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Enhancing the efficacy of radiotherapy with *Kalanchoe daigremontiana*: A nanotechnological approach

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ABSTRACT

Radiotherapy is widely utilized in the treatment of cancer, but this method is associated with dose-dependent limitation. Herbal compounds contain various secondary metabolites that increase the effectiveness of radiotherapy. However, a significant challenge associated with the use of herbal preparations is their poor biocompatibility. To address this limitation, the active ingredients of the leaves of *Kalanchoe daigremontiana* were extracted subsequently, phytoniosomes were prepared from the extract. The phytoniosomes were characterized through various analyses, Content analysis was performed with Quadrupole time-of-flight mass spectrometer (QTOF-MS) and encapsulation efficiency was evaluated with High-performance liquid chromatography (HPLC). Then, the cytotoxic effects of the phytoniosomes were evaluated on the Human Breast Epithelial Adenocarcinoma Cell Line (MCF-7), and Human Cervix epithelial Adenocarcinoma Cell Line (HeLa), using the MTT assay over multi-periods. Then radiotherapy studies have been carried out to increase this effect via Linear Accelerator (LINAC). Finally, to further understand the interactions between the phytoniosomes and cells, the niosomes were labelled with Fluorescein isothiocyanate (FITC) and fluorescence images were obtained. The results confirmed that the size of plain niosomes and phytoniosomes were 95.9 ± 0.52 and 126.0 ± 5.14 nm, respectively and localized around cytoplasm. Phytoniosomes combined with radiotherapy on cell lines are more effective than radiotherapy or phytoniosomes alone. The cell viability decreased significantly to 7.102 ± 3.51 for the MCF-7 cell line and to 8.183 ± 4.117 for the HeLa cell line). In conclusion, the combined use of these niosomal preparations with radiotherapy has the potential to be used as a tool for overcoming dose-dependent limitation in radiotherapy.

1. Introduction

Cancer is a global health problem due to its high morbidity rate and economic burden. The high number of cases have caused governments to allocate significant budgets to cancer treatment (Sung et al., 2021). In recent years, new perspectives have emerged in cancer treatment in the light of the development of nanotechnology and biotechnology (Hajizadeh et al., 2019). Biocompatibility, blood circulation number and stability of a drug molecule can be increased with various nanocarrier systems. In addition, these carrier systems can help drug molecules to enter cells easily (Goh et al., 2017). Niosomes, a subcategory of liposomes, have garnered significant attention among researchers in recent years due to their unique composition, which comprises of non-ionic surfactants. These surfactants are known to be biocompatible and biodegradable, making niosomes a promising candidate for various biomedical applications such as drug delivery, gene therapy, and diagnostic imaging. The first use of niosomes as a carrier system emerged in the early 1970s with the investigation of the potential for pharmaceutical use of non-ionic surfactants synthesized by Guy Vanlerberghe for the company L'Oréal at the University of Strathclyde (Handjani-Vila, Ribier, Rondot, & Vanlerberghie, 1979). Advantages like being; cheap, osmotically active, chemically stable, highly biocompatible and degradable, non-immunogenic, and having have a long storage life, low toxicity and controlled drug effectiveness and release. (Almeida, Nag, Rogers, & Delehanty, 2020; Jacob, Nair, & Al-Dhubiab, 2017).

Radiotherapy, a non-invasive and highly effective treatment option, is a widely used form of cancer treatment that involves the application of ionizing radiation to malignant tissue in order to induce cell death and control tumour growth. This therapy method is highly preferred in targeting various tissues including breast cancer, lung cancer, cervix

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cancer, and others (Deng et al., 2017; Zhang et al., 2021). Radiotherapy can be delivered externally, using a machine to direct the radiation at the affected area, or internally, by inserting a radioactive source into the body. Radiotherapy plays an important role in the management of cancer and is an effective way to control the disease and improve quality of life for many patients (Alfouzan, 2021; Dunlop et al., 2020). While radiotherapy is generally well-tolerated, it can cause side effects such as fatigue, skin irritation, nausea and cardiac problems (Taylor & Kirby, 2015). With continued advancements in technology and techniques, the role of radiotherapy in the treatment of cancer will likely continue to evolve and expand.

Human being has been used herbal ingredients to fight diseases since ancient times. Herbal ingredients can show antioxidant, antimicrobial, antiproliferative, cytotoxic effects, can also be applied to reduce the radiation toxicities especially with the secondary metabolites they contain in leaves, roots, stems and seeds (Ameri et al., 2023; Hejazi et al., 2015; Stojanović, Jovanović, & Zlatković, 2015). The genus Kalanchoe belongs to the Crassulaceae family and is widely localized in tropical and subtropical regions. Various species of Kalanchoe have been utilized in traditional medicine, with a notable reputation for their medicinal properties. These plants have been commonly employed for the treatment of various ailments, particularly in the Far East where they have been traditionally used for the treatment of cancer (Saravanan, Murugan, & Kumaravel, 2020). Recently, Kalanchoe plants have been reported to have cytotoxic and antiproliferative properties and preferred in the treatment of cancer. (Hsieh et al., 2012; Justyna Stefanowicz-Hajduk et al., 2022). Herbal extracts, low biocompatibility and cell absorption, constitute a large part of the drugs used in the treatment of cancer (Nirmala, Samundeeswari, & Sankar, 2011). In order to overcome these handicaps, plant-derived nanovesicular carriers have a promising potential and it is predicted that they will have an important place in multi-functional nano drug systems of the future (Yu, Deng, Liu, Zhang, & Wang, 2020).

Plant-derived nanovesicular carriers, such as liposomes, niosomes and polymeric nanoparticles, have emerged as promising drug delivery vehicles in recent years. These carriers are made from natural, biocompatible and non-toxic materials, making them suitable for use in biomedical applications (Amalraj, Jacob, Varma, & Gopi, 2020; Wei & Ba, 2020). Additionally, these carriers can be engineered to have specific properties, such as targeted drug delivery or sustained release. Further research is needed to fully enlighten the potential of these carriers in biomedical applications.

The objective of this study is to perform a comparative evaluation, for the first time in the literature, of the cytotoxic activities of Kalanchoe species extracts and their niosomal forms (phytoniosomes) against human cancer cell lines (Human Breast Epithelial Adenocarcinoma Cell Line, MCF-7 and Human Cervix epithelial Adenocarcinoma Cell Line, HeLa) and to assess their combined effect with radiotherapy. Recent research has demonstrated the potential of Kalanchoe species as a novel treatment option for cancer. The present study aims to enhance our understanding of biotechnologically modified herbal preparations and to determine the extent to which they can enhance the potential of combined therapies.

