kanser, Kitosan, Kurkumin.

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INTRODUCTION

Cancer disease, characterized by epigenetics and mutations, can be partially triggered by environmental factors. Immunotherapy, radiotherapy, chemotherapy and surgical interventions are used in the treatment of this disease, which is responsible for most of the deaths in the world. (Erez, Shchelochkov, Plon, Scaglia, & Lee, 2011). Of these, chemotherapy is the main treatment method used in the treatment of metastatic tumors. However, this treatment method can cause very serious side effects (such as neurotoxicity, organ damage, bone marrow suppression, etc.)(Feng, Wei, Lee, & Zhao, 2017; Prasanna, Ahmed, Mohiuddin, & Coleman, 2014).

It is known that some plant extracts have positive effects on human health. It has been shown in animal experiments that phytochemicals and microchemicals taken with food can be effective agents in preventing diseases(L. Li, Braiteh, & Kurzrock, 2005). Anti-inflammatory, anti-cancer and contraceptives can be counted among these effects. Due to their non-toxicity and pre-clinical antitumor activity, they may enable the development of new approaches in cancer treatment(Kawamori et al., 1999; L. Li et al., 2005). However, the biggest problem with these extracts is their low bioavailability. Although they are pharmacologically active, their low water solubility and rapid metabolism cause their systemic elimination to be shorter than desired. This has led researchers to increase the bioavailability of herbal extracts (Di Maio, Basch, Bryce, & Perrone, 2016; Feng et al., 2017).

One of the natural plant extracts that has been researched for a long time is the chemical curcumin (diferuloylmethane), an active ingredient obtained from the roots of Curcuma longa L. This chemical, which has been used in human diets and spices for centuries, has been determined to be pharmacologically safe. The compound has been reported to have strong antiproliferative and proapoptotic effects in vitro and has been shown to suppress carcinogenesis(Huang et al., 1997; L. Li et al., 2005; Lu et al., 1994; Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995).

To overcome the poor absorption of curcumin, researchers have tried to develop different strategies such as emulsion, complex, nanogel and micelle. Thus, they focused on increasing cell permeability, dissolution rate and solubility(Feng et al., 2017; Song, Cha, & Choi, 2016). In addition to the improvement in cellular uptake and cytotoxic effects of the extracts that were tried to be stabilized, positive effects on tumor cells have also been reported(Shinde & Devarajan, 2017; Singh, Sharma, & Gupta, 2015). The ability of encapsulation techniques to carry hydrophilic and hydrophobic drugs shows that they may have high potential in effective cancer treatments. Thus, the biological distribution and clearance of the desired molecule can be changed(Bingham, Olmsted, & Smye, 2010; Wang, Ishida, Ichihara, & Kiwada, 2005).

Chitosan is a hydrophilic, biocompatible and biodegradable polymer with low toxicity. Several studies have highlighted the potential of using chitosan as an absorption enhancing agent. Moreover, due to its bioadhesive properties, chitosan has also attracted great attention in novel bioadhesive drug delivery systems(Filipović-Grčić, Škalko-Basnet, & Jalšienjak, 2001).

This study was planned to evaluate the comprehensive anti-cancer activity of curcumin, extract encapsulations of biodegradable chitosan liposomes, and drug potential in promyelocytic leukemia (HL-60) cell line.

MATERIALS AND METHODS

Materials

The CCK-8 kit used in the study was from Abbkine; Trypsin EDTA, DMEM, penicillin-streptomycin antibiotics were purchased from Cytiva, and NaOH, chitosan, sodium alginate, acetic acid, $CaCl_2$, phosphate buffered saline (PBS) were purchased from Sigma. Other materials were available in our laboratory. *Curcuma longa L*. root was purchased from the local market. Flavonoid and phenolic compounds gas chromatography (GC) (Agilent/ 7890A) and scanning electron microscope (Sem-edx) (Carl Zeiss) analyzes were performed at CANKAM research center.

Curcumin extraction

Turmeric (*Curcuma longa L.*) plant root was first dried at 45 ºC for 2 nights to remove all moisture. The prepared root was crushed in a mortar and stored in a moisture-free environment. The extract was obtained using two different methods;

Extract 1-(TEX-1: Pure water- curcumin extraction): 2.00 g of dry turmeric plant powder was placed in a cartridge prepared from filter paper. The mouth was closed so that no material could escape.

Placed in Soxlet. The mechanism was set up by placing 220 mL of pure water into a 500 mL balloon. The heater was set at 300ºC. The soxhlet process siphoned back to repeat five times. (More than 6 hours, until clear). After the final cartridge application, the system was cooled and the extracts were removed from the collection container.