2. Materials and methods

2.1. Materials

In this study, all chemicals were used in analytical purity. Phosphate Buffered Saline (PBS), Chloroform (Chf), Methanol (MeOH), Ethanol (EtOH), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reagent, Dialysis sacks MWCO 12,000 Da, Tween 60, Sodium Dodecyl Sulphate (SDS), dimethyl sulfoxide (DMSO), DAPI, FITC were purchased from Merck (Germany). Cholesterol (Chol) was purchased from Avanti (Alabama). The reproductive portion of *Kalanchoe daigremontiana*, a cultivar, was harvested from the Istanbul district in Turkey during the month of April in the year 2022. A total of 50 g of fresh leaf tissue was extracted using either 95% ethanol or water as solvents. The extraction process involved stirring the plant material with the solvent for 24 h at room temperature at a rotation speed of 250 revolutions per minute. The resulting solution was subsequently filtered (15–20 μm pore size) and the solvent was removed via rotary evaporation under vacuum conditions. The concentrated extract was then stored in a vial at 4 °C for further analysis (J. Stefanowicz-Hajduk et al., 2020).

2.2. Preparation of the phytoniosomes

Phytoniosomes were prepared using thin film hydration method, described by (Un et al., 2015). A 1:1 M ratio of Tween 60 and cholesterol was utilized in all experiments. A solution was prepared by dissolving 10 mM Tween 60, cholesterol, and 1.0 mg/mL of the plant extract in 2 mL of chloroform in a round bottom flask. The chloroform was then evaporated using a rotary evaporator (BUCHI, Switzerland), resulting in the formation of a thin film on the bottom of the flask. Then 2 mL PBS (pH 7.4, 60 °C) was added on thin film. Subsequently, the solution was subjected to vigorous vortexing (10 min) to obtain narrow-sized vesicles, followed by bath sonication for 30 min. Lastly, the samples were dialyzed against distilled water for 12 Hours at +4 °C. The preparation of FITC-added niosomes was performed in a similar manner, with the inclusion of 50 μg/mL of FITC in the PBS portion of the procedure.

2.3. Particle size and zeta potential

The particle size and the zeta potential of the phytoniosomes were evaluated using a Zetasizer (Malvern, UK). All samples were measured 3 times.

2.4. Chromatographic analysis

2.4.1. Quadrupole time-of-flight mass spectrometer (QTOF-MS)

For ionization of the chromatographic eluates, an Agilent 6550 iFunnel high-resolution Q-TOF-MS Accurate-Mass, equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface, was employed in negative and positive ion mode. The instrument was operated under the following conditions: drying gas flow, 14.0 L/min; nebulizer pressure, 35 psi; gas drying temperature, 290 °C; sheath gas temperature, 400 °C; sheath gas flow, nitrogen at 14 L/min; and nozzle voltage 1000 V. The spectra were collected in targeted MS/MS mode using a scan range of m/z 50 to 1800 for inducing MS/MS data collection, with a collision energy of 20 eV during analysis (Sevimli-Gur et al., 2021). Integration and data evaluation were performed using the MassHunter Workstation software, and the MassHunter METLIN and the Accurate Mass Personal Compound Database and Library (METLIN_AM_PCDL) were employed to detect phenolic compounds (Agilent Technologies, Santa Clara, CA, USA).

2.4.2. High-performance liquid chromatography (HPLC) system

Freshly prepared phytoniosomes were dialyzed against distilled water overnight to eliminate unencapsulated extract. Chlorogenic acid, the ester of caffeic acid and quinic acid, are well-known phenolic compounds found in Kalanchoe extracts widely (Bogucka-Kocka, Zidorn, Kasprzycka, Szymczak, & Szewczyk, 2018; Chernetskyy et al., 2018). Chlorogenic acid was used to determine Encapsulation Yield (EY) of phytoniosomes. The dialyzed phytoniosomes were disrupted with ethanol to obtain free components. An Agilent 1260 Infinity II system (Agilent Technologies Santa Clara, CA, USA) was used for liquid chromatographic analysis. Chromatographic column sample solutions were determined by Agilent a Poroshell 120 EC-C18 column (3.0 × 100 mm, particle size 2.7 μm). The mobile phase consisted of phosphoric acid (% 0,2): water (%79.8): acetonitrile (%20). The flow rate was 1 mL/min, and the automatic sample feeding room was 25 °C. Detection and quantification were carried out at 330 nm for Chlorogenic acid.

The EY is calculated according to the following equation (Eq. (1)).

EY (%) = [Chlorogenic acid in phytoniosomes / Chlorogenic acid in extract] × 100 (Eq.1)

2.5. Cell culture

MCF7 and HeLa cell lineswere obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). cells were grown in media containing 10% foetal bovine serum (FBS) and 1.0% penicillin/streptomycin at 5.0% CO_2 incubator at 37 °C under aseptic cell culture conditions.

2.5.1. Cytotoxicity assay

MTT is a water-soluble tetrazolium salt utilized in various cellular viability assays. When MTT enters living cells, it undergoes enzymatic reduction by the mitochondrial enzyme succinate dehydrogenase, resulting in the formation of insoluble purple formazan crystals. The intensity of the resulting colour is directly proportional to the number of viable cells present in the sample. MCF-7 and HeLa cell lines grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% foetal bovine serum (FBS) and 1.0% penicillin/streptomycin (P/S) used to determine *in vitro* toxic effect of plant extracts, niosomes and phytoniosomes 7 different doses (1, 2.5, 5, 10, 25 50 and 100 μg/mL) 3 different time periods (24, 48 and 72 h) (Guler et al., 2014). 4×10^3 cells/well were seeded in 96-well-tissue plates and cultivated 3 day at normal cell culture conditions until reaching %80 confluence. Afterwards, different dose of samples was added on cell with fresh medium and incubate at 3 different time period, the cells were incubated in 110 μL/well of a 10% solution of MTT in medium for 4 h after the removal of the medium. After time periods of incubation, 100 μL of a 0.1 mg/mL SDS solution (0.01 M HCl) was added to the cells. Samples measured at 570 nm using a Multiscan Go spectrophotometer (Thermo Fischer, USA).

2.5.2. Radiotherapy

For the application of radiotherapy (RT), cell irradiation was conducted as follows: 4×10^3 cells were seeded into 96 well plates and allowed to incubate overnight. The medium was subsequently removed, and the phytoniosomes (1 μg/mL) were added on cell and incubated for 2 h. After that cells were exposed to 4 Gy doses of radiation delivered by a linear accelerator (LINAC) (Elekta Versa HD- High-Definition Dynamic Radiosurgery). This procedure was previously described (Barlas et al., 2016). After irradiation, replaced to incubator at 37 °C and 5.0% CO2 for 72 h. Subsequently, cell viability was determined through the standard MTT assay as previously described.

2.5.3. Fluorescent microscopy

Fluorescent microscopy is based on the property of certain molecules called fluorophores, which absorb light at a specific wavelength and emit light at a longer wavelength. The cellular internalization of FITC labelled niosomes and phytoniosomes was evaluated using a DM:4000B microscope and a DFC7000T camera (LEICA, Germany). A total of 8×10^3 cells were seeded in a chamber slide and incubated for 48 h under standard cell culture conditions. Following the incubation period, the media was replaced with treatment media containing non-toxic (1 μg/mL) concentrations of the samples. The cells were then incubated for an additional 2 h before being washed twice with PBS. Subsequently, images were acquired using a fluorescent microscope under bright green and DAPI filter illumination (Barlas, Aydindogan, Arslan, Timur, & Yagci, 2019). These images were subsequently merged using the Image J software program.

2.6. Statistical analysis

The study presented data as averages with standard deviations (±SD). All statistical analyses were performed using a one-way ANOVA with a post-test of Tukey's multiple comparison test. The study repeated all experiments a minimum of three times. (P values < 0.05 = significant and P values < 0.01 or 0.001 = highly significant).

3. Results

3.1. Characterization and stability

The study encompassed an assessment of nanovesicle size, zeta potential, and short-term size-dependent stability a period of 30 days, providing valuable insights into the intricate relationship between these vesicular systems and cells. Following the extraction of kalanchoe and subsequent preparation of phytoniosomes (TW60: Chol: Kalanchoe), dynamic light scattering (DLS) and zeta potential measurements were conducted. The size of plain niosomes and phytoniosomes was determined as 95.9 ± 0.52 nm and 126.0 ± 5.14 nm, respectively. Moreover, the zeta potential of the vesicles was measured as −24 ± 4.0 mV and −19 ± 2.0 mV for plain niosomes and phytoniosomes, respectively, as presented in Table 1. Size-dependent stability assessments were performed using DLS, as depicted in Fig. 1, revealing a slight increase in the size of both plain niosomes and phytoniosomes after the 30-day period. Notably, no significant alteration was observed in the surface load measurements conducted during this process.

3.2. Ingredient analysis and Encapsulation Yield (EY)

All content analyses of the extracts were made with QTOF-MS and active ingredients were listed (Supp Table 1). In the light of the analysis, Chlorogenic acid, one of the major compounds, was used to calculate the encapsulation efficiency. Encapsulation Yield of phytoniosomes was obtained by HPLC with measurements of phenolic compounds (Chlorogenic acid). Prior to the samples were given to the Agilent 1260 Infinity II system, both the standard curves for the formed Chlorogenic acid and the linear equations of the curves were found as $y = 146.43x - 0.8057$. ($R^2 = 0.9993$) for Chlorogenic acid with linearity between 0.01 and 0.5 ppm. Result showed that, EY of phytoniosomes was detected as 78.15% for Chlorogenic acid. Also, The Fourier Transform Infrared (FT-IR) spectra of phytoniosome, determined on a Thermo Scientific Nicolet 6700 FT-IR in a wide range of 500–4000 cm-1 as illustrated in Supp figure 1.

3.3. Cell viability assay

Recently, there has been a growing interest in natural compounds and their potential as preparations for cancer treatment. Researchers have focused on studying the biologically active ingredients found in these compounds. In this context, nanovesicular carrier systems have emerged as important tools to enhance the uptake and efficient delivery of herbal agents with low biocompatibility to tumour cells. In this particular study, the objective was to investigate the anti-carcinogenic effects of phytoniosomes. To achieve this, the cytotoxic effects of seven different doses and three different time periods were assessed in two distinct cell lines. The experimental setup involved treating the cells with plain niosomes, plant extracts, and phytoniosomes at specific concentrations. The viability of the cells was evaluated after 24, 48, and

Table 1
Size distribution and zeta potential of plane niosomes and phytoniosomes.

	Size (nm)	Polydispersity Index	Zeta Potential (mV)
Plain Niosome	95.9 ± 0.52	0.154 ± 0.012	−24 ± 4.0
Phytoniosome	126.3 ± 5.14	0.245 ± 0.048	−19 ± 2.0

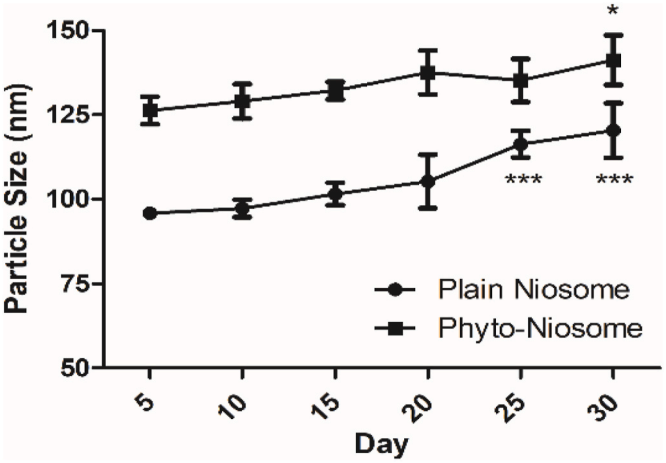


Fig. 1. The short-term stability of nanovesicles was assessed in a size-dependent manner, with the results being presented as the mean accompanied by the standard deviation (±SD). Statistically significant variations are indicated with asterisks (* denotes P < 0.05, *** denotes P < 0.001).

72 h using the MTT method. Fig. 2 illustrates the results obtained from the study. It was observed that phytoniosomes exhibited higher cytotoxicity in both cell lines. However, after 24 h of incubation, there was no significant difference between the cytotoxic effects of phytoniosomes and free extracts. In contrast, after 48 h, the results demonstrated that phytoniosomes reduced cell viability to 50,583 ± 3255 (P < 0,001) for HeLa cell line and 52,993 ± 1502 (P < 0,001) for MCF7 cell line at a concentration of 25 μg/mL. This critical concentration range decreased to 5 μg/mL after 72 h, 52,566 ± 2640 (P < 0,001) for HeLa cell line and 47,391 ± 3544 (P < 0,001) for MCF7 cell line. Furthermore, a dose of 100 μg/mL was found to induce cell death in nearly all cells of both tested cell lines.