Extract 2- (TEX-2: Ethanol-ammonia-curcumin extraction): 2.180 g of dry turmeric plant powder was put into a filter paper cartridge. The mouth was closed so that no material could escape. Placed in Soxlet. The mechanism was set up by placing 200 mL of ethanol and 20 mL of ammonia into a 500 mL volumetric flask. The heater was set at 300ºC. The soxhlet process siphoned back to repeat five times. (More than 6 hours, until clear). After the final cartridge application, the system was cooled and the extracts were removed from the collection container.

Phenolic, flavonoid content and Sem-edx analysis of extracts

In the study, total phenolic content was calculated by measuring at 760 nm according to the Folin-Ciocalteu method. Gallic acid equivalents of the samples and total flavonoid content were calculated by the Aluminum chloride method (Used as standard)(Yırtıcı, Ergene, Atalar, & Adem, 2022). Sem-edx analyzes were performed at CANKAM research center.

Microencapsulation of extracts

Encapsulations were made by changing the microencapsulation techniques previously reported by us and other researchers(Azevedo, Bourbon, Vicente, & Cerqueira, 2014; Ulusal, Ulusal, Dagli, & Toprak, 2024; Yaşar & Yaşar, 2024). 4.02g sodium hydroxide (NaOH) was added to 42mL Sodium alginate (1%) solution and mixed in a magnetic stirrer until transparent. Approximately 2.4mL of 20mM $CaCl_2$ was added to the solution dropwise (while dropping, it was mixed ultrasonically for about 5 minutes) and placed in a petri dish. Meanwhile, extracts 1 and 2 were dissolved in approximately 2mL of ethanol-water mixture (0.166g and 0.18g, respectively) and 4.5mL of 4% chitosan was added. It was stirred vigorously until completely dissolved. All solutions were dropped into sodium alginate using a syringe from a distance of 10cm. Spherical drops, which hardened in approximately 1 hour, were separated. It was passed through 20Mm $CaCl_2$ and pure water. The resulting capsules were placed in a petri dish and dried at 45 ºC. Capsules obtained from Exstart 1 and 2 were coded (capsule 1 and capsule 2, respectively).

Encapsulatin efficiency (EE) and Drug loading (DL)

A UV spectrophotometer was used for all yield (EE) and content (DL) calculations (Shimadzu). It was studied in 3 repetitions(R. Li et al., 2017; Ulusal et al., 2024; Yaşar & Yaşar, 2024). All extracts were scanned between 200 and 800 nm at 0.5 nm intervals. The obtained peaks were used in the calculations of the DL and EE values of the capsules (Peak: Found at 427nm for TEX-1 and TEX-2).

Quantitative analyzes were used to determine the content of the extract bound (trapped) in the capsule. It was centrifuged to determine the encapsulated amount and dispersed again using phosphate buffer. Finally, extract content determination was made (supernatant) and spectroscopic analysis was performed by dissolving the extract capsules in ethanol.

DL equation;

$DL(\%)=M_{EC}/M*100$ (M_{EC}: Turmeric plant extract mass held in capsule (coded TEX-1 and 2); M: Mass of the capsule)

Kapsül EE'si denklemi;

$EE(\%)= M_{EC}/M_{TEX-1/2}*100$ ($M_{TEX-1/2}$: Amount of extract used for encapsulation.)

Release of extract from encapsulated liposomes

The dialysis bag technique was used in the release kinetics of all capsule structures obtained in the study. (Ulusal et al., 2024). pH: 5.5 and 7.4 were preferred in all release studies due to the acidic environmental conditions of cancer cells (anaerobic respiration).

A dialysis bag (Dialysis Membrane-110, 2.4nm, HiMedia, India) and PBS (pH: 5.5 and 7.4) containing 30% ethanol were preferred as the release medium. All work was done in a shaking water bath (100rpm at 37 ºC). To determine the cumulative amount of released extract, 1 mL of suspension medium was withdrawn at regular intervals and replaced with fresh medium. Spectroscopy was performed at wavelengths determined depending on the type of each extract obtained with different

techniques. (TEX-1 and TEX-2: 427nm). In the final stage, extract releases were determined as % cumulative;

$$\text{Total Release (\%)} = (50\,Ci + \sum_{i=1}^{n} \text{C}i - 1)/Mo$$

(Ci: TEX-1 and TEX-2 concentrations measured at regular intervals; M0: Initial mass of TEX-1 and TEX-2).