3.4. Radiotherapy

The effectiveness of radiotherapy in the treatment of cancer is widely recognized in the medical community, with numerous studies demonstrating its efficacy in controlling cancer growth and improving patient outcomes. In the present study, the radio sensitizing effects of herbal compounds with cytotoxic properties in niosomal forms were investigated (Lin et al., 2012). The samples were first pre-incubated with cells for 2 h and then subjected to 4 Gy radiation using a linac. The results of the 72-h viability tests, as depicted in Fig. 3, showed that the cell

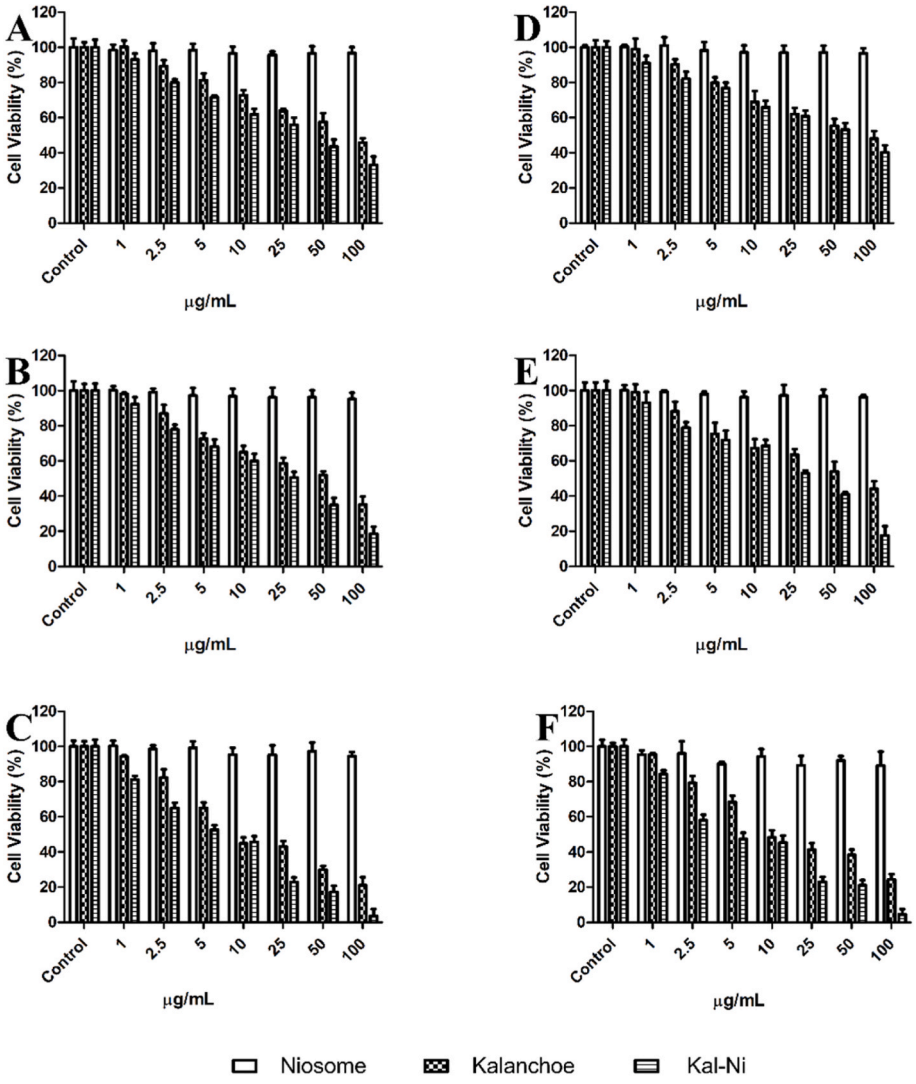


Fig. 2. Effect of plain niosomes, Kalanchoe extracts and Phytoniosomes on viability in HeLa (A, B, C) and MCF-7 (D, E, F) cell lines. 3 different time periods were used (A and D = 24 h B and E = 48 h C and F = 72 h). The mean accompanied by the standard deviation (±SD).

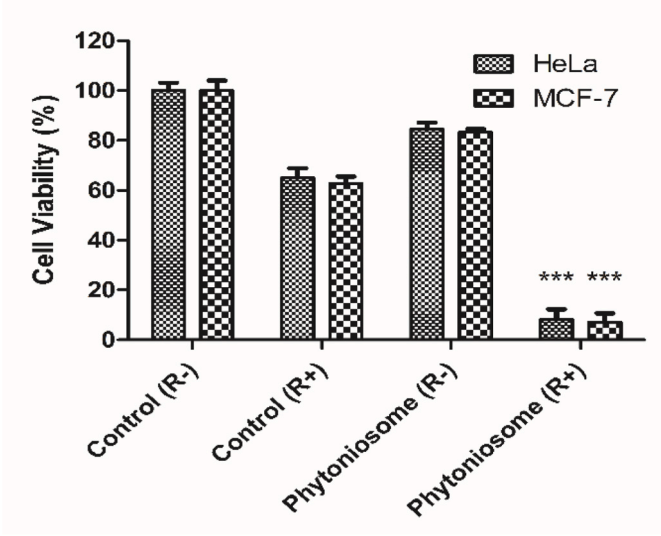


Fig. 3. MTT results for combined effect of Phytoniosomes and Radiotherapy. (R-) mean is no radiation and (R+) mean is 4 Gy Radiation. The mean accompanied by the standard deviation (±SD).

viability of the MCF-7 cell line was 62.709 ± 2.822 ($P < 0{,}001$) in the radiation-only group, whereas it was 65.042 ± 3.804 ($P < 0{,}001$) for the HeLa cell line. In the group treated with only phytoniosome (1 μg/mL), the viability for MCF-7 and HeLa cells was determined to be 84.261 ± 1.286 ($P < 0{,}01$) and 83.501 ± 2.622, ($P < 0{,}01$) respectively. Conversely, in the group where radiation and phytoniosome were applied concurrently, cell viability decreased significantly to 7.102 ± 3.51 ($P < 0{,}001$) for the MCF-7 cell line and to 8.183 ± 4.117 ($P < 0{,}001$) for the HeLa cell line, respectively.

3.5. Cell imaging

Fluorescent microscope images were taken to get more information about cell penetration capabilities and intracellular localization characteristics of the phytoniosomes. For this purpose, FITC-loaded carrier systems were treated with HeLa and MCF-7 cell lines for 2 h, then washed and DAPI dye was used to stain the nuclei of the cells, and the images were taken with a fluorescent microscope and overlapped with the Image-J program (Fig. 4). As a result, according to FITC dye, the phytoniosomes are concluded to easily penetrate into the cell and accumulate around the cytoplasm and there was no significant difference between HeLa and MCF-7 cell lines so this indicates that phytoniosomes did not show selectivity for these two cell lines.

4. Discussion

Critical parameters for effective nanoparticle performance in biological systems include size, surface charge, and stability. Encapsulation efficiency is a significant parameter for nanocarrier systems such as liposomes, niosomes, and polymeric micelles (Jain & Thareja, 2019). Size and surface charge analyses provide valuable insights into the characteristics of nanocarrier systems in both *in vitro* and in vivo settings. Cellular uptake of nanocarrier systems occurs through membrane fusion (Chernomordik & Kozlov, 2008; Yang et al., 2016), with surface charge playing a crucial role in this process. Charged liposomes demonstrate approximately 20-fold higher cellular uptake compared to uncharged liposomes (Düzgüneş & Nir, 1999). Furthermore, negatively charged vesicles exhibit greater encapsulation than positively charged ones (Tang et al., 2021). Additionally, nanovesicles around 100 nm demonstrate optimal properties as the intracellular uptake of 60 nm vesicles and the stability of vesicles around 200 nm are comparatively low (Bozzuto & Molinari, 2015). Previous studies were demonstrated that, Vesicle sizes are in the range of 400–600 nm when prepared by different methods, but these sizes can approach around 100 nm with the extrusion method (Nowroozi, Almasi, Javidi, Haeri, & Dadashzadeh, 2018). In addition, the polydispersity index values of vesicles prepared by the extruder method are lower than other studies (Demir et al., 2014). This is due to the controlled adjustment of the dimensions using filters with specific pore diameters. When plant extracts were added in formulation, a slight positive change in the zeta potential of the vesicles was

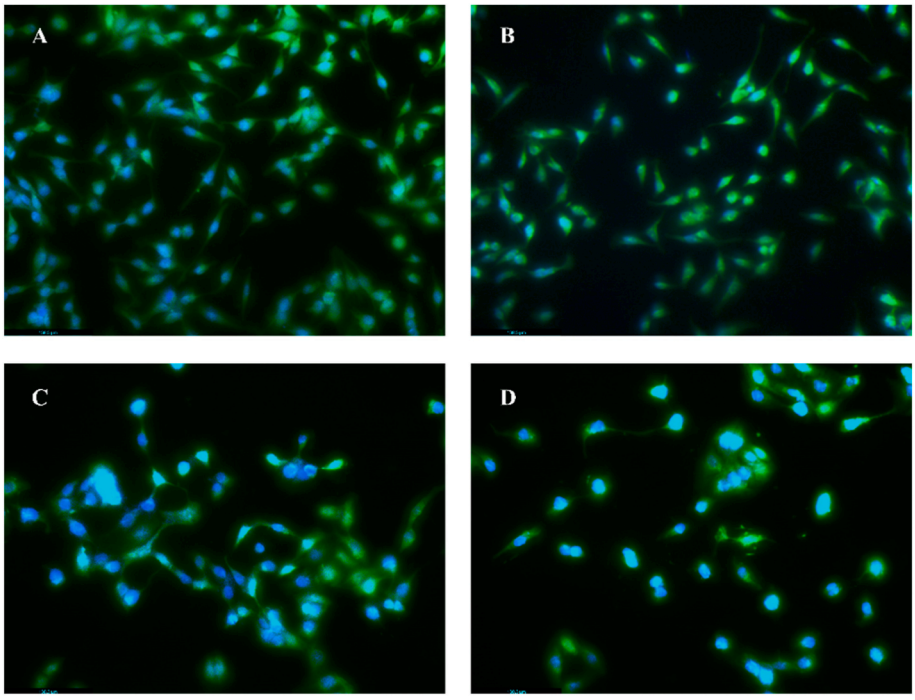


Fig. 4. Fluorescence microscope images of the FITC and DAPI labelled phytoniosomes (A) plane noisome (B) with HeLa cells. Fluorescence microscope images of the FITC and DAPI labelled phytoniosomes (C) plane noisome (D) with MCF-7 cells. The magnification factor of the microscope in use is set at a twenty-fold increase.

observed. This shift may be the result of the adsorption of positively charged flavylium cations on the liposome surface (Guldiken, Gibis, Boyacioglu, Capanoglu, & Weiss, 2021). According to stability test slight growth of both plain niosomes and phytoniosomes was observed, which may be due to vesicle fission or aggregation (Szabová, Mišík, Havlíková, Lízal, & Mravec, 2021). Particles with low charge density or lacking charge exhibit a propensity to aggregate over time, leading to the formation of clusters. In contrast, particles possessing higher charges display repulsive forces, impeding aggregation and ensuring the stability of particle suspensions. Generally, zeta potentials below −30 mV or above 30 mV are considered adequate to maintain interparticle repulsion and the overall stability of particle suspensions (Tenchov, Bird, Curtze, & Zhou, 2021).

HPLC device is used in the analysis of the main components of plant extracts because of its high sensitivity and high accuracy. In previous studies, very variable results can be seen in the measurement of the encapsulation efficiency of biologically active material by vesicular systems, and the results in this study are average values (Demir et al., 2014; Katuwavila, Perera, Karunaratne, Amaratunga, & Karunaratne, 2016; Zaremba-Czogalla et al., 2020). Such a wide range of results is due to the diversity of materials used to construct the vesicular systems and the differences in synthesis methods and conditions (Guimarães, Cavaco-Paulo, & Nogueira, 2021).

According to MTT assay, plain niosomes did not have a statistically significant effect on cell viability at all time periods because of its non-toxic components at low doses (Limongi et al., 2021). The fact that phytoniosomes are more effective than free plant extracts are due to that niosomes increase the biocompatibility of the extracts and provide better uptake into the cell thanks to their solubility, size and surface charge (Ben-Shabat, Yarmolinsky, Porat, & Dahan, 2020; Pandey, Bhairam, Shukla, & Gidwani, 2021).