Cytotoxicity test (in vitro)

Liposomes of two different curcumin extracts obtained from the root of the turmeric plant were evaluated with the cell counting kit CCK-8 (this test measures cell viability). All viability tests were performed on the promyelocytic leukemia cell line (HL-60). *All cells were incubated in RPMI-1640 (GIBCO) medium until confluency (>95%) (content: 10% fetal bovine serum-FBS-, 1% penicillin; conditions: 95% humidity; 5% CO_2; 37 °C). When sufficient cell numbers were reached, the cell medium was removed and centrifuged, and the cells were stained with Triptan blue. Planting was done in a 96-well plate (medium: approximately 5000 cells and RPMI-1640 medium). After 2 hours, the same (determined) concentrations of turmeric extract and encapsulated liposomes were added into the medium (*1000, 500, 250, 100, 50, 25, 10 ve 1 µg/mL*). It was added to the empty capsule to determine capsule toxicity. 10µL of CCK-8 reagent was added to the cell wells that were kept in an incubator with 5% carbon dioxide for 24 and 48 hours, and the incubation continued.* After waiting for a while (about 2 hours), the plates were evaluated using a microplate reader (Biotek H1 Synergy, USA) at a wavelength of 450nm. Cells without liposomes and oil extract were considered negative control (100% viability), and cells containing 100µM H_2O_2 incubated under the same conditions were considered positive control. The drug dose (IC_{50}) that killed half of the cells was calculated using Graphpad Prism 10.0.2.

RESULTS

According to TEX-1 FT-IR analysis results, a broad peak was obtained at 3284 cm^{-1} and peaks were obtained at 1636 cm^{-1} and 594 cm-1. Second extract FT-IR results (TEX-2) were obtained at 2978 cm^{-1}, 2348 cm^{-1}, 1607 cm^{-1}, 1433 cm^{-1}, 863 cm^{-1} and 685 cm^{-1}. These peaks showed the expected structures in TEX-1 and TEX-2. The results at 2978 and 3284 cm^{-1} showed the presence of O-H groups and methyl groups, as well as phenolic structures, in the extracts. FT-IR results indicated methyl, C=C, O-H, C-C, C-H, C=O phenolic and aromatic structures of curcumin molecules in all extract types (Figure 1).

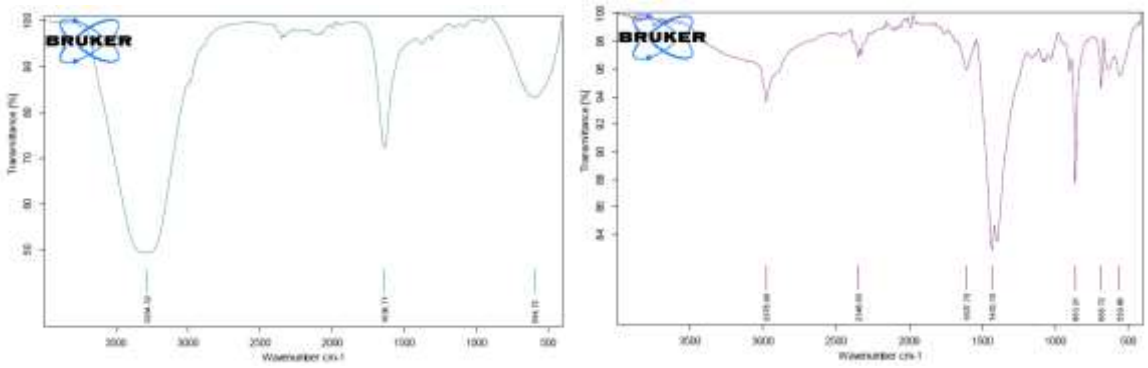


Figure 1. FT-IR analysis. TEX-1 (Left); TEX-2 (Right)

As a result of FT-IR analysis of the prepared encapsulated curcumin extract liposomes, when the encapsulation results with TEX-1 were examined, the molecular structures of curcumin, chitosan and alginate were determined. In the analysis results, a broad peak was obtained at 2978 cm^{-1}. Other peaks were obtained at 1566, 1433, 1068, 863, 686 and 561 cm^{-1}. In the FT-IR results of TEX-2 encapsulation, peaks were obtained at 3274, 1563, 1405, 1067, 861, 684, 558 cm^{-1} (Figure 2).

In addition, sem-edx analysis results and total phenolic and flavonoid content results of TEX-1 and 2 were as expected (Elemental spectrum, microscopy and total contents are shown in Figure 3.