Radiotherapy plays a vital role in the treatment of cancer, employing ionizing radiation to target and eliminate malignant tumours. The integration of radiotherapy with radiosensitizer, is a promising employed to achieve comprehensive cancer management without side effects. The results show that, the combined therapy model is more effective than the use of the two treatment modalities individually. Although there is limited literature on the radio sensitizing effects of anticarcinogenic herbal extracts, the mechanism of action remains elusive. Numerous studies have indicated that plant-derived porphyrins, flavonoids, and other secondary metabolites exhibit radiosensitivity (Tiwari & Mishra, 2020; Zhou, Liang, & Dai, 2016). Also, Recent evidence has put forward the importance of cancer cells' mitochondrial metabolism in response to radiotherapy. It has been demonstrated that reducing the cancer cells' mitochondrial biogenesis can increase the radiotherapy efficacy (Taghizadeh-Hesary, Houshyari, & Farhadi, 2023). Recently, Stefanowicz-Hajduk et al. (2022) demonstrated that *Kalanchoe daigremontiana* can impair mitochondrial membrane potential of cancer cells. Therefore, one may conclude that a possible mechanism for the radiosensitizing effect of *Kalanchoe daigremontiana* is its negative effects on cancer cells mitochondria. On the other hand, there are reports of herbal extracts exhibiting radioprotection (Goel et al., 2004), and it has been speculated that the increased cytotoxicity in the current study may also be due to the increased efficacy of phytoniosomes in cell lines with a weakened defense system after radiation exposure.

Fluorescence imaging, an emerging modality in the field of cancer treatment, involves the utilization of fluorescent probes to enable visualization and detection of cancerous lesions at the molecular and cellular levels. In this particular study, the fluorophore FITC was employed as a probe, and it was observed that nanovesicles exhibited accumulation within the cytoplasm. Prior studies have suggested that charged liposomal and niosomal formulations have the ability to traverse the cell membrane via micropinocytosis (Kang, Jang, & Ko, 2017), subsequently concentrating within the cytoplasmic region (Aneja et al., 2019; Bourbour et al., 2022). Additionally, the comparable cytotoxic effects of phytoniosomes against the two investigated cell lines indicate a mutual reinforcement of the findings from both studies.

5. Conclusion

In the present study, the extracts of a traditionally used and well-known plant were loaded into vesicular systems for the first time, and the effects of these phytoniosomes on living systems were evaluated over an extended period of 72 h. Then Radiotherapy studies were carried out using LINAC to understand the combined effects with radiation. Characterization experiments revealed that vesicular systems with an average size of around 100 nm and high encapsulation efficiency were obtained using Tween 60 as the surfactant. In vitro cytotoxicity investigations were executed to ascertain the influence of phytoniosomes on the cytotoxic potential of Kalanchoe extracts. The results of these studies indicated that phytoniosomes significantly enhanced the cytotoxic activity of the Kalanchoe extracts. Specifically, the IC50 value for the HeLa cell line at the end of 72 h was determined to be 16.5633 μg/mL, and for the MCF-7 cell line, the IC50 value was 18.7739 μg/mL for the same period. According to the results of radiotherapy, it was observed that the use of phytoniosomes together with radiation significantly increased the cytotoxic effect. Fluorescence imaging studies were performed to gain further insight into the interactions of vesicular systems with cells, and it was observed that the vesicles were able to penetrate cells and accumulate in the cytoplasm. In conclusion, the biotechnological modification of Kalanchoe extracts has been found to enhance their efficiency and has increased their potential as an anticancer drug in the field. This highlights the significance of modifications for the biocompatibility of natural compounds in similar studies. The outcome of the radiotherapy studies substantiates the premise that the efficacy of Kalanchoe extracts is significantly enhanced when used in tandem with radiotherapy, thereby indicating their increased potential as an anticancer agent. This formulation has the potential to be used as a tool for overcoming dose-dependent limitations in cancer therapy. On the other hand, it shows that due to the radiosensitive metabolites in herbal ingredients, their use as radioprotective should be investigated in detail and careful attention should be paid in further investigations.

Declaration of competing interest

There are no conflicts to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jrras.2023.100612.

References

Alfouzan, A. F. (2021). Radiation therapy in head and neck cancer. *Saudi Medical Journal, 42*(3), 247. https://doi.org/10.15537/smj.2021.42.3.20210660

Almeida, B., Nag, O. K., Rogers, K. E., & Delehanty, J. B. (2020). Recent progress in bioconjugation strategies for liposome-mediated drug delivery. *Molecules, 25*(23), 5672. https://doi.org/10.3390/molecules25235672

Amalraj, A., Jacob, J., Varma, K., & Gopi, S. (2020). Preparation and characterization of liposomal β-Caryophyllene (Rephyll) by nanofiber weaving technology and its effects on delayed onset muscle soreness (DOMS) in humans: A randomized, double-blinded, crossover-designed, and placebo-controlled study. *ACS Omega, 5*(37), 24045–24056. https://doi.org/10.1021/acsomega.0c03456

Ameri, A., Heydarirad, G., Choopani, R., Poshtmahi, S., Ameri, P., Talebi, F., … Taghizadeh-Hesary, F. (2023). Sumac-rose water mouthwash versus benzydamine to prevent radiation-induced oral mucositis in head and neck cancers: A phase II randomized trial. *Journal of Cancer Research and Clinical Oncology*, 1–13. https://doi:10.1007/s00432-023-04687-1.

Aneja, R., Grigoletto, A., Nangarlia, A., Rashad, A. A., Wrenn, S., Jacobson, M. J., et al. (2019). Pharmacokinetic stability of Macrocyclic peptide triazole HIV-1 inactivators alone and in liposomes. *Journal of Peptide Science, 25*(4), e3155. https://doi.org/10.1002/psc.3155

Barlas, F. B., Aydindogan, E., Arslan, M., Timur, S., & Yagci, Y. (2019). Gold nanoparticle conjugated poly (p-phenylene-β-cyclodextrin)-graft-poly (ethylene glycol) for theranostic applications. *Journal of Applied Polymer Science, 136*(12), Article 47250. https://doi.org/10.1002/app.47250

Barlas, F. B., Demir, B., Guler, E., Senisik, A. M., Arican, H. A., Unak, P., et al. (2016). Multimodal theranostic assemblies: Double encapsulation of protoporphyrine-IX/Gd 3+ in niosomes. *RSC Advances, 6*(36), 30217–30225. https://doi.org/10.1039/C5RA26737D

Ben-Shabat, S., Yarmolinsky, L., Porat, D., & Dahan, A. (2020). Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. *Drug delivery and translational research, 10*(2), 354–367. https://doi.org/10.1007/s13346-019-00691-6

Bogucka-Kocka, A., Zidorn, C., Kasprzycka, M., Szymczak, G., & Szewczyk, K. (2018). Phenolic acid content, antioxidant and cytotoxic activities of four Kalanchoë species. *Saudi Journal of Biological Sciences, 25*(4), 622–630. https://doi.org/10.1016/j.sjbs.2016.01.037

Bourbour, M., Khayam, N., Noorbazargan, H., Yaraki, M. T., Lalami, Z. A., Akbarzadeh, I., & Tan, Y. N. (2022). Evaluation of anti-cancer and anti-metastatic effects of folate-PEGylated niosomes for co-delivery of letrozole and ascorbic acid on breast cancer cells. *Molecular Systems Design & Engineering, 7*(9), 1102–1118.

Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine, 10*, 975. https://doi.org/10.2147/IJN.S68861

Chernetskyy, M., Woźniak, A., Skalska-Kamińska, A., Żuraw, B., Blicharska, E., Rejdak, R., … Weryszko-Chmielewska, E. (2018). Structure of leaves and phenolic acids in Kalanchoë daigremontiana Raym.-Hamet & H. Perrier. *Acta Sci. Polonorum Hortorum Cultus, 17*, 137–155. https://doi.org/10.24326/asphc.2018.4.13

Chernomordik, L. V., & Kozlov, M. M. (2008). Mechanics of membrane fusion. *Nature Structural & Molecular Biology, 15*(7), 675–683. https://doi.org/10.1038/nsmb.1455

Demir, B., Barlas, F., Guler, E., Gumus, P., Can, M., Yavuz, M., … Timur, S. (2014). Gold nanoparticle loaded phytosomal systems: Synthesis, characterization and in vitro investigations. *RSC Advances, 4*(65), 34687–34695. https://doi.org/10.1039/C4RA05108D

Deng, X., Wu, H., Gao, F., Su, Y., Li, Q., Liu, S., et al. (2017). Brachytherapy in the treatment of breast cancer. *International Journal of Clinical Oncology, 22*, 641–650. https://doi.org/10.1007/s10147-017-1155-5

Dunlop, A., Mitchell, A., Tree, A., Barnes, H., Bower, L., Chick, J., … McNair, H. (2020). Daily adaptive radiotherapy for patients with prostate cancer using a high field MR-linac: Initial clinical experiences and assessment of delivered doses compared to a C-arm linac. *Clinical and translational radiation oncology, 23*, 35–42. https://doi.org/10.1016/j.ctro.2020.04.011

Düzgüneş, N., & Nir, S. (1999). Mechanisms and kinetics of liposome–cell interactions. *Advanced Drug Delivery Reviews, 40*(1–2), 3–18. https://doi.org/10.1021/acscentsci.6b00172

Goel, H. C., Prasad, J., Singh, S., Sagar, R. K., Agrawala, P. K., Bala, M., … Dogra, R. (2004). Radioprotective potential of an herbal extract of Tinospora cordifolia. *Journal of Radiation Research, 45*(1), 61–68. https://doi.org/10.1269/jrr.45.61

Goh, W. J., Lee, C. K., Zou, S., Woon, E. C., Czarny, B., & Pastorin, G. (2017). Doxorubicin-loaded cell-derived nanovesicles: An alternative targeted approach for anti-tumor therapy. *International Journal of Nanomedicine, 12*, 2759. https://doi.org/10.2147/IJN.S131786

Guimarães, D., Cavaco-Paulo, A., & Nogueira, E. (2021). Design of liposomes as drug delivery system for therapeutic applications. *International Journal of Pharmaceutics, 601*, Article 120571. https://doi.org/10.1016/j.ijpharm.2021.120571

Guldiken, B., Gibis, M., Boyacioglu, D., Capanoglu, E., & Weiss, J. (2021). Ascorbic acid-induced degradation of liposome-encapsulated acylated and non-acylated anthocyanins of black carrot extract. *Journal of the Science of Food and Agriculture, 101*(13), 5707–5714. https://doi.org/10.1002/jsfa.11225

Guler, E., Barlas, F. B., Yavuz, M., Demir, B., Gumus, Z. P., Baspinar, Y., … Timur, S. (2014). Bio-active nanoemulsions enriched with gold nanoparticle, marigold extracts and lipoic acid: In vitro investigations. *Colloids and Surfaces B: Biointerfaces, 121*, 299–306. https://doi.org/10.1016/j.colsurfb.2014.05.026

Hajizadeh, M. R., Parvaz, N., Barani, M., Khoshdel, A., Fahmidehkar, M. A., Mahmoodi, M., et al. (2019). Diosgenin-loaded niosome as an effective phytochemical nanocarrier: Physicochemical characterization, loading efficiency, and cytotoxicity assay. *Daru Journal of Pharmaceutical Sciences, 27*(1), 329–339. https://doi.org/10.1007/s40199-019-00277-0

Handjani-Vila, R., Ribier, A., Rondot, B., & Vanlerberghie, G. (1979). Dispersions of lamellar phases of non-ionic lipids in cosmetic products. *International Journal of Cosmetic Science, 1*(5), 303–314. https://doi.org/10.1111/j.1467-2494.1979.tb00224.x

Hejazi, E., Nasrollahzadeh, J., Fatemi, R., Mohamadi, L. B. Y., Saliminejad, K., Amiri, Z., & Idali, F. (2015). Effects of combined soy isoflavone extract and docetaxel treatment on murine 4T1 breast tumor model. *Avicenna Journal of Medical Biotechnology, 7*(1), 16.

Hsieh, Y.-J., Yang, M.-Y., Leu, Y.-L., Chen, C., Wan, C.-F., Chang, M.-Y., et al. (2012). Kalanchoe tubiflora extract inhibits cell proliferation by affecting the mitotic apparatus. *BMC Complementary and Alternative Medicine, 12*(1), 1–10. https://doi.org/10.1186/1472-6882-12-149

Jacob, S., Nair, A. B., & Al-Dhubiab, B. E. (2017). Preparation and evaluation of niosome gel containing acyclovir for enhanced dermal deposition. *Journal of Liposome Research, 27*(4), 283–292. https://doi.org/10.1080/08982104.2016.1224897

Jain, A. K., & Thareja, S. (2019). In vitro and in vivo characterization of pharmaceutical nanocarriers used for drug delivery. *Artificial Cells, Nanomedicine, and Biotechnology, 47*(1), 524–539. https://doi.org/10.1080/21691401.2018.1561457

Kang, J. H., Jang, W. Y., & Ko, Y. T. (2017). The effect of surface charges on the cellular uptake of liposomes investigated by live cell imaging. *Pharmaceutical Research, 34*, 704–717.

Katuwavila, N. P., Perera, A., Karunaratne, V., Amaratunga, G. A., & Karunaratne, D. (2016). Improved delivery of caffeic acid through liposomal encapsulation, 2016 *Journal of Nanomaterials*. https://doi.org/10.1155/2016/9701870.