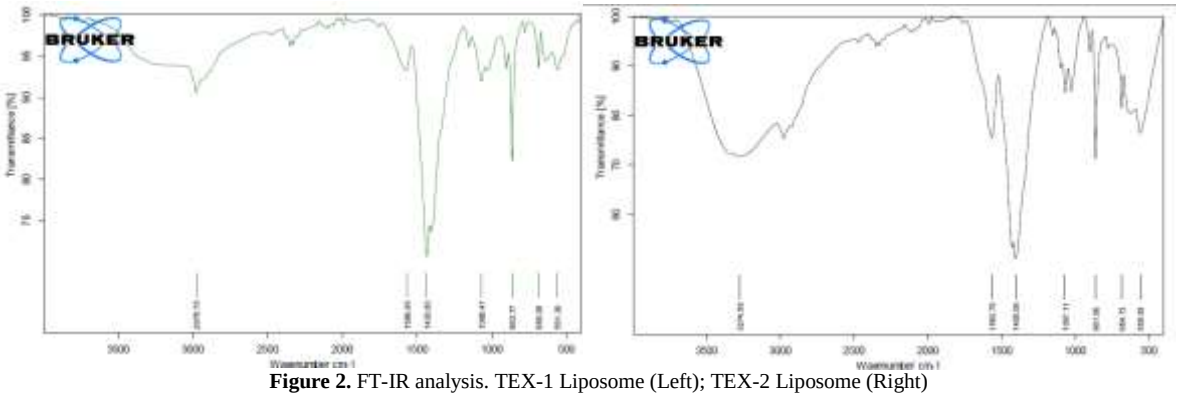


Figure 2. FT-IR analysis. TEX-1 Liposome (Left); TEX-2 Liposome (Right)

Edx (Weight%)	Na K	Ca K	Cl K	C K	O K	Conents	T. Phenolic (mg GAE/g)	T. Flavonoid (mg RE/g)
ZEK-1 Capsule	38,46	0,5	0,39	13,99	46,65	ZEK-1	21,66	10,08
ZEK-2 Capsule	40,71	0,56	0,38	12,23	46,13	ZEK-2	23,76	9,81

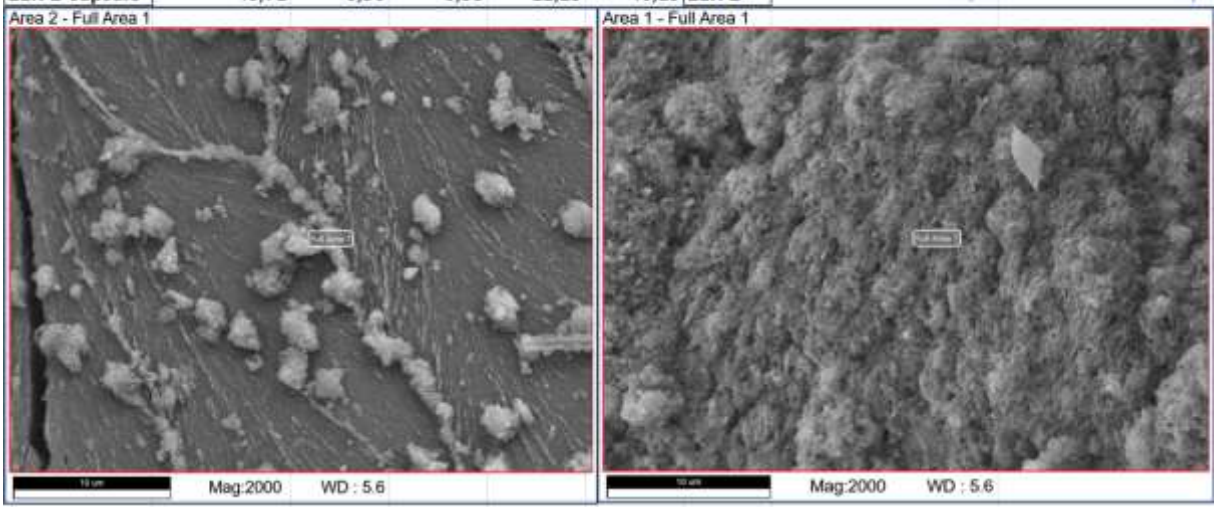
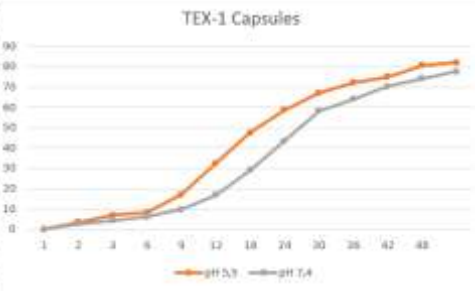