Limongi, T., Susa, F., Marini, M., Allione, M., Torre, B., Pisano, R., et al. (2021). Lipid-based nanovesicular drug delivery systems. *Nanomaterials, 11*(12), 3391. https://doi.org/10.3390/nano11123391

Lin, C., Yu, Y., Zhao, H.-g., Yang, A., Yan, H., & Cui, Y. (2012). Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiotherapy & Oncology, 104*(3), 395–400. https://doi.org/10.1016/j.radonc.2011.10.023

Nirmala, M. J., Samundeeswari, A., & Sankar, P. D. (2011). Natural plant resources in anti-cancer therapy-A review. *Research in Plant Biology, 1*(3), 1–14.

Nowroozi, F., Almasi, A., Javidi, J., Haeri, A., & Dadashzadeh, S. (2018). Effect of surfactant type, cholesterol content and various downsizing methods on the particle size of niosomes. *Iranian Journal of Pharmaceutical Research: IJPR, 17*(Suppl2), 1.

Pandey, R., Bhairam, M., Shukla, S. S., & Gidwani, B. (2021). Colloidal and vesicular delivery system for herbal bioactive constituents. *Daru Journal of Pharmaceutical Sciences, 29*(2), 415–438. https://doi.org/10.1007/s40199-021-00403-x

Saravanan, V., Murugan, S., & Kumaravel, T. (2020). Genotoxicity studies with an ethanolic extract of Kalanchoe pinnata leaves. *Mutation Research, Genetic Toxicology and Environmental Mutagenesis, 856*, Article 503229. https://doi.org/10.1016/j.mrgentox.2020.503229

Sevimli-Gur, C., Gezgin, Y., Oz, A., Al Sharqi, S., Gumus, Z. P., & Dunford, N. T. (2021). Biological activity of the extracts from pecan shelling industry byproducts. *Transactions of the ASABE, 64*(3), 869–877. https://doi.org/10.13031/trans.14439

Stefanowicz-Hajduk, J., Hering, A., Gucwa, M., Hałasa, R., Soluch, A., Kowalczyk, M., … Ochocka, R. (2020). Biological activities of leaf extracts from selected Kalanchoe species and their relationship with bufadienolides content. *Pharmaceutical Biology, 58*(1), 732–740. https://doi.org/10.1080/13880209.2020.1795208

Stefanowicz-Hajduk, J., Hering, A., Gucwa, M., Sztormowska-Achranowicz, K., Kowalczyk, M., Soluch, A., et al. (2022). An in vitro anticancer, antioxidant, and phytochemical study on water extract of kalanchoe daigremontiana Raym.-Hamet and H. Perrier. *Molecules, 27*(7), 2280. https://doi.org/10.3390/molecules27072280

Stojanović, G. S., Jovanović, S.Č., & Zlatković, B. K. (2015). Distribution and taxonomic significance of secondary metabolites occurring in the methanol extracts of the Stonecrops (Sedum L., Crassulaceae) from the Central Balkan Peninsula. *Natural Product Communications, 10*(6). https://doi.org/10.1177/1934578X1501000637

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 71*(3), 209–249. https://doi.org/10.3322/caac.21660

Szabová, J., Mišík, O., Havlíková, M., Lízal, F., & Mravec, F. (2021). Influence of liposomes composition on their stability during the nebulization process by vibrating mesh nebulizer. *Colloids and Surfaces B: Biointerfaces, 204*, Article 111793. https://doi.org/10.1016/j.colsurfb.2021.111793

Taghizadeh-Hesary, F., Houshyari, M., & Farhadi, M. (2023). Mitochondrial metabolism: A predictive biomarker of radiotherapy efficacy and toxicity. *Journal of Cancer Research and Clinical Oncology*, 1–23. https://doi.org/10.1007/s00432-023-04592-7.

Tang, J., Rakshit, M., Chua, H. M., Darwitan, A., Nguyen, L. T., Muktabar, A., … Ng, K. W. (2021). Liposome interaction with macrophages and foam cells for atherosclerosis treatment: Effects of size, surface charge and lipid composition. *Nanotechnology, 32*(50), Article 505105. https://doi.org/10.1088/1361-6528/ac2810

Taylor, C., & Kirby, A. (2015). Cardiac side-effects from breast cancer radiotherapy. *Clinical Oncology, 27*(11), 621–629. https://doi.org/10.1016/j.clon.2015.06.007

Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q. (2021). Lipid nanoparticles─ from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano, 15*(11), 16982–17015. https://doi.org/10.1021/acsnano.1c04996

Tiwari, P., & Mishra, K. P. (2020). Flavonoids sensitize tumor cells to radiation: Molecular mechanisms and relevance to cancer radiotherapy. *International Journal of Radiation Biology, 96*(3), 360–369. https://doi.org/10.1080/09553002.2020.1694193

Un, R. N., Barlas, F. B., Yavuz, M., Ag Seleci, D., Seleci, M., Gumus, Z. P., … Coskunol, H. (2015). Phyto-niosomes: In vitro assessment of the novel nanovesicles containing marigold extract. *International Journal of Polymeric Materials and Polymeric Biomaterials, 64*(17), 927–937. https://doi.org/10.1080/00914037.2015.1030663

Wei, X.-Q., & Ba, K. (2020). Construction a long-circulating delivery system of liposomal curcumin by coating albumin. *ACS Omega, 5*(27), 16502–16509. https://doi.org/10.1021/acsomega.0c00930

Yang, J., Bahreman, A., Daudey, G., Bussmann, J., Olsthoorn, R. C., & Kros, A. (2016). Drug delivery via cell membrane fusion using lipopeptide modified liposomes. *ACS Central Science, 2*(9), 621–630. https://doi.org/10.1021/acscentsci.6b00172

Yu, L., Deng, Z., Liu, L., Zhang, W., & Wang, C. (2020). Plant-derived nanovesicles: A novel form of nanomedicine. *Frontiers in Bioengineering and Biotechnology, 8*, Article 584391. https://doi.org/10.3389/fbioe.2020.584391

Zaremba-Czogalla, M., Jaromin, A., Sidoryk, K., Zagórska, A., Cybulski, M., & Gubernator, J. (2020). Evaluation of the in vitro cytotoxic activity of caffeic acid derivatives and liposomal formulation against pancreatic cancer cell lines. *Materials, 13*(24), 5813. https://doi.org/10.3390/ma13245813

Zhang, Z., Lu, M., Chen, C., Tong, X., Li, Y., Yang, K., … Qin, L. (2021). Holo-lactoferrin: The link between ferroptosis and radiotherapy in triple-negative breast cancer. *Theranostics, 11*(7), 3167. https://doi: 10.7150/thno.52028.

Zhou, Y., Liang, X., & Dai, Z. (2016). Porphyrin-loaded nanoparticles for cancer theranostics. *Nanoscale, 8*(25), 12394–12405. https://doi.org/10.1039/C5NR07849K