Figure 3. Sem-edx results of extracts and capsules (TEX-1 liposome: lower left; TEX-2 liposome: lower right) and total contents (upper)

Spectroscopic analyzes of TEX-1 and 2 encapsulations were performed at different pHs (pH: 5,5;7,4). According to the results of this analysis, it was determined that TEX-1 Liposomes released 67.03% of their content into the environment at pH 5.5 and 58.24% of their existing content at pH 7.4 in the first 24 hours. It was determined that TEX-2 liposome suspensions released 73.23% of their content at pH 5.5 and 66.89% of their content at pH 7.4 in the first 24 hours (Figure 4).

(h)	pH 5,5	pH 7,4
0	0	0
1	3,46	2,71
2	6,84	4,16
3	8,12	6,15
6	17,08	9,84
9	32,52	16,95
12	47,59	29,06
18	58,64	43,54
24	67,03	58,24
30	72,16	63,91
36	75,04	70,28
42	80,71	74,1
48	82,19	77,65



(h)	pH 5,5	pH 7,4
0	0	0
1	3,91	3,14
2	6,28	5,42
3	7,95	6,84
6	14,98	13,91
9	28,74	24,78
12	44,68	38,09
18	65,39	52,67
24	73,23	66,89
30	81,26	72,45
36	85,52	76,12
42	89,19	79,04
48	90,28	81,64

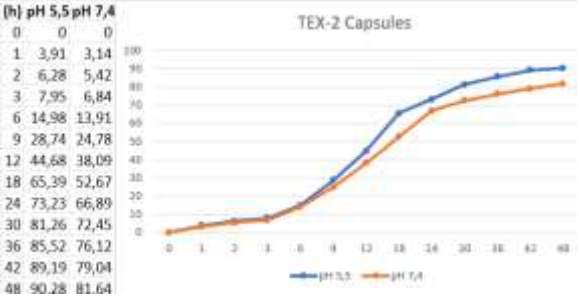


Figure 4. 48-hour release of liposomes (capsules), TEX-1 capsule (Left) and TEX-2 capsule (Right)

In the study, viability tests of different concentrations of TEX-1 and 2 liposomes (1, 10, 25, 50, 250, 500 and 1000µg/mL) were performed on HL-60 cell lines. Values and ratios (%) in HL-60 cell lines, relative to control (Figure 5).

Extracts obtained by two different methods were encapsulated with alginate/chitosan. The ability of liposomes to encapsulate TEX was evaluated. EE and DL for TEX microcapsule 1 were

82.40% and 3.21%, respectively, and EE and DL for microcapsule 2 were 88.16% and 4.68%, respectively.

C (ug/mL)	TEX1-24h	TEX1-48h	Capsule1-24h	Capsule1-48h
1000	40,42357274	30,66783831	26,06060606	15,21924223
500	51,38121547	40,50966608	29,76190476	16,45381013
250	61,04972376	42,35500879	38,59307359	24,45721584
100	79,65009208	57,38137083	46,51515152	36,7816092
50	80,93922652	67,13532513	52,18614719	45,33844189
25	89,59484346	73,11072056	65,15151515	60,23839932
10	93,8305709	78,82249561	79,80519481	76,01106854
1	94,29097606	89,98242531	94,95670996	92,3584504
Control	100	100	100	100
C (ug/mL)	TEX2-24h	TEX2-48h	Capsule2-24h	Capsule2-48h
1000	30,36253776	30,20746888	29,95642702	23,27898551
500	46,75226586	33,85892116	37,58169935	23,64130435
250	55,21148036	43,48547718	45,31590414	27,89855072
100	59,21450151	53,60995851	52,39651416	32,24637681
50	66,23867069	61,57676349	65,68627451	49,63768116
25	83,3081571	77,84232365	74,50980392	56,34057971
10	91,23867069	80,74688797	87,69063181	66,9384058
1	97,35649547	92,03319502	97,16775599	95,28985507
Control	100	100	100	100

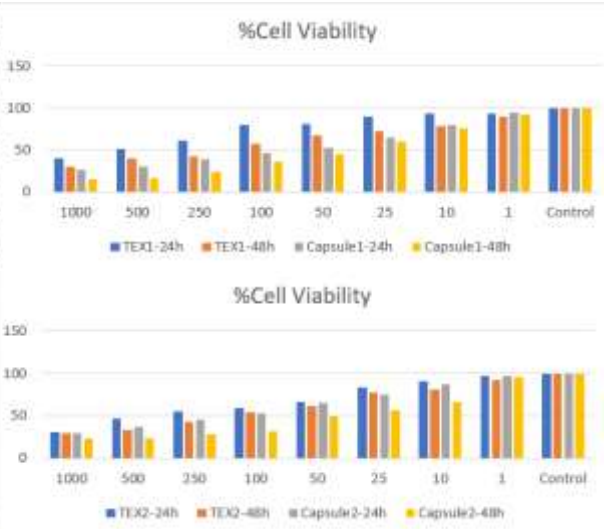


Figure 5. Viability effect of capsules on HL-60 cell line (24 and 48 h); TEX-1 and capsule 1 (upper); TEX-2 and capsule 2 (bottom)

IC_{50} dose analyzes were also performed on HL-60 cell lines. Both extracts and liposomes were evaluated for 48 h. The IC_{50} doses of TEX-1 and liposomes after 48 hours were determined as 242.3-126.1µg/mL, respectively, and the IC_{50} levels of TEX-2 and liposomes were determined as 81.2-19.3µg/mL, respectively (Figure 6).

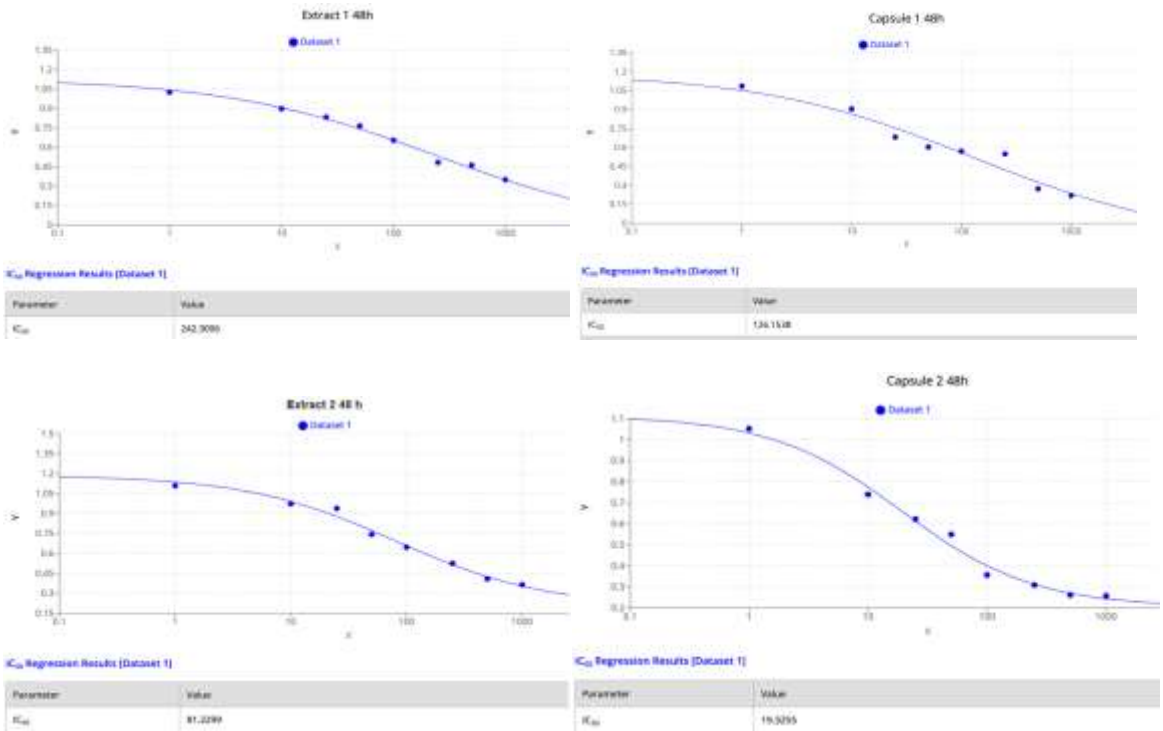


Figure 6. IC_{50} dose determination analysis results of extract and liposomes in HL-60 cell lines (48h: TEX-1 and capsule results (upper); TEX-2 and capsule results (bottom))

DISCUSSION

Cancer, the second cause of death in the world after cardiovascular diseases, is a major global health problem that poses a risk in the 0-74 age range (overall risk; 20.2%). 18 million cancer patients were diagnosed in 2018. Most of these patients are tried to be treated with surgery and radiation treatments. However, these treatment methods are inadequate in micrometastasis cases(Rahman et al., 2024). Additionally, healthy cells are also lost in chemotherapy. In recent years, the study of new anti-infective and anti-cancer agents has attracted the attention of many researchers in the field of ethnopharmacology(Rios & Recio, 2005; Taşkın, Tarakçıoğlu, Ulusal, Örkmez, & Taysı, 2019). The entomedical data approach is important in identifying these plants containing medicinally active compounds by researchers(Cordell, Beecher, & Pezzuto, 1991). If carrier systems are developed, more effective treatments can be provided with fewer drugs and side effects can also be reduced(R. Li et al., 2017).

In this study, the anti-proliferative effects of curcumin (turmeric extract), which is reported to have anti-inflammatory, anti-cancer and anti-microbial properties(Ranjan, Mukerjee, Helson, Gupta, & Vishwanatha, 2013), on HL-60 cell lines were examined. However, curcumin was encapsulated (capsules 1 and 2) because its compound is hydrophobic(L. Li et al., 2005; Marques, 2010). Thus, their bioavailability was improved.

Extracts were prepared from turmeric plant root with two different methods (pure water; ethanol-ammonia). Then, two different liposomes (capsules) were obtained by alinate/chitosan encapsulation of these extracts, and all of them were imaged with sem-edx. Research analysis results showed that the extracts and encapsulations performed as intended. All physicochemical analyzes were determined by FTIR analyses. The broad peak observed at 3284 cm^{-1} in the FT-IR results of pure water-curcumin extract showed that methyl, O-H and phenolic structures were present. The presence of aromatic rings at 1636 cm^{-1} indicates the presence of C=C and C=O. The peaks observed at 1068, 863, 686 and 561 cm^{-1} indicate the presence of C-C, C-O and aromatic structures. In the ethanol-ammonia extract FT-IR results, the peak observed at 2978 cm^{-1} indicates the presence of methyl, O-H and phenolic structures. The peak at 1608 cm^{-1} indicated aromatic rings, the peak at 1433 cm^{-1} indicated methyl and C-O bonds, and the peaks at 863, 685 cm^{-1} indicated the presence of C=C, C-O and aromatic structures. As a result of all these analyses, it was determined that there were phenolic and flavonoid compounds in both extract structures. Later GC analyzes also showed the presence of phenolic and flanoid compounds in TEX-1 and TEX-2 (21.66 mg GAE/g /10.08 mg RE/g; 23.76 mg GAE/g /9.81 mg RE/g, respectively). Liposome 1 and 2 FT-IR analyzes showed amine, O-H, C-H, phenolic structures (2978;3274 cm^{-1}, respectively). The peaks at 1566/1563 cm^{-1} indicated the presence of C=O, methyl structures. The peaks observed at 1068 and 1405 cm^{-1} showed C-C, C-O, aromatic structures. Our results also showed chitosan and alinate peaks. These results were consistent with the literature, and the sem-edx and elemental spectrum were supporting evidence(Afzal et al., 2022; Hettiarachchi, Dunuweera, Dunuweera, & Rajapakse, 2021; Lestari & Indrayanto, 2014; San et al., 2022).

The controlled drug delivery capabilities and cost/process benefits of capsules are interesting. Encapsulation is a technique that has the potential to overcome hydrophobic drug and dissolution issues. For this reason, capsule forms of pharmaceuticals continue to be researched. Chitosan, one of these encapsulation forms, has attracted great attention in bioadhesive drug delivery systems. By combining chitosan and liposomes, specific, long-lasting and controlled release can be achieved (Marques, 2010; Phaechamud & Darunkaisorn, 2016). In this study, alginate/chitosan encapsulation of TEX-1 and TEX-2 extracts obtained with different techniques was performed. Then, these capsules were applied to the HL-60 cell line in aqueous medium and the time-dependent release of TEX-1-2 after encapsulation was examined. The entire study was carried out at Ph 5.5 and 7.4, taking into account cancer cell metabolism(Gunduz et al., 2014). Both encapsulations were determined to release most of their available content at pH 5.5. This may be due to earlier deterioration of encapsulation integrity in an acidic environment. Our release analyzes showed that the release from microcapsules was not high in the first hours. Although this situation seems contrary to the "drug explosion" explanation mentioned in the literature(Yaşar & Yaşar, 2024), it may also be related to chitosan concentration, as reported by Ramgonda et al. Changing active surface and polymer ratios in capsule synthesis may change the release data. As a result, the chitosan concentration used in the microsphere formulation may play a release inhibitory role(Ramgonda, Masareddy, Patil, & Bolmal, 2021). Our study results also support this.

Capsules at pH 7.4 showed later and slower release than those at pH 5.5. This indicates that the degradation of chitosan is delayed at high concentrations.

TEX-1 encapsulation released 67.03% (pH 5.5) and 58.24% (pH 7.4) in the first 24 hours (in cell line release trials). Likewise, it was determined that it released 82.19% (pH 5.5) and 77.65% (pH 7.4) in the first 48 hours. In TEX-2 encapsulation trials, this situation is respectively; 73.23%; 90.28% (pH 5.5) and 66.89%; 81.64% (pH 7.4). Our results showed that the hydrophobic drug carrier capsules released more than half of their content in the first 24 hours. It had released 90% of its contents within 48 hours. Release rates started to decrease in the first 24-30 hours. These results were similar to the literature(Afzal et al., 2022; San et al., 2022; Ulusal et al., 2024; Yaşar & Yaşar, 2024).

It is important to know the EE and DL values in liposome studies in order to overcome the difficulty of using particularly expensive drugs. Therefore, performing EE and DL analyzes in encapsulation studies is important for the usability and effectiveness of drugs(Ulusal et al., 2024; Yaşar & Yaşar, 2024). In the analyses, the EE and DL percentages of liposomes 1 and 2 were found to be similar to the literature (respectively, 82.40%; 3.21% and 88.16%; 4.68%). Ulusal et al. coated the extracts obtained from the plant with alginate/chitosan and found the EE and DL values of the capsules they made with two different methods to be similar to our study (94.10%; 4.92% and 86.69%; 3.15%, respectively)(Ulusal et al., 2024). Similarly, Yaşar et al. found that EE and DL values in alginate/chitosan encapsulations of hibiscus plant extracts were similar (87.64%; 3.94% and 91.56%; 5.2%, respectively)(Yaşar & Yaşar, 2024). Li et al. determined the EE and DL values (88.75% and 3.96%, respectively) of the curcumin carrier coated with thiolated chitosan(R. Li et al., 2017). It is possible to increase similar literature studies. (Afzal et al., 2022; San et al., 2022). Our results showed that encapsulation and drug loading were achieved, consistent with the literature.

Researchers report that mammalian cell lines are important in evaluating the cytotoxic effects of chemical compounds. Therefore, the HL-60 cell line is an ideal examination tool(da Costa et al., 2008; Militão et al., 2006). In this study, liposomes with high drug capacity were prepared. The antiproliferative effect of liposomes and extracts on HL-60 cell lines and the IC_{50} values in these cell lines were calculated using the CCK-8 method. According to our results, TEX-1 and 2 extract IC_{50} values varied between 242.3-81.2 µg/mL, respectively, in the first 48 hours. After microencapsulation, it was determined that their effectiveness increased and IC_{50} values decreased to concentrations of 126.1-19.3 µg/mL, respectively. This result showed that the encapsulated drug activity was dose- and time-dependent. It was observed that gradual release could also be effective at lower doses as high TEX concentrations were reached. (Ulusal et al., 2024; Yaşar & Yaşar, 2024). Our anti-proliferative study showed that cell viability caused cellular cytotoxicity in a dose-dependent manner. TEX-1 extract was less effective than its capsule. Its effectiveness at all concentrations was lower than that of capsule forms. TEX-2 extract also showed lower efficacy than capsule forms (almost, except Capsule 24h). Grace et al. mentioned the strong inhibitory effect of the extracts they isolated from Curcuma longa in their experiments on cell lines(Yue et al., 2010). Similarly, Esmael et al reported that dendrosomal curcumin exhibited higher bioavailability than void curcumin and strongly inhibited cellular proliferation in a dose-dependent manner(Babaei et al., 2012). Results compatible with our results are frequently encountered in the literature(Delfine et al., 2017; Ulusal et al., 2024; Yaşar & Yaşar, 2024).

CONCLUSION

The therapeutic potential of microcapsules developed today provides new perspectives on applications in biochemistry, medicine and biotechnology. Today, the diversification and development of these encapsulation techniques continues. In this way, more effective use of preferred drugs during treatment is ensured and their bioavailability can be increased. In this study, hydrophilic, biocompatible and low-toxicity chitosan/alginate encapsulations of poorly soluble curcumin were made and the synthesized capsules were used as a drug carrier system on HL-60 cell lines. Cell viability tests showed that capsule forms were more effective in cell growth. It is thought that more successful results can be obtained by lowering the IC50 values. It was also concluded that Curcumin and turmeric extracts were effective on promyelocytic leukemia cell lines. These results can be a reference for future studies.

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